Histopathology Reporting

Guidelines for Surgical Cancer

David P. Boyle
Derek C. Allen
Editors

Fourth Edition

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ISBN 978-3-030-27827-4 ISBN 978-3-030-27828-1 (eBook)
https://doi.org/10.1007/978-3-030-27828-1

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

To Maria, Sophia, Eva and Theo.

Preface to the Fourth Edition

Since the previous edition, demand for cellular pathology services continues to increase annually whilst the availability of consultant pathologists has not kept pace. Increasing pressures are also associated with more complicated requirements relating to reporting: this, in turn, resulting from the central role that pathology plays in guiding patient management through accurate categorisation of disease entities and provision or prognostic and therapy predictive information. Whilst a seemingly inexorable challenge faced by the pathologist, it is also a time of fascinating development that has placed pathology at the centre of patient management.

The aim of the fourth edition of this text is to continue to ensure consistency of approach in specimen examination and diagnosis for consultant and trainee pathologists. The standardised approach to various cancer types is built upon to reflect the numerous updates across many systems. Whilst it is acknowledged that a cohort of general pathologists continues to report, subspecialisation is an ever increasing trend. Since the third edition, TNM8 has been published and the WHO classifications of tumours continue to be revised. Keeping up to date requires a great deal of time and a sustained approach that is difficult to maintain across several subspecialist areas. As such, this edition is enhanced by subspecialist pathologist co-authorship with updates across the various chapters. As well as presenting information and advances relating to approach to diagnosis, use of immunohistochemistry and molecular techniques, it is intended that the knowledge and experience gained through reporting a large volume of cases in a specific area, attendance at specialist meetings and discussion with clinical colleagues is also imparted in the following pages. The text is not intended to be an encyclopaedic tome but more an aide memoire and helpful guide to everyday reporting written by specialists and applicable to both specialist and general reporting.

The use of illustrations from Wittekind C, Greene L, Hutter RVP, Klimfinger M, Sobin LH. TNM Atlas: Illustrated Guide to the TNM Classification of Malignant Tumours, 5th edition. Springer-Verlag. Berlin, Heidelberg, 2005 is very gratefully acknowledged.

I would like to express my gratitude to my co-editor Dr. Derek Allen for allowing me the opportunity to edit and update the text, and I wish him a long and happy retirement. I am indebted to my pathology colleagues for imparting their expert, specialist input and collective wisdom in the various chapters. Special thanks are given to Geraldine Boyle for invaluable help in image production. Appreciation and thanks are also expressed to Wyndham Hacket

Pain, Prakash Marudhu, Manika Power and all the staff at Springer. Greatest thanks are given to my wife Maria and our kids Sophia, Eva and Theo for tolerating my long absences at the computer.

Belfast, UK David P. Boyle

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Introduction

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Histopathology reports on surgical cancer specimens are becoming increasingly complex for many reasons. With closer clinicopathological correlation and the use of novel immunohistochemical and molecular techniques, new entities and classifications of tumour emerge that are linked to prognosis and are predictive of response to various treatment modalities. Increasingly the surgical oncologist wants tissue biopsy proof of cancer diagnoses so that patients may be recruited to suitable treatment protocols and clinical trials. Multiple factors are required to produce histopathologic reports including assessment of prognostic indicators such as tumour grade, extent of organ spread, relationship to primary excision margins, lymph node and vascular spread. This multitude of required information acknowledges the central role of pathology in patient management and affirms the need for precision. Broadly, tumours must be typed, graded, staged and assessed for completeness of excision and presence in lymphovascular channels: in short, an assessment of tumour aggressiveness and extent. Accurate classification and information on tumour stage and prognosis requires increased time and detail in surgical pathology dissection and reporting. These necessary, but stringent demands are met by diagnostic surgical pathologists with varying degrees of success and standards of reporting. Maintaining high standards in reporting and ensuring that the appropriate details are provided is facilitated by the use of proforma driven standardised reporting and referral to standardised cancer datasets.

Standardised cancer datasets not only itemise core and non-core factors relevant to patient management, but also include key audit criteria as a means to improving quality standards. Examples include for colorectal cancer the mean lymph node harvest and percentage case involvement of the serosa and extramural vessels; expected rate of positivity in melanoma sentinel lymph node samples; expected rate of HER2 positivity in breast cancer cases. This approach in the United Kingdom is encouraged by the Royal College of Pathologists peer-reviewed and updated Cancer Datasets which are readily available on-line. Tissue Pathways for non-cancer specimens are also published. Both are available at https://www.rcpath.org/profession/guidelines/cancer-datasets-and-tissue-pathways.html. It also forms the basis of an international collaboration for standardised cancer reporting referencing the College of American Pathologists Cancer Protocols and Checklists (https://www.cap.org/protocols-and-guidelines/cancer-reporting-tools/cancer-protocol-templates) and the Royal College of Pathologists of Australasia Structured Reporting Cancer Protocols (https://www.rcpa.edu.au/Library/Practising-Pathology/Structured-Pathology-Reporting-of-Cancer/Cancer-Protocols). Furthermore the UK Royal

D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_1

College of Pathologists has published a series of Key Performance Indicators in Pathology (available at https://www.rcpath.org/profession/quality-improvement/kpis-for-laboratory-services.html) to enhance and provide a metric assessment of quality standards in the end-to-end laboratory service. They include timeframe and percentage achievement targets for availability and timeliness of clinical advice, participation in multidisciplinary meetings, coding of histopathology reports, use of cancer biopsy and resection report proformas, documentation of second opinions, critical results communication, reporting turnaround times, monitoring of outstanding reports, appraisal, continuing professional development, participation in appropriate interpretive EQA schemes, user satisfaction surveys, teaching, training, supervision and succession planning. This raft of standards are enabling ISO 15189 process focused CPA UK (Ltd.) laboratory accreditation to evolve by developing a more holistic patient/outcome based approach. Along with contemporary guidelines on staffing and workload levels they also form a basis for annual medical staff appraisal and revalidation in the UK. Similarly the Royal College of Pathologists has a code of practice for histopathologists and histopathology services.

From the pathologist's point of view, standard reports act as an important aide-memoire for the inclusion of necessary data and audit shows that quality standards of information increase accordingly. Also, once the pathologist is familiarised with them such reports are relatively time-efficient to dictate and transcribe. Standardised reports integrate well with both traditional secretarial transcription as well as voice recognition dictation systems. The referring clinician can extract from them the relevant data with ease and cancer registries can be facilitated—supplemented by automated download and the capacity for search of key audit criteria if the database is suitably computerised.

The approach taken herein is aimed at fostering the use of standard format reports in surgical cancer. The headings used are common to many cancers, and may be used as an aide memoire. The end product is concise, clear and relevant to patient management. The format is:

1. Gross Description
   - Specimen: description
   - Tumour:
     - Site
     - Size
     - Appearance
     - Edge
2. Histological type
3. Differentiation/grade
4. Extent of local tumour spread
5. Lymphovascular invasion
6. Lymph nodes
7. Excision margins
8. Other pathology
9. Other malignancy

These criteria are chosen for the following reasons:

Gross Description

Specimen

- *Specimen type*; *biopsy or resection*. Full standard format reports are most relevant to resection specimens although the principles and abridged forms are applicable to biopsies. Sometimes a resection is more conveniently reported as free text, or, in standard format but requiring clarification in the further comments section. If dictated as free text care must be taken to include the required diagnostic and prognostic parameters. Biopsy reports should at least comment on the following (if the data are available): tumour point of origin, type of cancer, differentiation or grade, extent of epithelial or subepithelial

spread, adjacent dysplasia or carcinoma in situ and involvement of lymphovascular channels. The proportion of tissue involved by tumour can be useful, e.g. prostate cancer. It is important epidemiologically that cancer registries can distinguish between biopsy and resection specimens to avoid duplication of statistical data leading to overestimates of cancer incidence and prevalence. This can be achieved by unique patient identification and careful indexing of 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 exfoliative or fine needle aspiration cytology, radiological imaging and serum marker levels (e.g. prostate specific antigen, PSA).

- Specimen type also has implications for excision margins and clinical adjuvant treatment and follow up, e.g. breast sparing excision biopsy versus mastectomy, diathermy snare polypectomy versus colonic resection.
- *Specimen weight and size*. This may also be an indicator of the underlying pathology, e.g. primary adrenal cortical neoplasms >50 g are usually carcinoma rather than adenoma, and abundant vesicular uterine curettings up to 100 g suggests complete hydatidiform mole with subsequent potential for persistent trophoblastic disease and choriocarcinoma. In other situations these parameters may be less relevant routinely but are often included. They may prove useful to correlate with clinical notes should a clinical or specimen discrepancy arise.

Tumour

Site

Location of tumour within the epithelium or stromal tissue can often give clues as to its nature. Mucous membrane lesions are often primary and epithelial or sometimes lymphoid in character. Mural lesions may be primary and mesenchymal or, similar to serosal disease, secondary and extrinsic. Site dictates which adjacent tissues are involved by direct spread (e.g. cervix carcinoma—ureter) and can indicate variable tumour differentiation and prognosis within a given structure (e.g. multifocal neoplasia within the urinary tract). It can also be used as an audit tool to monitor resection rates as in anterior resection versus abdominoperineal resection for rectal carcinoma. It can influence the diagnosis, e.g. epiphyseal versus diaphyseal bone tumours, renal pelvis (transitional cell) carcinoma versus renal cortical (clear cell) carcinoma. Laterality (right or left) is obviously extremely important in patient management. Some cancers also have a tendency for multifocal growth, e.g. transitional cell carcinoma of the urinary tract or thyroid papillary carcinoma.

Size

Size influences the diagnosis (gastrointestinal stromal tumours >5 cm are more likely to be malignant) and the prognosis and influences subsequent patient management and treatment options (malignant melanoma: ≤1 mm Breslow depth—pT1, >1–2 mm—pT2; sarcoma: prognosis relates to tumour size, extent of disease and location; breast carcinoma: Nottingham Prognostic Index = 0.2 × size (cm) + grade + lymph node stage, Adjuvant! Online). Gross measurements should ideally be made on the fresh tissue and checked against the histological slide allowing for tissue shrinkage with fixation and processing (e.g. 30% for oesophageal resections). Clinical measurements may be preferable but are not consistently supplied with specimens. Small measurements are done with a dome magnifier, loupe, the stage micrometer, eyepiece graticule or measured digitally. Whichever method is employed, it should be calibrated and reproducible. Guidelines are given (National Health Service Breast Screening Programme) to distinguish between invasive tumour size and whole tumour size to include in situ disease.

Appearance

Characteristic appearances are:

Luminal and polypoid
- Oesophageal spindle cell carcinoma.
- Uterine malignant mixed mesodermal tumour (carcinosarcoma).
- Gastrointestinal multiple lymphomatous polyposis or familial adenomatous polyposis.

Nodular
- Carcinoid tumour of bronchus or ileum.
- Malignant melanoma.

Sessile/plaque
- Early gastrointestinal carcinoma (stomach, oesophagus, colorectum).
- Lymphoma of gut.
- High-grade bladder carcinoma.

Ulcerated
- Usual carcinoma morphology.

Fleshy
- Malignant lymphoma, seminoma.

Pigmented
- Malignant melanoma.

Haemorrhagic
- Choriocarcinoma (gestational or testicular), renal cell carcinoma.

Cystic
- Ovarian carcinoma.
- Renal cell carcinoma.
- Thyroid papillary carcinoma.
- Secondary squamous carcinoma of head and neck.

Edge

Circumscribed	Mucinous carcinoma, medullary carcinoma and phyllodes tumour of breast, pancreatic endocrine tumours, some gut cancers.
Irregular	Infiltrating carcinoma. In general an irregularly infiltrating tumour margin is more aggressive than a pushing margin.

Histological Type

For the most part this mirrors the World Health Organization (WHO) International Classification of Tumours but refers to other classifications where appropriate. The classifications have also been partially edited to reflect those diagnoses that are more commonly encountered or discussed as differential diagnoses.

Histological type influences:

1. Prognosis—e.g. breast carcinoma
   - Excellent: tubular, cribriform, mucinous.
   - Good: tubular mixed, alveolar lobular.
   - Intermediate: classical lobular, invasive papillary, medullary.
   - Poor: ductal (no special type), mixed ductal and lobular, solid lobular.
2. Management—e.g. lung carcinoma
   - Non-small cell carcinoma: surgery ± radio-/chemotherapy depending on stage.
   - Small cell carcinoma: chemo-/radiotherapy.
3. Tumour distribution
   - Thyroid papillary carcinoma: potentially multifocal.
   - Ovarian epithelial borderline tumours: bilaterality, peritoneal implants, pseudomyxoma peritonei, appendiceal mucinous neoplasia.
4. Associated conditions
   - Thyroid medullary carcinoma: multiple endocrine neoplasia syndromes (MEN).
   - Duodenal periampullary carcinoma: familial adenomatous polyposis (FAP).

Differentiation/Grade

Three tier systems (well/moderate/poor differentiation or Grade 1/2/3, bladder carcinoma WHO grading) have traditionally been used based on subjective assessment of similarity to the ancestral tissue of origin (e.g. Broder classification incorporating keratinisation and intercellular bridges in squamous carcinoma and tumour gland formation in adenocarcinoma), cellular pleomorphism,[1]

[1]Cellular pleomorphism: this largely relates to nuclear alterations in size (anisonucleosis), shape, polarity, chromasia, crowding and nucleolar prominence. Cytoplasmic differentiation may also be taken into account.

mitoses[2] and necrosis.[3] This is strengthened when the individual criteria are formally evaluated and assimilated into a score that gives strong prognostic information (breast carcinoma, sarcoma). However a subjective three tier system is not advantageous when the majority of lesions fall into one category (e.g. colorectal carcinoma is predominantly moderately differentiated) and there is a lack of prognostic stratification. It is also compounded by poor reproducibility and tumour heterogeneity. This has resulted in emergence of two tier systems to identify prognostically adverse cancers (poorly differentiated/high-grade versus well to moderately differentiated/low-grade in colorectal carcinoma). In addition specific grading systems exist, e.g. Fuhrman nuclear grade in renal cell carcinoma and the Bloom and Richardson grade in breast cancer. Poor differentiation (G3) overlaps with and is sometimes combined with an undifferentiated (G4) category. Mixed differentiation with regard to tumour subtype and grade is relatively common. Overall tumour grade may be based on the worst rather than predominant grade. Carcinosarcoma (syn sarcomatoid carcinoma, spindle cell carcinoma) represents carcinoma with spindle cell change, and variable monophasic/biphasic and homologous or heterologous mesenchymal differentiation arising from malignant pluripotential stem cells and the process of epithelial-mesenchymal transition (EMT).

[2]Mitoses: the assessment of mitotic activity either as a stand-alone mitotic activity index or as part of a grading system is a strong prognostic factor as in breast carcinoma. However, care must be taken: (a) delayed fixation may significantly alter numbers of mitoses resulting in lower counts but also makes them more difficult to identify; (b) hyperchromatic, pyknotic, apoptotic bodies should be ignored and only clearly defined mitotic figures counted. Strict criteria should be used such as absence of the nuclear membrane and clear hairy extension of nuclear material ± increased basophilia of the cell cytoplasm; (c) counts should be related to a fixed field area against which various high power microscope objectives can be calibrated. In general a ×40 objective is used.

[3]Tumour necrosis: apoptotic (single cell) or coagulative (confluent with pyknotic nuclear material in eosinophilic debris).

Extent of Local Tumour Spread

Blocks

Due to tumour heterogeneity and variation in direct extension across the invasive front, multiple blocks of tumour and adjacent structures should be taken to ensure a representative sample. A useful general principle is one block per centimetre diameter of tumour mass with targeting of specific areas, e.g. area of greatest invasion, solid foci in ovarian tumours, haemorrhagic foci in testicular tumours (choriocarcinoma).

- Colorectal carcinoma: 4 or 5 blocks to show the tumour in relation to mucosa, wall, serosa, mesentery and extramural vessels.
- Thyroid nodule: 8–10 blocks including the capsule to distinguish follicular adenoma from minimally invasive follicular carcinoma.
- Ovarian tumours: 1 block/centimetre diameter to account for the spectrum of benign, borderline and malignant changes in one lesion, particularly mucinous tumours.

Border

Pushing/infiltrative.

Lymphocytic Reaction

Prominent/sparse.

Tumours with a pushing border and prominent lymphocytic reaction are regarded as having a better prognosis than those with a diffusely irregular infiltrating margin and sparse lymphocytic reaction, e.g. colorectal carcinoma, head and neck carcinoma, malignant melanoma, medullary carcinoma of breast, advanced gastric carcinoma.

Perineural Spread

Carcinoma of prostate, gall bladder and extrahepatic bile duct, pancreas and salivary gland

adenoid cystic carcinoma where it is also a useful diagnostic feature of malignancy. In prostatic cancer there is some evidence that perineural invasion relates to the presence of extracapsular spread of disease and in other cancers it increases the likelihood of local recurrence.

Breslow Depth/Clark Level

Malignant melanoma. Direct linear measurement from granular cell layer (mm) and anatomical level of invasion of the vertical component are strong prognostic indicators.

TNM (Tumour Node Metastasis) Classification

The TNM classification is an international gold standard for the assessment of spread of cancer and the revised 8th edition has been published by the UICC (International Union Against Cancer) taking into account new prognostic information, investigations and treatments. The system has evolved over 70 years as a tool for the careful collection of accurate data pertaining to cancer spread which can then be consistently related to planning of treatment, prognosis, evaluation of treatment and exchange of information between clinicians and centres. Virtues are that it translates into hard data some of the subjective language used in descriptive pathology reports and also encourages the pathologist to be more analytical in approach. It also improves pathologist to clinician communication. The post-surgical histopathological classification is designated pTNM and is based on pre-treatment, surgical and pathological information.

pT	Requires resection of the primary tumour or biopsy adequate for evaluation of the highest pT category or extent of local tumour spread.
pN	Requires removal of lymph nodes sufficient to evaluate the absence of regional node metastasis (pN0) and also the highest pN category.
pM	Requires microscopic examination of distant metastases which is often not available to the pathologist and therefore designated on clinical or radiological grounds. If available (e.g. a multidisciplinary meeting) the TNM categories can be stratified into clinical stage groupings which are used to select and evaluate therapy, e.g. carcinoma in situ is stage 0 while distant metastases is stage IV. However for the most part the pathologist concentrates on pT and pN which gives reasonably precise data to estimate prognosis and calculate end results. Stage grouping is mostly based on the anatomical extent of disease but for some tumour sites or entities other factors are included: histological type (thyroid), age (thyroid), grade (bone, soft tissue), tumour markers (testis) and risk factors (gestational trophoblastic tumour).

Multiple synchronous tumours (diagnosis at same time or within 6 months of each other): classify the tumour with the highest pT category and indicate the number of tumours in brackets, e.g. pT2 (4). In simultaneous bilateral cancers of paired organs each tumour should be classified independently. Systemic or multicentric cancers potentially involving many discrete organs are categorised only once in any individual, e.g. malignant lymphoma, leukaemia, Kaposi's sarcoma and mesothelioma. If there is doubt about the assigned T, N or M category in a particular case then the lower (i.e. less advanced) category is chosen. Note that in practice the multidisciplinary meeting may choose to upgrade the category to ensure that the patient receives adequate therapy, particularly in younger and fit individuals. When size is a criterion for the pT category, it is a measurement of the actual unfixed invasive component although measurement is often based on pathologic assessment of fixed tissue. Adjacent in situ change is not counted and if the fixed specimen shows a significant discrepancy with the clinical tumour measurement the latter is chosen.

Direct spread into an adjacent organ is recorded in the pT classification and is not considered distant metastasis whereas direct spread into a regional lymph node is considered in the pN category. The number of resected and positive nodes is recorded. Metastasis in a non-regional node is pM disease.

pT	Primary tumour
pTX	Primary tumour cannot be assessed histologically
pT0	No histological evidence of primary tumour
pTis	Carcinoma in situ
pT1, pT2, pT3, pT4	Increasing size and/or local extent of the primary tumour histologically
pN	Regional lymph nodes
pNX	Regional lymph nodes cannot be assessed histologically—not submitted. If submitted but less than the recommended number for a regional lymphadenectomy designate as uninvolved with the number harvested in brackets, e.g. pN0(6)
pN0	No regional lymph node metastasis histologically
pN1, pN2, pN3	Increasing involvement of regional lymph nodes histologically

Main categories can be subdivided for further specificity, e.g. pT1a or pT1b to signify unifocality or multifocality.

X classification is used when primary tumour (pTX) or regional lymph nodes (pNX) cannot be assessed histologically. The X suffix is not valid for distant metastasis (pM) assessment.

The TNM classification is applied to a range of malignant tumour types including carcinoma, malignant mesothelioma, malignant melanoma, gastrointestinal endocrine and stromal tumours, gestational trophoblastic tumours, germ cell tumours and retinoblastoma. A notable exception is lymphoma which is classified according to the Lugano classification, a modification of the Ann Arbor classification.

TNM Optional Descriptors

L	Lymphatic invasion
LX	Cannot be assessed
L0	Not present
L1	Present
V	Venous invasion
VX	Cannot be assessed
V0	Not present
V1	Microscopic
V2	Macroscopic (lumen or wall alone)

Note that lymphovascular invasion does not qualify as local spread of tumour in the pT classification (except liver and testis).

Pn	Perineural invasion
PnX	Cannot be assessed
Pn0	Not present
Pn1	Present

Prefix

y	Tumour is classified during or after initial multimodality therapy
r	Recurrent tumour, staged after a disease free interval
a	Classification first determined at autopsy

Suffix

m	Multiple primary tumours at a single site
mi	Nodal micrometastasis ≤2 mm
i	Nodal isolated tumour cells (ITC) ≤0.2 mm
sn	Sentinel nodes

Where appropriate other internationally recognised staging systems are also given, e.g.

Malignant lymphoma	Lugano classification
Gynaecological cancers	International Federation of Gynaecology and Obstetrics (FIGO)

Lymphovascular Invasion (LVI)

Definition

LVI usually relates to microscopic tumour emboli within small thin walled channels in which distinction between post-capillary venule and lymphatic channel is not possible—hence the general term LVI is used. In colorectal carcinoma, lymphatic invasion and to a lesser extent vascular invasion may be powerful predictors of lymph

node metastases in pT1 disease and should be differentially assessed. It is important to identify an endothelial lining to differentiate from retraction space artifact, which often comprises a rounded aggregate of tumour sited centrally and free within a tissue space. Other helpful features of vascular invasion are the presence of red blood cells, thrombosis and a point of attachment to the endothelium. In difficult cases, judicious use of immunochemical markers (CD34—lymphatic and vascular endothelium, CD31, D2-40 (podoplanin)—lymphatic endothelium) may be helpful, but in general, adherence to strict morphological criteria is recommended. Elastic special staining may also be helpful for venous spaces, as is recognition of surrounding muscle.

Significance

There is controversy as to the significance of LVI but in practice most pathologists view tumours with prominent LVI as those that are most likely to show longitudinal submucosal spread/satellite lesions and lymph node involvement. Extratumoural LVI is regarded as more significant than intratumoural LVI and is most frequently encountered at the invasive edge of the tumour. LVI in tissue well away from the tumour is a strong marker of local and nodal recurrence in breast carcinoma, and is a criterion indicating the need for postoperative adjuvant therapy. Regarding breast, when present in the overlying skin it denotes the specific clinicopathological entity of inflammatory breast carcinoma which is staged pT4. LVI is a strong determinant of adjuvant chemotherapy in testicular germ cell tumours. LVI also forms part of the pT classification for testicular and liver tumours, and, if present in a distant organ (e.g. lymphangitis carcinomatosa of the lung in pancreatic cancer) it is classified as disseminated disease (pM1).

Vascular Involvement

Some tumours (hepatocellular carcinoma, renal cell carcinoma) have a propensity for vascular involvement and care should be taken to identify this on specimen dissection and microscopy as it also alters the tumour stage. Extramural vascular invasion is a significant adverse prognostic factor in colorectal carcinoma but can be difficult to define. Sometimes one is reliant on circumstantial evidence of a tumour filled longitudinal structure with a wall partly formed of smooth muscle, lying at right angles to the muscularis propria and adjacent to an arteriole. Widowed arteries can be a useful indicator of venular involvement in a number of situations. The significance of vessel wall infiltration without luminal disease is uncertain but probably indicates potential access to the circulation.

Lymph Nodes

As discussed above the assessment of regional lymph nodes in a surgical cancer resection requires sufficient numbers to be able to comment on the absence of regional metastases and also the highest pN category, i.e. the total node yield and the number involved are important. In gastric carcinoma this means sampling and examining ideally at least 15 regional nodes. Thus lymph node yields can be used to audit both care of dissection by the pathologist, adequacy of resection by the surgeon and the choice of operation, e.g. axillary node sampling versus clearance. This is also influenced by use of preoperative neoadjuvant treatment. All nodes in the specimen should be sampled and although ancillary techniques exist (e.g. xylene clearance, revealing solutions) there is no substitute for time spent at careful dissection with a readiness to revisit the specimen after discussion at the multidisciplinary meeting. Care should be taken not to double count the same lymph node. The TNM target numbers recommended for a regional lymphadenectomy appropriate to a particular site should be kept in mind on dissecting the specimen: node hunting should not cease on reaching the target number. The pathologist should also remember to count those nodes in the histological slides that are immediately adjacent to the tumour as they are sometimes ignored yet may be more likely to be involved.

What Is a Node?

- A lymph node is a discrete mass of fibrovascular tissue enclosed within a dilated lymphatic vessel usually identified by its transient lymphoid population and subcapsular sinus.
- Direct extension of the primary tumour into lymph nodes is classified as a lymph node metastasis (TNM rule).
- A tumour nodule (satellite) in the connective tissue of a lymph drainage area without histological evidence of residual lymph node could be discontinuous spread, lymphovascular invasion or a totally replaced lymph node. If considered a totally replaced node (usually having the form and smooth contour of a lymph node) it is classified, along with all other similar such nodes, in the pN category as a regional lymph node metastasis. If a vessel wall is identified through routine or ancillary staining, it should be classified as venous or lymphatic invasion. In appendiceal and colorectal carcinomas the presence of tumour satellites confers pN1c status if all regional nodes are negative on pathologic examination.

When size is a criterion for pN classification (e.g. breast carcinoma) measurement is of the metastasis, not the entire node (TNM rule). Size is also the whole measurement of a conglomerate of involved lymph nodes, and, includes perinodal tumour.

Isolated Tumour Cells and Micrometastases

The significance of nodal micrometastases >0.2 to ≤2 mm (designated (mi), e.g. pN1 (mi)), and, isolated tumour cells (ITC) ≤0.2 mm (designated (i+), e.g. pN0 (i+)) demonstrated by immunohistochemistry is not entirely resolved and handled differently according to tumour type in TNM8, e.g. in colorectal adenocarcinoma the finding of isolated tumour cells may confer no deleterious prognostic effect in otherwise node negative disease (therefore nodes with only isolated tumour cells are considered negative pN0). However in Merkel cell carcinoma and cutaneous malignant melanoma nodal ITC are classified as pN1.

In practical terms an accommodation within available resources must be made. Most busy general laboratories will submit small nodes (<5 mm) intact or bisected, and a mid-slice of larger ones. Additional slices may be processed as required if the histology warrants it. Sentinel nodes are discussed in the next section. Sometimes there is circumstantial evidence of occult metastases, e.g. a granulomatous response that will promote the use of immunohistochemistry and further levels in the search for micrometastases/ITCs. The prognostic significance of micrometastases has yet to be completely clarified for the majority of cancers and changes in prognosis are difficult to determine as treatment modalities change and improve over time. This area needs further clarification from large international trials which examine clinical outcome related to the immunohistochemical and molecular (RT-PCR) detection of minimal residual disease in lymph nodes and bone marrow samples considered tumour negative on routine examination. Detection by non-morphological techniques such as flow cytometry or DNA analysis is designated (mo1+), e.g. pN0 (mol+) or pM0 (mol+) in lymph node or bone marrow respectively. In the interim the rationale behind asigning (i+) and (mol+) to the pNO category is because they do not typically show evidence of metastatic activity, e.g. proliferation, stromal reaction or penetration of vascular or lymphatic sinus walls.

Sentinel Node

The sentinel lymph node is the first lymph node to receive lymphatic drainage from a primary tumour. More accurately it may refer to more than one node through which lymphatic channels originating in the tumour drain. If it is tumour positive other regional lymph nodes are likely to be involved, but not involved if the sentinel node is negative. It is tracked by vital dye or radioactive colloid mapping. It is cut into 2 mm serial slices perpendicular to the nodal long axis to maximise exposed surface area, all processed and examined histologically. This

may be supplemented by appropriate immunohistochemistry, e.g. cytokeratins, melanoma markers. Examination may utilise alternate methods such as the EORTC (European Organisation for the Research and Treatment in Cancer) protocol.

Limit Node

The limit node is the nearest node(s) to the longitudinal and/or apical resection limits and suture ties. Some specimens, e.g. transverse colon, will have more than one and they should be identified as such. Involvement of this node may confer a worse prognosis.

Extracapsular Spread

Extracapsular spread is an adverse prognostic sign and an indicator for potential local recurrence (e.g. bladder cancer), particularly if the spread is near to or impinges upon a resection margin, e.g. axillary clearance in breast carcinoma. Perinodal tumour is also included in measurement of metastasis maximum dimension.

Excision Margins

The clearance of excision margins has important implications for patient follow up, adjuvant therapy and local recurrence of tumour. Positive resection margins in a breast cancer may mean further local excision, conversion to a total mastectomy and/or radiotherapy to the affected area. Measurements should be made on the gross specimen, checked against the histological slide and verified using a microscopic rule. A very useful practical aid is a hand-held Perspex dome magnifier or loupe with graticule. Painting of the margins by ink supplemented by labelling of the blocks is important and can be helpful in verifying visualisation of a true margin. Paint adheres well to fresh specimens but also works on formalin fixed tissue. India ink or alcian blue are commonly used. Commercially available multicoloured inks are helpful, particularly if there are multiple margins as in breast carcinoma. Care is required in interpretation as ink may run into tissue crevasses mimicking surgical margins: correlation with a macroscopic image of blocks taken is helpful in this regard. The relevance of particular margins varies according to specimen and cancer type.

1. *Longitudinal margins*. Involvement can be by several mechanisms:
   (a) *Direct spread*. In rectal carcinoma the longitudinal margin in an anterior resection is considered satisfactory if the anastomosis is 2–3 cm beyond the macroscopic edge of the tumour, i.e. direct longitudinal spread is minimal. However, there may be involvement if the tumour is extensively infiltrative, poorly differentiated or of signet ring cell type, or shows prominent LVI. Appropriate limit blocks should be taken. In addition to the resection specimen limits separate anastomotic rings are also usually submitted.
   (b) *Discontinuous spread*. In oesophageal and gastric carcinoma there is a propensity for discontinuous lymphovascular submucosal and mural spread, and margins should be checked microscopically even if some distance from the primary tumour.
   (c) *Multifocal spread*. In transitional cell carcinoma of the urinary tract, malignant lymphoma of the bowel and papillary carcinoma of the thyroid, potential multifocality must be borne in mind.
2. *Circumferential radial margin* (*CRM*). These non-peritonealised margins are important in relation to local recurrence and morbidity and may influence additional treatment modalities such as radiotherapy and chemotherapy, e.g. mesorectal CRM and rectal carcinoma. It is recommended practice to measure how far the carcinoma has spread beyond the organ wall and how far it is from the CRM. Example variations are: oesophageal carcinoma and the adventitial margin, cervical carcinoma and the paracervical/parametrial margin, renal carci-

noma and the perinephric fat/fascial margin. Lymph node mestastasis at a CRM is also considered positive. The significance of some other examples is less well defined but comment should be made, e.g. the mesenteric edge in colonic carcinoma.

3. *Quadrant margins*. Examples are a skin ellipse for carcinoma or malignant melanoma. Usually the longitudinal axis margins are sufficiently clear and the nearest to the tumour are the transverse axis and deep aspects. It is important to check clearance not only of the infiltrating tumour but also adjacent field change, e.g. epidermal dysplasia or radial spread of a malignant melanoma. Actual measurement of margin clearance can be important in assessing the need for further local excision, e.g. malignant melanoma. An alternative technique is multiple serial transverse slices demonstrating the entirety of the transverse axis margins with the longitudinal axis tips also embedded in-toto ("toast-racking"). In highly critical sites where margins are tighter (e.g. periorbital), specimens may be embedded to examine the entire peripheral margin.
4. *Serosa or peritoneum*. This is a visceral "anatomical margin" and breach of it allows carcinoma to access the abdominal and pelvic cavities. Its importance has been re-emphasised, as for example at the upper anterior aspect of the rectum where there is potential for peritoneal disease as well as local mesorectal recurrence posterolaterally. Standard practice may for some cancers also involve measuring the distance from the invasive edge of the tumour to the serosa, e.g. uterine adenocarcinoma.
   (a) *Colonic carcinoma*. Prognostic distinction is made between carcinoma in a subserosal position (pT3) and carcinoma being at and ulcerating the serosal surface (pT4). The serosa is considered involved if tumour is actually on or ulcerating the lining mesothelial cells.
   (b) *Lung carcinoma*. Visceral pleural involvement is infiltration of the superficial (outer) elastic layer. Visceral pleural involvement without breach of the superficial layer appears to make no prognostic difference.
5. *Multiple margins*. As in breast carcinoma (lateral/medial, superior/inferior, superficial/deep) this requires differential painting and block labelling, according to a previously agreed protocol for specimen orientation markers, e.g. surgical sutures or clips. Alternatively the surgeon may submit multiple site orientated shave margins marked as to their inner and outer (new in-vivo margin) aspects.
6. *Involvement*. Inadequate clearance of excision margins varies according to the tissues and tumours concerned and may provoke lively comment from surgical colleagues at MDM:
   (a) *Breast carcinoma*. Invasive <5 mm; in situ (ductal) <10 mm. In clinical practice a non-involved margin of 1–2 mm is acceptable.
   (b) *Rectal carcinoma*. Mesorectum; ≤1 mm (either by direct extension or discontinuous in a node or lymphovascular channel).

TNM Resection Classification

R	Residual tumour
RX	Presence of residual tumour cannot be assessed
R0	No residual tumour
R1	Microscopic residual tumour (proven by tumour bed biopsy or cytology)
R2	Macroscopic residual tumour

Residual disease takes into consideration not only locoregional tumour but also any remaining distant metastases. It can also be applied following surgery, radiotherapy, or chemotherapy, alone or in combination. For a number of tumour sites there are semiquantitative histological regression grading systems applicable to post multimodal treatment, e.g. oesophageal and rectal cancers, and bone and soft tissue sarcomas. Due to the variation in these schemes it is recognized that there is a need for an internationally standardized

grading system that is reproducible and clinically relevant. The marker of response to therapy should be the amount of residual tumour tissue present rather than the fibrosis, as the latter may not be a consequence of treatment but tumour related stromal desmoplasia. It should be noted that clinical response to neoadjuvant therapy does not always directly correlate with evidence of tumour regression in the resection specimen.

Other Pathology

This heading reminds the pathologist to add additional information as necessary and to look for and comment on related findings, relevant predisposing and concurrent lesions, associated conditions and useful markers.

Some examples are:

- Gastric carcinoma, incomplete (type IIb) intestinal metaplasia, gastric atrophy, dysplasia, synchronous MALToma, *Helicobacter pylori*.
- Colorectal carcinoma, adenomatous polyps, familial adenomatous polyposis, periampullary carcinoma and duodenal adenoma.
- Thyroid medullary carcinoma, multiple endocrine neoplasia (MEN) syndromes.
- Hepatocellular carcinoma, hepatitis B/C infection, cirrhosis, Budd–Chiari syndrome, varices.

Other general comments are included such as diagnostic criteria, immunophenotype, prognostic indicators and clinical and treatment parameters.

Other Malignancy

The TNM classification predominantly concerns carcinoma but also includes malignant mesothelioma, malignant melanoma, gastrointestinal endocrine and stromal tumours, gestational trophoblastic tumours, germ cell tumours and retinoblastoma. This section notes the more common non-carcinomatous cancers such as uterine smooth muscle/stromal tumours, malignant lymphoma/leukaemia and sarcoma. Summary diagnostic and prognostic criteria are given where relevant.

Ancillary Techniques

Various ancillary techniques are important in the histopathology of surgical cancer and should be employed as appropriate. Some of these are commented on at various points in the protocols, e.g. under sections “Histological Type” and “Other Pathology”.

Photography

At the bench line diagrams and specimen digital macrophotography are crucial means of correlating block samples, disease stage and margin status, and, communication between dissector, reporting pathologist and the clinical multidisciplinary team.

Cytology

Although not the primary subject of this book, *fine needle aspiration cytology* (*FNAC*) is an important technique that also compliments histologic examination and forms part of overall case assessment and is therefore included here. FNAC using 25-22 gauge needles has become the first order investigation in many cancers due to its speed, cost effectiveness, proficiency and convenience for both clinician and patient. It does not only provide specific inflammatory (e.g. Hashimoto’s thyroiditis) and malignant diagnoses (e.g. thyroid papillary carcinoma), but can sort patients into various management groups: viz., inflammatory and treat, benign and reassure, atypical and further investigation (by core/open biopsy or excision), or malignant with specific therapy (surgery, chemotherapy, radiotherapy). It can be used to refute or confirm recurrence in patients with a known previous diagnosis of

malignancy and to monitor response to therapy or change in grade of disease. It provides a tissue diagnosis of cancer in patients unfit for more invasive investigations or when the lesion is relatively inaccessible, e.g. in the lung periphery, mediastinum, abdomen, pelvis and retroperitoneum. It must be integrated with the clinical features and investigations (serology, radiology) and can be complemented by other techniques, e.g. core biopsy. It potentially provides material for routine morphology, histochemical and immunocytochemical techniques, electron microscopy, cell culture and flow cytometry. The direct smear and cytospin preparations can be augmented by formalin fixed paraffin processed cell blocks of cell sediments and needle core fragments (minibiopsies) which can combine good morphology (the cores providing a tissue pattern) and robust immunohistochemistry. It can be applied to many organs: salivary gland, thyroid gland, palpable lymphadenopathy, breast, skin, prostate, subcutaneous tissues and deep connective tissues. Radiologically guided FNAC is useful for non-central respiratory cancers and tumours in the mediastinum, liver, pancreas, kidney, retroperitoneum, abdomen and pelvis. Endoscopic FNAC is also being used more frequently, e.g. transbronchial, transrectal, transduodenal and transgastric/transoesophageal for lymph node staging or tumours covered by intact mucosa. Body cavity fluid cytology (both aspirates of free pleural, pericardial and peritoneal fluid and peritoneal/pelvic washings) continues to play an active role in the diagnosis, staging and monitoring of cancer. Yield of information is maximised by a combination of morphology and immunohistochemistry on direct smear/cytospin preparations (using air dried Giemsa and wet fixed Papanicolaou/H and E stains) and cell blocks (cell sediments and fragments).

Exfoliative cytology: along with cytological brushings and washings is also pivotal in the assessment of various cancers, e.g. lung cancer, where the information obtained is complementary to that derived from direct biopsy and aspiration cytology. It can provide diagnostic cells not present in the biopsy specimen (for reasons of sampling error, tumour type or accessibility), correlate with it or allow subtyping that is otherwise obscured in artifacted biopsy material. Common sites of application are bronchus, mouth, oesophagus, stomach, bile duct, large intestine, bladder, renal pelvis and ureter.

Liquid based preparations: with good morphology and preservation of immunogenicity are increasingly complementing or replacing traditional cytological methods.

Frozen Sections

Used in a range of scenarios:

- Check excision of parathyroid glands versus thyroid nodules or lymph nodes in hyperparathyroidism.
- Operative margins in gastric carcinoma, partial hepatectomy, head and neck and urinary cancers.
- Cancer versus inflammatory lesions at laparotomy.
- Organ suitability for transplantation and examination of potentially malignant lesions.
- Lymph node metastases in head and neck, urological, and gynaecological cancers prior to radical dissection.
- Mohs' micrographical surgery in resection of basal cell carcinoma of the face.
- Frozen sections should be used sparingly due to problems of interpretation and sampling in the following cancers: malignant lymphoma, ovarian carcinoma, minimally invasive thyroid carcinoma, pancreas and extrahepatic bile duct carcinoma.

Histochemical Stains

Histochemical stains can be valuable, examples being: PAS ± diastase or mucicarmine for adenocarcinomatous differentiation, PAS-positive inclusion bodies in malignant rhabdoid tumours and alveolar soft part sarcoma, PAS-positive glycogen in renal cell carcinoma.

Immunohistochemistry

Immunohistochemistry: invaluable in assessing tumour type, prognosis and potential response to treatment, i.e. as *diagnostic*, *prognostic and predictive biomarkers*. It also has a role as a surrogate marker of an inherited mutation, e.g. demonstration of defective mismatch repair proteins in hereditary non-polyposis colorectal cancer (HNPCC). Can also act as a surrogate marker for high risk HPV infection (p16).

Tumour Type

- Further detail is given in their respective chapters but typical cancer type immunoprofiles are given in Table 1.1.
- Select antibody panels are also of use in differential diagnosis in a number of circumstances (Table 1.2).
- The cytokeratin subtypes CK7 and CK20 have an important role to play in tumour characterisation and can provide a good initial immunochemical triage (Table 1.3).

Table 1.1 Immunoprofile of cancer types

System	Tumour/condition	Marker panel
Head and neck	Salivary gland tumours	Epithelium: AE1/AE3 and myoepithelium: S100, calponin, CK5/6, p63. Grade: Ki-67
	Thyroid gland carcinoma	Papillary and follicular: thyroglobulin, TTF1, CK19, galectin 3. Medullary: calcitonin, CEA, chromogranin, Ki-67
	Squamous cell carcinoma	AE1/AE3, CK5/6, p63, p16 (HPV related oropharyngeal), EBER (EBV related nasopharyngeal)
Gastrointestinal	Oesophageal carcinoma	Squamous: AE1/AE3, CK5/6, p63
		Adenocarcinoma: CAM 5.2, CK7, ±CK20 (>50%)
	Barrett's dysplasia	p53, Ki-67, AMACR
	Gastric adenocarcinoma	CEA, EMA, CK7, ±CK20 (>50%), CDX-2. E cadherin+/ HER2 ± (intestinal type), E cadherin/HER2—(diffuse type)
	Small bowel adenocarcinoma	CEA, EMA, CK20, ±CK7 (50%)
	Colorectal adenocarcinoma	CEA, EMA, CK20, CDX-2, β catenin ± CK7 (5–10% poor differentiation or MSI-H). MMR abs
	Hepatocellular carcinoma	AFP, Hep Par1, CEA (polyclonal/canalicular), CD10, CAM 5.2, CK8, CK18
	Pancreaticobiliary carcinoma	CEA, CA19-9, CA125, CK7, CK19, p53 ± CK20, CDX-2. Loss of DPC4
	Gastrointestinal stromal tumours	DOG1, CD117, CD34, Ki-67, ±Sm actin, desmin, S100, protein kinase c theta
	Gastrointestinal endocrine tumours	Chromogranin, synaptophysin, CD56, CDX-2, Ki-67 ± CAM 5.2, gastrin, insulin, glucagon
	Anal squamous carcinoma	AE1/AE3, CK5/6, p63, p16
Respiratory	Small cell carcinoma	CAM 5.2 (paranuclear dot), CD56, synaptophysin, TTF-1, Ki-67. Chromogranin—carcinoid
	Non-small cell carcinoma	Adenocarcinoma: CAM 5.2, Ber EP4, CK7, TTF-1, napsinA, CD15, MOC 31
		Squamous cell: AE1/AE3, CK5/6, p63, 34βE12
	Malignant mesothelioma	Positive: CAM 5.2, AE1/AE3, CK5/6, CK7, calretinin, WT1, thrombomodulin, HBME1, p53, EMA
		Negative: CEA, BerEP4, CD15, MOC 31, desmin

Table 1.1 (continued)

System	Tumour/condition	Marker panel
Gynaecological	Ovarian carcinoma	Serous: PAX-8, CK7, CA125, WT1, Ki-67, ER, p16
		Mucinous/endometrioid: CEA, CK7, ±ER, CK20, CDX-2, PAX-8 (not in mucinous lesions)
	Sex cord stromal (also testicular tumours)	Inhibin, melan-A, SALL 4, vimentin, calretinin, CD99, ±CAM 5.2, AE1/AE3, EMA, Ber EP4, Ki-67
	Uterus, mesenchymal	Leiomyomatous: desmin, h-caldesmon, Sm actin, ±CD10, Ki-67, ER
		Stromal: CD10, Ki-67, ±desmin, h-caldesmon, Sm actin, ER
	Endometrial carcinoma	Endometrioid: PAX-8, ER, vimentin, CAM 5.2, AE1/AE3, CK7 ± CK20, CD10
		Serous: PAX-8, p53, Ki-67, p16, HMGA2, PTEN
	Cervix—CGIN	Ki-67, p16, bcl-2±
	Cervical adenocarcinoma	CEA, CK7, ±CK20 (ER/vimentin negative), p16
	Cervical squamous carcinoma	AE1/AE3, CK5/6, p63, p16
	Hydatidiform mole	$p57^{kip2}$ in partial/complete moles (present/absent)
Genitourinary	Renal clear cell carcinoma	PAX8, CAM 5.2, AE1/AE3, CD10, EMA, vimentin, CD15, RCC ab, CA-IX
	Renal papillary carcinoma	PAX8, CK7, Vimentin, AMACR, BerEP4. CD117 negative
	Renal chromophobe carcinoma	CK7, Ber EP4, E-cadherin, MOC 31 (decreased CD10, vimentin, RCC ab). CD117 positive
	Urothelial carcinoma	GATA3, p63, 34βE12, AE1/AE3, CK7, CK20, p53, uroplakin III
	Prostate carcinoma	PSA (polyclonal), PSAP, AMACR, NKX3.1, AR and CK7/20, 34βE12, p63 basal cell negative
	Testicular germ cell tumour	Seminoma: PLAP, CD117, OCT3/4, SALL 4, D2-40, HCG (syncytiotrophoblast giant cells), cytokeratins±
		Embryonal carcinoma: CAM 5.2, CD30, OCT3/4, SALL 4, D2-40, ±PLAP and EMA negative
		Yolk sac tumour: CAM 5.2, AFP, SALL 4, glypican-3, ±PLAP. Negative for CD30, CD117, OCT3/4, D2-40
		Choriocarcinoma: HCG, CK7 (cytotrophoblast). OCT3/4 negative
		Sex cord stromal tumours: inhibin, calretinin, Melan-A, S100, CD99, cytokeratins
Breast	Breast carcinoma	ER, PR, HER2, CK7. Also Sm actin, CK5/6, p63, CK14, CK8/18, E-cadherin (see Chap. 23)
Soft tissue	Spindle cell sarcoma	Vimentin, CD34, Sm actin, desmin, h-caldesmon, CAM 5.2, AE1/AE3, EMA, S100, CD99, TLE1, HHV8, DOG1, CD117, β catenin, ALK, HMB45
	Small round blue cell tumours	CD45, tdt, S100, CD99, Fli1, desmin, myogenin, WT1, NB84, CAM 5.2, CD56, CK20
	Adrenal carcinoma	Inhibin, melan-A, synaptophysin, Ki-67, cytokeratin±, and EMA, CEA negative
	Phaeochromocytoma	Chromogranin, synaptophysin, S100 sustentacular cells, Ki-67, cytokeratins±
Skin	Cutaneous carcinoma	Basal cell: Ber EP4+/EMA−
		Squamous cell: Ber EP4−/EMA+
	Malignant melanoma	S100, melanA, HMB45, Ki-67, SOX10
	Merkel cell carcinoma	Synaptophysin, CD56, CK20, CAM 5.2, Ki-67. TTF1 negative

Table 1.1 (continued)

System	Tumour/condition	Marker panel
Haemopoietic	Malignant lymphomas and leukaemias	CD45, CD20, CD3 and CD4, CD5, CD8, CD10, CD13, CD15, CD19, CD21, CD23, CD30, CD34, CD43, CD56, CD57, CD61, CD68, CD117, κ and λ (IHC and ISH), cyclin D1, bcl-2, bcl-6, bcl-10, ALK, Ki-67, EBV LMP1, EBER, granzyme B, myeloperoxidase, MUM1, Pax5, Oct2, BoB1, EMA, TIA1, Factor VIII, chloroacetate esterase, neutrophil elastase, MNDA

Adapted from McManus DT. Miscellaneous specimens. In: Allen DC, Cameron RI, editors. Histopathology specimens. Clinical, pathological and laboratory aspects. 2nd ed. London: Springer; 2012
Queries about immunohistochemical staining may be answered at http://www.immunoquery.com
Sm actin smooth muscle actin, *TTF1* thyroid transcription factor, *CK* cytokeratins: specific (e.g. CK7, 20) or cocktails (CAM 5.2: CKs 8, 18, 19; 34βE12: CKs 1, 5, 10, 14; AE1/AE3: CKs 10, 15, 16, 19/1–8), *AFP* α-fetoprotein, *HCG* human chorionic gonadotrophin, *PLAP* placental alkaline phosphatase, *Hep Par1* hepatocyte antibody, *RCC ab* renal cell carcinoma antibody, *CD56* neural cell adhesion molecule (NCAM), *Ki-67* MIB 1, *ER* oestrogen receptor, *PR* progesterone receptor, *PSA* prostate specific antigen, *PSAP* prostate specific acid phosphatase, *AMACR* (*P504S*) alpha-methylacyl co-enzyme A racemase, *tdt* terminal deooxynucleotidyltransferase, *ALK* anaplastic lymphoma kinase, *LMP1* latent membrane protein (EBV), *EBER* EBV encoded RNA (in-situ hydridisation), *MSI-H* high level of microsatellite instability, *MMR abs* mismatch repair antibodies MLH1, PMS2, MSH2, MSH6, *DPC4* deleted in pancreatic cancer, *DOG1* discovered on gastrointestinal stromal tumours 1, *CDX2* caudal homeobox gene

Table 1.2 Select antibody panels in differential diagnosis

Differential diagnosis	Antibody panel
Poorly differentiated tumour	
Carcinoma/melanoma/lymphoma[a]/ germ cell tumour/GIST/PEComa/ sarcoma (epithelioid variants)/ granulocytic sarcoma	CAM 5.2, AE1/AE3, S100, melanA, HMB45, CD45, CD30, ALK, PLAP, OCT3/4, SALL 4, CD117, DOG-1, desmin, CD34, CD68
Mesothelioma/pulmonary adenocarcinoma	CK7, CK5/6, calretinin, WT1, EMA, CEA, Ber EP4, MOC 31, TTF1
Small round cell tumour	
Small cell carcinoma/Merkel cell carcinoma/melanoma/lymphoma/ leukaemia (lymphoblastic)/Ewing's: PNET/rhabdomyosarcoma/ neuroblastoma/intra-abdominal desmoplastic small cell tumour	CAM 5.2, synaptophysin, CD56, TTF1, CK20, S100, CD45, tdt, CD99, Fli1, desmin, myogenin, NB84, WT1, Ki-67
Bladder/prostate carcinoma	CK7, CK20, p63, 34βE12, uroplakin III, PSA, PSAP, AR, NKX3.1, AMACR, GATA3
Renal carcinoma/adrenal cortical neoplasm/phaeochromocytoma	PAX8, CAM 5.2, AE1/AE3, CD10, EMA, RCC ab, inhibin, melan-A, CD56, synaptophysin, S100, chromogranin
Hepatocellular carcinoma/ cholangiocarcinoma/metastatic colorectal carcinoma	CAM 5.2, AE1/AE3, AFP, Hep Par 1, CEA (polyclonal/canalicular), CD10, CK7, CA19-9, CK20, CDX-2
Paget's disease of nipple/melanoma/ Bowen's disease	CAM 5.2, AE1/AE3, EMA, CK7, HER2, p63, S100, melan-A (CK20 for anovulval Paget's)

Table 1.2 (continued)

Differential diagnosis	Antibody panel
Metastatic carcinoma of unknown primary site: site indicative antibodies	Thyroglobulin, TTF1, CK19, galectin3: differentiated thyroid carcinoma
	CEA, calcitonin: medullary carcinoma thyroid
	TTF1, napsin A: lung adenocarcinoma
	PSA (polyclonal), PSAP, AMACR: prostate carcinoma
	CDX-2: gastrointestinal carcinoma
	CA19-9: pancreas, upper gastrointestinal carcinoma
	CA125: ovarian serous (also WT1) and sometimes pancreas, breast carcinoma
	GCDFP-15, ER, PR: breast carcinoma
	PLAP, CD117, OCT3/4, SALL 4, CD30, AFP, HCG: germ cell tumour
	AFP, Hep Par 1, CD10, CEA (polyclonal/canalicular): hepatocellular carcinoma
	RCC ab, CD10, vimentin, EMA: renal cell carcinoma
	CK7, CK20: see Table 1.3
Neuroendocrine tumours	Chromogranin, synaptophysin, CD56, Ki-67. Synaptophysin is a robust panmarker of neuroendocrine tumours. Chromogranin stains low-grade/well differentiated endocrine (carcinoid) tumours more strongly, and high-grade endocrine (small cell/large cell) carcinoma weakly. CD56 and Ki-67 are the converse of this.

[a]Including anaplastic large cell lymphoma (ALCL)

Table 1.3 CK7, CK20 tumour expression

Immunoprofile	Carcinoma
CK7+ CK20+	Gastric/oesophageal adenocarcinoma
	Pancreatic adenocarcinoma
	Transitional cell carcinoma
	Ovarian mucinous adenocarcinoma
CK7+ CK20−	Lung adenocarcinoma
	Breast adenocarcinoma
	Ovarian serous and endometrioid adenocarcinoma
	Endometrial/endocervical adenocarcinoma (usually CK20 negative)
	Mesothelioma
CK7− CK20+	Colorectal adenocarcinoma
CK7− CK20−	Prostate adenocarcinoma
	Renal clear cell adenocarcinoma
	Hepatocellular carcinoma
	Lung carcinoma (non-adenocarcinoma types)

Antibodies should not be used in isolation but a panel employed with positive and negative in-built and external controls. This is due to a spectrum of co-expression seen with a number of antibodies, e.g. EMA (carcinoma, plasmacytoma, Hodgkin's disease and anaplastic large cell lymphoma) and CD15 (Hodgkin's disease and lung adenocarcinoma). *Interpretation should also be closely correlated with the morphology*. The antibodies in Table 1.1 are only part of a rapidly enlarging spectrum of new generation, robust antibodies that can be used with formalin fixed, paraffin embedded tissues, and show enhanced demonstration of expression by heat mediated antigen retrieval techniques such as microwaving and pressure cooking, and, highly sensitive polymer based detection systems. It is important to determine that the immunopositive reaction is in an appropriate location (e.g.

membrane staining for HER2, nuclear staining for ER, TTF1), is not simply related to entrapped normal tissues (e.g. infiltration of skeletal muscle fibres), and is of appropriate staining intensity. In some circumstances the number of positive cells is important, e.g. Ki-67 index.

Prognosis

- HER2, p53 oncogene expression, Ki-67 (MIB-1) proliferation index.

Potential Treatment Response

- Oestrogen/androgen expression and hormonal response in breast (e.g. Tamoxifen) and prostate cancer.
- HER2 expression and Herceptin (trastuzumab) therapy in breast cancer and gastric cancer.
- CD20 expression and Rituximab therapy in non-Hodgkin's malignant lymphoma.
- CD117 expression and Imatinib (Glivec) therapy in GISTs.

Electron Microscopy

Electron microscopy has a well established role in certain areas such as assessment of renal disease. Use in cancer diagnosis has declined with the advent of other diagnostic ancillary tests, particularly immunochemistry and molecular analysis. It requires specialized equipment and expertise and may be more appropriately provided on a regional or network basis. However, it can still potentially play a diagnostic role where morphology and immunochemistry are inconclusive. Specific features can be sought in:

- Carcinoma (tight junctions, short microvilli, secretory vacuoles, intermediate filaments).
- Melanoma (pre-/melanosomes).
- Vascular tumours (intra-cytoplasmic lumina, Weibel-Palade bodies).
- Neuroendocrine carcinoma (neurosecretory granules).
- Mesothelioma (long microvilli).
- Smooth muscle/myofibroblastic tumours (longitudinal myofilaments with focal dense bodies).
- Rhabdomyosarcoma (basal lamina, sarcomere Z line formation).
- Perineural/meningeal lesions (elaborate complex cytoplasmic processes).

Molecular and Chromosomal Studies

Evolving areas of diagnostic use of molecular and chromosomal studies are *clonal immunoglobulin heavy/light chain restriction* and *T cell receptor gene rearrangements* in the confirmation of malignant lymphoma, and, the characterisation of various cancers (particularly malignant lymphoma, sarcoma and some carcinomas, e.g. renal) by *specific chromosomal translocation changes*. Gene rearrangement studies can be carried out on formalin fixed paraffin embedded material but fresh tissue put into suitable transport medium is required for metaphase cytogenetic chromosomal analysis—although reverse transcriptase polymerase chain reaction (RT-PCR) methods are being developed for paraffin material. Genotypic subtypes of various malignancies, e.g. rhabdomyosarcoma, have been defined with differing clinical presentation, prognosis and response to therapy. *Detail is given in* Table 1.4 but some examples are:

Follicle centre lymphoma, follicular	t(14;18)
Mantle cell lymphoma	t(11;14)
Synovial sarcoma	t(X;18) (p11.2;q11.2)
Myxoid liposarcoma	t(12;16)(q13;p11)
Alveolar rhabdomyosarcoma	t(2;13)(q35;q14)

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/over expression of gene products (most lymphomas, e.g.

Table 1.4 Translocations in cancer types

Translocation	Tumour 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	Breakapart 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 tumour	Breakapart FISH assay for EWS target; rt-PCR for specific fusion partners	Diagnosis
t(12;22)(q13;q12) EWS-ATF1 fusion	Clear cell sarcoma	Breakapart 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 rtPCR 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 rtPCR 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 rtPCR 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	Burkitt's lymphoma	Breakapart c-myc FISH assay, Southern Blot of hmwDNA	Diagnosis of Burkitt's but also found in subset DLBCL where it has prognostic implications
C-myc translocated to Ig heavy chain and deregulated expression			
t(14;18)(q32;q21) and variants	Follicular lymphoma	FISH, PCR	Diagnosis of follicular lymphoma but also seen in subset DLBCL where it has prognostic implications
BCL2 translocated to Ig heavy chain and deregulated expression		BCL2 immunohistochemistry	
t(11;14)(q13;q32) and variants	Mantle cell lymphoma	FISH, PCR	Diagnosis of mantle cell lymphoma
Cyclin D1 translocated to Ig heavy chain and deregulated expression		Cyclin D1 immunohistochemistry	
t(2;5)(p23;q35)	Anaplastic large cell	FISH breakapart ALK probe	Diagnosis of anaplastic large cell lymphoma/inflammatory myofibroblastic tumour
NPM–ALK fusion	Lymphoma	rt-PCR	
TPM3 clathrin or other gene fusion targets	Inflammatory myofibroblastic tumour	Immunohistochemistry ALK	Potentially predictive of response to crizotinib in non-small cell lung cancer
TMPRSS2–ERG or other ETS family members	Prostatic adenocarcinoma	FISH translocation assay or rt-PCR	??Prognostic

From McManus DT. Miscellaneous specimens and ancillary techniques. In: Allen DC, Cameron RI, editors. Histopathology specimens: clinical, pathological and laboratory aspects. 2nd ed. London: Springer; 2012

? and ?? dictates the current degree of uncertainty of application of this technique

cyclin D1 or BCL2), or result in a novel chimaeric fusion gene product (most sarcomas, e.g. EWS-FLI 1). Translocations can be detected by dual colour interphase FISH assays to a single target gene with breakapart probes designed to span the breakpoint or by using dual target probes to detect fusion signals. Mutliplex 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 localisation 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 tumour 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.

Somatic mutation analysis has a number of applications in *differential diagnosis*, *prediction of prognosis and treatment response*. *Detail is given in* Table 1.5 but some examples are:

Colorectal cancer	K-RAS, microsatellite instability levels, BRAF
Melanoma, thyroid cancer	BRAF
Lung adenocarcinoma	EGFR, ALK
GISTs	c-kit, PDGFR
Renal cancer	Xp11

Table 1.5 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
BRAF mutations	Melanoma	PCR/direct sequencing or tests for V600E mutation	Predictive of response to vemurafinib.
	Thyroid carcinoma		Diagnosis? Predictive of stage
EGFR mutations	Lung adenocarcinoma	PCR/direct sequencing	Predictive: mutation positive more likely to respond to gefitinib treatment
c-kit/PDGFRA mutations	Gastrointestinal stromal tumours	PCR/direct sequencing	Predictive: exon 11 mutations more likely to respond than exon 9 to imatinib
HER2 over expression/ amplification	Breast carcinoma, gastric carcinoma	Algorithmic IHC/FISH, CISH or DDISH	Predictive: strong (+++) IHC and moderate IHC(++)/ISH positive more likely to respond to trastuzamab
EML4-ALK translocations	Lung adenocarcinoma	IHC, ALK breakapart FISH assay	Predictive of response to erlotinib
Mismatch repair gene immunohistochemistry	Colorectal carcinoma, endometrial carcinoma	IHC	In context of family history/ fulfilment of revised Bethesda criteria suggests HNPCC
MSI testing		PCR, electrophoresis	Some evidence as prognostic marker (good) and detrimental 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

From McManus DT. Miscellaneous specimens and ancillary techniques. In: Allen DC, Cameron RI, editors. Histopathology specimens: clinical, pathological and laboratory aspects. 2nd ed. London: Springer; 2012

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. Whilst 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 signalling 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 centres 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 signalling networks rather than individual target genes.

In situ hybridisation techniques may be used to detect viral nucleic acid (e.g. EBV in post transplant lymphoproliferative disorders, HPV subtyping in cervical biopsies), lymphoid clonality (κ, λ light chain mRNA), and karyotypic abnormalities such as HER2/neu amplication in breast cancer and n-myc in neuroblastoma.

Flow cytometry has a diagnostic role in subtyping of leukaemia and malignant lymphoma, and may help distinguish between partial and complete hydatidiform moles.

Quantitative Methods

There is a role for the use of quantitative methods as diagnostic aids. These include stereology, morphometry, automated image analysis, DNA cytophotometry and flow cytometry: some of these techniques are fully integrated into diagnostic laboratory systems whilst others are more often utilised in research settings. In general, adverse prognosis is related to alterations in tumour cell nuclear size, shape, chromasia, texture, loss of polarity, mitotic activity index, proliferation index (Ki-67 or S-phase fraction on flow cytometry), DNA aneuploidy and spatial density. Most of these techniques show good correlation with carefully assessed basic histopathological criteria and, rather than replacing the pathologist and basic light microscope, serve to emphasise the importance of various parameters and sound morphological technique. Areas of potential incorporation into pathological practice are:

- Morphometric measurement of Breslow depth of melanoma invasion, osteoid seams in osteomalacia, and muscle fibre type and diameter in myopathy.
- Mitotic activity index in breast carcinoma, GISTs, gynaecological and soft tissue sarcomas.
- DNA ploidy in partial versus complete hydatidiform mole.

With the advent of more advanced computer hardware and sophisticated software, artificial intelligence and automated tissue analysis are being explored:

- Automated cervical cytology.
- Inference and neural networks in prostatic cancer and colonic polyps.
- Bayesian belief networks and decision support systems in breast cytology.
- MACs (malignancy associated changes) in prostate cancer based on alterations in nuclear texture.
- Bioinformatics facilitates analysis of gene and tissue microarrays used to test the level of

expression for multiple genes in relatively few samples, or, the staining pattern of relatively few markers on a large number of samples, respectively. This allows more standardised scoring of current prognostic markers on samples from multiple patients, and also facilitates discovery of new prognostic cancer biomarkers.

This whole area of translational research is rapidly developing and evolving and it remains to be resolved as to which facets will eventually be incorporated into routine practice.

Error, Audit, Quality Assurance, Clinical Governance and Digital Microscopy

Errors in a subjective discipline such as diagnostic pathology are inevitable but rates are surprisingly low (1–2%). Whether cognitive (oversight or interpretational) or operative (non-analytical) they may be purely academic (e.g. a difference in nomenclature) or clinically significant (e.g. a false positive diagnosis of cancer). Any surgical pathologist hopes to avoid the latter and the potential consequences for the patient. Errors are discovered by various routes: inconsistency in clinical outcome with individual case review, review at regular multidisciplinary cancer meetings, topic related audit, systematic selective surgical case review, or, prospective in-house or external case referral for opinion. Clinical governance defines standards of care with open acknowledgement, communication and correction of errors. Professionals are encouraged to quality assure, sometimes double report, check their work in a team context supporting colleagues, and identify any indicators of underperformance. Advice from the Royal College of Pathologists is that pathologists should not report outside their field of expertise, and that there should be judicious use of various forms of double reporting, e.g. to address particular local needs, in the context of review for multidisciplinary team meetings, and for some diagnoses where mandated by specialist organisations (available at www.rcpath.org/profession/publications.html). In the UK the Royal College of Pathologists Professional Standards Unit publishes protocols and advises on issues of professional performance with the capacity to investigate and recommend remedial action in individual cases. Consequently most pathologists adopt several strategies to maintain standards including participation in continuing professional development (CPD) and interpretive external quality assurance (EQA) schemes. CPD entails attendance at local, national and international conferences and seminars, journal reading and other educational activities relevant to the pathologist's practice with reinforcement of strengths and identification of knowledge gaps. This approach is inherent to annual appraisal and medical revalidation which should be consolidative and developmental in nature. EQA schemes are general or specialist in type with pre-circulation of slides or access to web-based digitally scanned images and clinical histories. The pathologist submits diagnostic answers which are marked in comparison to the participants' consensus diagnoses. Results are confidential to the pathologist but an individual with repeated outlying marks may be flagged up to the scheme co-ordinator so that appropriate advice can be given. Definition of what constitutes a specialist pathologist is complex but at least involves spending a significant amount of professional time practising in the relevant area and participation in the relevant multidisciplinary team meeting and an appropriate EQA scheme. Pragmatic experience informs who to send the referral case to and just "who can do the business". The rapidly improving technology of digitised virtual microscopy with scanning of whole slide images is developing roles as an alternative to slide circulation in EQA schemes, case referral to experts, live remote diagnostic reporting, image incorporation into diagnostic reports, facilitation of cross site multidisciplinary meetings, education and training, and in research and archiving. Automated immunohistochemistry, multiblocks and scanning of tissue microarrays will augment morphology by assessment of multiple prognostic markers. Thus the way ahead is charted for the surgical pathologist building on

the foundation of clinical knowledge allied to morphological expertise, supplemented by various ancillary techniques, and showing a slide around to colleagues whose opinion you value.

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Part I

Gastrointestinal Cancer

- Oesophageal Carcinoma
- Gastric Carcinoma
- Ampulla of Vater and Head of Pancreas Carcinomas
- Small Intestinal Carcinoma
- Colorectal Carcinoma
- Appendiceal Lesions
- Anal Canal Neoplasia (with Comments on Pelvic Exenteration)
- Gall Bladder Carcinoma
- Perihilar and Distal Extrahepatic Bile Duct Carcinoma
- Liver Carcinoma

Oesophageal Carcinoma

2

Damian McManus

The geographical incidence of oesophageal cancer varies greatly with high risk areas in China and Asia where the predominant tumour type is squamous cell carcinoma contrasting with a sharp increase in distal oesophageal adenocarcinoma in lower risk areas in the Northern and Western hemispheres. Risk factors include smoking, alcohol ingestion, obesity, metabolic syndrome, gastrooesophageal reflux, and Human Papilloma Virus (HPV) infections. There is a very marked male predominance in oesophageal adenocarcinoma. Risk factors and response to treatment show differences between the two histological subtypes, reflected in their mutational signatures and driver gene mutations.

Oesophageal cancer usually presents with progressive dysphagia initially for solids and ultimately liquids. Investigation is by endoscopy and biopsy, and chest X-ray to detect any enlargement of the heart, mediastinal lymph nodes or lung lesion that may be causing extrinsic compression. For biopsy proven cancer, staging for local and distant disease includes EUS (endoluminal ultrasound) for tumour depth and lymph node spread, CT (computerized tomography) scan of chest and abdomen and combined CT/PET (positron emission tomography) scan for locoregional and non-regional metastases. Oesophagogastric junctional adenocarcinoma and select cases (T3 or bulky tumours) of distal oesophageal adenocarcinoma have laparoscopy with peritoneal staging washings. Bronchoscopy may be undertaken in patients with clinical or radiological features suspicious of tracheobronchial invasion. Thoracoscopy may be required to sample suspect mediastinal lymph nodes. The potential for synchronous upper aerodigestive tract tumours is borne in mind. Patients with identified distant metastases or involved lymph nodes in three compartments (neck, mediastinum, abdomen) are not suitable for curative treatment.

Treatment of oesophageal cancer may be palliative (chemoradiotherapy, laser ablation, stent) in bulky high stage disease, or, curative in intent with earlier lesions. Radical chemoradiation for squamous cell carcinoma is similar in efficacy to chemotherapy and surgery. Peri-operative chemotherapy for adenocarcinoma may downstage the tumour, followed by radiological restaging, and then surgery. Chemoradiation followed by surgery is used in some countries (trials, e.g. Neo-AEGIS are ongoing in the UK). Choice of operative procedure depends on the general health of the patient, the tumour site and extent, the choice of planned oesophageal substitute (stomach, jejunum, colon), and preference of the surgeon. Ideally, longitudinal clearance margins of 5–10 cm should be achieved and an appropriate two field lymphadenectomy performed to improve staging and local disease control. Transthoracic or

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_2

transdiaphragmatic hiatal approaches are available with the latter particularly suitable for localised distal oesophageal lesions and resulting in less operative morbidity than thoracotomy. Minimally invasive oesophagectomy (MIO) procedures are being developed using combined thoracoscopic and laparoscopic techniques. There is currently no preferred evidence based method of choice. Early lesions confined to the mucosa may be amenable to local surgical techniques, e.g. endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD). It is recognized that these "big biopsy" specimens have a triple purpose: diagnostic, staging and potentially therapeutic. Follow up radical surgery may be necessary if there is submucosal involvement, lymphovascular invasion or deep margin involvement in the EMR specimen.

Gross Description

Specimen

- Biopsy/EMR/ESD/partial oesophagectomy/total thoracic oesophagectomy (TTO)/oesophagectomy with limited gastrectomy/oesophagogastrectomy.
- Procedure: endoscopic/MIO/transthoracic or transhiatal.
- Number of fragments EMR/ESD dimensions(mm)/length of oesophagus and proximal stomach (mm). Measurements are better assessed on the fresh specimen as formalin fixation causes up to 30% contraction. The external surface is also inspected for the presence of adventitial fat, lateral mediastinal pleura, pericardium and distally abdominal peritoneum.

Tumour

Site

- Mid/lower oesophagus/oesophagogastric junction/cardia (Fig. 2.1).
- Distances (mm) to the proximal and distal resection limits and the oesophagogastric junction. The junction can vary in location or be obscured by tumour. Anatomically distal oesophagus has an external layer of adventitia or abdominal peritoneum whereas proximal stomach is orientated to serosa. Tumour is clearly oesophageal if its bulk or epicentre is above the oesophagogastric junction as defined by internal or external landmarks, e.g. the mucosal squamocolumnar Z line, where the tubular oesophagus ends and the saccular stomach begins, or orientation to adventitial fat. This may also be corroborated histologically with the finding of Barrett's metaplasia/dysplasia or squamous epithelium/dysplasia in the oesophagus. Note that under TNM8 tumours of the oesophagogastric junction, and those in the proximal stomach with an epicenter within 2 cm of the junction and involving the oesophagus, are staged as oesophageal cancer. From a clinical point of view, tumours involving the junction are classified as Siewert I (distal oesophagus growing down), II (truly junctional) or III (gastric cardia growing up). In addition squamous cell, small cell and undifferentiated carcinomas involving the junction are regarded as oesophageal in origin.

Size

- Length × width × depth (mm) or maximum dimension (mm). Cure is unlikely in tumours that are >10 cm in length.
- Superficial carcinoma is often small (<2–3 cm long) but advanced carcinoma frequently involves long segments of oesophagus.

Appearance

- Polypoid: spindle cell carcinoma with good prognosis.
- Warty/verrucous: verrucous carcinoma.
- Nodular/plaque: superficial carcinoma (the gross and endoscopic appearances may be classified similar to that of early gastric cancer; see Chap. 3).

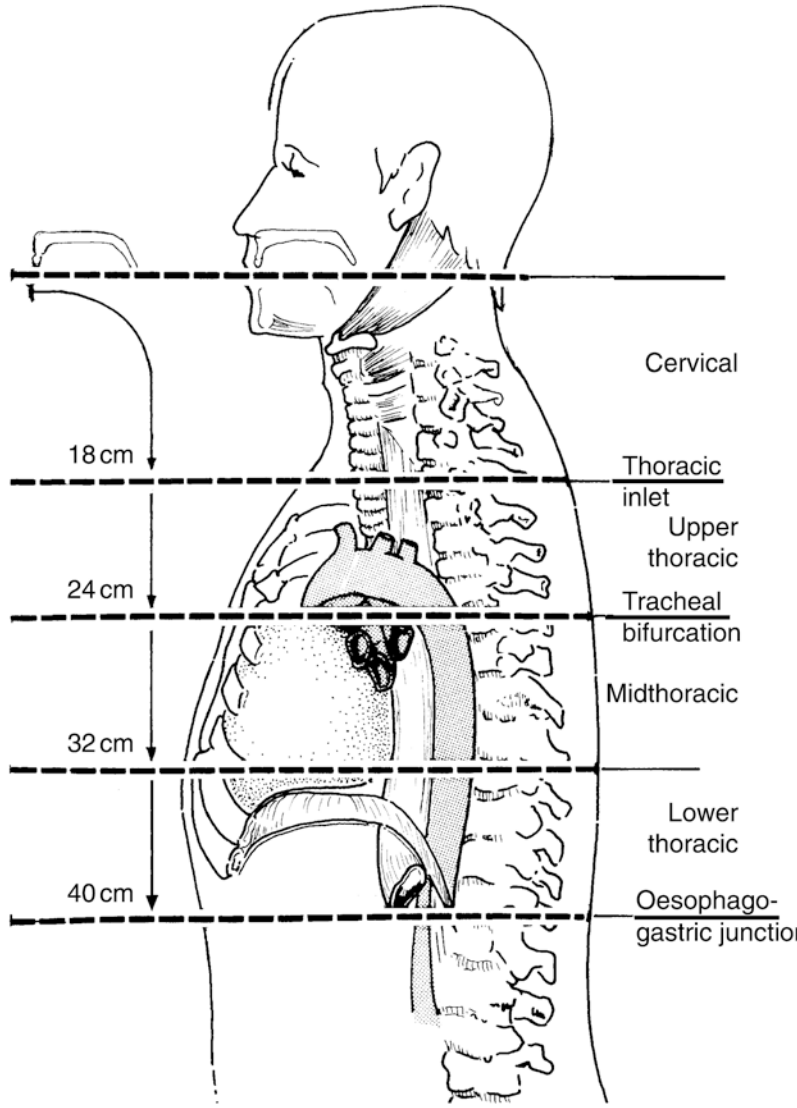

Fig. 2.1 Oesophagus. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

- Fungating/stricture/ulcerated/infiltrative: usual types.
- Multifocal (10%).
- Regression and scarring post adjuvant chemo-/radiotherapy.

Edge

- Circumscribed/irregular.

Other Pathology

- Fistula/perforation either spontaneous, post neoadjuvant therapy or post endoscopy.
- Diverticulum.
- Achalasia.
- Barrett's metaplasia: velvety mucosa distinct from the pale squamous mucosa and proximal to the junction.
- Male preponderance (3:1).

Histological Type

Adenocarcinoma

- *50–60% of cases*.
- In the *distal oesophagus/oesophagogastric junction* on the basis of specialised enteric type *Barrett's metaplasia* and *dysplasia.* The incidence of this tumour has greatly increased (×3–5 in the last 20 years). Various suggested factors are heredity, improved socio-economic conditions with obesity from a Western diet rich in processed foods, antibiotic eradication of acid suppressing pangastric cag-A (cytotoxin associated gene product) positive *Helicobacter pylori* with restoration of gastric acidity and increased gastro-oesophageal reflux disease, proton pump inhibitor therapy and bile reflux. Most are tubular or papillary and of *intestinal type,* some are signet ring cell or mucinous. There is *poor prognosis* as presentation is at a late stage, typically with adventitial, lymph node and perineural invasion.

Squamous Cell Carcinoma

- *30–40% of cases*.
- *Mid-oesophagus*, older age. Risk factors are smoking and excess alcohol.
- Usually moderately differentiated and keratinising.
- *Verrucous:* exophytic and keratotic with a pushing deep margin of cytologically bland bulbous processes. Slow growing but anecdotally may become more aggressive especially after radiation.
- *Basaloid:* poor prognosis and *aggressive*. Deeply invasive nested pattern of palisaded basaloid cells with central necrosis, atypia and mitoses.

Spindle Cell Carcinoma (Polypoid Carcinoma/Carcinosarcoma)

- A spindle cell squamous carcinoma that undergoes varying degrees of stromal mesenchymal homologous or heterologous differentiation.
- Men, sixth decade.

Adenosquamous Carcinoma

- Mixed glandular and squamous cell differentiation, *aggressive.*

Undifferentiated Carcinoma

- Absent squamous cell or glandular differentiation and a *high-grade lesion*.
- In poorly differentiated lesions, adenocarcinoma may be BER-EP4, CDX2 positive, and squamous cell carcinoma negative for these markers but CK5/6, p63 and p40 positive. However, there can be some overlap in immunophenotypic expression. Characterisation is of use as squamous cell carcinomas are often more responsive to neoadjuvant chemoradiation and can be associated with synchronous or metachronous upper aerodigestive tract tumours.

Mucoepidermoid/Adenoid Cystic Carcinoma

- Of oesophageal submucosal duct origin with a tendency to local recurrence and metastases in 50% of cases.

Small Cell Carcinoma

- A poorly differentiated/high-grade neuroendocrine carcinoma, either primary or secondary from lung, or as part of a mixed differentiation oesophageal cancer. It is of *poor prognosis*. Distinguish from poorly differentiated basaloid squamous cell carcinoma or adenocarcinoma by synaptophysin/CD56/TTF-1 and paranuclear dot CAM 5.2 expression in small cell carcinoma.

Malignant Melanoma

- Primary or secondary. Primary requires adjacent mucosal junctional atypia. Comprises *0.1% of oesophageal malignancy*—polypoid, ulcerated, satellite nodules, pigment, *poor prognosis*.

Metastatic Carcinoma

- *Direct spread*: stomach, thyroid, hypopharynx, bronchus and lung
- *Distant spread*: breast, malignant melanoma.

Differentiation

Well/moderate/poor/undifferentiated, or Grade 1/2/3/4.

- Influence on prognosis is uncertain unless the tumour is anaplastic, e.g. undifferentiated carcinoma, small cell carcinoma or basaloid carcinoma.
- For squamous cell carcinoma, differentiation features are keratinisation and intercellular bridges, and, for adenocarcinoma the percentage tumour gland formation (well/G1 > 95%: moderate/G2 50–95%: poor/G3 < 50%). Undifferentiated carcinomas cannot be categorised as either squamous cell or adenocarcinoma and are classified as Grade 4 (as is small cell carcinoma).
- Heterogeneity of grade and differentiation within individual tumours is not uncommon, e.g. mixed intestinal and signet ring patterns.

Extent of Local Tumour Spread

Border: pushing/infiltrative.

Lymphocytic reaction: prominent/sparse.

Depth (pT stage) and distance (mm) to the nearest painted perioesophageal circumferential resection margin (CRM).

Superficial ("early") squamous cell carcinoma of the oesophagus is defined as intraepithelial or invasive squamous cell carcinoma confined to the mucosa or submucosa, with or without lymph node spread (pTis, pT1). It is of more *favourable prognosis* than the usual muscle invasive deep or *"advanced" carcinoma (beyond the muscularis propria)* with differing 5 year survival rates: 60–90% vs. 5–10%. Carcinoma invading *submucosa* does less well (25–35% lymph node metastases, 55% 5 year survival) than that confined to the mucosa alone (88% 5 year survival irrespective of lymph node status). *Depth of invasion* is the most important *prognostic indicator* on multivariate analysis and requires histological assessment as there is variable correlation with gross, radiological and endoscopic appearances. Note that on biopsy, distinction between dysplastic glands or squamous epithelium abutting an irregular muscularis mucosae and true invasion can be difficult: look for single cells and nests of infiltration (± a desmoplastic stromal reaction). This is further complicated by the relatively common finding of duplication of the muscularis mucosae in Barrett's metaplasia. The edge of a well differentiated adenocarcinoma may manifest only as mildly atypical glands devoid of stromal reaction but undermining oesophageal squamous epithelium. However, the pitfall of squamous re-epithelialisation overlying Barrett's mucosa (± dysplasia) related to ablation treatment must also be borne in mind.

The TNM8 classification applies only to carcinomas (Fig. 2.2).

pTis	Carcinoma in situ/high-grade dysplasia
pT1	Tumour invades lamina propria or muscularis mucosae (pT1a) or submucosa (pT1b)
pT2	Tumour invades muscularis propria
pT3	Tumour invades adventitia
pT4	Tumour invades adjacent structures
pT4a	Pleura, pericardium, azygos vein, diaphragm, or peritoneum
pT4b	Aorta, vertebral body, trachea.

About 50% of distal oesophageal carcinomas spread into the proximal stomach with potential for serosal involvement.

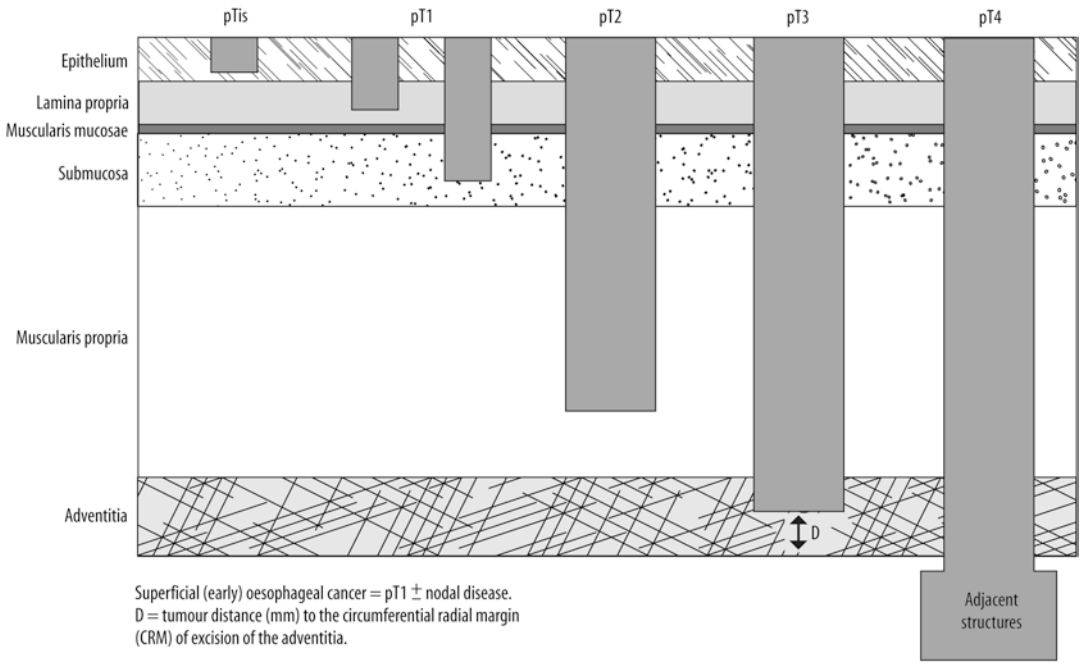

Fig. 2.2 Oesophageal carcinoma. Invasion of adjacent structures includes pleura, pericardium, azygos vein, diaphragm and peritoneum (pT4a) and aorta, vertebral body or trachea (pT4b). Adapted from *Histopathology Reporting: Guidelines for Surgical Reporting*, *2nd ed.,* © 2006, Springer

In cases with post neoadjuvant therapy tumour regression, ypT is determined by the *deepest residual viable tumour* and not more deeply placed acellular keratin debris where tumour may have been. A *three grade classification of tumour response to neoadjuvant therapy* (no or minimal residual tumour/moderate residual tumour/no response) is a *prognostic indicator* with complete regression and absence of lymph node or distant metastases being favourable signs. Histological tumour regression shows variable correlation with other measures of clinical response such as improvement in dysphagia, or decrease in tumour metabolic FDG (fluorodeoxyglucose) avidity on PET scan.

Lymphovascular Invasion

Present/absent.

Intra-/extratumoural.

The presence of lymphovascular invasion (LVI) is a *strong prognostic indicator*. In advanced carcinoma lamina propria and submucosal LVI are not infrequent, resulting in carcinomatous emboli several centimetres beyond the gross tumour edge. These skip metastases (15% of cases) are not classified separately under TNM8. Perineural invasion is also characteristic.

Lymph Nodes

The significance of lymph node micrometastases ($\leq$ 2 mm diameter) is uncertain but *involvement of lymph nodes*, particularly if multiple, is a *strong prognostic indicator*. Lymph node metastases occur *early in the disease course* (60% at the time of presentation) and are the commonest cause of treatment failure. Histological assessment is required as specificity of lymph node involvement on EUS is limited. Involvement of stomach and later liver, lungs and adrenal gland is not infrequent. Up to *30% of metastases are clinically occult* and discovered at CT/PET examination.

Site/number/size/number involved/limit node/extracapsular spread.

Regional nodes: those in the oesophageal drainage area including coeliac axis nodes and paraoesophageal lymph nodes in the neck, but not the supraclavicular lymph nodes. A regional lymphadenectomy will ordinarily include a minimum of 7 regional lymph nodes (Fig. 2.3).

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
pM1	Distant metastasis (commonest sites are mediastinum, lung and liver)

Excision Margins

Distances (cm) to the proximal and distal limits of excision. There is good evidence that proximal resection margin involvement in particular increases the risk of recurrence.

Distance (mm) to the painted perioesophageal CRM. Involvement (tumour present to within 1 mm) is an index of the degree of tumour spread and extent of surgical resection with potential for local recurrence or residual mediastinal disease and decreased survival. There is some uncertainty in evidence surrounding the use of 1 mm to define margin involvement: Royal College of Pathologists guidance recommends recording distance to CRM for all tumours (microscopic residual tumour (R1) refers to tumours <1 mm from the CRM).

Oesophageal carcinoma may show *multifocality* (10–25%), direct or discontinuous submucosal and lymphovascular spread and *intramural metastasis* (15%). This has obvious implications for examination of resection margins (sometimes by intraoperative frozen section) and potential for local recurrence.

Other Pathology

Diverticula, achalasia, coeliac disease and Plummer-Vincent syndrome (middle aged to

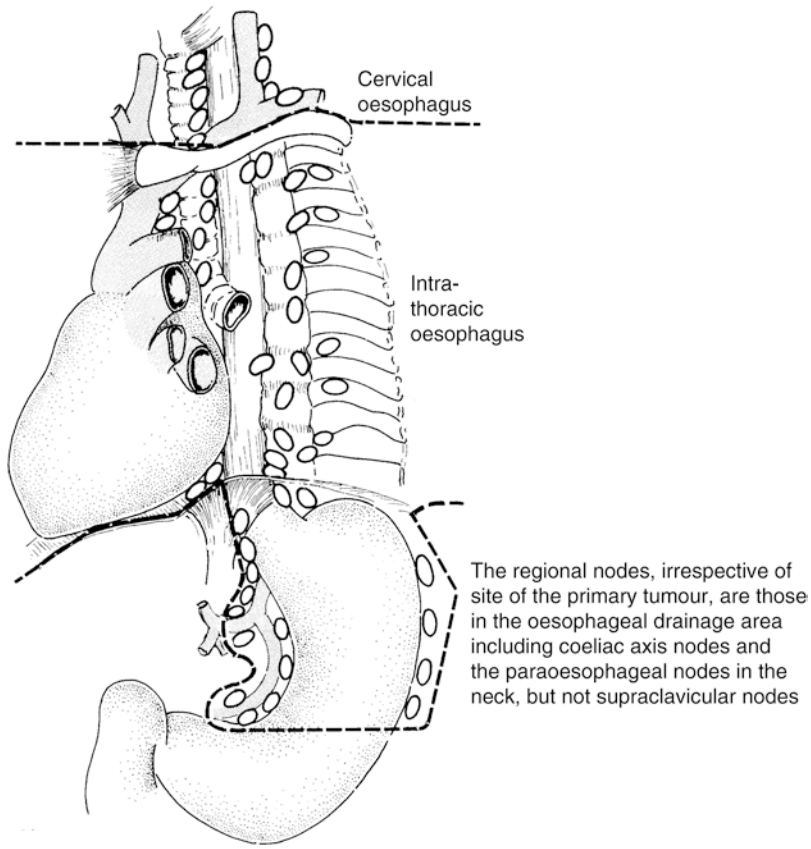

Fig. 2.3 Oesophagus: regional lymph nodes. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

elderly females, iron deficiency anaemia, post cricoid pharyngo-oesophageal web) have an increased incidence of oesophageal carcinoma. Squamous cell carcinoma is usually in the mid-thoracic oesophagus while Barrett's related adenocarcinoma is commoner, being the most frequent malignant tumour of the distal oesophagus. The *incidence* of *Barrett's related adenocarcinoma* and *oesophagogastric junctional tumours* has markedly *increased.*

Barrett's metaplasia: defined as replacement of the lower oesophageal squamous mucosa by metaplastic glandular epithelium due to *gastrooesophageal reflux disease*. The Barrett's segment can be *classical (>3 cm long)* or *short (<3 cm long),* with long segment disease having an increased risk of malignancy. The clinical extent of the Barrett's change is described according to its length of circumferential disposition and total segment length, i.e. C2M6 is circumferential over 2 cm in a 6 cm long segment. Ultra-short segment Barrett's is now regarded as junctional metaplasia, a separate condition related to *Helicobacter pylori* infection or acid reflux. An approximate guide is that 10% of patients with hiatus hernia and/or gastrooesophageal reflux develop Barrett's metaplasia. Population based studies of progression rates in Barrett's oesophagus suggest progression rates of <0.5% per annum, with progression rates highest in older males The *specialised intestinal* or *enteric variant of Barrett's metaplasia* is the usual precursor to dysplasia rather than the atrophic gastric fundic or non-specialised cardia types.

The *biological behaviour* of *low-grade dysplasia* is uncertain with potential for regression after high dose proton pump inhibitor (PPI) treatment, or progression. It requires reassessment and if a higher grade lesion is excluded, PPI treatment is instigated with subsequent 6 monthly endoscopic surveillance. It has been suggested that low grade dysplasia is overdiagnosed and underestimated as a risk factor for progression. If a high proportion of new Barrett's cases are diagnosed as low grade dyplasia then progression rates are typically lower than for centres diagnosing low grade dysplasia more frequently. Progression rates are also higher when two or more observers *independently* diagnose a case as low grade dysplasia.

Persistent low grade dysplasia, double read preferably by experienced or specialist gastrointestinal pathologists is now accepted as an indication for referral for ablation treatment.

There is a *strong association* between *high-grade dysplasia* and concurrent or subsequent *adenocarcinoma,* indicating the need for immediate clinicopathological reassessment, short-term follow up, and consideration given to its eradication (see below). The recognition of significant dysplasia requires *confirmation* by a second experienced pathologist or positive repeat biopsy. Useful clues to the presence of dysplasia are mucosal villosity and persistence of cytological dysmaturation into the surface epithelium. Features suggestive of *adenocarcinoma* include cribriform architecture and high-grade dysplastic epithelium with any or several of: superficial ulceration, infiltration by polymorphs, necrotic eosinophilic luminal and nuclear debris, and undermining of squamous mucosa. Infiltration of lamina propria and stromal desmoplasia indicate established malignancy. Observer agreement rates are reasonably good for high-grade dysplasia. However, it is important to distinguish *florid regenerative changes* in oesophageal squamous and glandular mucosae from dysplasia. This must take into account erosion, ulceration and the degree of inflammation that is present, as well as cytoarchitectural changes, e.g. nuclear enlargement with nucleolar prominence and basal cell hyperplasia. *Squamous epithelial regrowth* after anti-reflux, laser or photodynamic ablative therapy can produce variably atypical and confusing cytoarchitectural changes, as does chemoradiation therapy. Maturation towards the epithelial surface is reassuring. Over expression of p53 or AMACR and a high Ki-67 proliferation index (especially in the surface epithelium) can sometimes help to highlight or confirm mucosal dysplasia and its potential for progression to adenocarcinoma in dysplastic Barrett's mucosa. It should be noted that the *primary diagnosis of Barrett's metaplasia* (CLO–Columnar Lined Oesophagus) is heavily dependent on the *endoscopic findings* and *site of biopsy*, i.e. an origin from the anatomical oesophagus. Pathognomonic histological features are metaplastic glandular

epithelium associated with native oesophageal structures, e.g. submucosal glands or ducts. Glandular mucosa with squamous epithelial islands is also a useful clue. Specialised enteric differentiation is reasonably distinctive whereas fundic gastric mucosa is more often associated with hiatus hernia.

Surveillance for dysplasia in Barrett's mucosa is recommended as annual or biennial endoscopy with quadrantic, segmental (every 2 cm) biopsies. More sophisticated endoscopic techniques are now more commonly available, e.g. magnification/chromoendoscopy, spectroscopy. *Target biopsy* of any gross lesion (ulcers, nodules, plaques, strictures) is important as this is more likely to yield significant pathology. In the absence of tumour, or in a medically unfit patient with an early mucosal lesion, *local ablative therapy* can be used, e.g. EMR of any mucosal nodule, and high radiofrequency (HALO), laser or photodynamic therapy to the background Barrett's segment. In general, mucosa confined disease can be managed by endosopic treatment and subsequent surveillance. Detailed multidisciplinary team discussion and *careful patient selection* are crucial when considering organ conserving treatment. Adverse histological prognostic factors in any EMR specimen or signs of clinical progression may signify the need to proceed to radical surgery. Close patient follow up is required. *Oesophagectomy* tends to be reserved for cases with submucosal invasion as it has up to a 25% risk of lymph node metastases, where there are clinically suspected regional lymph node metastases, or there has been unsuccessful endoscopic therapy, or high-grade dysplasia that is beyond treatment by HALO. Non-regional disease precludes radical surgery.

Field change squamous cell dysplasia/carcinoma in situ: often encountered adjacent to or overlying squamous cell carcinoma. A precancerous phase and the biological course of these premalignant changes is uncertain but better established in countries such as China and Japan where the incidence of oesophageal carcinoma is greater. This has led to the establishment of endoscopic and cytological screening programmes targeted at the detection of early stage carcinoma (10–15% only of cases in the West). As with glandular dysplasia, a two-tiered system of *low-grade* (≤50% of the epithelial width) and *high-grade dysplasia* is used. Dysplasia is found more frequently overlying and adjacent to superficial than advanced squamous cell carcinoma. It may be identified at endoscopy as friable, erythematous plaques or nodules. *Some 25% of high-grade dysplasia lesions progress to carcinoma*. If confined to the mucosa at endoscopy and EUS, *local mucosal resection* (EMR) or *ablation* (HALO, laser) may be used. Histologically it must be distinguished from inflammatory regenerative or reflux changes, viral infection (herpes simplex, CMV), pseudoepitheliomatous hyperplasia (e.g. overlying a granular cell tumour), chemoradiotherapy changes and established cancer. A further diagnostic clue is that squamous dysplasia lifts off with the biopsy forceps in intact strips bound by basement membrane whereas squamous cell cancer fragments.

Concurrent squamous cell carcinoma: of bronchus and oropharyngolaryngeal ring has an *incidence of 10–15%.* Bronchoscopy and upper airways endoscopy may be required prior to radical treatment to exclude a lung cancer spreading to involve the oesophagus. Conversely careful scrutiny of the chest CT scan is also necessary to identify any evidence of spread of an oesophageal cancer to contiguous mediastinal structures (pT4 disease) that might exclude consideration of radical surgery.

Chemo-/radiotherapy necrosis and tumour regression: cell apoptosis, vacuolation and degeneration, necrosis, inflammation, fibrosis, residual aggregates of keratin with a giant cell reaction, and perforation may all be seen leaving only residual microscopic tumour. The degree of *tumour response to treatment is graded* according to the five tier *Mandard score*. As discussed above, a three grade scoring system is easier to use in practice and more reproducible. Post surgical pathological staging is determined by the *deepest focus of residual tumour,* and not tissue reaction or acellular keratin where tumour may have been present pretreatment.

Chemoradiation may be selected as an alternative radical treatment for squamous cell carcinoma

of the middle third of the oesophagus (total field length for primary and nodal metastases must be <10 cm) and chemotherapy *followed by surgery* for suitable patients with operable tumours. Distal oesophageal and Siewert I junctional adenocarcinomas may be treated by *preoperative chemotherapy* followed by surgery or *radical chemo-radiotherapy* . Trials are ongoing to compare peri-operative chemotherapy with chemoradiation to determine if increased pathological response rates and complete resection are reflected in improved survival. It is estimated that some *50–60% of tumours show quite marked morphological changes of regression,* with squamous cell carcinoma being more radiotherapy and chemoresponsive than adenocarcinoma. More sophisticated preoperative staging (e.g. CT/PET scan and EUS with FNAC) is being assessed as a means of predicting those patients likely to benefit from preoperative neoadjuvant therapy, and in selecting patients with locoregional confined disease for primary resection. Patients are restaged radiologically after neoadjuvant therapy is com pleted prior to curative intent radical surgery. This is in case there has been non-response and tumour progression in the interim. Ablative laser therapy and insertion of an expansile metal stent are additional *palliative measures* for bulky obstructive non-resectable tumour.

Spindle cell carcinoma: cytokeratin and mesenchymal markers (vimentin, desmin, actin) are helpful in spindle cell carcinoma (syn. carcinosarcoma). These tumours show a *biphasic spectrum of differentiation*: malignant epithelial (squamous) and mesenchymal tissues (usually sarcoma not otherwise specified, sometimes cartilage, bone, striated muscle). There is either intimate intermingling or juxtaposition of the components which are present in variable amounts (the epithelial component may be microscopic or in situ). *Prognosis is intermediate to good* because they are exophytic intraluminal lesions which present at a relatively early stage despite their size (50% 5 year survival).

Prognosis

Prognosis of oesophageal cancer is poor (5 year survival 5–10% in the Western Hemisphere), and relates to *tumour type* (small cell carcinoma, basaloid carcinoma are adverse), *grade* (equivocal), *diameter* (in superficial carcinoma), but most importantly *depth of invasion* and *stage. Lymph node status* and whether the *longitudinal* and *circumferential radial margins* are positive (55% recurrence rate, 25% 5 year survival) or negative (13% recurrence rate, 47% 5 year survival in one series) are important prognostic variables. Early oesophageal squamous cell carcinoma does significantly better than advanced disease. Early (pT1) adenocarcinoma may show less lymph node disease and local recurrence than equivalent squamous cell lesions. However, for the majority of cases, although adenocarcinoma may have slightly better overall 5 year survival (25%), the two main pathological types have little differential influence on prognosis.

Other Malignancy

Malignant Lymphoma/Leukaemia

- Rare: more usually secondary to gastric or systemic/mediastinal lymph node disease.
- Primary lymphoma is large B cell in type, or extranodal marginal zone lymphoma (MALToma).
- Consequences of immunosuppression due to the tumour or its treatment may be seen, e.g. CMV, herpetic or fungal oesophagitis.

Leiomyoma/Leiomyosarcoma/GISTs

- *leiomyomas* greatly outnumber *leiomyosarcomas* (feature of malignancy: >5 cm diameter, necrosis, mitoses >5/50 high-power fields, cellular atypia, infiltrative margins). Most leiomyomas are small, identified by endoscopy and arise from the muscularis mucosae or inner muscularis propria. They can be multiple, intraluminal or intramural. They may harbor significant numbers of CD117 positive interstitial cells of Cajal which may cause diagnostic confusion, particularly on small biopsy specimens. Oesophageal gastrointestinal stromal tumours *(GISTs)* are rare (DOG1/

CD117/CD34 positive, desmin negative) and potentially malignant with liver metastases.

Sarcoma

- Rare: 90% are *leiomyosarcoma* (desmin, h-caldesmon positive).
- Embryonal rhabdomyosarcoma (childhood: desmin/myo D1/myogenin positive).
- Kaposi's sarcoma (AIDS): human herpes virus 8 (HHV 8) positive.
- Synovial sarcoma: children/adults, polypoid mass in upper oesophagus.
- Exclude the more common possibility of a spindle cell carcinoma (polypoid carcinoma/carcinosarcoma) with cytokeratin positive spindle cells and varying degrees of homologous or heterologous mesenchymal differentiation.

Others

- Rare: granular cell tumour (S100 positive, overlying pseudoepitheliomatous hyperplasia), well differentiated neuroendocrine (carcinoid) tumour: chromogranin, synaptophysin, CD56 positive, Ki-67 $\leq$ 2%, good prognosis.

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Gastric Carcinoma

3

Damian McManus

Gastric cancer accounts for 8% of cancers worldwide with high incidence (Eastern Asia, Eastern Europe, Central/Latin America) and low incidence (North America, Northern Europe, Africa) areas. The former tends to distal tumours of intestinal type, the latter to more proximal tumours with a significant proportion of diffuse cancers. Risk factors are smoking, diet (high in salt-preserved/smoked foods—low in fresh fruit/vegetables), bile reflux and *Helicobacter pylori* infection. About 10% show familial clustering and 1–3% are on a direct hereditary basis. There is also an increased risk of upper gastrointestinal carcinoma in patients who have had previous radiotherapy/chemotherapy for testicular cancer or Hodgkins's lymphoma.

Gastric cancer can present with anaemia, haematemesis, weight loss, abdominal pain or dyspeptic symptoms. Investigation is by endoscopy with biopsy. Decreased distensibility and wall motility either on endoscopy or barium meal examination are suspicious of linitis plastica (diffuse or poorly cohesive carcinoma). Multiple biopsies (a minimum of 8) are required as up to 10% of endoscopically suspicious lesions will require re-biopsy. Staging for local and distant disease includes endoscopic ultrasound (EUS: tumour depth and lymph node spread) and CT scan of chest, abdomen and pelvis. Staging laparoscopy with cytological washings and biopsy of any nodules or plaques in the serosa or omentum is important in staging as CT has limited sensitivity for peritoneal metastasis. Positive peritoneal cytology (4.4–11% positive rate) and or peritoneal metastasis generally indicates incurable metastatic disease. PET scanning is more useful for cancers of the OG junction but is not routinely requested for primary gastric cancer staging in the UK.

Non-regional disease is an indicator for palliative treatment including chemotherapy, and surgery if there is anatomical dysfunction, e.g. gastric outlet obstruction or anaemia. Curative intent surgery can be localised (endoscopic mucosal resection—EMR; endoscopic submucosal dissection—ESD) or radical: the extent of the latter depending on the patient's age, fitness, tumour type, stage and location. Distal (antral) tumours are treated by subtotal gastrectomy and proximal tumours by total gastrectomy. The aim is to achieve a complete (R0) resection with proximal, distal and circumferential margin clearance. Limited gastric resection is used for palliation or in the very elderly. Extent of lymphadenectomy is tailored accordingly. Surgery is often preceded by a course of neoadjuvant chemotherapy and radiological re-staging. Postoperative adjuvant chemotherapy may be indicated following full clinicopathological staging of the surgical specimen and multidisciplinary

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_3

team discussion. A minority of patients with metastatic or recurrent disease are suitable for trastuzumab therapy.

Gross Description

Specimen

- Cytological brushings, washings or aspirate/endoscopic biopsy/EMR(endoscopic mucosal resection)/ESD(endoscopic submucosal dissection)/partial (proximal or distal) or total gastrectomy/oesophagogastrectomy/lymphadenectomy ± omentectomy.
- Number of biopsy fragments/EMR/ESD dimensions (mm).
- Length (mm) along greater curvature.
- Length (mm) of oesophagus and duodenum.

Tumour

Site

- Distal oesophagus/cardia/fundus/ corpus/antrum/pylorus/duodenum (Fig. 3.1).
- Lesser curve/greater curve.
- Anterior/posterior wall.
- Multifocal 6%: in particular early gastric cancer and malignant lymphoma.

Antrum (50%) and lesser curve (15%) are traditionally the most frequent sites. However, the incidence of *distal gastric carcinoma is decreasing* while that of the *proximal stomach and cardia is markedly increasing*. This is in part due to eradication of *Helicobacter pylori* infection and loss of its acid suppression effect with more reflux changes. *Proximal cancer* presents at a more *advanced stage* than equivalent-size distal lesions with a *worse prognosis* and similarities in behaviour to distal oesophageal adenocarcinoma. Adenocarcinomas in the vicinity of the oesphagogastric junction are clinically designated as either Siewert I: distal oesophagus coming down, Siewert II: junctional, or Siewert III: gastric cardia going up. Under TNM8, cancers involving the oesophagogastric junction (OGJ) that have their epicenter within the proximal 2 cm of the cardia (Siewert types I/II) are staged as oesophageal cancers. Cancers whose epicenter is more than 2 cm distal from the OGJ, even if the OGJ is involved, are staged using stomach cancer TNM and stage groups.

Size

- Length × width × depth (mm) or maximum dimension (mm).

Appearance

- Polypoid/plaque/ulcerated/infiltrative/mucoid/linitis plastica (leather bottle stomach)/scirrhous/fleshy.

Advanced (muscle invasive) gastric cancer is classified macroscopically according to Borrmann classification as types:

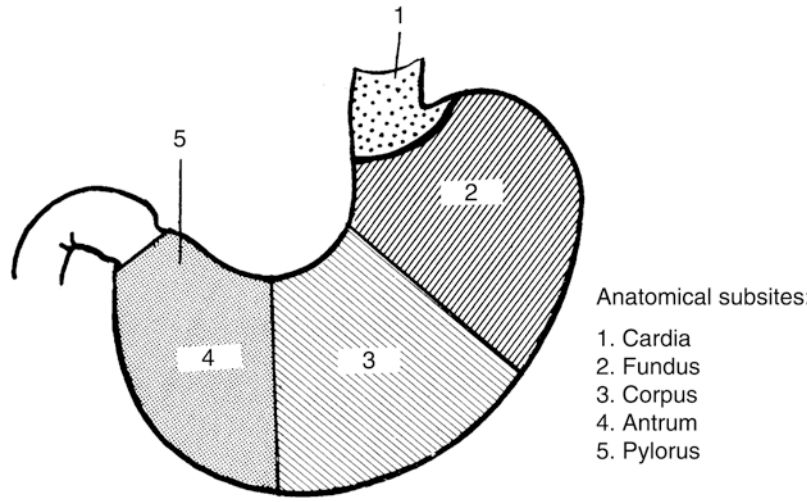

Fig. 3.1 Stomach. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

I	Polypoid
II	Fungating
III	Ulcerated
IV	Infiltrative

Types I, II/III and IV tend to correspond to tubulo/papillary, intestinal, and signet ring cell (linitis plastica) adenocarcinomas respectively, although there is overlap between the categories. Polypoid/exophytic tumours are regarded as being of better prognosis than ulcerated/deeply infiltrative cancers.

Edge

- Circumscribed/irregular.

Histological Type

An amalgam of the WHO and Lauren classifications is used.

Adenocarcinoma

Intestinal	50%	Antrum
Diffuse	20%	Body of stomach, young or elderly patients
Mixed	25%	
Solid	5%	

The WHO system classifies gastric cancer as: tubular, papillary, mucinous, poorly cohesive (including signet ring cell and its variants), and mixed carcinomas. Comprehensive molecular evaluation of primary gastric adenocarcinomas as part of the cancer Genome Atlas project has proposed four molecular subtypes (Epstein-Barr virus (EBV) positive, microsatellite unstable, genomically stable and tumours with chromosomal instability) that may be identified using immunohistochemistry and in situ hybridization. Genomically stable tumours show considerable overlap with diffuse type/poorly cohesive adenocarcinoma whereas tumours with chromosomal instability show intestinal type morphology.

Intestinal carcinomas: have tubuloacinar (common), papillary or mucinous (colloid) patterns, and form polypoid or ulcerative lesions with expansile margins. They are associated with *atrophic gastritis, intestinal metaplasia* and *dysplasia*. By definition tubular adenocarcinoma is well differentiated and may be difficult to diagnose due to wide separation of glands in a non-desmoplastic stroma. Undermining of structures can be helpful, e.g. muscularis mucosae or oesophagogastric junction squamous epithelium. Equally, papillary adenocarcinoma is exophytic with well differentiated epithelial fronds supported by a fine fibrovascular stroma. Biopsies may only sample the surface component and distinction from high-grade dysplasia can be problematic. The endoscopic and EUS/CT appearances and sharp demarcation from adjacent mucosa must be taken into account. The definition of a mucinous adenocarcinoma, whether glandular, colloid or signet ring cell requires mucin production in >50% of the tumour cells or area.

Diffuse carcinomas: comprise single cells with clear or eosinophilic *signet ring cell* cytonuclear appearances, and form *linitis plastica* with infiltrating margins. A point of origin from dysplasia is often difficult to demonstrate as the tumour emanates from the mid-mucosal proliferative zone (from non-metaplastic foveolar or mucous neck cells), or, deep lamina propria invading submucosa, muscularis, serosa and with *transperitoneal spread*. The cells do not express the adhesion protein E-cadherin. A minority (8–10%) of gastric cancers are hereditary. In a young patient, occult presentation with an inherited autosomal dominant (germline mutation in E-cadherin CDH1) diffuse gastric cancer, which in females can be associated with breast lobular carcinoma, should be considered. Alternatively intestinal gastric cancer can develop in a young patient as part of the hereditary non-polyposis colon cancer syndrome (HNPCC) and rarely Familial Adenomatous Polyposis (FAP).

Adenocarcinoma Variants

Invasive micropapillary carcinoma: small clusters of cells in clear spaces simulating vascular channels. It shows a high propensity for lymphovascular and lymph node metastases.

Hepatoid carcinoma: glandular and hepatocellular differentiation with marked vascular invasion and poor prognosis. ± AFP immunoexpression, polyclonal CEA positive. Enteroblastic adenocarcinoma or clear cell adenocarcinoma producing AFP has also been described.

Parietal cell carcinoma: rare, solid sheets of cells with eosinophilic granular cytoplasm.

Medullary carcinoma, lymphoepithelial carcinoma: medullary carcinoma has a solid syncytial morphology, regular vesicular nuclei with pinpoint nucleoli, and a pushing circumscribed margin associated with a dense peritumoural lymphoplasmacytic infiltrate. It has a high level of microsatellite instability (MSI-H), and is similar to the right sided colonic cancers found in HNPCC. Conversely, lymphoepithelial carcinoma has single cells, small clusters and glands and an infiltrating margin with numerous intratumoural or tumour infiltrating lymphocytes (TILs). It has a 77% 5 year survival and can be associated with EBV infection. Anecdotally these EBV positive cases are more chemoresponsive than usual gastric cancer.

Adenosquamous Carcinoma and Squamous Cell Carcinoma

- Rare: need gland formation, keratinisation and intercellular bridges. They show vascular invasion and are *aggressive.*

Undifferentiated Carcinoma

- Cytokeratin positive but no glandular or squamous cell differentiation.

Neuroendocrine Tumours

- Well differentiated/low-grade neuroendocrine (carcinoid) tumour, or, poorly differentiated/high-grade neuroendocrine (small cell/large cell) carcinoma (see Section "Other malignancy").

Malignant Lymphoma

- Low-grade MALToma with potential for high-grade transformation.
- Less commonly: diffuse large B cell lymphoma, follicle centre cell lymphoma, mantle cell lymphoma, T cell lymphoma.

Metastatic Carcinoma

- *Direct spread*: pancreas, oesophagus, transverse colon.
- distant spread: small cell carcinoma lung, malignant melanoma, breast, kidney, choriocarcinoma, ovary, germ cell tumour.
- Metastatic infiltrating lobular carcinoma of breast can mimic signet ring cell carcinoma of stomach and a known clinical history of a previous breast primary is crucial to the diagnosis. Metastatic breast carcinoma is typically GATA3 positive and CDX2/CK20 negative. Note that a significant minority of gastric adenocarcinomas may also be oestrogen receptor positive but do not show the diffuse strong positivity seen in breast lobular cancer.

Differentiation

Well/moderate/poor/undifferentiated, or, Grade 1/2/3/4.

For adenocarcinoma based on the percentage tumour gland formation (well/G1 > 95%: moderate/G2 50–95%: poor/G3 < 50%).

Undifferentiated gastric carcinoma (grade 4) shows no glandular differentiation and requires positive cytokeratin stains to distinguish it from malignant lymphoma or sarcoma. Signet ring cell carcinoma is regarded as poorly differentiated (grade 3) and small cell carcinoma as undifferentiated (grade 4).

Goseki grade—based on mucin secretion and tubule formation:

I	Tubules well differentiated, mucin poor
II	Tubules well differentiated, mucin rich
III	Tubules poorly differentiated, mucin poor
IV	Tubules poorly differentiated, mucin rich.

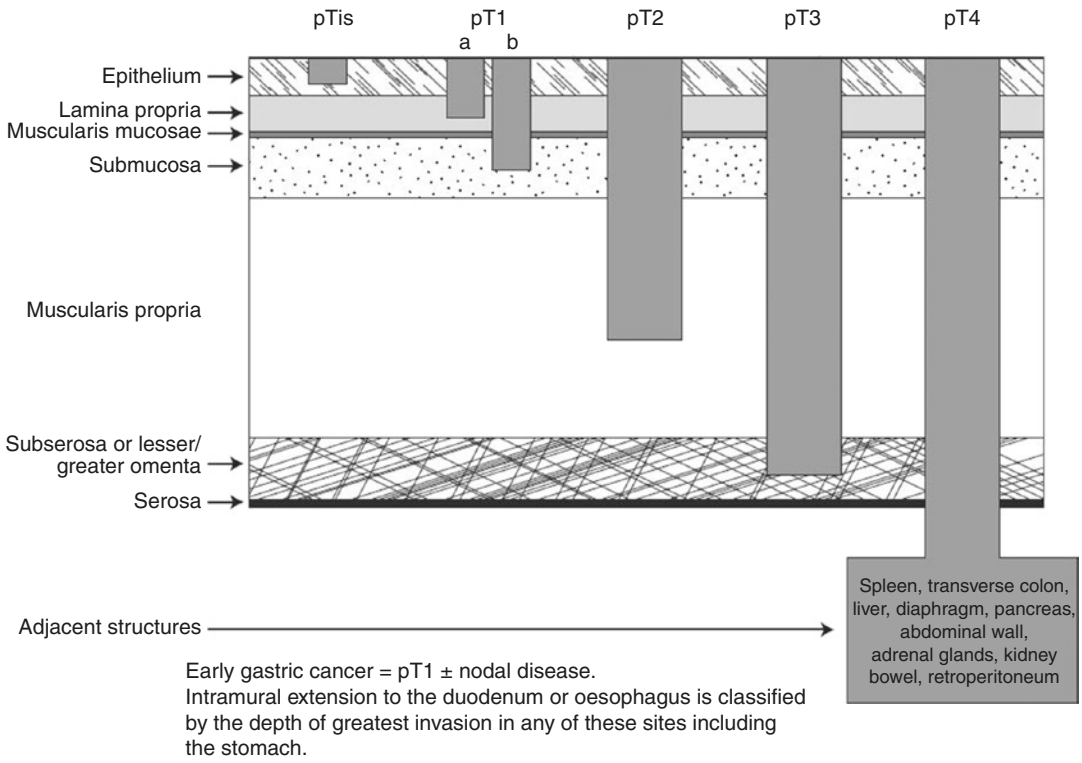

Fig. 3.2 Gastric carcinoma. Tumour perforation of serosa is pT4a. Tumour invasion of listed adjacent stomach structures is pT4b. Reproduced, with permission, from *Histopathology Reporting: Guidelines for Surgical Reporting*, *2nd ed.,* © 2006, Springer

Well differentiated mucin poor cancers have better 5 year survival rates (50–80%) than moderately or poorly differentiated mucin rich tumours (18–46%). Mucin subtyping into gastric (MUC5AC, MUC6) and intestinal (MUC2, CDX2, CD10) may facilitate identification of more aggressive cancers although this is not yet fully clarified.

Extent of Local Tumour Spread

- Border: pushing/infiltrative.
- Well circumscribed tumours have longer patient survival than infiltrating cancers (except in early gastric cancer).
- Lymphocytic reaction: prominent/sparse.
- Gastric cancer is considered as either *early (pT1)* or *advanced (≥ pT2)* as there is *prognostic discrepancy* between the various levels of invasion. 5 year survival figures are: pT1(85–95%), pT2(60–80%), pT3(40–50%).

The TNM8 classification applies only to carcinomas (Fig. 3.2).

pTis	Carcinoma in situ: intraepithelial tumour without invasion of the lamina propria/ high-grade dysplasia
pT1	Tumour invades lamina propria (pT1a) or submucosa (pT1b)
pT2	Tumour invades muscularis propria
pT3	Tumour invades subserosa or mesenteric/ omental fat
pT4	Tumour perforates serosa (pT4a) or invades adjacent structures (pT4b) (spleen, transverse colon, liver, diaphragm, pancreas, abdominal wall, adrenal gland, kidney, small intestine, retroperitoneum).

The lesser omentum includes the gastrocolic and gastrohepatic ligaments, and involvement of their peritoneal covering constitutes pT4 disease.

Discontinuous greater omental or peritoneal tumour nodules, or positive peritoneal cytology are classified as metastatic disease (pM1).

Intramural extension to the oesophagus or duodenum is classified by the depth of greatest invasion in any of these sites.

Diffuse gastric carcinoma may not elicit a desmoplastic stroma and the depth of mural invasion, which is often extensive and can be characterised by small, inapparent non-mucinous tumour cells in the muscularis propria and adventitia, may be underestimated. Equally margin status can be incorrectly assessed. Stains (PAS ± diastase, cytokeratins, CEA, EMA) should be used to show its full extent and also to distinguish tumour cells from histiocytes in both the mucosa and lymph node sinus network. Tumour regression following neoadjuvant chemotherapy can also markedly alter the volume of residual viable cancer making accurate pathological staging problematic.

Lymphovascular Invasion

Present/absent.

Intra-/extratumoural.

Venous, lymphatic and perineural invasion are adverse prognostic factors.

Intestinal gastric adenocarcinoma tends to *venous invasion* with spread to liver, lung, adrenal glands and bone. *Diffuse gastric carcinoma* favours *lymphatic* and *direct transperitoneal spread.* Bilateral ovarian metastases from diffuse gastric cancer comprise the majority of *Krükenberg tumours.* Uterine body, cervix and colorectum can also be involved by metastatic disease. About 50% of patients present with extragastric spread of tumour.

Lymph Nodes

Site/number/size/number involved/limit node/extracapsular spread (Figs. 3.3 and 3.4).

Regional nodes: perigastric, hepatoduodenal, lymph nodes along the left gastric, common hepatic, splenic and coeliac arteries. Other intra-abdominal lymph nodes (retropancreatic, mesenteric, paraaortic) are distant metastases (pM1). A regional lymphadenectomy will ordinarily include a minimum of 16 lymph nodes but numbers depend on the extent of surgery. In a *D1 resection* only perigastric lymph nodes are excised. In a *D2 (radical) gastrectomy* there is additional lymph node dissection along the hepatic artery, coeliac plexus, greater omentum, gastrosplenic omentum, portal vein, and splenic artery. A *D3 resection* includes lymph nodes from the hepatoduodenal ligament, superior mesenteric vein, aorta/vena cava to the inferior mesenteric artery and retropancreatic area. D2 nodes are generally located >3 cm from the tumour. The surgeon may choose to submit these in separately labelled containers and a minimum yield of 25 nodes is commonly targeted.

pN0	No regional lymph node mestatasis
pN1	1 to 2 involved regional nodes
pN2	3 to 16 involved regional nodes
pN3	More than 16 involved regional nodes.

Survival at 5 years decreases with increasing numbers of involved lymph nodes—N1/N2: 46% to N3: 30% of patients.

Excision Margins

Distances (mm) to the radial, proximal and distal limits of excision and serosa.

Gastric carcinoma (especially diffuse signet ring cell) may show a *multifocal distribution* and submucosal skip lesions. Margins need to be checked histologically even if well away from the main tumour mass on gross examination. Diffuse carcinoma present to within 5 cm of the resection margin has an adverse prognosis. Distal intestinal cancers tend to stop at the pylorus while diffuse carcinoma may involve the first part of the duodenum. Proximal (cardia)

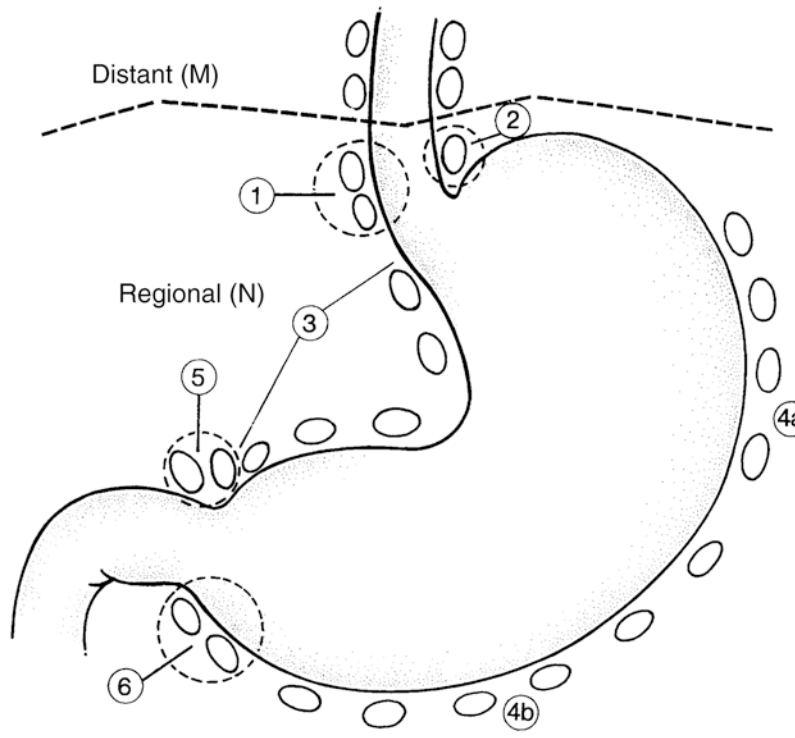

Fig. 3.3 Stomach: regional lymph nodes. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

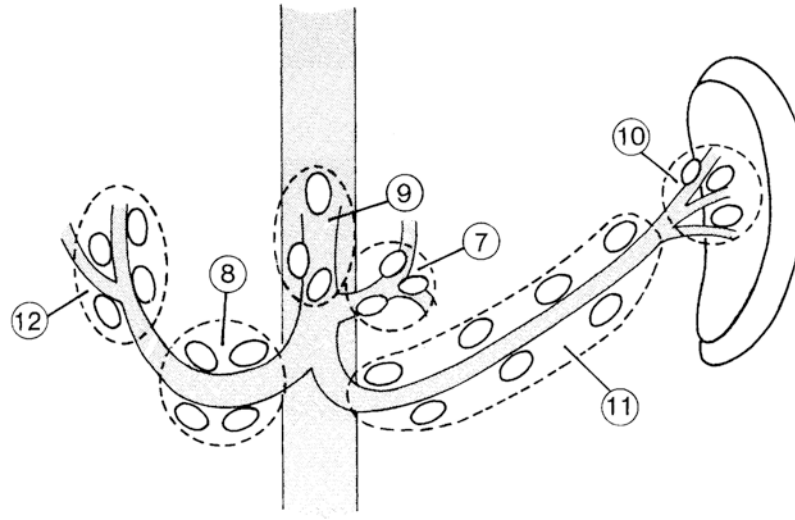

Fig. 3.4 Stomach: regional lymph nodes. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

tumours often involve distal oesophagus (Siewert III—see Section 1).

The radial margin is the non-peritonealised lesser or greater omental margin closest to the tumour. It can be inspected and inked prior to blocking although it can be difficult to identify in individual cases.

Other Pathology

Early Gastric Cancer (EGC)

Forming *10–15% of gastric cancers* in the Western hemisphere and limited to the mucosa ± submucosa ± lymph node involvement. The *5 year survival is 85–95%* compared with 20–35% for advanced gastric cancer. Designation as EGC is on a resection specimen as endoscopic biopsies are constrained by sampling limitations.

Macroscopic/Endoscopic Classification of EGC

Type I	Protruded	10%
Type IIa	Raised	Superficial 80%
Type IIb	Flat	
Type IIc	Depressed	
Type III	Excavated	10%

Mixed types are common. Types I and IIa tend to be well differentiated (tubular, papillary) whereas types IIb, IIc and III also include ulcerated intestinal, poorly differentiated and signet ring cell tumours, although there is considerable overlap between macroscopic and microscopic appearances.

Lesser curve is the commonest site but *10% are multifocal* and require mapping of the resection specimen.

Tumours with lymph node metastases do worse than those without and tend to be large (> 5 cm, 80% positive lymph nodes), or show *submucosal invasion (19% positive lymph nodes)* rather than being confined to the mucosa (4% positive nodes).

Two prognostic paradoxes contrast with advanced gastric carcinoma:

1. Diffuse type EGC has a better prognosis than intestinal type EGC due to vascular spread in the latter
2. EGC with a broad, expansile deep margin destroying muscularis mucosae is more aggressive than EGC with an irregular infiltrating margin fenestrating the muscle. The tendency for the former to progress to advanced carcinoma is thought to relate to higher DNA aneuploidy rates. These tumours form a minority (10%) of EGC but have higher rates of lymphovascular invasion, lymph node metastases (25%) and lower 10 year survival rates (65%): i.e. well differentiated cancers with a pushing margin do worse than poorly differentiated tumours with an infiltrating margin.

Treatment is usually by *partial gastrectomy* but after appropriate clinicopathological staging *local excision by EMR/ESD* is possible. *Risk factors* predictive of lymph node metastases and the need for *further surgery* are: size >3 cm, surface ulceration (>50%), poor differentiation, deep submucosal (sm2/sm3) invasion, lymphovascular invasion and incomplete excision with deep margin involvement.

Predisposing Lesions

Gastritis[1]*:* ± *Helicobacter pylori (HP).* Demonstrated by histochemical (cresyl violet, Giemsa, Warthin-Starry) or antibody stains. HP can assume a coccoid rather than spiral form in resection specimens. HP positive patients have × 3–6 increased cancer risk especially those with the cytotoxic (cag-A) genotype of HP.

Intestinal metaplasia: type IIb/III (sulphomucin rich). Demonstrated by high iron diamine alcian blue or Gomori's aldehyde fuchsin alcian

[1]Classified and semi-quantitatively graded (none/mild/moderate/marked) using the Sydney classification. It classifies and grades chronic gastritis based on an assessment of *histological* (neutrophils, chronic inflammation, atrophy, intestinal metaplasia), *topographical* (antral/corpus predominant or pangastritis) and *aetiological* (HP, drugs) factors.

blue stains. The large intestinal variant of metaplasia is more strongly associated with mucosal dysplasia and intestinal pattern gastric adenocarcinoma. Mucin subtyping is not routinely done as it is not considered a sufficiently strong predictive factor although the extent of intestinal metaplasia is broadly indicative.

Atrophy: ± pernicious anaemia with gastric parietal cell and intrinsic factor antibodies. *10–20% develop carcinoma*.

Dysplasia: low/high-grade, either in flat (commonest), sessile or polypoid mucosa, and in metaplastic (intestinal) or non-metaplastic (gastric foveolar) mucosa. Gastrointestinal epithelial neoplasia is categorised according to the Vienna Consensus Classification (Table 3.1).

There is a *strong association (30–80%)* between *high-grade dysplasia* and *adenocarcinoma* either concurrently or within 1–2 years of diagnosis. Distinction between high-grade dysplasia/carcinoma in situ and lamina propria invasion can be difficult. In Europe and the USA there needs to be invasion of the lamina propria or muscularis mucosae before the term (intramucosal) adenocarcinoma is used: i.e. both cytological and architectural derangement. Eastern hemisphere pathologists require less stringent criteria. In practice an array of histological features indicates progression to *adenocarcinoma.* These include single cells or clusters of atypical cells in the lamina propria, glandular complexity (cribriform or laterally anastomosing epithelium), or stromal desmoplasia. Diagnosis of dysplasia in a biopsy should be followed by *reassessment* with multiple biopsies to exclude concurrent adenocarcinoma. Dye spraying can facilitate endoscopic identification. Imaging, e.g. EUS may help define a mass or infiltrative lesion. If this is absent flat low-grade dysplasia may be *monitored endoscopically,* while polypoid low-grade dysplasia and high-grade dysplasia in flat or polypoid mucosa should be considered for either *local endoscopic* or *formal surgical resection*. With local resection careful histological assessment of the specimen and discussion at the multidisciplinary meeting are needed to exclude any requirement to proceed to more radical surgery. Care must be taken to distinguish dysplasia from regenerative change in inflammation and ulceration, reactive gastropathy, e.g. foveolar hyperplasia in bile reflux and drug ingestion (NSAIDs, aspirin), and crypt juxtaposition in small intestinal metaplasia. A lack of surface epithelial maturation, budding/branching and cystically dilated deep glands are useful pointers to dysplasia.

Table 3.1 Vienna consensus classification of gastrointestinal neoplasia

Category	Neoplasia/dysplasia
1.	Negative
2.	Indefinite
3.	Non-invasive low grade Low grade adenoma/dysplasia
4.	Non-invasive high grade
4.1	High grade adenoma/dysplasia
4.2	Non-invasive carcinoma (carcinoma in-situ)
4.3	Suspicious of invasive carcinoma
5.	Invasive—either intramucosal[a], submucosal or beyond

The WHO (2010) classification uses the term intraepithelial neoplasia interchangeably with dysplasia and the categories: negative, indefinite, low-grade, high-grade/intramucosal neoplasia (carcinoma) and invasive neoplasia (carcinoma)

[a]A more recent proposed modification suggests categorising intramucosal carcinoma as 4.4 as these sub-categories show poor intra/inter observer reproducibility and all require at least endoscopic or surgical local resection. Choice of procedure will depend on the lesion size, depth of invasion (as assessed by endoscopy, radiology and endoscopic ultrasound), histological grade and general features (e.g. age, fitness)

Polyps

- *Hyperplastic:* often antral and regenerative in nature. A 1–3% risk of malignancy (either within the polyp or elsewhere in the stomach), particularly if large (>2 cm) and multiple.
- *Fundic gland cyst*: the commonest gastric polyp. Associated with FAP in a young patient, but usually in older patients receiving proton pump inhibitor therapy (due to parietal cell hyperplasia consequent upon secondary hypergastrinaemia). Rarely the polyps show surface dysplasia in FAP.
- *Adenomatous:* 8% of cases with a 30–40% risk of malignancy related to the size (>2 cm),

villous architecture and grade of dysplasia. These are usually an intestinal type adenomatous polyp as opposed to foveolar or pyloric.
- *Rare*: FAP, Peutz-Jeghers, Cowden's syndromes, inflammatory fibroid polyp.

Ménétrier's disease and lymphocytic gastritis: hyperplastic gastropathy can be associated with adenocarcinoma.

Synchronous gastric lymphoma of mucosa associated lymphoid tissue (MALToma) : also *Helicobacter* related.

Tumours covered by intact mucosa: such as diffuse gastric carcinoma (signet ring cell) or stromal tumours, can be difficult to demonstrate by routine biopsy and multiple biopsies with jumbo forceps may be required. Cytological brushings and washings or endoscopic FNAC may be helpful.

Gastrointestinal cytology: may yield positive information in the following situations:

(a) FNA of submucosal/mural/extrinsic lesions including enlarged locoregional lymph nodes found on staging CT/EUS
(b) FNA of pancreatic mass lesions and brushings of common bile duct/pancreatic duct strictures
(c) Brush cytology of oesophageal and colonic strictures not amenable to usual biopsy.

Endoscopic biopsies: multiple (a minimum of 8) biopsies should be taken from ulcerated carcinomas including the ulcer base and mucosal edges. Distinction must be drawn between adenocarcinoma and pseudomalignant changes in glandular epithelium, endothelial cells and stromal cells in erosions and ulcer base tissue. Biopsy from the base of a deeply penetrating benign peptic ulcer may yield hepatocytes or pancreatic acinar cells not to be misinterpreted as gastric adenocarcinoma. Gastric xanthoma (CD68 positive, cytokeratin and mucin negative) can also mimic diffuse gastric carcinoma and immunohistochemistry is helpful in such situations.

Immunophenotype

Gastric carcinoma is variably neutral and acidic mucin positive (PAS-AB, mucicarmine), cytokeratin (CAM 5.2, CK7/±20), EMA and CEA positive, ± CDX-2. Diffuse carcinoma is E-cadherin negative and intestinal pattern adenocarcinoma is positive. In cases with metastatic *or recurrent disease,* biopsy or resection tissues are assessed for *HER2 overexpression* as a guide to potential response to monoclonal antibody therapy. As for breast carcinoma, immunohistochemistry (supplemented by fluorescent, chromogenic or dual colour/dual hapten in situ hybridization for equivocal) is used with a positive result indicated by strong membrane staining in >30% of the cells. Expression can be heterogeneous within a tumour requiring adequate sampling, and staining can be limited to the basolateral membranes. Variation is recognized in positivity rates depending on the staining platform and antibody used. About 10–20% of gastric cancers are positive (intestinal type 33%, diffuse type 5% only).

Prognosis

Prognosis of gastric cancer is poor, the majority of cases presenting with advanced disease. It relates to *histological type, grade* and crucially, *stage*. Intestinal gastric carcinoma has higher 5 year survival rates than diffuse gastric carcinoma, e.g. for pT3 lesions 42% versus 17%. Intestinal gastric carcinoma may be considered for partial gastrectomy because of its expanding margins, whereas total gastrectomy is advised for diffuse carcinoma. Additional important prognostic indicators are lymph node status, lymphovascular invasion, peritoneal and resection margin involvement, and an infiltrative versus an expansive tumour margin. These factors tend to outweigh other parameters such as the Lauren and Ming classification or Goseki grade. *Prior to proceeding to radical surgery, complete clinical staging is necessary to exclude any non-regional disease.* This involves CT ± PET scan and peritoneal laparoscopy with cytology and biopsy.

Preoperative neoadjuvant and palliative chemotherapy have roles to play. EGC does considerably better (see above) and may be amenable to endoscopic mucosal resection or submucosal dissection (EMR/ESD). This is more commonly utilized in Japan than the UK but is now being adopted in particular for frail elderly patients. ESD may achieve better curative resection rates than EMR but requires greater operative skill, longer procedural time and is subject to increased risk of complications.

Other Malignancy

Gastric Carcinoid Tumours

A well differentiated/low-grade neuroendocrine tumour, and chromogranin, synaptophysin positive, CD56±. Mitoses are usually <2 per 10 high power fields (hpfs) and Ki-67 index is ≤2% (i.e. a grade 1 (G1) tumour).

They are either small and multiple (types 1 and 2), or larger, solitary and sporadic (type 3).

1. *Multiple (benign)* : commonly type 1 lesions associated with autoimmune atrophic gastritis and endocrine cell hyperplasia (nodules <150 μm). Rarely Zollinger Ellison syndrome and MEN 1 (type 2 lesions)

   Gastric atrophy → hypochlorhydria → hypergastrinaemia → ECL (enterochromaffin like) cell hyperplasia → microcarcinoidosis (multiple, mucosal, 1–3 mm).

   Can be *monitored by endoscopy* and treated conservatively with biopsy excision of small polyps up to 1 cm diameter. Polyps 1–2 cm in size are treated by *polypectomy* or *local resection* as they are of uncertain or low malignant potential with an overall metastatic rate of approximately 2–5%.
2. Single (type 3 lesions/aggressive): 13% of cases overall with a 22–75% metastatic rate and 25% mortality. If the lesion is large (> 2 cm) or ulcerated consider definitive *surgical resection*

   Malignancy relates to:
   - Any functioning tumour
   - Angioinvasion
   - Non-functioning tumour ≥2 cm diameter and with invasion beyond the submucosa
   - Atypical features (atypia, necrosis, increased mitoses (≥2/10hpfs) and/or Ki-67 index (>2%)) i.e. a G2/G3 tumour
   - *70–80% 5 year survival*.
   - Indolent growth with spread to nodes, liver, bone and skin.

TNM8 (excludes high grade neuroendocrine carcinomas): pT1 ≤ 1 cm and not into muscularis propria; pT2 > 1 cm or into muscularis propria; pT3 into subserosa; pT4 involves serosa or adjacent structures.

The European Neuroendocrine Tumour Society designates subserosa as pT2 and serosa as pT3.

Gastrointestinal neuroendocrine tumour cells express functional somatostatin receptors and tumours can be detected by octreotide (somatostatin antagonist) scan, and treated with similar agents.

Gastrointestinal Mesenchymal or Stromal Tumours (GISTs)

Site: stomach (60–70%), small intestine (25–35%), colorectum and oesophagus (10%). Submucosal, mural or serosal subsites.

Myogenic: 10% of cases are desmin/h-caldesmon/smooth muscle actin positive and DOG-1/CD117 (c-kit) negative, representing true leiomyoma or leiomyosarcoma (rare).

Neural: 10% of cases are S100/synaptophysin positive and DOG-1/CD117 (c-kit) negative, representing Schwannoma (has a characteristic peritumoural lymphoid infiltrate), granular cell tumour, neurofibroma (can be associated with von Recklinghausen‘s disease, MEN syndrome and GISTs elsewhere in the gastrointestinal tract).

Stromal: DOG-1, CD117 (c-*kit*: tyrosine kinase receptor), CD34 positive, and absent or incomplete myogenic/neural differentiation. Putative precursors are the interstitial cells of Cajal, which are gastrointestinal pacemaker cells located in the deep submucosa and myenteric plexus. Note that there can be heterogeneity and focal expression of antigens. In general, antigen positivity is DOG-1 and CD117 (>95%), CD34 (70–85%), smooth muscle actin (20–40%), h-caldesmon (60–80%) and nestin (90–100%). DOG-1/CD117 negative GISTs may be identified by positive protein kinase c theta and PDGFR (platelet derived growth factor receptor) positive mutation analysis. *C-kit mutation analysis* (exon 11 in 70% of cases) is also helpful in confirming the *diagnosis, prediction of progression* and *response to drug treatment* (exon 11 is more responsive than exon 9). Mutation analysis is generally recommended if immunohistochemistry is equivocal, and in cases of borderline/malignant GISTs. Note that other malignant tumours can also be CD117 positive, e.g. seminoma, malignant melanoma and some metastatic carcinomas (e.g. breast, ovary, colorectal, small cell carcinoma). DOG-1 (transmembrane protein Discovered On GIST1) is a highly sensitive and specific marker for GIST but may also show weak to moderate expression in some other tumours, e.g. colorectal, endometrioid and acinic cell carcinomas, spindle cell malignant melanoma and malignant peripheral nerve sheath tumours.

GANT (gastrointestinal autonomic nerve tumour) is now regarded as a variant of GIST and assessed accordingly. Multinodular/plexiform GISTs may show loss of expression of succinate dehydrogenase b. Such GISTs usually do not show c-kit or PDGFRA mutations and may be paediatric type/sporadic or associated with Carney Stratakis syndrome or Carney's triad (gastric GIST, pulmonary chondroma, extra-adrenal paraganglioma). Type 1 neurofibromatosis may be associated with multiple synchronous GISTS especially of the small bowel that are also wild type for KIT/PDGFRA.

Malignancy, which is less frequent than in small intestinal stromal tumours, cannot be accurately predicted from the histology. However, *indicators of malignancy* (strongest asterisked) are:

- Size (> 5 cm)∗
- Cellularity (cell density increases in sarcoma)
- Atypia
- Cell type (epithelioid is worse than spindle cell)
- Necrosis (coagulative in type)∗
- Margins (circumscribed versus infiltrative, e.g. invasion into mucosal lamina propria)∗
- Mitoses >5/50 high power fields∗
- Location in fundus or gastro-oesophageal junction.
- Loss of CD117/DOG-1 expression and over-expression of p53.

Clinical risk: gastric GISTs are categorized as no, very low, low, moderate or high *metastatic risk* on the basis of *size* and *mitoses* (Table 3.2). The risk of recurrence is however higher for SDH deficient GISTs and the modified Miettinen criteria do not apply. Treatment is *complete surgical (open or laparoscopic) resection* by either local (sleeve) or radical gastric resection depending on the tumour size and location. Neoadjuvant therapy is not licensed in the UK but is sometimes used on an individual basis to produce tumour

Table 3.2 Risk factors in gastric GISTs—risk of progressive disease, metastases or tumour related death

	Tumour dia (cm)	Mitoses/50hpfs	Percentage risk
No risk	≤2	< 5	0
Very low risk	>2–5	< 5	1.9
Low risk	>5–10	< 5	3.6
Moderate risk	>10	< 5	10
	>2–5	> 5	16
High risk	>5–10	> 5	55
	>10	> 5	86

regression to facilitate choice and ease of operative technique, e.g. anorectal, oesophagogastric or duodenal GISTs.

Metastases: histological grading of established sarcoma is contentious and tumour size is a suggested index of metastatic risk. *Metastases are commonly to peritoneum, liver, pancreas, retroperitoneum* and *lungs.* Metastases are metabolically active on PET but become negative on treatment. Metastatic disease responds well to *medical therapy (Glivec (imatinib))* resulting in hyalinization, myxoid and cystic degeneration. It gives several disease free years but usually therapeutic escape occurs with *recurrent peritoneal disease or size progression of liver metastases.* This may relate to the cytostatic rather than tumouricidal effects of the treatment, or newly acquired mutations in the tumour cells. Up to 13% of patients show no primary response to therapy and disease progression within 6 months.

Biopsy proof can be difficult as GISTs are extramucosal lesions (submucosal and mural) often with surface ulceration. FNAC at endoscopy may be helpful in establishing a diagnosis of a spindle cell lesion. The biopsy forceps may also be directed to the base of the ulcer where there is already mucosal loss.

See Chap. 5, Section "Other Malignancy".

Malignant Lymphoma

Secondary to systemic/lymph node disease or primary (commoner) in the stomach, it is the *commonest site* for *extranodal non-Hodgkin's malignant lymphoma* (40% of cases). Primary disease bulk is centred on the stomach and its regional lymph nodes.

Gastric malignant lymphoma: can present as single or multiple lesions, a sessile plaque or thickened folds found incidentally at endoscopy, an ulcerated tumour or a thickened non-expansile stomach. The majority are of B cell MALT (mucosa associated lymphoid tissue) type, the low-grade variant being characterised by a proliferation of small to medium sized centrocyte like cells, destructive lymphoepithelial lesions, monotypic immunoglobulin expression in surface plasma cells, invasion between or into reactive follicles (follicular colonisation) and/or immunoglobulin gene rearrangements on PCR. There is evidence that *localized low-grade lesions* (i.e. without deep submucosal or muscle invasion) may regress on *anti-Helicobacter medication* and they usually pursue an *indolent time course* with potential metastases to other extranodal sites, e.g. gastrointestinal tract and Waldeyer's ring. They may also transform to or present as *high-grade lesions* necessitating *chemotherapy and/or surgery,* which are also applicable to extensive low-grade disease. Potential *resistance to anti-helicobacter treatment* is indicated by tumour cell bcl-10 expression, presence of the t(11;18) translocation (30% of cases), large cells or disease in the deep submucosa or beyond. EUS may help to define the latter. Advanced stage disease and t(11;18) tumours should be considered for radiotherapy or chemotherapy. The cytological composition of MALToma can be heterogeneous and clear distinction between a mucosal or lymph node origin may be arbitrary, especially in high-grade disease. From a practical point of view establishing *a diagnosis of malignant lymphoma, B cell phenotype, low- or high-grade character* and *full clinicopathological staging* are the salient features relevant to management.

Immunohistochemistry may also be helpful in establishing *monoclonality* (κ, λ light chain restriction), demonstrating *lymphoepithelial lesions* (cytokeratins), and excluding other *lymphoma subtypes*. Low-grade MALToma is usually CD45/CD20 positive but CD5, CD10 and CD23 negative. Aberrant expression of CD43 (MT1) and MNDA are also helpful. In situ hybridization can be very useful to demonstrate light chain restriction in infiltrates showing plasma cell differentiation. Cytokeratins and leukocyte common antigen (CD45) are also necessary to distinguish high-grade lymphoma from undifferentiated carcinoma, and signet ring or plasmacytoid change in malignant lymphoma from signet ring cell gastric adenocarcinoma. Low- and high-grade areas may coexist in gastric lymphoma and there can be adjacent synchronous or metachronous (up to several years later)

adenocarcinoma associated with MALToma. About 40–60% of gastric malignant lymphomas are of high-grade large B cell type and diagnosis is usually straightforward (cytological atypia/destructive monomorphous infiltrate), with confirmatory immunohistochemistry for lymphoid markers and negative epithelial markers. Distinction between low-grade malignant lymphoma and lymphoid hyperplasia, as in *Helicobacter pylori* gastritis or peptic ulcer, can be problematic and diagnosis depends on the density and atypia of the lymphoid infiltrate and degree of gland distortion and loss. *Immunoglobulin gene rearrangements* provide supportive evidence although monoclonality does not always correlate with potential for progression to malignancy and can be seen in a minority of cases of chronic gastritis. Sometimes designation of low-grade malignant lymphoma is only attained after several biopsy episodes and when there is a lack of response of the lymphoid infiltrate to eradication of *Helicobacter pylori*. Persistence of monoclonality over time may be helpful. Various scoring systems exist for making a diagnosis of low-grade MALToma and assessing its response to *Helicobacter pylori* eradication. Minimal residual disease shows persistent basal lymphoid aggregates whereas response gives a diminished lymphoid infiltrate and fine fibrosis in the lamina propria.

Overall prognosis is reasonably good *(40–60% 5 year survival),* low-grade malignant lymphomas following an indolent course (65–95% 5 year survival). However, about 50% of high-grade malignant lymphomas are aggressive with spread beyond the stomach (40–55% 5 year survival). *Prognosis* relates to both *grade* and *stage* of disease at the time of presentation. Treatment of extensive low-grade and high-grade disease is with *chemotherapy* and/or *surgery*, the latter particularly if there are anatomical considerations, e.g. multifocality, bulky ulcerated luminal disease, anaemia or gastric outlet obstruction. Initial full clinical staging is carried out (CT scan, bone marrow biopsy).

Other forms of malignant lymphoma are unusual in the stomach, e.g. follicle centre cell lymphoma, mantle cell lymphoma, Burkitt's lymphoma, anaplastic large cell lymphoma and T cell lymphoma. EBV positive diffuse large B cell lymphoma and EBV positive extranodal NK/T cell lymphoma may occur at extranodal sites including stomach. Hodgkin's lymphoma is rare, but may be seen in advanced recurrent disease post treatment.

Leukaemia

- Stomach can be involved in up to 25% of cases.
- CD34, CD43, CD68, CD117/chloroacetate esterase/myeloperoxidase positive cells (myeloid/granulocytic sarcoma).

Miscellaneous Rare Malignancy

- Kaposi's sarcoma: visceral involvement can be present in 30–60% of AIDS patients.
- Angiosarcoma, rhabdomyosarcoma, alveolar soft part sarcoma, teratoma, choriocarcinoma, yolk sac tumour.
- Metastatic malignant melanoma in the stomach is now seen with increasingly powerful chemo-/immunosuppressive therapies leading to unusual patterns of metastatic disease.

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4 Ampulla of Vater and Head of Pancreas Carcinomas

Paul J. Kelly

Ampullary adenocarcinoma represents 0.5% of gastrointestinal malignancies and is strongly associated with mucosal adenoma(s), either sporadic in older patients or related to Familial Adenomatous Polyposis (FAP) or Lynch syndrome in younger patients. Pancreatic cancer occurs mainly in patients 60–80 years of age and in the developed countries of Northern Europe and North America (particularly in African Americans). In the UK some 9000 new cases are reported each year, accounting for 3% of all new cancer diagnoses. Risk factors are hereditary (family history of pancreatic cancer or BRCA gene positivity), smoking, obesity, high intake of saturated fatty acids, low intake of fruit and vegetables, lack of exercise, diabetes, alcohol intake and a history of chronic pancreatitis (risk ×10).

Pancreatic and ampullary cancers classically present with anorexia, weight loss and dark urine with pale stools due to painless obstructive (cholestatic) jaundice. Investigation includes liver function tests (increased bilirubin, alkaline phosphatase) and serum CA19–9. Tissue diagnosis is obtained in a majority, but not all patients, using a combination of oesophagogastroduodenoscopy (OGD), endoscopic retrograde cholangiopancreatography (ERCP) and endoluminal ultrasound (ELUS) providing a range of biopsy, brush, aspiration cytology and fine needle core biopsy samples. Ultrasound can confirm extrahepatic duct obstruction. Staging for local and distant disease also includes three-dimensional imaging studies, including triphasic, contrast enhanced CT or MRI. ELUS also provides information regarding staging and disease resectability. Radiological imaging is the most important diagnostic tool for pancreatic cancer and determines operability. Staging laparoscopy may also be done prior to consideration of radical surgery to identify small (<1 cm), peritoneal or hepatic metastasis, Radical surgery is contraindicated by liver, peritoneal and distant metastases and so staging laparoscopy may also be done during planning stages to identify small (<1cm), peritoneal or hepatic metastases. The fitness of the patient and any comorbidity are also important factors.

Pancreatic neuroendocrine tumours may present as a consequence of a functional hormonal syndrome due to elevated serum hormone levels, or symptoms relating to the mass effect within the ampullary region or pancreas. Localisation of the primary lesion and metastases is by octreotide isotope scan, multiphasic CT or MRI scans, or ELUS. Treatment entails complete local excision of the primary tumour and regional lymph nodes; a combination of surgery and medical treatment can be used for metastatic disease.

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_4

Gross Description

Specimen

- Endoscopic brushings or biopsy/transduodenal or percutaneous fine needle aspiration cytology (FNAC) or needle core biopsy, including fine needle core biopsy.
- Whipple's procedure (partial gastrectomy, duodenectomy and partial pancreatectomy). A pylorus preserving pancreaticoduodectomy may be used for small periampullary tumours thus maintaining the storage and release functions of the distal stomach and proximal 3 cm of duodenum.
- Total pancreatectomy (partial gastrectomy, duodenectomy, total pancreatectomy and splenectomy).
- Weight (g) and size/length (mm) of component parts, number of fragments, core lengths (mm).

Carcinomas of the ampulla and head of pancreas are considered together because of their anatomical juxtaposition, overlap and common potentially operative resection (Whipple's procedure). *A majority of ampullary cancers are operable but only a minority of pancreatic carcinomas.*

Tumour

Site

- Non-ampullary duodenal mucosa/duodenal papilla/intra-ampullary /pancreatic head (60–70% of pancreatic carcinomas)/terminal common bile duct. It can be difficult to distinguish adenocarcinoma of the pancreas and adenocarcinoma of the terminal common bile duct from adenocarcinoma of the ampulla of Vater as they can share similar histological features. Careful examination of the exact *anatomical location of the tumour epicentre* is required during macroscopic assessment supported by circumstantial evidence for a point of origin, e.g. an adenomatous lesion in the ampullary mucosa or dysplasia/intraepithelial neoplasia in the pancreatic/bile duct epithelium.

Size

- Length × width × depth (mm) or maximum dimension (mm).
- Ampullary cancers >25 mm diameter have a decreased 5 year survival. Pancreatic exocrine cancers >30 mm are often inoperable. Pancreatic neuroendocrine tumours >20–30 mm show greater local and vascular invasion and metastatic potential.

The size of pancreatic tumours should be carefully mapped out during macroscopic examination and confirmed histologically for staging purposes.

Appearance

- Polypoid/nodular/diffuse/ulcerated: ampullary tumours.
- Scirrhous/mucoid/cystic: pancreatic exocrine tumours.
- Circumscribed/pale: pancreatic neuroendocrine tumours.

Edge

- Circumscribed/irregular.

Histological Type

Ampulla

- Comprise *20% of peripancreatic tumours* and *10–50% of cancers resected* by pancreaticoduodenectomy.
- *Premaligant lesions:* include intestinal type adenomas (commonest), intraductal papillary neoplasms, and flat intraepithelial neoplasia (dysplasia) of the ampullary epithelium.
- *Adenocarcinoma:* intestinal-type, pancreaticobilary type or mixed; differentiation based on morphology and immunohistochemistry (CK20, CDX2, MUC2 and MUC1). Most cases are usually of well to moderately differentiated *intestinal* pattern arising from adenomatous dysplasia in the peri-/intraampullary mucosa. Endoscopic biopsy underestimates the nature and extent of disease yielding a positive diagnosis of malignancy in only about 40% of

cases. It samples the surface dysplasia but not the underlying adenocarcinoma which is better demonstrated as a mass lesion on imaging studies (ELUS, CT scan). A useful clue in biopsies of the periampullary region and duodenal papilla is the presence of tumor microemboli in lamina propria lymphovascular channels or dysplasia of the ampullary duct epithelium.

- *Papillary adenocarcinoma:* intraluminal, exophytic, well differentiated; of better prognosis and can be multifocal in the extrahepatic biliary tree.
- *Mucinous adenocarcinoma*: mucin in >50% of the tumour. It may co-exist with usual ampullary cancer subtypes.
- *Signet ring cell adenocarcinoma*: 2% of cases.
- *Others:* adenosquamous, clear cell, small cell, hepatoid, squamous cell and undifferentiated carcinomas.
- *Metastatic carcinoma:* usually by direct spread, e.g. stomach, pancreas, terminal common bile duct, renal carcinoma. Some 10–15% of ampullary adenocarcinomas arise from the terminal portion of either the main pancreatic or common bile ducts, and therefore, have a biliary phenotype making distinction from invasion by pancreatic adenocarcinoma difficult.

Pancreas

Classification is based on the *gross appearance* of the tumour (solid/cystic/intraductal), and the *line of cellular differentiation* which is either exocrine (ductal/acinar) or neuroendocrine.

Solid tumours: ductal adenocarcinoma, acinar cell carcinoma, pancreaticoblastoma, and neuroendocrine, solid-pseudopapillary tumours.

Cystic tumours: serous cystadenoma (30%), mucinous cystic neoplasm (MCN) (40%), intraductal papillary mucinous neoplasm (IPMN) (30%), and degenerative forms of all the above solid types.

Exocrine

- *Ductal adenocarcinoma: 80–90% of cases* comprising a tubuloacinar pattern of malignant ductal epithelium in a desmoplastic stroma. There is often perineural invasion and dysplasia of the adjacent duct epithelium (20–30%). *Pancreatic intraepithelial neoplasia (PanIN)* is synonymous with dysplasia and is a microscopic papillary or flat, non-invasive epithelial neoplasm comprising cuboidal columnar epithelial cells with variable degrees of cytoarchitectural atypia. It usually arises in pancreatic ducts <5 mm diameter, is multifocal and seen adjacent to existing adenocarcinoma being regarded as a precursor to it. Initially subdivided as PanIN 1, 2 and 3 (equivalent to mild, moderate and severe dysplasia respectively) but now two-tiered system preferred (PanIN 1 and 2 = low grade; PanIN 3 = high grade). Low grade PanIN is relatively common in biopsy specimens and resection specimens. High grade PanIN is often found in the vicinity of pancreatic carcinomas. Note that PanIN can be mimicked by florid reactive atypia or cancerisation of ducts by invasive ductal carcinoma.
  Multifocality 15–40%.
  Male preponderance.
- *Ductal adenocarcinoma variants:*
  - *Colloid carcinoma: 1–3% of cases* and mucin in >80% of the tumour. Almost always arise in association with an intestinal-type IPMN.
  - *Adenosquamous:* at least 30% represents the squamous cell component—poor prognosis.
  - *Microglandular/signet ring cell*: poor prognosis. Exclude a gastric carcinoma secondary deposit.
  - *Others:* oncocytic, clear cell, hepatoid, medullary (sporadic or HNPCC).
- *Undifferentiated/anaplastic carcinoma*: *2–7% of cases* and variably termed pleomorphic/anaplastic/giant cell/sarcomatoid carcinoma. It shows spindle cells, pleomorphic cells, mitoses and lymphovascular invasion. There is a variant with osteoclast-like giant cells (CD68 positive—poor prognosis).
- *Intraductal neoplasms of the pancreas*: intraductal papillary neoplasms (IPMN) are clinically detectable grossly visible, non-invasive,

epithelial neoplasms of the pancreatic ductal system and are distinct from pancreatic intraepithelial neoplasia (PanIN). Often diagnosed incidentally on imaging, and are precursors of malignancy requiring surveillance and/or surgical resection. Macroscopically classified as main pancreatic duct, branch duct (better prognosis), or combined type if both main and branch ducts are involved and result in varying duct dilatation (>10 mm) and cytoarchitectural atypia. Histologically, IPMNs are classified by epithelial subtype as gastric, intestinal, pancreaticobiliary or oncocytic and are benign/borderline or malignant according to the degree of dysplasia ± invasion. Main duct involvement and intestinal and pancreaticobilary subtypes associated with higher risk of associated invasive malignancy. About *20–30% are associated with colloid or ductal adenocarcinoma,* 80% are in the head of pancreas and multifocal within the duct system. *Intraductal tubulopapillary neoplasms (ITPN)* are rarer, lack overt mucin production seen in IPMNs and typically exhibit high grade dysplasia. Macroscopically presents as a solid or nodular intraductal mass with less obvious cystic change. 50% occur in the head of pancreas.

- *Serous cystic tumours:* elderly patients, in the body or tail (50–75%); F > M (2:1) and *mostly benign* (macro/microcystic /oligocystic/solid serous adenoma). It comprises glycogen-rich, clear cuboidal epithelium lining fluid filled microcysts with a central scar. Diagnosis can be aided by analysis of aspirated cyst fluid which, in distinction from mucinous cystic tumours and pseudocysts, has low viscosity and zero levels of leucocyte esterase. *Surgical excision is curative but rarely required*. It is also seen in 35–75% of patients with Von-Hippel Lindau syndrome. Serous cystadenocarcinoma is rare (1–3% of serous cystic neoplasms) and is defined by the presence of distant metastases.
- *Mucinous cystic neoplasm (MCN):* benign/borderline/malignant spectrum of appearance and behaviour tending to malignancy. Unlike IPMNs, MCNs are not connected to the pancreatic ductal system. Vast majority occur in women (>90%) with mean age at diagnosis between 40 and 50 years. Localised to body or tail in 95% of patients and can be either unilocular or multilocular. There is characteristic ovarian type stroma in the wall (helpful to distinguish from pancreatic pseudocyst where the epithelial lining is lost). Prognosis relates to the presence of associated invasive malignancy and degree of invasion (which can be focal within a lesion) into the pancreatic and extrapancreatic tissues. The carcinoma is usually ductal in character, occasionally adenosquamous or undifferentiated (with or without giant cells). Resection is recommended for all MCNs in surgically fit patients and is curative for almost all noninvasive MCNs. Those with an invasive component have 50% 5 year survival figures.
- *Solid pseudopapillary tumour (syn. solid-cystic-papillary tumour):* adolescent girls/young women and of *low malignant potential* (10% metastasise to liver and peritoneum) but *usually benign*. Occur anywhere in the pancreas. Macroscopically large, round, circumscribed, soft and may appear encapsulated. Soft, brown to haemorrhagic cut surface depending on degree of cystic change. It comprises pseudopapillae covered by several layers of uniform, endocrine like epithelial cells and a vascularised, hyalinised stroma with necrosis and cystic change. Alpha-1-antitrypsin/vimentin/CD10/β-catenin (nuclear)/E-cadherin/progesterone positive, ± synaptophysin. May show pancytokeratin positivity but CK7 and 19 are negative. Frank high grade malignant transformation is extremely rare.
- *Acinar cell carcinoma: 1–2% of cases* in the head of pancreas. Large, circumscribed lobulated/multinodular appearance +/− focal intraductal growth. Highly cellular with uniform cells arranged in acinar, sheeted/solid or gyriform patterns. PAS positive cytoplasmic granules (zymogen) often present resembling normal pancreas. Uniform nuclei +/− prominent nucleolus. Mitoitc figures often easily identified, unlike pancreatic neuroendocrine tumours (see below) which may

appear similar. It is enzyme antibody positive, e.g. lipase, amylase, trypsin. Lymph node and liver metastases can be present in 50% of cases at diagnosis with *aggressive behaviour*.

- *Mixed differentiation carcinoma:* acinar/ductal, acinar/neuroendocrine or ductal/neuroendocrine are rare and behave as for ductal carcinoma. The neuroendocrine component must be at least 30% of the tumour.
- *Pancreaticoblastoma:* malignant in children and favourable prognosis if resected before metastases occur (nodal/hepatic in 35% of cases). It is also chemoresponsive, and consists of epithelial (acini, squamous nests) and mesenchymal (spindle cell) components.

Neuroendocrine/Endocrine Tumours

- By definition pancreatic neuroendocrine neoplasms (PanNENs) have significant neuroendocrine differentiation and show expression of synaptophysin usually and also chromogranin. All pancreatic neuroendocrine neoplasms are now considered to have malignant potential. They are subdivided in to pancreatic neuroendocrine tumours (PanNETs) and neuroendocrine carcinomas (PanNECs) based on morphology and grade (see below). PanNENs account for 2–5% of pancreatic tumours. An increasing incidence may relate to improvements in diagnostic imaging.
- PanNETS have well differentiated morphology. Typically circumscribed, pale and consist of solid/trabecular/gyriform/acinar cell patterns with hyaline (+/− amyloid-like) stroma, limited pleomorphism and nuclei have salt and pepper chromatin pattern. They may be grade 1, 2 or 3 (see below). Grade 3 PanNETs are recently recognised and should not be misdiagnosed as Grade 3 PanNECs.
- May be functional or non-functional based on ability to secrete specific hormones and produce specific clinical syndromes/symptoms. The majority (>60%) are non-functional. Functional tumours can be subclassified according to the hormone produced: insulinomas (70%), glucagonoma, somatostatinoma, VIPoma, PPoma, gastrinoma. Other functional PanNENs may secrete ectopic hormones including ACTH, serotonin, parathyroid hormone and cholecystokinin. Ectopic hormone production may be more common in PanNECs. Immunohistochemical labeling for a hormone does not necessarily confirm that a tumour is functional. CK19 positivity in PanNETs may be associated with a worse prognosis.
- Functional hormonal syndromes:
  - *Gastrinoma:* pancreatic head, duodenum, gastric antrum, Zollinger-Ellison syndrome (multiple gastroduodenal ulcers, endocrine (carcinoid) tumourlets or microadenomas (<5 mm)).
  - *Insulinoma:* body and tail—psychiatric/neurological symptoms/hypoglycaemia.
  - *Vipoma:* body and tail—watery diarrhoea, hypocalcaemia and achlorhydria.
  - *Glucagonoma:* body and tail—diabetes mellitus/skin rash/stomatitis.
  - S*omatostatinoma:* head >tail—diarrhoea, hyerpglycaemia, gallstones, hypochlorhydra. Ampullary somatostatinoma typically lack the features of somatostatinoma syndrome
- *PanNECs* are poorly differentiated, high grade malignant tumours. Morphologically present as small cell carcinoma, large cell neuroendocrine carcinoma, or carcinomas composed of cells with intermediate features. These tumours are by definition grade 3 exhibiting >20 mitoses per 2 mm^2 and have a Ki67 proliferative index of >20% and typically >55% (see grading below).

Over 50% of patients with pancreatic neuroendocrine tumours present with liver metastases at diagnosis. It is important on a needle core biopsy of a liver metastasis to not only demonstrate the neuroendocrine nature of the tumour, as opposed to the more commonly occurring diagnoses of metastatic colorectal or pancreatic ductal carcinomas, but also to provide its grading for prognosis and tailored oncological management. Immunohistochemistry can be used to try to establish a primary site, especially for grade 1/2 tumours, e.g. pancreatic: PAX6, polyclonal

PAX8, PR, PDX1; intestinal: CDX2; pulmonary: TTF1.

Association with multiple endocrine neoplasia (MEN) syndrome: the pancreas is involved in 80–100% of type 1 MEN syndrome, gastrinoma being the commonest (50%) lesion. Associated abnormalities are hyperplasia or tumours of parathyroid, pituitary and adrenal glands. Also seen in Von-Hippel Lindau syndrome.

Mixed Neuroendocrine Non-neuroendocrine Neoplasms (MINENs)

- *<1% of cases* comprising bivalent amphicrine cells or adjacent foci of mixed differentiation (the neuroendocrine component being at least 30% of the tumour). Neuroendocrine component is typically high grade. The term MINEN is now preferred over MANEC (mixed adeno-neuroendocrine carcinoma).

Metastatic Carcinoma

- *Direct spread:* stomach, colorectum, biliary tract, kidney, abdominal mesothelioma/malignant lymphoma.
- *Distant spread:* pleomorphic carcinoma of the pancreas has to be distinguished from metastatic malignant melanoma, sarcoma, choriocarcinoma and large cell lung carcinoma. Small cell lung carcinoma and renal carcinoma can also involve the pancreas.

Differentiation

Ductal and ampullary carcinoma: pancreatic ductal and ampullary adenocarcinoma can be graded according to the percentage tumour gland formation (well/G1 > 95%: moderate/G2 50–95%: poor/G3 < 50%) and mitotic activity (<5, 5–10, >10/10 hpfs).

By convention and definition signet ring cell adenocarcinoma and undifferentiated carcinoma (no glandular differentiation) are grade 3 and grade 4, respectively. Well differentiated pancreatic adenocarcinoma is relatively rare and can be difficult to distinguish from non-neoplastic ducts. Malignant glands are of variable size, shape and angularity with atypical nuclear and nucleolar features. Cell cytoplasm is tall and pale to clear in character. Other morphological patterns that occur but are not recognised as specific entities include clear cell, foamy gland, large duct pattern and cystic papillary pattern. The latter two patterns may be confused with IPMNs. *Perineural, lymphovascular and fat invasion are diagnostically helpful*.

Intraductal papillary neoplasms: are currently classified by a 3 tier system based on degree of dysplasia as low, intermediate and high. There is increasing support to move to a 2-tier system of low (low and intermediate) and high grade IPMNs:

Low-grade	Single layer of cells, mild nuclear atypia no mitoses
Intermediate	Moderate nuclear atypia, nuclear stratification, crowding and loss of polarity <5 mitoses/10 high-power fields
High-grade	Severe architectural and cellular atypia, mitoses >5/10 high-power fields

Pancreatic neuroendocrine neoplasms: in general, gastrointestinal neuroendocrine tumours are graded based on their proliferative activity; *low-grade or well differentiated tumour* (G1: 0–1 mitoses/10hpfs,[1] Ki-67 index 0–<3%; G2: 3–20 mitoses/10hpfs, Ki-67 index 3–20%[1]), G3: >20 mitoses/10hpfs, Ki-67 index >20%. A 5% threshold for G1 versus G2 has been suggested for pancreatic NETs. The WHO 2017 classification of pancreatic NETs recognises a subdivision of G3 tumours incorporating G3 pancreatic NETs and G3 NECs. *G3 NETs have well differentiated morphology akin to G1 and G2 tumours but the Ki67 index is > 20% but usually < 55%. G3 NECs are poorly differentiated carcinomas, as in the older schemes, and usually demonstrate abundant mitotic activity and have a Ki67 index that is usually > 55% and typically > 75%.* Distinction of G3 NECs from G3 NETs is of critical importance for determining treatment and prognosis. There can be inconsistent correlation of cytological features and growth pattern with biological behaviour, but

[1] A high power field equating to 0.2 mm^2 (10hpfs = 2 mm^2)

grading is broadly indicative of outcome. Each case needs detailed clinicopathological discussion at a specialist multidisciplinary meeting.

Extent of Local Tumour Spread

Border: pushing/infiltrative.

Lymphocytic reaction: prominent/sparse.

The TNM8 classification applies to carcinomas of the Ampulla of Vater, exocrine pancreas and pancreatic neuroendocrine tumours.[2]

Ampulla

pTis	Carcinoma in situ
pT1a	Tumour limited to the ampulla or sphincter of Oddi
pT1b	Tumour invades beyond the sphincter of Oddi (perisphinteric invasion) and/or duodenal submucosa
pT2	Tumour invades the muscularis propria of the duodenal wall
pT3a	Tumour invades 0.5 cm or less into the pancreas
pT3b	Tumour invades more than 0.5 cm into the pancreas or extends into peripancreatic tissue or duodenal serosa without involvement of the coeliac axis or superior mesenteric artery
pT4	Tumour with vascular involvement of the superior mesenteric artery, coeliac axis or common hepatic artery (Figs. 4.1, 4.2 and 4.3).

Pancreas

pTis	Carcinoma in situ
pT1a	Tumour 0.5 cm or less in greatest dimension
pT1b	Tumour greater than 0.5 cm but less than 1 cm in greatest dimension
pT1c	Tumour between 1 cm and 2 cm in maximum dimension
pT2	Tumour more than 2 cm but no more than 4 cm in greatest dimension
pT3	Tumour more than 4 cm in greatest dimension
pT4	Tumour involves coeliac axis, superior mesenteric artery or coeliac axis (Figs. 4.4, 4.5 and 4.6).

[2]The European Neuroendocrine Tumour Society (ENETS) designates pancreatic neuroendocrine tumours as: pT1 < 2 cm (less than 5 mm = microadenoma/benign), pT2 > 2 cm and ≤ 4 cm, pT3 > 4 cm, pT4 invading adjacent large vessels or stomach, spleen, colon or adrenal gland. The ENETS TNM staging system is endorsed by The Royal College of Pathologists

Lymphovascular Invasion

Present/absent.

Intra-/extratumoural.

Perineural space involvement is common in pancreatic carcinoma and *lymphovascular invasion* is present in up to 50% of cases with spread to local regional lymph nodes at the time of diagnosis. *Invasion of portal vein* has *adverse independent prognostic significance*. Therefore, assessment of any segmental resection of portal/superior mesenteric vein removed en bloc with the pancreaticoduodenectomy (5–10% of cases) is important for prognosis and staging. Sites of *distant metastases* are the liver, peritoneum, lung, adrenal, bone, skin and central nervous system. Metastases to ovary can mimic primary ovarian mucinous neoplasms. Regional lymph node involvement is also present in 35–50% of ampullary carcinomas.

Lymph Nodes

Site/number/size/number involved/limit node/extracapsular spread.

Regional nodes: peripancreatic, pancreaticoduodenal, common bile duct, pyloric and proximal mesenteric. A regional lymphadenectomy will ordinarily include a minimum of 12 lymph nodes.

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

Up to 50% of patients have involved regional lymph nodes at presentation.

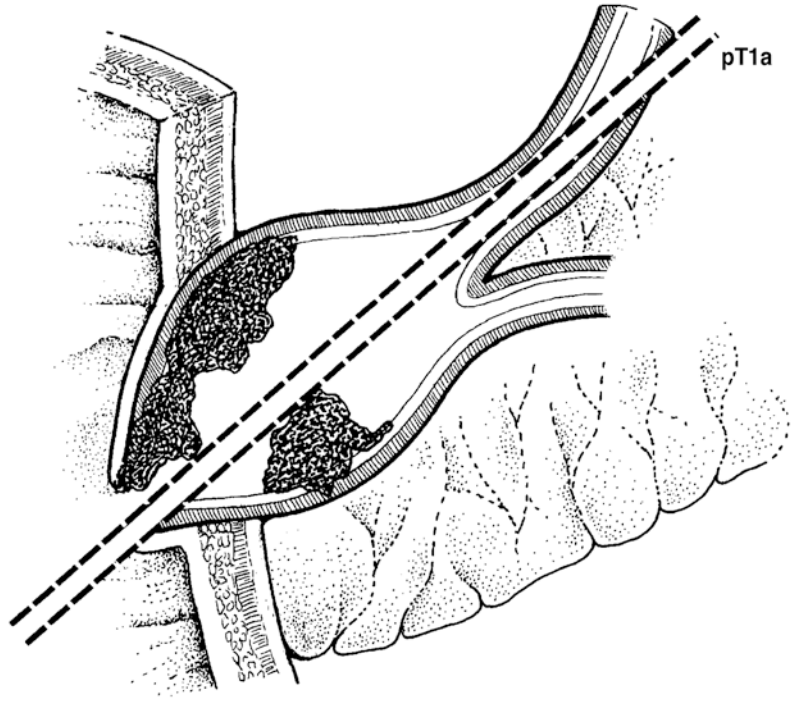

Fig. 4.1 Ampulla of Vater carcinoma. Adapted from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

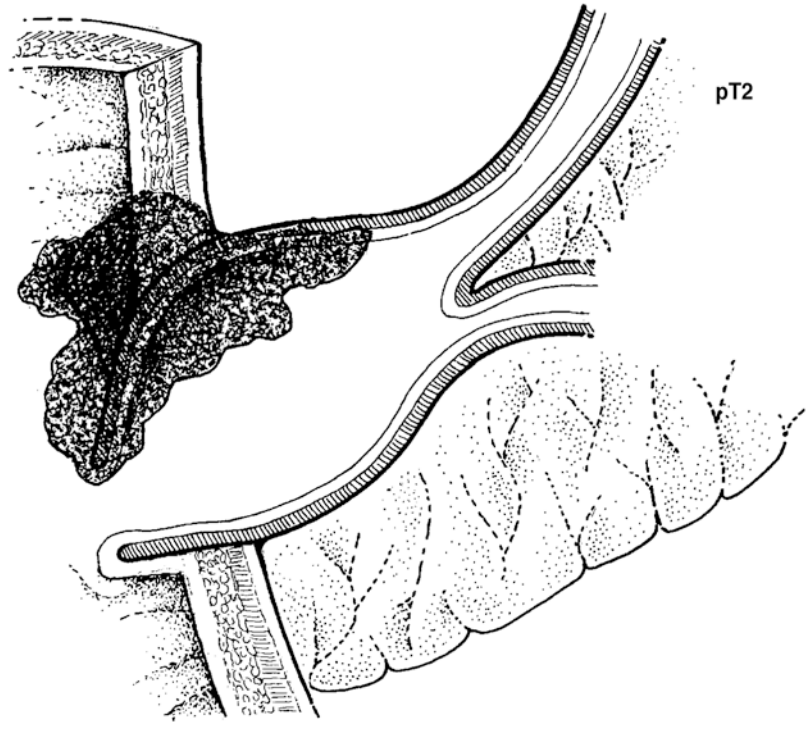

Fig. 4.2 Ampulla of Vater carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

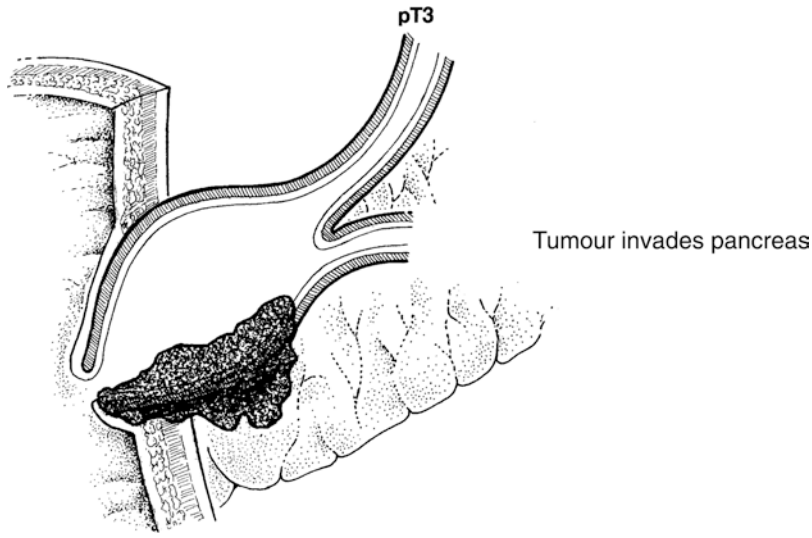

Fig. 4.3 Ampulla of Vater carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

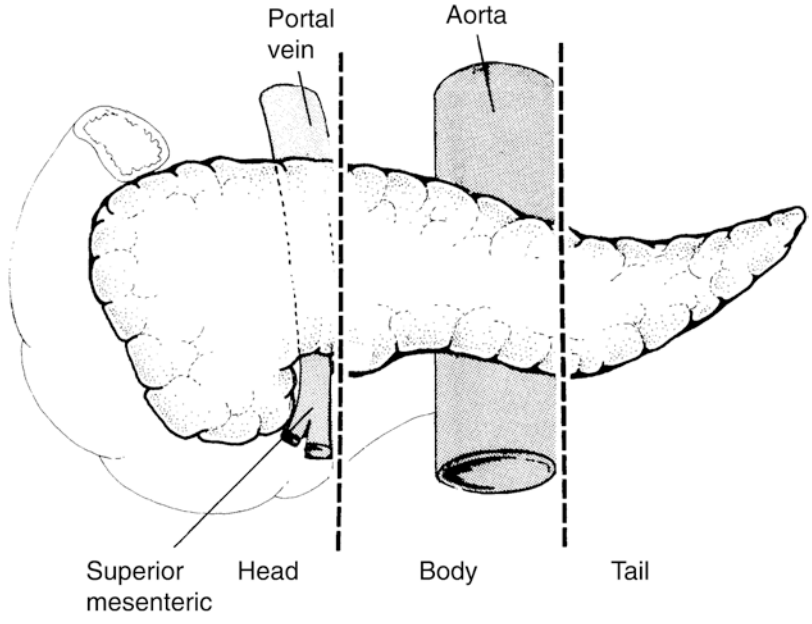

Fig. 4.4 Pancreas. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

Excision Margins

Distances (mm) to the following transected or dissected margins.

Transected: proximal (gastric/duodenal), distal (duodenal), common bile duct, distal pancreatic, superior mesenteric artery (also referred to as medial or uncinate).

Dissected: posterior pancreatic surface and superior mesenteric vein. The anterior surface is not a true margin but direct involvement (0 mm) is an adverse feature.

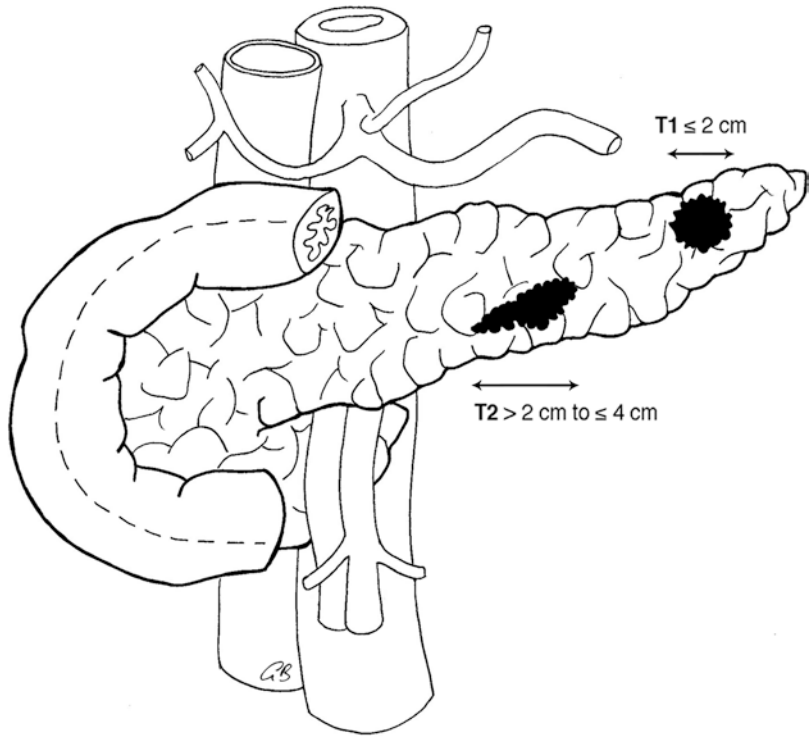

Fig. 4.5 Pancreatic carcinoma, pT1 and pT2

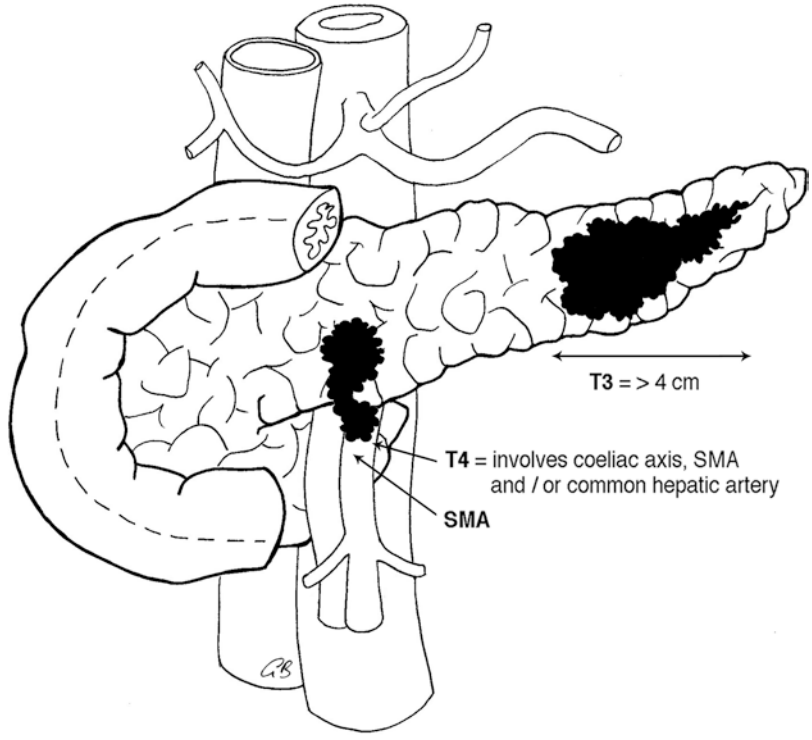

Fig. 4.6 Pancreatic carcinoma, pT3 and pT4

Tumour ≤1 mm constitutes microscopic margin involvement (R1) for carcinomas with exception of the anterior surface of the head of pancreas (0 mm). For PanNETs margins an incomplete excision applies if the margin is directly involved (0 mm).

Tumour <1 mm from the margin is reported as *positive (R1) in > 75% of cases.* Due to the dispersed tumour growth pattern at the periphery of pancreatic cancer, assessment of complete excision is more problematic than for other gastrointestinal cancers. Disparities exist in quoted R1 rates in various studies. This may relate to differences in opinion as to what constitutes a true surgical margin in pancreatic specimens and, in the past, a lack of a systematic approach to the pathological examination of these specimens.

The commonest site for *local recurrence of invasive carcinoma* after a Whipple's procedure is the *posterior pancreatic surface* and at the superior mesenteric vessel margins. These should be inked accordingly and the distance to tumour measured histologically. Local recurrence from intraductal tumour is more likely at a ductal resection margin.

Other Pathology

Ampulla

- *Duodenal adenoma(s), Familial Adenomatous Polyposis:* periampullary carcinoma is one of the commonest causes of death in FAP.

Pancreas

- 3–10% of pancreatic carcinomas are *hereditary:* either a positive family history of pancreatic carcinoma, hereditary pancreatitis, BRCA1, BRCA2, PALB2 or CDKN2A (p16) mutations, Peutz-Jeghers syndrome, or in the setting of HNPCC.
- *Disseminated intravascular coagulation (DIC), thrombophlebitis migrans:* clinically present in 25% of cases, particularly with mucin secreting tumours.
- *Gastrointestinal neuroendocrine syndromes:* e.g. Zollinger-Ellison syndrome (diarrhoea, gastric hyperacidity with gastric/duodenal/jejunal ulcers), Werner-Morrison syndrome/WDHA syndrome (watery diarrhoea, hypokalaemia, achlorhydria).
- *Chronic pancreatitis and autoimmune pancreatitis (Type 1 or Type 2):* can mimic pancreatic cancer clinically, on imaging and at operative inspection and palpation.
- *Chronic pancreatitis:* shows acinar atrophy, distortion and regenerative changes with stromal fibrosis and clusters of residual islet tissue that can *mimic pancreatic carcinoma.* Similar changes are also seen upstream and adjacent to pancreatic carcinoma due to duct obstruction indicating that interpretation and sampling can be problematic. Duct structures in chronic pancreatitis tend to retain their rounded contour and lobular architecture, lack significant malignant cytological change, show no invasion of nerve sheaths or peripancreatic fat, or juxtaposition to thick walled muscular vessels.
- *Pancreatic cancer:* jaundice of short duration in a patient older than 60 years is suspicious of malignancy. Other indicators are elevated serum CA19–9 (usually in cancers >30 mm diameter), duct stricture at ERCP, or a mass lesion on CT scan/ELUS. *Radiological imaging* is important in establishing *contraindications to surgery* (distant metastases or major vessel involvement, e.g. coeliac artery or common hepatic artery), or *other potentially operable diagnoses*, e.g. serous, mucinous/papillary neoplasms. A tissue diagnosis may be obtained by positive duct cytology brushings or transduodenal/percutaneous FNAC or needle core biopsy. This is particularly important to direct appropriate *palliative or neoadjuvant chemo(radio)therapy* in patients with *non-resectable or borderline resectable disease*, and to exclude *other treatable malignancies*, e.g. malignant lymphoma in peripancreatic nodes. In a proportion of cases (15–20%) a firm diagnosis will not be obtained and must be assumed on the basis of clinical

probability allowing radical surgery to proceed in a medically fit patient with resectable disease on imaging. As a result of these diagnostic difficulties there is inevitably a low risk (1–5%) of a *false positive clinical diagnosis of cancer*, and it not being present in the resection specimen.

Immunophenotype

- *Neuroendocrine:* chromogranin, synaptophysin, CD56. A high Ki-67 index and CK19 positivity are a guide to metastatic potential. Poorly differentiated/high-grade (G3) neuroendocrine carcinoma shows reduced staining intensity for chromogranin A, and well differentiated/low-grade tumours for CD56.
- *Hormonal*: specific peptides—insulin, glucagon, gastrin, pancreatic polypeptide, VIP, ACTH, somatostatin.
- *Exocrine carcinoma*: cytokeratins (including CK7, CK8, CK18, CK19, ±CK20), CEA, CA19–9, CA-125, MUC I—expressed in >80% of ductal lesions, ± CDX-2, p53, loss of SMAD4/DPC4.

Prognosis

Prognosis in pancreatic ductal carcinoma is poor. Average life expectancy at diagnosis is 4–6 months with a relative survival to 1 year of 20%. Poor prognosis relates to *tumour site* (body and tail are worse than head, as the latter may present early with obstructive jaundice), *size* (> 45 mm is adverse), *histological grade* and *stage*. Complete surgical resection offers the only potential for curative treatment. However, approximately 10% of patients are eligible for potentially curative surgery at diagnosis. Overall 5 year survival rates of up to 30% can be achieved with surgical removal and adjuvant chemotherapy. *Suitability for resection* is determined by local extent of the tumor and a lack major vessel (coeliac artery or common hepatic artery) compromise/involvement. Patients with borderline resectable tumours may receive *neoadjuvant chemotherapy +/− radiotherapy in order to downstage disease and convert to a resectable state. Postoperative adjuvant chemotherapy is commonly used after resection of pancreatic and ampullary carcinomas*. Serum CA19–9 levels can be of use in monitoring response to therapy and as a surveillance tool during follow up to detect recurrent disease. The majority of patients present late with symptoms due to advanced disease in lymph node and retroperitoneal tissues. *Treatment is often palliative* with relief of ductal biliary obstruction by open or laparoscopic bypass, or endoscopic stent insertion, and, chemotherapy for select patients. *Non-invasive MCN and IPMN are potentially curable* by complete surgical resection. Those with an invasive component have 27–65% 5 year survival depending on the extent and histological type of the invasive component. *Pancreatic neuroendocrine tumours* may present with their associated metabolic or gastrointestinal syndrome and have an *indolent time course* being of low to intermediate-grade malignancy. *Ampullary carcinoma is more favourable* than pancreatic or bile duct carcinoma with a 5 year survival of 25–50%. This can improve to 80–85% if the tumour is at an early stage and confined to the sphincter of Oddi (pT1). Transduodenal wide local excision may be adequate for carefully selected ampullary tumours, e.g. adenoma, but only after careful staging and exclusion of an underlying mass lesion requiring radical surgery.

Neoadjuvant Treatment

Neoadjuvant chemotherapy +/− radiotherapy is increasingly used to treat patients with locally advanced or borderline resectable disease. Evaluation of margin status following neoadjuvant therapy can be problematic due to tumour regression. Extensive sampling and careful evaluation of margins is required for post neoadjuvant specimens. Several regression grading schemes exist, e.g. College of American Pathologists scheme:

Grade 0	No viable cancer cells	Complete response
Grade 1	Single or rare small groups of cancer cells	Near complete response
Grade 2	Residual cancer with evident tumour regression but more than single cells or rare small groups of cancer cells	Partial response
Grade 3	Extensive residual cancer with no evident regression	Poor response

Other Malignancy

Leukaemia

Malignant Lymphoma

- Usually spread from paraaortic/peripancreatic nodal lymphoma.
- Extramedullary plasmacytoma.

Sarcoma

- Rare
- Leiomyosarcoma, liposarcoma, fibrosarcoma, osteosarcoma.
- Exclude secondary from gastrointestinal tract (spindle cell carcinoma/GIST/sarcoma) or retroperitoneum.

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Small Intestinal Carcinoma

5

Damian McManus

Small intestinal carcinoma is uncommon with more arising in the duodenum than in the jejunum and ileum combined. African Americans are a high risk group. Other risk factors are smoking, alcohol, chronic inflammation (Crohn's disease, coeliac disease) and Familial Adenomatous Polyposis (FAP). Presentation can vary with poorly localized central abdominal pain, obstruction with vomiting, colicky pain, constipation and distension. There may be a palpable mass or gastrointestinal bleeding.

Up to 60% of duodenal polyp/mass lesions are benign (e.g. Brunner's gland hyperplasia/adenoma, nodular gastric heterotopia (first part duodenum—D1) and duodenal adenoma). Malignant lesions are relatively uncommon, usually in D2, and occurring in patients 60–79 years of age. About 16% of these represent metastases from other sites, e.g. lung, breast, colon and pancreas, and they have a poor prognosis despite surgical resection. Radical surgery is considered for a primary lesion in the absence of widespread metastases. Assessment is by upper gastrointestinal endoscopy and CT scan. Palliative treatment may be considered as a first line approach including duodenal stenting to overcome bowel obstruction by a compressing extrinsic tumour mass. Tissue diagnosis is important to exclude other treatable malignancies, e.g. malignant melanoma, or malignant lymphoma in adjacent paraduodenal lymph nodes.

Localised jejunal and distal ileal mass lesions require a segmental resection and right hemicolectomy respectively, with en-bloc resection of the relevant mesenteric pedicle. Distal ileal lesions may be seen and biopsied at colonoscopy. More proximal small intestinal lesions may be characterized by push endoscopy, barium follow through studies or CT scan. Serosal disease, e.g. seedlings of metastatic carcinoma can be visualized and biopsied at laparoscopy or exploratory laparotomy.

Gross Description

Specimen

- Endoscopic/laparoscopic or open biopsy/resection.
- Whipples pancreaticoduodenectomy, segmental bowel resection, right hemicolectomy: depending on the tumour site in the proximal/mid-/distal small bowel, respectively. Needle core biopsy of a small intestinal or mesenteric mass is usually avoided due to the risk of capsule rupture and tumour seeding, jeopardising complete primary resection, suitability for which is assessed by CT scan.
- Weight (g) and size/length (cm), number of fragments.

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_5

Tumour

Site

- Duodenum (particularly periampullary)—70%: see Chap. 4.
- Jejunum/ileum—30%.
- Mucous membrane/muscularis/extra-mural.
- Serosal/mesenteric/nodal/single/multifocal.
- Mesenteric/anti-mesenteric border.
- Meckel's diverticulum.

Size

- Length × width × depth (mm) or maximum dimension (mm).

Appearance

- Polypoid/sessile/ulcerated/diffusely infiltrative/fleshy/pigmented/yellow/ stricture/intussusception ± secondary ischaemic necrosis of the tumour tip/intussusceptum (portion passing into another segment) or intussuscipiens (receiving segment).

Duodenal carcinomas tend to be papillary or polypoid, *ileojejunal carcinomas* ulcerated and annular with constriction of the bowel wall (napkin ring like). Presentation can be non-specific, e.g. anaemia or weight loss, with poorly defined central abdominal pain or signs of subacute obstruction. There may be a detectable mass either on abdominal examination or CT scan. *Well differentiated endocrine (carcinoid) tumour* is nodular, yellow, solitary or multifocal, causing bowel obstruction due to fibrosis or acting as the apex of an intussusception. On CT scan it forms a characteristic dense spiculate mesenteric mass associated with coarse flecks of calcification. *Malignant lymphoma* can be subtle in the edge of a perforated jejunitis, unicentric or multifocal, and a fungating, fleshy mural or mesenteric mass. There may or may not be a preceding history of coeliac disease. *Metastatic carcinoma* variably forms serosal seedlings, nodules or plaques. *Gastrointestinal stromal tumours (GISTs)* are mural lesions which can be dumbbell shaped with luminal and extramural components. They can also be separate from the bowel wall and mesenteric in location. Deposits of *malignant melanoma* may be darkly pigmented, and *choriocarcinoma* haemorrhagic.

Edge

- Circumscribed/irregular.

Histological Type

Adenocarcinoma

- *Enteric pattern:* the usual type and well or moderately differentiated.
- *Anaplastic:* poorly differentiated forms occur more frequently than in colorectal cancer.
- *Mucinous carcinoma:* mucin forms >50% of the tumour area.
- *Signet ring cell carcinoma:* mucin in >50% of the tumour cells.

Diagnosis of primary small intestinal adenocarcinoma is by *exclusion of spread from more common sites,* e.g. colorectum and stomach. As in the large intestine there is some evidence for a dysplasia (adenoma)—carcinoma sequence in the adjacent mucosa. *Prognosis is poor* due to late presentation at an advanced stage.

Neuroendocrine (Carcinoid) Tumour

- Yellow/nodular/solitary or multifocal (25%).
- A well differentiated/low-grade neuroendocrine tumour which is positive for chromogranin/synaptophysin/CD56±/CDX-2. Mitoses are usually <2 per 10 high power fields (hpf) and Ki-67 index ≤2% (i.e. a G1 tumour).
- Typically an insular pattern of uniform cells in a dense fibrous stroma with vascular thickening.
- 20% have *carcinoid syndrome* implying liver metastases. It is characterized by facial flushing, asthma and thickening of cardiac valves due to release of vasoactive peptides (e.g. serotonin) into the systemic circulation.
- *Tumour grade* is based on cell type, atypia, necrosis and proliferative activity:

mitoses/10hpfs, Ki-67 index (see Chap. 4. Differentiation).
- *Low-grade malignancy:* any functioning well differentiated tumour; any tumour with angioinvasion; non-functioning tumour ≥2 cm or with invasion beyond the submucosa.
- *High-grade malignancy:* tumour with a high mitotic rate, cellular atypia or necrosis and poorly differentiated neuroendocrine large cell/small cell carcinomas. Chromogranin±, synaptophysin/CD56/Ki-67 positive.

Prognosis: well differentiated neuroendocrine (carcinoid) tumour has an overall *50–65% 5 year survival rate*. It is better for small lesions (metastatic rate: < 1 cm (2%), 1–2 cm (50%), > 2 cm (80%)) confined to the wall (85% 5 year survival) than those invading the serosa or beyond (5% 5 year survival). Metastases are to *regional lymph nodes and liver* (multiple, solid/cystic), and also bone, skin and thyroid. The above comments relate mostly to classical EC (enterochromaffin) cell ileojejunal well differentiated neuroendocrine tumours. Duodenal lesions (5–8% of cases) are sporadic or arise in association with MEN (multiple endocrine neoplasia) or neurofibromatosis syndromes. They have a better prognosis, occur mainly in D1/D2 and include *non-functioning G cell tumours, gastrinomas* (Zollinger Ellison/MEN syndromes), *somatostatinoma* (look for psammoma bodies) and *gangliocytic paraganglioma*.

Others

- Rare: adenosquamous, sarcomatoid carcinoma; aggressive.

Metastatic Carcinoma

- *Direct spread:* colorectum, ovary, stomach, pancreas.
- *Distant spread:* lung, breast, malignant melanoma and choriocarcinoma.

The bulk of disease in metastatic spread is *extramural* but tumour can invade muscularis and mucous membrane causing obstruction or perforation, and *mimicking a primary lesion* on macroscopic and microscopic examination. Adjacent mucosal dysplasia is a useful pointer and adenoma is present in 24% of primary lesions. Small bowel is a common site of metastatic malignancy with formation of peritoneal seedlings, multiple nodules, plaques and strictures causing obstruction.

Metastatic Malignant Melanoma

- Pigmented/multifocal.
- Amelanotic/oligomelanotic.
- Malignant melanoma requires confirmation with S100, HMB-45, SOX10, melan-A.

Differentiation

Adenocarcinoma: well/moderate/poor/undifferentiated, or, Grade 1/2/3/4 based on the percentage tumour gland formation (well/G1 > 95%: moderate/G2 50–95%: poor/G3 < 50%). Signet ring cell carcinoma is grade 3, small cell and undifferentiated carcinoma (no gland formation) grade 4.

Well differentiated neuroendocrine tumour/high-grade neuroendocrine carcinoma: see Chap 4. Differentiation.

Malignant lymphoma: behaviour is dependent upon tumour subtype.

Sarcoma: low-/high-grade based on the degree of cellularity, atypia, necrosis and mitoses.

Extent of Local Tumour Spread

Border: pushing/infiltrative.

Lymphocytic reaction: prominent/sparse.

TMN8 classification for carcinoma (does not apply to tumours of the ampulla of Vater: see Chap. 4—Extent of local tumour spread).

pTis	Carcinoma in situ
pT1	Tumour invades:
pT1a	Lamina propria or muscularis mucosae
pT1b	Submucosa

pT2	Tumour invades muscularis propria
pT3	Tumour invades through muscularis propria into subserosa or into non-peritonealised perimuscular tissue (mesentery or retroperitoneum[a]) without serosal penetration
pT4	Tumour perforates visceral peritoneum or directly invades other organs/structures (includes other loops of small intestine, mesentery, or retroperitoneum and abdominal wall by way of serosa; for duodenum only, invasion of pancreas) (Fig. 5.1).

[a]The non-peritonealised perimuscular tissue is, for jejunum and ileum, part of the mesentery and, for duodenum in areas where serosa is lacking, part of the retroperitoneum

TNM8 classification for well differentiated neuroendocrine (carcinoid) tumours.

Duodenal/Ampullary/Jejunum/Ileal (italics indicate specific rules for that site).

pT1	Tumour invades lamina propria or submucosa, and is ≤1 cm in greatest dimension *Ampullary*: Tumour ≤1 cm in greatest dimension and confined within the sphincter of Oddi
pT2	Tumour invades muscularis propria or is >1 cm in greatest dimension *Ampullary*: Tumour invades through sphincter into duodenal submucosa or muscularis propria, or > 1 cm in greatest dimension
pT3	*Duodenal/ampullary*: Tumour invades the pancreas or peripancreatic adipose tissue *Jejunum/ileum*: Tumour invades through the muscularis propria into subserosal tissue without penetration of overlying serosa
pT4	Tumour perforates serosa or invades other organs or adjacent structures.

Lymphovascular Invasion

Present/absent.

Intra-/extratumoural.

Vessel wall fibrosis/stenosis in well differentiated neuroendocrine tumour.

Metastatic carcinoma often shows quite extensive lymphovascular invasion in the various layers of the bowel wall.

Lymph Nodes

Site/number/size/number involved/limit node/extracapsular spread.

Regional nodes:

Duodenum: pancreaticoduodenal, pyloric, hepatic (pericholedochal, cystic, hilar), superior mesenteric.

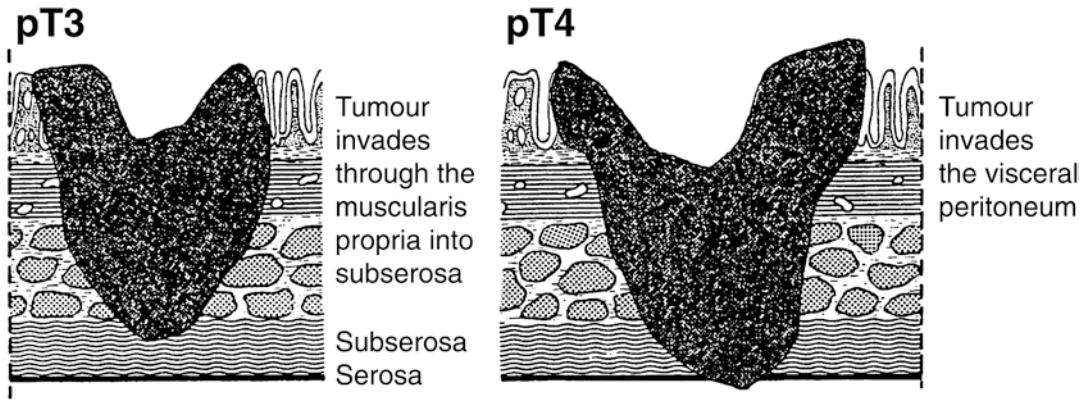

Fig. 5.1 Small intestinal carcinoma: pT4 also includes direct invasion of other organs or structures. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

Ileum/jejunum: mesenteric including the superior mesenteric nodes; and for the terminal ileum only—ileocolic, posterior caecal.

Small bowel adenocarcinoma (a regional lymphadenectomy will ordinarily include a minimum of 6 lymph nodes):

pN0	No regional lymph node metastasis
pN1	Metastasis in 1–2 regional lymph node(s)
pN2	Metastasis in ≥3 regional lymph nodes.

Well differentiated neuroendocrine tumour (a regional lymphadenectomy will ordinarily include a minimum of 12 lymph nodes):

Duodenal/ampullary	
pN0	No regional lymph node metastasis
pN1	Regional lymph node metastasis

Ileum/jejunum	
pN0	No regional lymph node metastasis
pN1	Regional nodal metastasis in less than 12 nodes
pN2	Large mesenteric masses (>2 cm) and or extensive nodal deposits (12 or greater) especially those that encase the superior mesenteric vessels

Excision Margins

Distances (mm) to the nearest longitudinal limit of resection and the painted deep radial (non-peritonealised soft tissue) mesenteric margin. In a segmental bowel resection it is usual to clear a 5 cm length of intestine on either side of the tumour with en bloc resection of a wedge of mesentery.

Other Pathology

There is increased incidence of adenocarcinoma in:

- *Familial Adenomatous Polyposis:* particularly periampullary related to duodenal adenomas. It is a significant cause of mortality in FAP.
- *Hereditary non-polyposis colon cancer (HNPCC).*
- *Peutz-Jegher's polyposis*: beware of epithelial misplacement/pseudo-invasion mimicking carcinoma; rare.
- *Crohn's disease.*
- *Coeliac disease.*
- *Ileostomy, ileal conduits.*
- *Meckel's diverticulum*: rare, but can also be a site for well differentiated neuroendocrine tumour, GIST, or leiomyosarcoma.

Coeliac disease/ulcerative jejunitis/gluten induced intestinal or enteropathy associated T cell lymphoma (EATL): change in or lack of responsiveness to a gluten free diet in a previously well maintained patient, or presentation as an ulcerative/perforated jejunitis, or an abdominal mass in an older patient can indicate onset of EATL. The lymphoma previously classified as Type II EATL is now designated as monomorphic epitheliotropic intestinal T cell lymphoma (MEITL). It is no longer thought to be associated with antecedent coeliac disease.

Stricture: carcinoid, metastases.

Intussusception: carcinoid, malignant lymphoma.

Multifocal: carcinoid, malignant lymphoma, malignant melanoma, metastases.

Meckel's diverticulum: carcinoid, adenocarcinoma, leiomyomatous tumours.

Immunophenotype

Small bowel adenocarcinoma is CAM 5.2, AE1/AE3, CEA, CK20 (40%), CDX-2 positive. About 50% are also CK7 positive.

Prognosis

Small bowel adenocarcinoma is unusual, being 50 times less common than large bowel carcinoma. Some 70% occur in the duodenum, particularly

the periampullary region. *Presentation is late* due to the fluid content of the bowel. Many patients already have transmural spread and lymph node metastases and the majority subsequently dies from their disease. *Five year survival rates are approximately 10–30%* with the most important prognostic indicator being *depth of spread* or *stage* of disease. Localised, regional and metastatic disease have 5 year survival rates of 57%, 34% and 3% respectively. Duodenum is less favourable than ileum and jejunum. *Surgical resection* is the curative intent treatment of choice.

Other Malignancy

Malignant Lymphoma

- Comprises *30–50% of small bowel malignancy*. Single or multifocal, primary or secondary to lymph node/systemic disease. Primary disease centres on the bowel with or without spread to regional lymph nodes.
- *MALToma*:
  - Low/high-grade.
  - B cell (70–90% of bowel lymphomas are of B cell lineage).
  - Centrocyte like cells with variable numbers of blasts (>20% = high-grade).
  - Lymphoepithelial lesions.
  - Monotypic immunoglobulin expression.
  - ± eosinophilia.
  - High-grade lesions are on a spectrum with *diffuse large B cell lymphoma* which forms *40–60% of small intestinal lymphoma cases*, with or without a component of MALT lymphoma.
- *Burkitt's type/B lymphoblastic*:
  - Children, or adults with HIV (non-endemic/non-EBV related).
  - Terminal ileum/ileocaecal valve—a high-grade lymphoma.
  - CD20, CD10, CD79a, tdt positive (lymphoblastic lymphoma). High Ki67 index (>90%) and a starry sky appearance.
- *Multiple lymphomatous polyposis*:
  - Centrocytic lymphoma or mantle cell lymphoma—splenic, small and large bowel disease with numerous intestinal polyps.
  - An intermediate-grade lymphoma—*aggressive disease* with advanced stage at presentation.
  - CD20, CD5, cyclin D1 positive.
- *EATL*:
  - Aggressive, forming 5% of GI lymphomas usually in the proximal jejunum. Pleomorphic medium to large cell infiltrate (CD3 positive) (previously classified as Type I EATL, now simply EATL) and adjacent enteropathic mucosa (villous atrophy/increased surface intraepithelial lymphocytes) showing 'pre-lymphomatous' changes or type 2 refractory coeliac disease, *viz* clonal intraepithelial lymphocytes with T cell receptor gene rearrangements and an aberrant immunophenotype (loss of CD4/CD8 expression) .
- *Follicle centre cell (follicular) lymphoma*:
  - More usually spread from lymph node disease rather than primary.
  - However, duodenal type follicular lymphoma is now recognised as a specific variant of follicular lymphoma with distinctive clinical and biological features. It is usually located in the second part of the duodenum and often forms polyps
- *Immunoproliferative small intestinal disease (IPSID):*
  - Mediterranean countries.
  - Alpha chain disease.
- *Post-transplant lymphoproliferative disorders:*
  - Polyclonal/monclonal/disparate morphology and behaviour.
  - Some regress on decreasing immunosuppression therapy, e.g. cyclosporin (see Chap. 35).

Prognosis: is better for low-grade B cell lymphomas (44–75% 5 year survival) than high-grade B or T cell lymphomas (25–37% 5 year

survival). Adverse prognostic indicators are *perforation, high-grade histology, T cell phenotype, multiple tumours, large size, serosal penetration and advanced stage* (Ann Arbor System: see Chap. 35).

Gastrointestinal Mesenchymal or Stromal Tumours (GISTs)

- Spindle cells, epithelioid cells, skeinoid collagen fibres. Note also that extra-intestinal mesenteric or retroperitoneal lesions can occur.
- Ileum 50%, jejunum 40%, duodenum 10%.

Myogenic: 10% of cases are desmin/h-caldesmon/smooth muscle actin positive and DOG-1/c-kit(CD117) negative, representing true leiomyoma or leiomyosarcoma.

Neural: 10% of cases are S100/synaptophysin positive and DOG-1/c-kit(CD117) negative, representing neurilemmoma (Schwannoma: characteristic peritumoural lymphoid infiltrate) or neurofibroma (can be associated with von Recklinghausen's disease/MEN syndrome and GISTs elsewhere in the gut).

Stromal: DOG-1/c-kit(CD117: tyrosine kinase receptor)/CD34 positive, with absent or incomplete myogenic/neural differentiation. Putative precursors are the interstitial cells of Cajal, which are gut pacemaker cells located in the deep submucosa and myenteric plexus. In general antigen positivity is DOG-1 and CD117 (95%), CD34 (70–85%), smooth muscle actin (20–40%), h-caldesmon (60–80%) and nestin (90–100%). *C-kit mutation analysis* (exon 11 in 60–70% of cases) is helpful in confirming the *diagnosis* and in prediction of *prognosis* and *response to drug treatment*. Exon 11 changes are more responsive than tumours with alterations in exon 9. Mutation analysis is generally recommended if immunohistochemistry is equivocal, and in cases of borderline/malignant GISTs and in small intestinal GISTs. Note that other malignant tumours can also be CD117 positive, e.g. seminoma, malignant melanoma and some metastatic carcinomas, e.g. breast, ovary, colorectal, small cell carcinoma. DOG-1 (transmembrane protein Discovered On GIST1) is a highly sensitive and specific marker for GIST but may also show weak to moderate expression in some other tumours, e.g. colorectal, endometrioid and acinic cell carcinomas, spindle cell malignant melanoma and malignant peripheral nerve sheath tumours.

GANT (gastrointestinal autonomic nerve tumour) is regarded as a variant of GIST and assessed accordingly. A small minority of patients with GISTs may have any of: a positive family history of GISTs, Carney triad (gastric GIST, pulmonary chondroma, extra-adrenal paraganglioma) or type 1 neurofibromatosis(often multiple and usually wild type for KIT, PDGFRA and BRAF mutations).

Malignancy relates to: size (> 2–5 cm), cellularity, atypia, cell type (epithelioid is worse than spindle cell), necrosis, margins and mitoses. *Prognosis* (approximately *50% 5 year survival)* is also stage dependent. DNA ploidy, Ki-67 proliferation indices, over expression of p53, loss of DOG-1/CD117 immunoexpression and morphometry also correlate with these parameters.

Clinical risk: small intestinal GISTs are categorised as being no risk, low, moderate or high *metastatic risk* on the basis of size and mitoses (see Table 5.1).

Prognosis: in GISTs is dependent on *patient age, tumour size (> 5 cm), tumour site* and *mitotic activity*. A robust criterion in stomach tumours is *>5 mitoses/50 high-power fields,* but *mid- and hindgut lesions are more aggressive* (even if <5 cm diameter and with low mitotic counts) *than foregut tumours*. Behaviour can also be unpredictable, with clinicopathological factors at best being only broadly indicative. The old terminology "gastrointestinal stromal tumour of uncertain malignant potential" has been replaced by clinical risk stratification. The modified

Table 5.1 Risk factors in ileojejunal GISTs – risk of progressive disease, metastases or tumour related death

	Tumour dia (cm)	Mitoses/50hpfs	Percentage risk
No risk	≤2	<5	0
Low risk	>2–5	<5	4.3
Moderate risk	>5–10	<5	24
High risk	>10 Any size	<5 >5	52 73–90

Miettinnen criteria are in widespread use but do not apply to SDH (Succinate Dehydrogenase) deficient GISTs that may occur in the paediatric age group, young females or as part of Carney Stratakis Syndrome or Carney's triad.

Metastases: are commonly to peritoneum, liver, pancreas, retroperitoneum and *lungs.* Lymph node metastases can occur but are rare and most often associated with GISTs showing SDH deficiency. Metastases are CT/PET scan positive but become negative on treatment. Metastatic disease generally responds well to *targeted tyrosine kinase inhibitor therapy (Glivec (imatinib))* resulting in tumour shrinkage, myxoid, hyaline and cystic degeneration. It gives several disease free years, or tumour down staging that can facilitate operative resection, but usually therapeutic escape occurs with *recurrent peritoneal disease or size progression of liver metastases*. This may relate to new acquired genetic mutations in the tumour cells. About 13% of patients are resistant to therapy with disease progression within 6 months.

Kaposi's Sarcoma

- HIV: 50% of high risk patients have visceral involvement.

Leukaemia

- 14.8–25% of cases.
- Granulocytic sarcoma (CD34/CD43/CD 68/CD117/chloroacetate esterase and myeloperoxidase positive). Molecular testing may also assist in more precise subclassification.

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Colorectal Carcinoma

6

Maurice B. Loughrey

Colorectal cancer accounts for about 10% of all new cancers worldwide and is the fourth commonest in men (after lung, prostate, stomach) and the third in women (after breast and cervix). Its geographical incidence varies greatly and is high but stabilising in the industrialised countries of Europe, Australia, North America and Japan. Risk factors are: obesity with a diet of highly calorific food rich in animal fats, low fruit and fibre and high red meat intake, lack of exercise, smoking, alcohol and chronic inflammation (e.g. inflammatory bowel disease). It is estimated that some 10–35% of colorectal cancers can be attributed to inherited susceptibility, with 5% on the basis of specific predisposing syndromes such as familial adenomatous polyposis (FAP) and Lynch syndrome, formerly known as hereditary non-polyposis colorectal cancer. The incidence of colorectal cancer amongst younger individuals is increasing, the reasons for which remain unclear.

Presentation of colorectal cancer is variable depending on the tumour site and growth pattern: this may manifest as bright red bleeding per rectum, abdominal pain or mass, change in bowel habit, tenesmus (feeling of incomplete faecal evacuation), or as iron deficiency anaemia due to chronic blood loss from the ulcerated surface of the lesion. A significant minority (10–15%) present as a surgical emergency due to intestinal obstruction or perforation, either directly through the tumour itself, or of the dilated bowel proximal to the stenotic tumour. National bowel cancer screening programmes are based on detection of occult blood loss, through the faecal occult blood (FOB) test or increasingly the faecal immunochemical test (FIT), which has the benefit of being specific for human blood. Screening is targeted at asymptomatic middle-aged patients with the aim of identifying and removing precursor lesions and early stage cancers.

Investigation of colorectal cancer is by endoscopy and biopsy with staging of biopsy proven cancers by CT scan of chest, abdomen and pelvis for evaluation of local and distant spread. MRI scan of rectal cancers complements this and clinical examination by imparting more accurate information about regional lymph node disease, extramural venous invasion and the status of the tumour edge and any suspicious nodes in relation to the mesorectal envelope and its fascial plane. This assessment of disease in relation to the mesorectal surgical margin influences decisions on neoadjuvant and operative management and impacts upon rates of local recurrence. Ideally, colonoscopy visualises the entirety of the colorectum to assess for synchronous lesions. CT colonogram and/or flexible sigmoidoscopy can be of use in a medically unfit patient or where there is a distal stricture not passable by the colonoscope or amenable to biopsy. A tight stricture

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_6

may be negotiated by a gastroscope which is of a narrower calibre.

Curative colorectal cancer surgery excises the primary lesion with adequate longitudinal and deep radial margins and *en bloc* resection of the relevant colonic mesenteric lymphovascular pedicle or the mesorectum. The type of resection is determined by the site, distribution and any multiplicity of lesions detected at preoperative colonoscopy. Planned elective laparoscopic surgery is the preferred option. Open abdominal surgery (laparotomy) may be necessary for extensive disease or if the presentation is as an acute emergency. Anterior resection with total mesorectal excision (TME) is the operation of choice for rectal tumours. This may be preceded by neoadjuvant short course radiotherapy for stage cT3 disease with a clear mesorectal fascial surgical margin on MRI, or neoadjuvant long course chemoradiotherapy for more advanced stage tumours (cT3/4) where the mesorectal fascial surgical margin is potentially compromised either by locally advanced primary tumour or by involved mesorectal lymph nodes or extramural venous invasion. Patients who have had a complete clinical response and excellent radiological response to such therapy (up to 25%) may be considered, after careful discussion with the patient, for close observational follow-up, holding surgery in reserve should tumour recurrence develop. Low rectal tumours may require abdominoperineal excision and sacrifice of the anal sphincter, if a distal clearance of at least 1 cm below the tumour cannot be achieved.

In the setting of unresectable tumour, palliative intent surgery delivers a more limited resection. Some patients with obstructing cancers undergo piecemeal resection, partial laser ablation or stenting to restore intestinal continuity and avoid the risk of perforation. This may allow elective resection to be planned for a later date. Stenting is contraindicated in low rectal tumours and right-sided colonic obstruction due to difficulties in stent placement and migration. Clinical follow up of colorectal cancer is by CT scan and colonoscopy, supplemented by serum CEA levels which can be elevated in recurrent or metastatic disease. Assay of serum CEA is not recommended for making a diagnosis of primary colorectal cancer. CT/PET scan can be of use in detecting extrahepatic or extrapulmonary metastases which may be a contraindication to consideration of distant metastasectomy. Otherwise limited metastatic disease, involving liver or lung, can be targeted aggressively by metastasectomy and/or ablation therapy.

Gross Description

Specimen

- Rectal/sigmoidoscopic/colonoscopic biopsy, local resection (EMR (endoscopic mucosal resection)/ESD(endoscopic submucosal dissection)/TEMS(transanal endoscopic microsurgery)/TAMIS(transanal minimally invasive surgery)), right or left hemi-/transverse/sigmoid/total colectomy/anterior resection (AR) or abdominoperineal excision of rectum (APER)/panproctocolectomy.
- Weight (g) and size/length (cm), number of fragments.

Tumour

Site

- Caecum/ascending colon/hepatic flexure/transverse colon/splenic flexure/descending colon/sigmoid colon/rectum/multifocal

  Tumour site strongly influences clinical presentation e.g. caecal carcinoma—anaemia, right iliac fossa mass; sigmoid colon carcinoma—alteration in bowel habit; rectal cancer—bright red blood per rectum, tenesmus.
- *For rectum: above/at/below the peritoneal reflection.* Tumours below the reflection have

a *higher rate of local recurrence* and tumours above/at the reflection anteriorly may *involve peritoneum*. The lateral angled descent of the peritoneum results in variation of the anatomical relationships with the upper rectum orientated to mesorectum posteriorly and laterally and peritoneum anteriorly. The mid-rectum is surrounded by mesorectum, whereas the lower rectum is below the level of the mesorectum encircled by pelvic sphincteric and levator ani muscle. Elsewhere in the colon the bowel is orientated to serosa and mesentery but the ascending and descending colons have a posterior non-peritonealised retroperitoneal bare area which is a surgical resection margin. Sigmoid colon ends where the external longitudinal muscle bands (taeniae coli) blend with the rectal muscularis propria.

- Distances (cm) to the dentate line and nearer longitudinal resection limit. Clinical (endoscopic/radiological: MRI scan) and anatomical definition of the rectum varies but, in general, distances from the anal verge are: lower rectum 0–5 cm, mid rectum 5–10 cm, and upper rectum 10–15 cm.
- Grading of plane of mesorectal excision (AR and APER specimens). Mesorectal fascia plane of surgery is optimal and results in a smooth, intact and symmetrically bulky mesorectum. Intramesorectal surgery results in defects in the mesorectum without exposure of muscularis propria. Muscularis propria plane of surgery implies the least optimal surgery, with limited mesorectum and exposure of muscularis propria in areas. This is associated with greater risk of circumferential margin involvement and local recurrence.
- Grading of plane of resection of the sphincters (APER only). Options are extralevator, sphincteric and intrasphincteric. Extralevator dissection provides a cylindrical specimen and maximises the chance of clear circumferential resection margin. Sphincteric dissection provides the classical waisted specimen with the circumferential margin formed by the surface of the sphincter muscles. Intrasphincteric surgery is suboptimal and implies significant disruption of the sphincter muscle or full thickness perforation exposing the lumen. This is associated with greater risk of circumferential margin involvement and local recurrence.

Size

- Length × width × depth (mm) or maximum luminal dimension (mm).
- Not shown to be an independent prognostic indicator but allows correlation with preoperative CT and MRI imaging and can be a gauge as to any effect of neoadjuvant therapy.

Appearance

- Polypoid/annular/ulcerated/mucoid/stricture/plaque.
- No independent influence on prognosis. Proximal cancers tend to be exophytic masses, other sites more likely to be ulcerated and annular.
- Expanding metallic mesh stent *in situ*: suitable for lesions from the distal transverse colon to the upper rectum. Outside of these locations there can be complications due to inadequate placement of the stent and its subsequent migration.

Perforation

- Present/absent. *Perforation has a higher incidence of local recurrence and poorer prognosis.* Perforation through the tumour is regarded as stage pT4a under TNM8 because of the potential contact with peritoneum (Fig. 6.1). This does not include proximal ischaemic back pressure perforation (e.g. caecum) due to an obstructing distal cancer. In this case the pT stage is determined by the degree of local spread of the distally sited cancer.

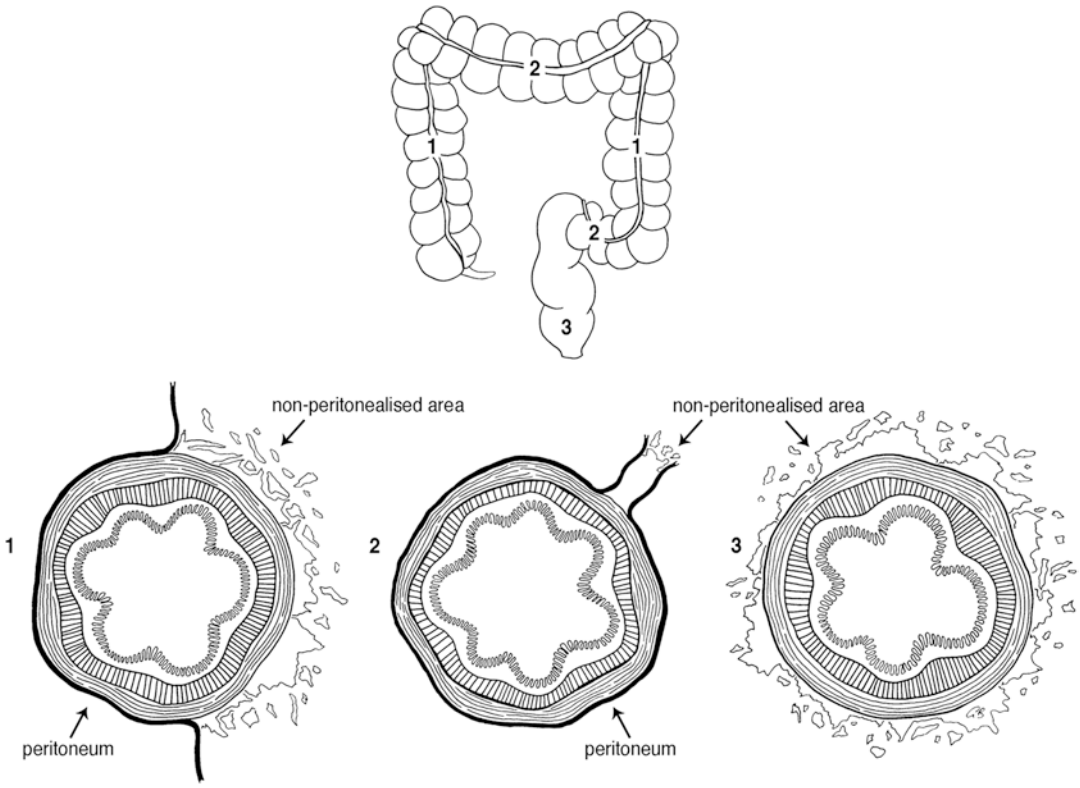


Fig. 6.1 The serosal covering of the large intestine differs along its course. This determines inking of specimens and block selection for pertinent histologic examination. (1) The ascending and descending colon lack peritoneum on their posterior surfaces. (2) The sigmoid colon is suspended on a narrow mesentery and so has a small non-peritonealised surface posteriorly. (3) The lower rectum lies beneath the peritoneal reflection and in corresponding resections will be completely surrounded by a circumferential non-peritonealised margin

Histological Type

Adenocarcinoma

- *85% of cases are colorectal adenocarcinoma of usual type.*
- *Diagnostic criteria are:* (a) neoplastic epithelial changes, in (b) a desmoplastic stroma, with (c) invasion beneath the muscularis mucosae. In practice a combination of (a) and (b) is the most useful indicating a biopsy derived from the ulcerated surface of the tumour. (c) is seen in material derived from the tumour edge. Neoplastic epithelial changes are both architectural (angular tubules/ cribriform nests/segmental or garland necrosis with dirty lumenal debris/single cells) and cytological (nuclear/nucleolar enlargement, overlap and stratification). Some 10–20% of biopsies will show only high-grade dysplasia or features suspicious for, but not totally diagnostic of, malignancy. A significant proportion of these cases will not require further biopsy as surgical resection will be merited on the basis of finding glandular neoplasia in the context of appropriate clinical and imaging features e.g. an ulcerated mass lesion or irregular stricture. In the setting of a rectal tumour, when neoadjuvant therapy is planned, repeat flexible sigmoidoscopy is recommended to obtain a diagnostic sample prior to therapy.

Mucinous Adenocarcinoma

- *10% of cases.*
- Tumour area is >50% extracellular mucin.
- Of no proven prognostic impact although this may reflect heterogeneity of this tumour group, comprising better prognosis microsatellite instability (MSI)-high tumour and worse prognosis microsatellite stable (MSS) tumours.

Signet Ring Cell Adenocarcinoma

- >50% signet ring cells.
- *Poor prognosis.*
- Distinguish from secondary carcinoma e.g. gastric signet ring cell carcinoma or prostate carcinoma.

Others

- *Micropapillary adenocarcinoma:*
  - Characterised by tumour cell clusters in stromal spaces mimicking lymphatic channels; associated with adverse features including lymph node metastates.
- *Medullary carcinoma:*
  - Characterised by sheets of malignant cells with vesicular nuclei, prominent nucleoli and abundant eosinophilic cytoplasm, and prominent intratumoral and peritumoral infiltration by lymphocytes and plasma cells. Strong association with MSI-high status.
- *Serrated adenocarcinoma:*
  - Prominent glandular serrations, sometimes accompanied by extracellular mucinous differentiation.
- *Adenoma-like adenocarcinoma:*
  - Pushing growth pattern and minimal desmoplastic reaction is observed. Extremely difficult to make a diagnosis of invasive malignancy on biopsy.
- *Adenosquamous carcinoma:*
  - Requires glandular and squamous (keratinisation/intercellular bridges) features. *Aggressive.*
- *Squamous cell carcinoma:*
  - Rectal: can be seen in ulcerative colitis/Crohn's disease/schistosomiasis/amoebiasis. Exclude spread from an anal carcinoma or cervical carcinoma. Need intercellular bridges ± keratinisation with no gland/mucin formation.
- *Undifferentiated carcinoma:*
  - Lack morphological evidence of squamous or glandular differentiation, or any other cell lineage. Differ from medullary carcinomas in not having a syncytial growth pattern or prominent lymphoplasmacytic infiltrates.
- *Neuroendocrine tumours:*
  - Either well differentiated/low-grade neuroendocrine tumour (grade 1, grade 2) or, rarely, poorly differentiated/high-grade neuroendocrine (small cell/large cell) carcinoma (grade 3). See Section "Other Malignancy".
- *Mixed differentiation:*
  - MiNEN: mixed neuroendocrine-non-neuroendocrine neoplasms; includes MANEC: mixed adenoneuroendocrine carcinoma e.g. usual adenocarcinoma with neuroendocrine carcinoma.
- *Metastatic carcinoma:*
  - *Transcoelomic spread:* stomach, ovary, endometrium, gastrointestinal tract, pancreas.
  - *Direct spread:* prostate, anus, cervix, kidney, stomach.
  - *Distant spread:* breast (infiltrating lobular), malignant melanoma, lung cancer.

Metastatic disease can infiltrate bowel wall and protrude into the mucosa *mimicking a primary lesion endoscopically and macroscopically*—a relevant previous history, extrinsic or mural disposition of the tumour, and a lack of mucosal tumour origin are crucial to the diagnosis.

Differentiation

Well/moderate/poor/undifferentiated, or, grade 1/2/3/4 based on the percentage tumour gland formation (well/G1 > 95%: moderate/G2 50–95%: poor/G3 < 50%). Undifferentiated carcinoma (grade 4) shows no gland formation.

Low-grade (well/moderate differentiation) or *high-grade (poor differentiation)*: due to established difference in prognosis and better reproducibility, a two-tier grading system is recommended. Grading applies only to adenocarcinoma, not otherwise specified and is not applicable to mucinous, signet ring cell or other subtypes of adenocarcinoma.

- *80–90% of cases are low-grade or well/moderately differentiated.*
- Foci of so-called tumour budding or poorly differentiated clusters at the advancing edge are insufficient to classify as high grade or poorly differentiated.

Extent of Local Tumour Spread

Border: pushing/infiltrative.

Lymphocytic reaction: prominent/sparse.

An expanding growth pattern/margin with a Crohn's-like inflammatory response is an indicator of better prognosis than an infiltrating, irregular margin with no inflammation. The former pattern is strongly associated with MSI-high colon cancers.

Degree of mesorectal/mesocolic spread measured from the outer border of the muscularis propria (> 5 mm) may influence prognosis. This measurement can also facilitate audit of preoperative radiological staging of extramural tumour spread.

The TNM classification applies only to carcinomas.

pT1	Tumour invades submucosa
pT2	Tumour invades muscularis propria
pT3	Tumour invades beyond muscularis propria into subserosa or non- peritonealised pericolic/perirectal tissues
pT4	Tumour invades the serosal surface or adjacent organs and/or perforation of visceral peritoneum[a].

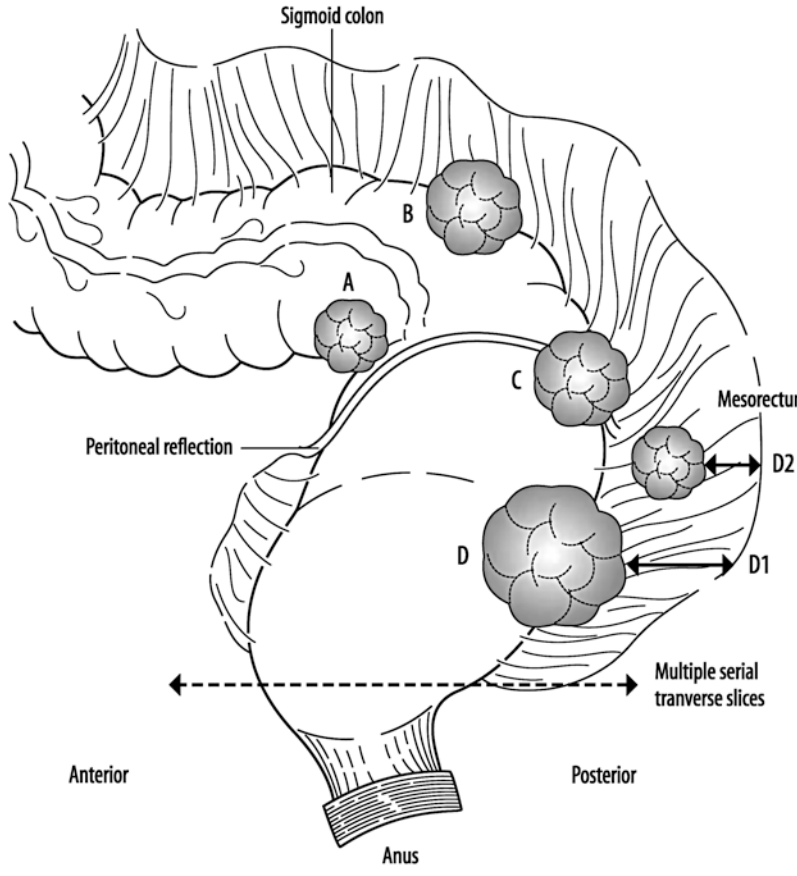
[a]Other descriptors (current TNM8): pT4a (visceral peritoneum), pT4b (other organs/structures)

Serosal involvement is tumour either at or ulcerating the serosal surface as this is prognostically worse than tumour in a subserosal inflammatory reaction. About 10% of patients develop peritoneal or ovarian (Krükenberg) deposits and there is a higher rate of distant metastases than in direct invasion of adjacent organs or structures alone. Serosal pT4b disease may include direct involvement of other segments of colon e.g. sigmoid colon by a caecal carcinoma. If no tumour is present in adhesions to other structures e.g. bladder, classify as pT3. Separate *mesenteric, omental or distant peritoneal deposits are metastatic (stage pM1c under TNM8) disease.* In low rectal cancer involvement of internal sphincteric muscle is pT3 and external voluntary sphincteric levator ani muscle is pT4b (TNM8). Intramural extension to adjacent bowel e.g. caecal carcinoma to ileum does not affect the pT stage.

A *minimum of five blocks of tumour and bowel wall* is necessary to assess the pT stage adequately and to find extramural vascular invasion or foci of discontinuous tumour within mesenteric fat. The specimen is cut into serial transverse slices 4–5 mm thick, laid out in order, photographed and with relevant slices selected for blocking.

Multiple carcinomas should be assessed and staged individually.

Direct implantation spread can be seen at anastomoses, peritoneal and abdominal wall wounds. Anastomotic site recurrence is unusual if the longitudinal margin clearance in the primary specimen is >5 cm (Figs. 6.2, 6.3 and 6.4).



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 5 mm thick
Blocks for histology are:

Above the reflection	A tumour, rectal wall and serosa
	B tumour, rectal wall and serosa tumour, rectal wall and mesentery
At the reflection	C tumour, rectal wall and serosa tumour, rectal wall and mesorectum
Below the reflection	D tumour, rectal wall and mesorectum
	D1 distance (mm) of the deepest point of continuous tumour extension to the nearest point of the painted CRM
	D2 distance (mm) of the deepest point of discontinous tumour extension (or in a lymphatic, node or vessel) to the nearest point of the painted CRM

Fig. 6.2 Rectal carcinoma. Reproduced, with permission, from *Histopathology Reporting: Guidelines for Surgical Reporting, second ed.,* © 2006, Springer

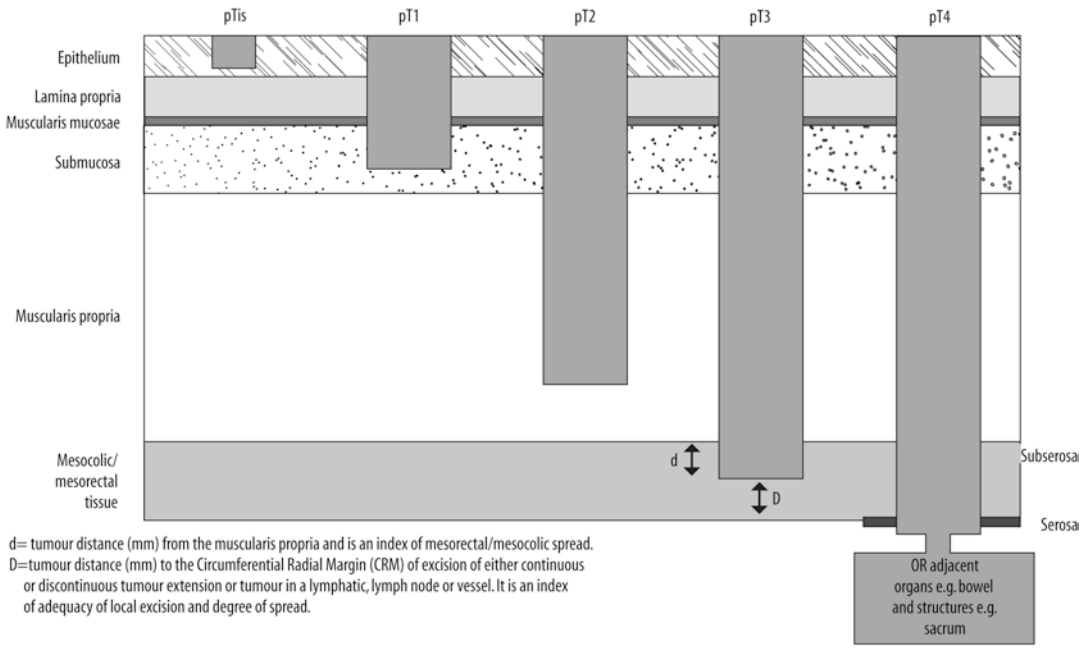


Fig. 6.3 Colorectal carcinoma. Reproduced, with permission, from *Histopathology Reporting: Guidelines for Surgical Reporting*, *second ed.,* © 2006, Springer

Venous, Lymphatic and Perineural Invasion

Present/absent.

Intra-/extramural.

Extramural venous invasion is an adverse prognostic factor. Orientation perpendicular to the muscularis propria is distinctive in appearance on MRI scan and macroscopically at the pathology dissection bench. At microscopy, the "protruding tongue" sign with smooth muscle fibres in its wall, adjacent "widowed" or "orphaned" small arteries and elastin and desmin stains can help identification. V2 is evident macroscopically and V1 only microscopically. Intramural venous invasion should also be reported. "Small vessel" lymphovascular invasion, intramural or extramural, can represent involvement of either lymphatics or small venules and both are classified under L1 (TNM8). Perineural invasion (Pn1), intramural or extramural, should be reported if identified.

All of these features, indicating engagement of metastatic pathways, have been demonstrated to have adverse prognostic association and their identification should merit consideration of adjuvant chemotherapy.

Lymph Nodes and Tumour Deposits

- Number of regional lymph nodes identified.
- Number of regional lymph nodes involved by tumour.
- Highest node involved (yes/no).
- Number of tumour deposits (number up to five, or >5).

Regional nodes: pericolic, perirectal, those located along the ileocolic, colic, inferior mesenteric, superior rectal and internal iliac arteries. A regional lymphadenectomy will ordinarily include at least12 lymph nodes. *Lymph node yield varies greatly even after careful dissection.* It is related to patient age (fewer in elderly patients), variation in individual anatomy, site (mesorectum yields fewer lymph nodes/ileocaecal angle mesentery more), the extent of resec-

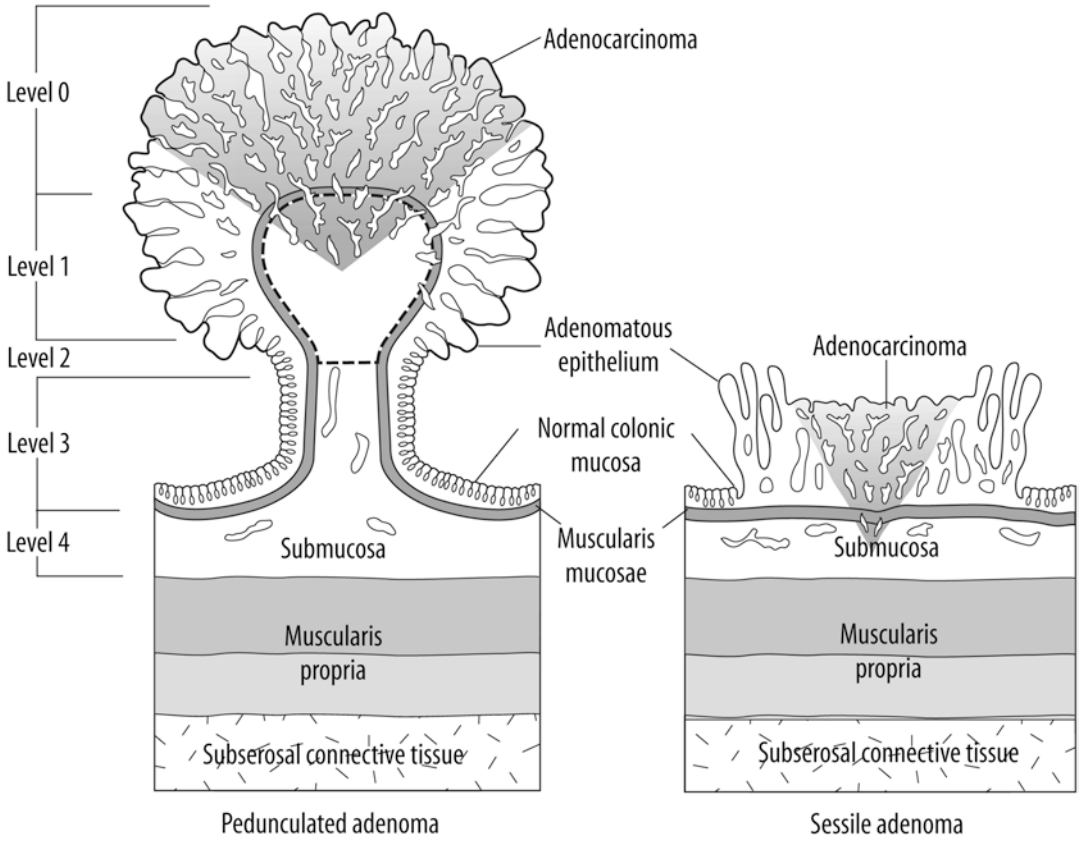


Fig. 6.4 Malignant colorectal polyp. Levels of invasion in a pedunculated adenoma *(left)* and a sessile adenoma *(right).* The *stippled areas* represent zones of carcinoma. Note that any invasion below the muscularis mucosae in a sessile lesion represents level 4 invasion, i.e. invasion into the submucosa of the bowel wall. In contrast, invasive carcinoma in a pedunculated adenoma *(left)* must traverse a considerable distance before it reaches the submucosa of the underlying bowel wall. The *dotted line* in the head of the pedunculated adenoma represents the zone of level 1 invasion. (Haggitt RC, et al. Prognostic factors in colorectal carcinomas arising in adenomas: implications for lesions removed by endoscopic polypectomy. Gastroenterology 1985;89:328–336. ©1985 Reproduced, with permission, from American Gastroenterological Association

tion performed, and history of preoperative neoadjuvant therapy. The latter leads to lymph node shrinkage. Tumour involvement of external iliac, common iliac or superior mesenteric artery nodes indicates distant metastatic disease (pM disease).

ALL *regional lymph nodes should be sampled for histology:*

- Essentially ALL fatty tissue should be carefully examined and ALL available lymph nodes harvested with a *median count of 12 lymph nodes per specimen* achieved, both departmentally and for individual dissectors. Techniques such as fat clearance and methylene blue staining can increase lymph node yield but there is no clear evidence that this approach increases numbers of positive lymph nodes or upstages the tumour. The "high tie" or apical node should be identified separately as involvement indicates worse prognosis. More than one vascular pedicle suture tie may mean more than one apical node needs to be identified as such. Lymph nodes are increased in number and *randomly distributed in the anterior, bilateral, posterior and distal mesorectal compartments* of rectal TME specimens, emphasising the need for a complete

surgical mesorectal excision and comprehensive dissection in the laboratory. In addition, in up to *48% of cases with nodal involvement, metastases are present within small (<0.5 cm) lymph nodes* which should therefore not be overlooked on macroscopic or microscopic examination. This also highlights the difficulty in determining an accurate clinical N stage on pre-treatment MRI scans. Furthermore, mesorectal lymph node metastases do not follow a predictable sentinel lymph node pathway.

- Small (<4 mm) lymph nodes should be submitted entirely for histology and larger nodes sampled by a central slice along the longest axis. Processing all lymph node tissue for histology may marginally increase the yield of involved lymph nodes at the expense of a marked increase in required blocks per case
- Micrometastatic disease in lymph nodes (deposits ≥0.2 mm and < 2 mm) have a higher risk of disease recurrence in early stage colorectal cancer compared to node-negative cancers, but there is no increased risk of disease recurrence in cases with 'isolated tumour cells' (single tumour cells or groups <0.2 mm). Therefore, any lymph node with a tumour deposit measuring ≥0.2 mm (but not <0.2 mm) is considered an involved node. Examination of multiple serial sections is advised in this assessment if necessary to derive the most accurate size of tumour deposit.
- Under TNM8, tumour deposits (satellites) are discrete macroscopic or microscopic nodules of cancer in the pericolorectal adipose tissue's lymph drainage area of a primary carcinoma that are discontinuous from the primary and without histological evidence of residual lymph node or identifiable venous, lymphatic or neural structures. Identification of these should prompt classification as venous invasion (V1/2), lymphatic invasion (L1) or perineural invasion (Pn1) rather than as tumour deposit. The presence of tumour deposits changes the nodal stage to pN1c, but only if all regional lymph nodes are negative on pathological examination. *Stage pN1c is only applied in the setting of node negative disease, under TNM 8*. If any nodes are involved by tumour, the number of tumour deposits is NOT added to the final involved node count to derive the final pN substage. Neither size nor contour are part of the definition of tumour deposits. The "3 mm rule" of TNM 5 no longer applies.
- Comment if an involved lymph node lies adjacent to (≤ 1 mm) the mesorectal circumferential radial margin or mesocolic margin as under TNM this equates to involvement of that margin. In the latter it raises the possibility of other involved lymph nodes left in place in any residual mesenteric pedicle. The biological significance of proximity to an intact mesorectal fascia where there is no residual soft tissue external to it in the bony pelvis is less certain.
- Direct invasion by tumour into a lymph node is regarded as a lymph node metastasis.

pN0	No regional lymph node metastatic disease
pN1	Metastatic disease in 1–3 regional lymph nodes
pN1a	Metastasis in 1 regional lymph node
pN1b	Metastases in 2–3 regional lymph nodes
pN1c	Tumour deposit(s), i.e. satellites,∗ in the subserosa, or in non-peritonealised pericolic or perirectal soft tissue *without* regional lymph node metastatic disease
pN2	Metastatic disease in 4 or more regional lymph nodes
pN2a	Metastases in 4–6 regional lymph nodes
pN2b	Metastases in 7 or more regional lymph nodes

Distant Metastatic Disease

Liver, lung and peritoneum are the commonest sites of distant metastatic disease. Other sites include *ovaries, vagina* and *bladder* where the *metastases can mimic primary adenocarcinoma of those organs*. Immunophenotypic profiles may aid distinction: e.g. ovarian cancer is CK7 positive, CK20/CDX-2 variable and weak for CEA, whereas colorectal cancer is usually strongly CEA/CK20/CDX-2 positive and CK7 negative. Occasional colorectal cancers express CK7 and a

minority are CK20 negative, particularly the MSI-high subset. Some metastatic deposits can also lose their expected immunogenic profile. Note that intestinal type (on morphology and immunophenotype) adenocarcinomas do arise as primary lesions in various organ sites, e.g. urinary bladder—exclusion of a colorectal metastasis then largely rests on a *relevant prior history*, *clinical investigation* and *radiological imaging*.

Given different prognostic impact and approaches to management, TNM8 subdivides stage pM1 disease into pM1a (confined to one organ without peritoneal disease), pM1b (metastatic disease in more than one organ without peritoneal disease) and pM1c (peritoneal metastatic disease, regardless of other organ involvement). pM1a/b disease may be amenable to metastasectomy with curative intent e.g. isolated tumour deposits in liver/lung. pM1c disease has worst prognosis and is largely incurable.

Excision Margins

Doughnuts/anastomotic rings/staple gun transections—involved/not involved.

Distances to the nearer longitudinal resection limit (mm), mesorectal CRM (mm), and mesocolic resection margin (mm).

Longitudinal spread beyond the gross edge of a colorectal carcinoma is unusual (<5% of cases) and anterior resection of rectal carcinoma is generally considered satisfactory if a macroscopic clearance of 3 cm beyond the lesion edge is feasible. Block the nearest longitudinal surgical margin if ≤3 cm from the tumour edge, or if >3 cm but with any of: an unusually infiltrative tumour margin, extensive lymphovascular or mesorectal invasion, or morphology such as signet ring cell, small cell or undifferentiated carcinoma.

CRM involvement = direct or discontinuous tumour spread, or tumour within a lymphatic channel, lymph node or vein ≤ 1 mm from the painted margin (under TNM guidance). Distance to this margin is best assessed from transverse serial slices of the resection specimen. At the multidisciplinary meeting they are correlated with the pretreatment MRI scan images as radiological demonstration of *rectal cancer threatening the surgical mesorectal fascial margin is an indicator for neoadjuvant long course chemoradiotherapy rather than short course radiotherapy.*

Note that a non-peritonealised CRM exists not only in the mesorectum but also in relation to the posterolateral aspect of the ascending colon. The prognostic significance of mesocolic margin involvement has not been fully clarified but is clearly an index of advanced local spread of disease and possibly of adequacy of surgery, again emphasising the need for complete resection of the mesocolic pedicle.

Other Pathology

Predisposing Conditions

Inflammatory: chronic ulcerative colitis or Crohn's disease (1% of colorectal carcinoma). Carcinoma in ulcerative colitis occurs in patients with *disease of pancolic distribution* (>50% colorectal involvement → 15% lifetime risk of cancer) and *long duration* (>10–30 years). It may be associated with preceding or concurrent *mucosal dysplasia* above, adjacent to or distant from the tumour. Carcinomas may be multiple, right-sided and, in up to one third of cases, difficult to define on endoscopic and gross examination. This is due to aberrant growth patterns with tumour arising in polypoid, villous or flat mucosal dysplasia in a background of mucosa already distorted by the effects of chronic inflammation, e.g. inflammatory polyps and strictures. Therefore *colonoscopic interval biopsy of flat mucosa and target biopsy of any mass lesions* is employed to detect dysplasia as a marker of potential carcinoma which may be occult and submucosal in location. Dysplasia-associated lesion or mass (DALM) is no longer applied as distinction between colitis-associated and sporadic neoplasms is notoriously difficult and of limited relevance compared to the local resectability status and presence or absence of background "flat" (endoscopically invisible) dysplasia.

Due to variation in observer reproducibility mucosal dysplasia should be assessed by two experienced pathologists according to the Vienna classification (Table 2.1). Endoscopically invisible high grade dysplasia should trigger surgical intervention, given high risk of underlying invasive cancer. Endoscopically invisible low grade dysplasia is more contentious and may be conservatively managed. Prognosis of colorectal cancer in ulcerative colitis is variable as some lesions present late masked by the symptoms of ulcerative colitis. Conversely, others are found early at regular (annual/biennial after 5–10 years disease duration) surveillance colonoscopy. Patients at high risk of developing colorectal cancer are those with: extensive ulcerative colitis or Crohn's disease with moderate or severe inflammation, a colonic stricture or mucosal dysplasia in the last 5 years, primary sclerosing cholangitis, or a family history of colorectal cancer in a first degree relative aged under 50 years. Early colonoscopy with chromoendoscopy is the recommended method of surveillance.

Neoplastic: aberrant crypt foci, adenoma(s), sessile serrated lesion(s), familial adenomatous polyposis, Lynch syndrome (see below), serrated polyposis syndrome, other rare polyposis syndromes, previous or synchronous carcinoma(s).

The dysplasia—carcinoma sequence indicates that development of adenocarcinoma increases with the *size of adenoma, its degree of villous architecture and grade of dysplasia, multiplicity of lesions and age of the patient.* High risk factors for developing colorectal cancer are five or more adenomas and lesion size >1 cm. A maximum diameter >2 cm confers approximate cancer risk of 50%. High risk factors in a rectal adenoma at flexible sigmoidoscopy are also good indications in individual patients for full colonoscopic survey and follow-up to detect right-sided colonic neoplasms.

In the UK, *dysplasia is applied to epithelial proliferation of any degree of complexity that is mucosa-based, i.e. above the muscularis mucosae. Adenocarcinoma is reserved for those lesions that show invasion beyond the muscularis mucosae into submucosa.* Terms such as carcinoma *in situ* are avoided due to the relative lack of mucosal lymphatics, the rarity of lymph node metastases with such lesions and the fear of over treatment with unnecessary radical resection. However, it is not always possible to demonstrate invasion through the muscularis mucosae in biopsy specimens and neoplastic epithelial changes with a desmoplastic stromal response in an appropriate clinical, endoscopic and radiological context are sufficient for a designation of adenocarcinoma. *Sampling error* must always be borne in mind in that a dysplastic fragment may not show the adjacent invasive component. Undoubtedly there are also malignant polyps for which terminology such as carcinoma *in situ* or intramucosal carcinoma is appropriate. In these circumstances there should be active discussion with the surgeon, emphasising that the process is "mucosa confined", and comments made on the *adequacy of local excision* and *absence of lymphatic invasion, venous invasion or any other adverse features (see below)*. The endoscopist should always make clear if a specimen is a complete polypectomy or a diagnostic biopsy from the surface of a larger lesion.

Malignant Polyps

Therapeutic polypectomy is generally considered achieved if the adenocarcinoma:

(a) Is well/moderately differentiated.
(b) Is clear of the stalk base.
(c) Lacks lymphatic or venous invasion.

Resection is considered if the adenocarcinoma:

(a) Is poorly differentiated.
(b) Involves the polypectomy margin.
(c) Shows lymphatic or venous invasion.

Other features which may be taken into consideration in assessing the need for surgical intervention to remove regional lymph nodes are depth of invasion (Haggitt level, Kikuchi level or distance beyond muscularis mucosae), width of

invasion (mm) and the presence or absence of tumour budding.

Resection is more likely indicated if the patient is young and medically fit to obviate the risk of lymph node metastases which can occur with pT1 lesions, potentially varying from 3% to 8% and 15% in the right colon, left colon and rectum, respectively. In an older patient with comorbidity the polypectomy site may be revisited, tattooed and reviewed at a later date. The polyp may recur and be amenable to mucosal resection, or, adenocarcinoma may ensue requiring further consideration of surgery depending on the fitness of the patient. A not uncommon finding, particularly in sigmoid lesions, is stalked adenomas that twist and prolapse resulting in *mucosal misplacement* or glandular herniation into submucosa *mimicking invasive carcinoma*. The presence of haemosiderin, lack of stromal desmoplasia, surrounding lamina propria and cytoarchitectural abnormalities similar to those of the overlying adenoma are helpful pointers to a benign lesion. In the rectum of a younger patient mucosal prolapse or solitary rectal ulcer syndrome must be considered if reactive glands are present within muscle and a misdiagnosis of adenocarcinoma avoided.

Local transanal resection is considered for large adenomas or early stage rectal cancers. It can be either mucosal and piecemeal (e.g. tubulovillous adenoma), or full depth mucosa or wall thickness (cancer). It is preferably employed for either a large adenoma not amenable to saline lift excision or snaring, or "early" pT1, Kikuchi sm1 (superficial third of the submucosa) invasive disease. *Indications for subsequent more radical surgery are*: incomplete tumour excision, deep submucosal (Kikuchi sm3) or muscularis propria invasion, lymphatic or venous invasion or the presence of a poorly differentiated invasive component (Fig. 6.4).

Most sporadic colorectal cancers arise on the basis of the p53/APC pathway (70%) but 30% (including MSI-H and microsatellite stable cancers) are associated with the *serrated pathway*, arising in sessile serrated lesions (older females, right-sided, *BRAF*-mutated) or traditional serrated adenomas (left-sided, *BRAF* or *RAS*-mutated).

Lynch syndrome (formerly known as hereditary non-polyposis colorectal cancer, HNPCC): autosomal dominant condition with 90% penetrance and a *70% risk of developing cancer by the age of 70 years. MLH1* and *MSH2* are the two most frequently mutated genes, *MSH6* and *PMS2* less commonly. Lynch syndrome accounts for *3–5% of colorectal cancer cases.* Amsterdam criteria requiring three affected family members across two generations with at least one <50 years of age at presentation are less relevant given small family sizes and often unavailable family histories. Lynch syndrome is found in about 15% of patients with mismatch repair deficient (dMMR) colorectal cancers. dMMR colorectal cancers are more commonly sporadic, caused by age-related somatic hypermethylation of MLH1, associated with *BRAF* mutation (absent in Lynch syndrome cancers so useful in screening pathway). In Lynch syndrome, numbers of adenomas are low but they can progress quickly to cancer, forming carcinomas tending to be right sided and multiple with an *18% incidence of synchronous or metachronous tumours.* dMMR colorectal cancers, sporadic and Lynch-related, are often mucinous or poorly/undifferentiated (medullary-like) in character and have expanding or circumscribed margins, prominent peritumoral and tumour-infiltrating lymphocytes (TILs), show usually paired loss of mismatch repair antibody nuclear expression (usually MLH1/PMS2, or, MSH2/MSH6), and a *high level of microsatellite instability (MSI-H).* They are less likely to show regional nodal or distant metastatic spread. Overall prognosis is better than in mismatch repair proficient colorectal cancers, at least in stages 1–3, but they are less responsive to conventional 5-fluorouracil/oxaliplatin-based chemotherapy. There is often a *family history of cancer in other viscera*, e.g. stomach, small intestine, pancreas, endometrium, breast, ovary, ureter and renal pelvis, and the risk of colonic cancer in a first degree relative of an affected individual is about 50%.

Familial adenomatous polyposis (FAP): sporadic in 30–50% of cases or *familial and autosomal dominant* with a high degree of penetrance (chromosome 5q21). The site of gene mutation

can determine the phenotype and type of surgery required. A minimum of 100 colorectal adenomas is required for a morphological diagnosis and these can vary from unicryptal dysplasia to macroscopic lesions. Usually thousands of adenomas are present and, if left untreated, one or more cancers occur on average *20 years earlier (90% by 50 years of age) than usual colorectal carcinomas* and more often in the left colon than the right. *Prophylactic colectomy with surveillance of the rectal stump or panproctocolectomy is undertaken*. FAP is also associated with adenomas and periampullary carcinoma in the duodenum (5% risk of cancer and a significant cause of mortality), gastric fundic gland cyst polyps and desmoid tumours (fibromatosis) as part of Gardner's syndrome (including epidermoid cysts and bone osteomas). Attenuated FAP has fewer lesions (30 adenomas) that are more often right sided with rectal sparing.

National Bowel Cancer Screening Programmes: in the UK, programme details vary by region, but most offer *60–74 year olds two yearly faecal occult blood (FOB) testing* or *faecal immunochemical testing (FIT),* the latter having better uptake and better specificity, being specific for human blood. If FOB or FIT positive, follow up by *colonoscopy* is offered with a view to detecting and removing more early stage cancers and precursor adenomas, increasing 5 year survival rates. 1–3% of these asymptomatic individuals have a positive FOB/FIT and in 50% of these colonoscopy or CT colonography shows an abnormality. This is either adenoma or carcinoma (80% of cases) or other pathology (20% of cases e.g. chronic inflammatory bowel disease) with a demonstrable shift to earlier stage cancers. Future plans for the programmes include age extension to 50–74 years and evaluation of additional screening by one-off sigmoidoscopy, typically at age 55 years.

Associated Conditions

- Presentation with *obstruction is an adverse prognostic factor* leading either to direct tumour perforation (pT4), proximal dilatation with ischaemic perforation of the caecum or to obstructive enterocolitis. The latter can mimic inflammatory bowel disease with continuous and skip lesions of transmural inflammation either adjacent to or distant from the carcinoma.

Immunophenotype

- CK20/CEA/MUC2/CDX2/SAT-B2 positive
- CK7 negative

Note some rectal, right sided colonic (usually MMR-deficient) and Lynch syndrome-associated cancers may lose CK20 and/or CDX2 positivity and 10% of cases can be CK7 positive, particularly rectal. These are complicating factors in typing metastatic adenocarcinoma of unknown origin or in biopsy diagnosis of colorectal cancer with aberrant morphology.

Neoadjuvant/Adjuvant Therapy

Postoperative adjuvant therapy, typically 5-fluorouracil/oxaliplatin/capecitabine-based, is used for chemotherapy naïve, stage 3 colorectal adenocarcinomas and "bad Dukes' B" adenocarcinomas, i.e. those node negative cancers but with any of: perforation, involved serosa or extramural venous invasion.

Prior to definitive surgery with curative intent, rectal cancer is assessed for evidence of local (by MRI scan), as well as distant (by CT scan), spread. This informs the need for neoadjuvant short course radiotherapy (for early stage cT3 disease) or long course chemoradiotherapy (for more locally advanced tumours with compromise of the mesorectal fascial surgical margin). Low risk tumours (upper rectal, cT1/cT2/N0) are generally treated by surgery alone (see introduction).

Neoadjuvant therapy can induce in a significant number of cases (30–50%) marked changes of tumour regression histologically: cell vacuolation, degeneration, apoptosis, necrosis, inflammation, and fibrosis leaving only microscopic

residual tumour foci. The *tumour regression score (TRS), assessed after surgical resection, has prognostic significance and can be graded* as: no viable cancer cells (TRS 0), single cells or rare small groups of cancer cells (TRS 1), residual cancer with evident tumour regression (TRS 2) or no evident tumour regression (TRS 3).

Prognosis of colorectal cancer and response to neoadjuvant therapies are patient dependent and can also partly relate to tumour genotype e.g. whether the cancer is DNA mismatch repair proficient or deficient, *KRAS* or *BRAF*-mutant. More thorough tumour genotyping, facilitated by next generation sequencing, allied to more sophisticated preoperative staging promises to allow selection of those patients who will benefit most from targeted neoadjuvant therapy, with individual tailoring of the drug and surgical management regimes accordingly.

Recurrent or Metastatic Disease

Significantly elevated serum CEA levels correlate with either *recurrent* or *metastatic disease*. After adequate local resection of the colorectal primary, oligometastatic disease in liver and/or lungs can be excised to good effect or subjected to chemoembolisation, *targeted radiofrequency and/or microwave ablation*. With widespread unresectable liver or peritoneal metastases *palliative chemotherapy* has a role to play—the primary may also be reduced in bulk, or a *stent* inserted across it to avoid obstruction and potential perforation with peritonitis. Due to difficulties in stent placement and subsequent migration this procedure is most suitable for lesions in the distal transverse or sigmoid colon.

Targeted therapy with cetuximab or panitumumab, antibodies against *epidermal growth factor receptor (EGFR)*, are recommended, in combination with conventional chemotherapy, for previously untreated metastatic colorectal cancer. Treatment is ineffective in tumours with *K-RAS* mutation downstream of the EGFR receptor and molecular testing of tumour tissue to establish *K-RAS* wild type status (approximately 50% of cases) is required prior to this therapy being offered.

BRAF mutation status prognostically stratifies both MSI-H and MSS colorectal cancer groups, with MSS, *BRAF*-mutant tumours having the worst prognosis.

Stage

TNM: see above.

Dukes' stage is no longer compatible with TNM staging, since the introduction of stage pN1c disease (tumour deposits) in TNM7, and is no longer reported routinely in pathology reports, but is still commonly used in clinical practice.

Dukes'	
A	Tumour limited to the wall, nodes negative
B	Tumour beyond the muscularis propria, nodes negative
C1	Nodes positive, apical node negative
C2	Apical node positive
D	Non-regional metastatic disease.

Resection

RO	Tumour completely excised locally
R1	Microscopic involvement of margin by tumour (to within 1 mm)
R2[a]	Macroscopic tumour left behind and/or gross involvement of margin.

[a]*R2should be reported only after consideration of the intraoperative findings*

Prognosis

Adverse Prognosis

- Tumour perforation (pT4) and obstruction.
- Some histopathological subtypes (e.g. signet ring cell)
- Poor differentiation.
- Male sex.
- Young and old age.

Prognosis relates most strongly to tumour TNM stage (particularly peritoneal and lymph node metastatic spread), differentiation and adequacy of local excision. Overall five year survival is 35–40%.

Stage	Dukes' 5 year survival	Incidence
A	95%	15%
B	75%	35%
C	35%	25%
D	25%	25%

A *positive resection margin* (usually the CRM) has strong prognostic significance for *local recurrence* and *death*. It is one of the most important causes of morbidity in rectal cancer. Low rectal cancers also have higher recurrence rates than mid- or upper rectal tumours. For every 100 patients with colorectal cancer it is estimated that 50 will be cured, 10 will die from pelvic recurrence, 5 from lymphatic spread and 35 from haematogenous spread. Sites of spread are *regional lymph nodes, liver (75%), lung (15%), bone and brain (5% each).*

Other Malignancy

Gastrointestinal Stromal Tumours (GISTs)

- GISTs are rare in the colorectum. See also Chaps. 3 and 5.
- Usually *aggressive* with adverse hisological features (Miettinen criteria) and liver/peritoneal metastases.
- Low risk GISTs in the colorectum are very rare.
- Distinguished from benign, usually small, leiomyomas e.g. in rectum.

Neuroendocrine Tumour/Carcinoma

- Well differentiated/low-grade neuroendocrine tumour, much less common than in terminal ileum, are strongly positive for chromogranin and synaptophysin with a low Ki67/MIB1 index (grade 1, ≤2%) and mitotic rate (< 2 per 10 high power fields/2mm^2).
  (a) chronic ulcerative colitis → enteroendocrine cell hyperplasia → "microcarcinoids".
  (b) grade 1 neuroendocrine tumour, <1 cm diameter.
  (a) and (b) are benign and managed by *endoscopic snare polypectomy*, usually with no further clinical consequences.
  (c) ulcerated tumour: malignancy relates to; size ≥2 cm diameter or invasion beyond submucosa, or angioinvasion, with these features necessitating *resection.*
- Lesions 1–2 cm diameter are potentially malignant and may be best treated by *wide local excision.*
- Right colonic neuroendocrine tumours tend to be large and ulcerated carcinomas (grade 3) with adverse prognosis but the commoner rectal "hind gut carcinoid" is usually solitary and < 1 cm in diameter. The latter are of L cell origin and show variable expression of neuroendocrine markers (chromogranin negative, synaptophysin positive).

Neuroendocrine differentiation can be present in up to 50% of usual type colorectal adenocarcinomas and is not prognostically significant. However a poorly differentiated high-grade neuroendocrine "small cell type" carcinoma component indicates aggressive behaviour. Pure high-grade neuroendocrine carcinoma of small cell type does occasionally occur as a large locally advanced primary tumour in the rectum. Metastasis from a lung cancer must be excluded by negative radiological imaging, and CDX2 immunoexpression of the primary intestinal carcinoma.

Malignant Lymphoma

- Predisposing conditions are chronic ulcerative colitis and AIDS (which can also result in Kaposi's sarcoma).
- Solitary or multifocal.
- Of probable mucosa associated lymphoid tissue (MALT) origin (>70%) with a heterogeneous, polymorphous cell population: *low-grade* < 20% blasts, *high-grade* > 20% blasts and on a spectrum with *diffuse large B cell lymphoma.*

- B (> 90%) or T cell ± high content of eosinophils.
- Prognosis relates to the grade and stage of disease.

Others: mantle cell lymphoma ("lymphomatous polyposis") which is of intermediate-grade and aggressive. Rarely follicular lymphoma spreading from systemic nodal disease. Burkitt's type/B-lymphoblastic in children or adults (with AIDS) in the terminal ileum/ileocaecal valve are high-grade lymphomas.

Leukaemia

- 50% of children with acute leukaemia who die in relapse.
- Chronic lymphocytic leukaemia in the elderly and usually found incidentally in a resection done for other reasons e.g. diverticular disease or colorectal cancer.
- Myeloid/granulocytic sarcoma (CD117/myeloperoidase positive).
- Single/multiple deposits.

Malignant Melanoma

- Primary or secondary; metastases are commoner.

Kaposi's Sarcoma

- AIDS/inflammatory bowel disease/human herpes virus 8 (HHV 8).
- 50% show visceral involvement, 8% in the hindgut.

Teratoma

- Rare; primary in caecum, sigmoid and rectum but exclude spread from ovary or sacrococcygeal area. Choriocarcinoma must be distinguished from adenocarcinoma with trophoblastic differentiation.

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Appendiceal Lesions

7

Ciaran O'Neill

Potentially malignant and malignant appendiceal tumours include well differentiated neuroendocrine (carcinoid) tumour (85% of cases), low-grade appendiceal mucinous neoplasm (LAMN), and adenocarcinoma. Goblet cell carcinoid, also designated crypt cell adenocarcinoma, has particular propensity to involve the appendiceal base or result in transcoelomic peritoneal spread.

Appendiceal tumours are usually encountered because of symptomatic acute appendicitis, an inflammatory appendix mass, or as part of a colectomy for other reasons. They are also seen in the context of CT scan investigation of lower abdominopelvic symptoms or mass lesions, e.g. ovarian cystic tumours. Related to this the patient may present with abdominal distension due to widespread pseudomyxoma peritonei associated with a ruptured appendiceal LAMN, or, ascitic fluid or peritoneal seedlings secondary to carcinomatosis peritonei.

Gross Description

Specimen

- Appendicectomy/right hemicolectomy.
- Length and diameter (mm).
- Mucocoele/perforation/diverticulum/appendicitis/appendicular mass.

Tumour

Site

- Tip/base/diverticulum/body.

Size

- Length × width × depth (mm) or maximum dimension (mm).

Appearance

- Polypoid/sessile/plaque/ulcerated/serosal mucin.

Histological Type

Neuroendocrine tumours (NET): Well-differentiated NET/Poorly differentiated neuroendocrine carcinoma (NEC) (small cell carcinoma)/Poorly differentiated large cell NEC/Goblet cell carcinoid (see below)/Combined classical and goblet cell carcinoid.

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_7

Well differentiated neuroendocrine tumour

- 0.5–1.5% of appendicectomy specimens.
- *85% of appendiceal tumours*.
- Usually a coincidental finding of yellow pale tumour at the tip although it may contribute to appendicitis when at the appendix base (10%).
- A well differentiated/low-grade neuroendocrine tumour and variably chromogranin, synaptophysin, CD56(±) positive depending on EC (usual enterochromaffin) or L cell origin. Low Ki-67 index (≤ 2%) and mitotic count (< 2 per 10 high power fields).

Usual type: 70% of cases and at the appendiceal tip. Solid nests/cords/ribbons/acini of uniform cells often with invasion of the muscularis ± serosa and lymphatics. *Benign behaviour* with *appendicectomy the treatment of choice*.

Rarely cases spread to abdominopelvic peritoneum, regional nodes and liver with 75-85% and 35% 5 year survival figures, respectively. These are usually >20 mm dimension and may have increased Ki-67 labeling. Radical surgery may be considered in these circumstances. *Extensive invasion of the mesoappendix (> 3 mm)* and the *appendiceal base* are also adverse indicators.

Goblet cell carcinoid (GCC)/crypt cell carcinoma: Goblet cell carcinoid has been used to refer to a range of appendiceal tumours with mixed endocrine and adenocarcinomatous differentiation. The term crypt cell carcinoma has been used in isolation or in conjunction with GCC to convey a more aggressive tumour with potentially poor outcome. GCC comprise clusters, strands or glandular collections of mucus secreting epithelial cells with a goblet cell or signet ring morphology. There is a range of appearances with the degree of cytological atypia, stromal desmoplastic reaction, and extent of infiltration impacting on prognosis (see Section "Differentiation" for Tang classification). Usually only a minor population of endocrine cells is present demonstrated by immunohistochemistry. GCC has potential for extra-appendiceal spread (20% of cases) and occasionally involvement of regional lymph nodes and liver. It shows a propensity for *transperitoneal spread* to involve the ovaries and direct spread through the appendix base and into the caecum. Localised, regional and distant disease have 5 year survival rates of 86%, 74% and 18% respectively. *Right hemicolectomy* should be considered if there is extensive spread with an infiltrative growth pattern, involvement of the appendix base or poorly differentiated features.

Distinguish from: (1) secondary colorectal carcinoma involving the appendix either directly (e.g. from caecal pouch) or via the peritoneum (signet ring cell carcinoma of rectosigmoid area), and, (2) primary colonic type mucinous adenocarcinoma of the appendix which is aggressive in behaviour and requires radical surgery.

Adenoma

- Analogous to lesions in the colorectum: tubular/tubulovillous or villous.
- Rare (<1% of appendicectomies).
- Localised (polypoid) or diffuse.
- May require follow up colonscopy.

Low Grade Appendiceal Mucinous Neoplasm (LAMN)

LAMNs may present clinically with appendicitis, often accompanied by mucocoele detected radiologically or at laparoscopy. The neoplastic epithelium is reminiscent of that in a colorectal adenoma but is often monolayered and either flattened or undulating. In addition to the intraepithelial neoplasia there may be other features such as obliteration of muscularis mucosa, band-like submucosal fibrosis, dissection of mucin (with or without accompanying epithelium) into or through muscularis propria. Intraperitoneal spread of mucin may occur due to mucinous dissection or secondary to appendicitis complicated by rupture.

High Grade Appendiceal Neoplasm (HAMN)

Mucinous neoplasm similar to LAMN but with high grade cytologic features, and confined to the appendix without invasion. These are associated with a greater likelihood (~six-fold) of epithelial cells within extra appendiceal mucin than LAMNs and may run a more aggressive course including development of recurrent disease in the peritoneum and decreased survival.

Adenocarcinoma

- 0.1% of appendicectomy specimens.
- Identified as primary by a mucosal adenoma/LAMN lesion.
- Histologically of usual colorectal type, but often cystic and well differentiated mucinous in character.
- Rarely signet ring cell carcinoma: distinguish from goblet cell carcinoid (focally chromogranin/synaptophysin positive) and metastatic gastric/breast carcinoma (infiltrating lobular type). In this respect it is necessary to know of previous operations to the stomach and breast and these sites may have to be investigated. Breast carcinoma may also be ER/PR/GATA3 positive and CK7 positive/CK20 negative whereas gastrointestinal cancers are usually CEA positive and CK20 positive/CK7 negative.
- Treatment is right hemicolectomy with regional lymphadenectomy. Prognosis reflects the histological grade of tumour and TNM stage. There is an overall 5 year survival rate of 60–65% for treatment with hemicolectomy, but this falls to 20% for appendicectomy alone.

Metastatic Carcinoma

- *Peritoneal spread:* colorectum, ovary, stomach.
- *Distant spread:* lung, breast.

Differentiation

Mucinous appendiceal adenocarcinomas can be well, moderately or poorly differentiated. Presence of a signet ring cell component infers poor differentiation.

Neuroendocrine tumours are classified based on their mitotic count in 10 high power fields (HPF) (10HPF equates to 2 mm^2) and Ki-67 index based on a 2000 cell sample. 10 high power fields (HPF) equates to 2 mm^2. Counting is directed to areas of highest mitotic activity and highest nuclear labelling.

Grade	Mitotic count (10HPF)	Ki-67 index (%)
G1	<2	≤2%
G2	2–20	>2–20%
G3	>20	>20%

Grade 1 and 2 tumours are usually well differentiated and retain expression of chromogranin A and synaptophysin. Necrosis suggests higher grade lesions and this is often more florid in grade 3 tumours. These tumours are poorly differentiated and may show reduced neuroendocrine immunohistochemical expression.

GCC can be classified according to morphologic criteria as defined by Tang *et al*:

A. Cohesive groups of goblet cells with minimal cytologic atypia, no desmoplasia and minimal appendiceal wall distortion.
B. Dyscohesive groups of goblet cells with a signet ring cell component. Can be arranged in irregular clusters or as single cells. Significant cytologic atypia and desmoplasia with associated destruction of the appendiceal wall.
C. At least focal evidence of goblet cell morphology. A component (greater than one low power field or 1 mm^2) not otherwise distinguishable from a poorly differentiated adenocarcinoma, which may appear as either gland-forming, confluent sheets of signet-ring cells, or undifferentiated carcinoma.

The percentages of GCC metastatic at presentation are 33%, 88% and nearly 100% for groups A, B and C respectively. Disease specific 5 year survival is 100%, 38% and 0% for groups A, B and C respectively. Right hemicolectomy is often advocated for B and C tumours.

Extent of Local Tumour Spread

Limited to the appendix, into mesoappendix, appendiceal base and caecum.

Adenocarcinoma

pTx	Primary tumour cannot be assessed
pT0	No evidence of primary tumour
pTis	Carcinoma in situ: intraepithelial (within basement membrane) or invasion of the lamina propria (intramucosal) with no extension through muscularis mucosae into submucosa
pT1	Tumour invades submucosa
pT2	Tumour invades muscularis propria
pT3	Tumour invades into subserosa or mesoappendix
pT4	Tumour perforates visceral peritoneum, including mucinous peritoneal tumour or acellular mucin on the serosa of the appendix or mesoappendix and/or directly invades other organs or structures. (a) Tumour perforates visceral peritoneum including mucinous peritoneal tumour or acellular mucin on the serosa of the appendix or mesoappendix. (b) Tumour directly invades other organs or structures

LAMN**:** pTis (LAMN) refers to a LAMN which is confined to the appendix (defined as involvement by acellular mucin or mucinous epithelium that may extend into the muscularis propria). Therefore neither pT1 nor pT2 staging (as above) applies to LAMN. In stage pT3, tumour extends through muscularis propria into subserosa or mesoappendix (however it is not officially stipulated whether "tumour" also applies to acellular mucin. *It would seem likely given the definitions for pTis and pT4 that pT3 would include acellular mucin*). In stage pT4a, tumour penetrates the visceral peritoneum, including acellular mucin or mucinous epithelium involving the serosa of the appendix. In pT4b tumour directly involves adjacent organs or structures, including acellular mucin or mucinous epithelium (does not include luminal or mural spread into adjacent cecum). Correct staging requires submission of the entire appendectomy specimen and also meticulous attention during dissection to minimise "carry over" of mucin which may lead to erroneous upstaging.

The presence of acellular mucin in the abdominal cavity is pM1a. Intraperitoneal deposits with mucinous epithelium are pM1b and non-peritoneal metastases are pM1c.

Neuroendocrine tumours: Classification only applies to well differentiated neuroendocrine tumours (G1/G2). High grade neuroendocrine carcinomas, mixed adenoneuroendocrine carcinomas and goblet cell carcinoid are staged as for adenocarcinomas.

pTx	Primary tumour cannot be assessed
pT0	No evidence of primary tumour
pT1	Tumour ≤2 cm in greatest dimension
pT2	Tumour >2 cm but ≤4 cm
pT3	Tumour >4 cm or with subserosal invasion or involvement of the mesoappendix
pT4	Tumour perforates the peritoneum or directly invades other adjacent organs or structures (excluding direct mural extension to adjacent subserosa, e.g. abdominal wall and skeletal muscle).

In addition to the TNM stage it is currently recommended that the European Neuroendocrine Tumour Society (ENETS) staging system which also applies to high grade neuroendocrine tumours is reported. The European Neuroendocrine Tumour Society designates: pT1 ≤ 10 mm, pT2 ≤ 20 mm and/or mesoappendix/subserosa invasion ≤3 mm, pT3 > 20 mm and/or invasion into mesoappendix/subserosa >3 mm. Greater likelihood of lymphovascular invasion is implied with mesoappendiceal infiltration >3 mm.

Lymphovascular Invasion

A cocktail of D2-40 (lymphatic marker) and CDX2 (marker of intestinal epithelial cells) may prove useful.

Lymph Nodes

Site/number/size/number involved/limit node/extracapsular spread.

Regional nodes: ileocolic. A regional lymphadenectomy will ordinarily include a minimum of 12 lymph nodes.

N1a	Single regional node metastasis
N1b	Metastases in 2–3 regional nodes
N1c	Tumour deposit(s), i.e. satellites, in the subserosa, or in non-peritonealised pericolic or perirectal soft tissue *without* regional node metastasis
N2	Metastasis in 4 or more regional lymph nodes

Excision Margins

Distances (mm) to the caecal base/proximal limit of excision and edge of the mesoappendix.

Other Pathology

Carcinoid syndrome: rarely occurs due to the scarcity of appendiceal carcinoid tumours metastatic to the liver.

Serrated Polyps

Appendiceal serrated lesions have similar appearances to sessile serrated adenomas/lesions of the colorectum. However they feature KRAS mutations rather than BRAF mutations whereas the converse is the case with sessile serrated adenomas/lesions of the colorectum. The behaviour of appendiceal serrated polyps has not been fully elucidated and it has not been established if their course parallels that of colorectal serrated lesions. They should be designated as serrated polyps with a comment on dysplasia and attention drawn in the report to their potentially distinct behaviour.

Mucocoele: this is a clinical or gross descriptive term only and is not a component of the histologic diagnostic nomenclature. Underlying causes of mucocoele are diverse and range from benign cystic dilatations to adenocarcinoma and anything in between capable of mucin production.

Pseudomyxoma Peritonei

Diffuse pseudomyxoma peritonei (PMP) is due to intraperitoneal accumulation of mucus from mucinous neoplasia and is characterized by the redistribution phenomenenon: mucus and cells follow the normal peritoneal fluid flow and are redistributed to sites of fluid absorption. Anatomical distribution therefore tends to spare some of the peritoneal surfaces with preferential location in the greater omentum, pelvis, paracolic gutters and subdiaphragmatic space.

Pseudomyxoma peritonei may be classified thus:

1. Acellular mucin.
2. Pseudomyxoma peritonei with low grade features: low grade mucinous carcinoma peritonei (equivalent to the older terminology disseminated peritoneal adenomucinosis).
3. Pseudomyxoma peritonei with high grade features: high grade mucinous carcinoma peritonei (equivalent to the older terminology peritoneal mucinous carcinomatosis).
4. Pseudomyxoma peritonei with signet ring cells.

Previously, it was thought that ovarian mucinous tumours were the source of pseudomyxoma peritonei and immunohistochemistry was often

applied in an attempt to distinguish appendiceal from ovarian origin. However it is now thought that an ovarian origin is unusual, an exception being mucinous neoplasia developing in ovarian teratoma. Occasionally pseudomyxoma peritonei may be due to mucinous carcinomas from other sites, e.g. colorectal, urachus, stomach or gall bladder. However, in the vast majority of cases it is appendiceal in origin, predominantly secondary to LAMNs.

Diffuse pseudomyxoma peritonei may be helped by cytoreductive debulking procedures such as peritoneal stripping and removal of involved organs (Sugarbaker technique), with *hyperthermic intraperitoneal chemotherapy (HIPEC)*. In the UK and Ireland mitomycin C is used which has lower toxicity than oxaliplatin based therapy, more often used in continental Europe. However, it is largely refractory to treatment, *slowly but relentlessly progressive* and causes death by bowel obstruction (45% 10 year survival). The outlook also varies according to whether the epithelial content is of low-grade or high-grade morphology, with 5 year survival rates of 63% and 23%, respectively. Where compete cytoreduction is achieved the corresponding figures can improve to 84% and 48%.

Other Malignancy

Malignant Lymphoma

- Primary (rare) or secondary to systemic/lymph node disease.
- Burkitt's lymphoma: ileocaecal angle in childhood and *aggressive high-grade* disease.

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8 Anal Canal Neoplasia (with Comments on Pelvic Exenteration)

Ciaran O'Neill

The incidence of anal carcinoma is increasing in the Western hemisphere (2.5–5-fold in recent decades) due to cigarette smoking, Human Papilloma Virus (HPV) infection (which is present in ~85% of anal squamous cell carcinoma) and immunosuppression, e.g. HIV. Chronic localized inflammation also increases risk of carcinoma, e.g. Crohn's related fistulae. Females are more frequently affected by carcinoma than males. Squamous cell carcinoma outnumbers adenocarcinoma by a ratio of 10–20:1. The 5 year survival figures for localised and extensive disease are 65–80% and 15% respectively.

Anal tumours present as a mass or feeling of fullness. Symptoms can be non-specific and develop late in the disease course. Patients may be reluctant to consult their doctors for investigation. Anal Intraepithelial Neoplasia (AIN) is often an incidental microscopic finding in minor surgical excision specimens or adjacent to carcinoma in local excisions.

For therapeutic reasons clear clinicopathological distinctions must be made and ideally invasive anal neoplasia cases should be discussed at a specialist regional anal neoplasia multidisciplinary meeting:

- Rectal type adenocarcinoma arising from the distal rectum or the colorectal zone of the upper anal canal can spread downwards and present as an anal tumour. Treatment is surgical (APER) preceded by neoadjuvant therapy.
- Anal canal squamous cell carcinoma can spread upwards or downwards presenting as low rectal or perianal/anal margin tumour, respectively. Treatment is primarily radio-/chemotherapy with surgery for non-responsive or recurrent disease. More recently local excision for early stage superficial lesions may be recommended.
- Perianal/anal margin squamous cell carcinoma can be confined to the skin or spread to involve the distal anus. Treatment is local surgical excision for the former. Extended local excision or more radical surgery, or radiotherapy alone or in combination are indicated for the latter.
- Anal canal adenocarcinoma, malignant melanoma or sarcoma are surgically resected.
- Investigation of anal tumours is by anoproctoscopy and biopsy with endoanal ultrasound, MRI and CT scans to stage biopsy proven disease.

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_8

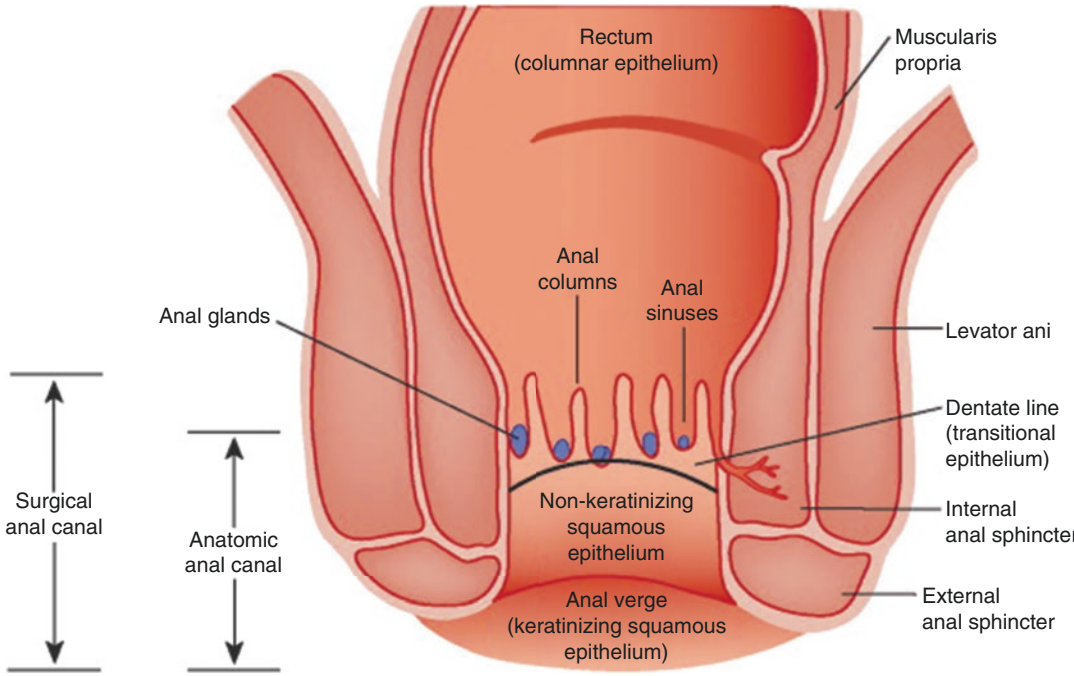

Fig. 8.1 The anatomy of the anal canal (Odze and Goldblum (2009). Reproduced with permission from Elsevier)

Gross Description

Specimen

- Biopsy/resection (wide local excision or abdominoperineal (APER)).
- Weight (g) and size/length (mm), number of fragments.

Tumour

Site

- Mucous membrane/muscularis/extra-mural.
- Low rectal/anal canal/perianal margin or skin. Perianal skin is within 5 cm of the anal margin.
- Anatomy:
  - Upper zone: colorectal mucosa
  - Anal transitional zone (ATZ): the epithelium here is 4–9 cells thick and the cells vary from cuboidal basally to columnar more superficially. In contrast to colorectal mucosa, ATZ epithelium (and underlying anal glands) has a CK7+/CK20- phenotype
  - Lower zone: stratified squamous epithelium continuous with appendage bearing perianal skin (Fig. 8.1)

Size

- Length × width × depth (mm) or maximum dimension (mm).

Appearance

- Polypoid/sessile/ulcerated/stricture/pigmented/fleshy/mucoid.

Edge

- Circumscribed/irregular.

Histological Type

Anal Margin/Perianal Skin

As for non-melanocytic skin carcinoma (Chap. 20), in particular well differentiated keratinising squamous cell carcinoma and variants including verrucous carcinoma. Also basal cell carcinoma, Bowen's disease, Paget's disease.

Anal Canal

Anal canal squamous cell carcinoma: previous anal squamous cell carcinoma classification has been complex with various classifications with many subtypes resulting in poor interobserver reproducibility with a lack of prognostic and therapeutic differences amongst subtypes. Currently, the majority are squamous cell carcinoma (this includes basaloid tumours, squamous tumours with focal ductal differentiation, large cell keratinising and non-keratinising—such histologic subtyping is difficult and of little clinical importance). Further subtypes worthy of mention include small cell anaplastic and verrucous carcinoma.

- Small cell anaplastic (distinct from small cell neuroendocrine carcinoma) has a poorer prognosis than generic squamous cell carcinoma. It is composed of a fairly homogenous population of small tumour cells with nuclear moulding and a high mitotic rate, and shows diffuse infiltration.
- Verrucous carcinoma (giant condyloma or Buschke-Lowenstein tumour) has combined endophytic and exophytic growth with minimal atypia and infrequent mitoses which are basally confined. Typically invade locally and usually do not metastasise. Have a more favourable prognosis and are treated primarily by surgical excision.

Immunohistochemical analysis with p16 (a surrogate marker for HPV infection) is not currently recommended for routine reporting of invasive anal squamous cell carcinoma. This is variously due to intratumoural heterogeneity and lack of consensus regarding positivity thresholds. Furthermore, at present modulation of radiation dosage is not affected by HPV expression. However, p16 expression remains a valuable marker in the assessment of high grade anal intraepithelial neoplasia.

Neoplasms traditionally are designated as either anal canal or anal margin depending on their localization. Anal margin neoplasms effectively behave as their cutaneous equivalents. The interface between anal canal and anal margin is not well defined anatomically with the WHO suggesting that anal canal tumours are those which *cannot* be seen in their entirety when gentle traction is placed on the buttocks. Similarly perianal tumours are proposed to be those that are entirely visible with gentle traction and within 5 cm of the anal margin.

Others

Anal gland adenocarcinoma: rare. Often show mucinous differentiation and comprises small tubular acini. In contrast to anorectal adenocarcinoma it is CK7 positive/CK20 negative/CDX2 negative. Late diagnosis and poor prognosis.

Extra-mammary Paget's disease: in *20% an underlying adnexal or rectal adenocarcinoma* is found. The *majority of cases are primary* and remain *confined to the epidermis.*

Well differentiated neuroendocrine tumour: <2 cm is treated by local excision, if ≥2 cm consider more radical surgery.

Poorly differentiated/high-grade neuroendocrine carcinoma: small cell or large cell—both rare.

Malignant melanoma: primary mucosal origin with adjacent junctional atypia that can be destroyed by surface ulceration. Spindle cell or epithelioid cell types. *1.5% of anal malignancy*—aggressive with early spread and death in months from liver and lung metastases. *Five year survival is <20%, median survival 19 months*. No significant survival advantages for radical surgery over local palliative excision.

Metastatic carcinoma: direct spread—adenocarcinoma of rectal type arising from the colorectal mucosa of the upper anal zone cannot be distinguished from usual low rectal carcinoma and is grouped with it. Other possibilities are prostatic adenocarcinoma and cervical carcinoma.

Differentiation

Well/moderate/poorly differentiated (this 3 tier system is recommended by both the WHO and American Joint Committee on Cancer, AJCC).

Poor prognosis related to apparent poor differentiation has not been definitively substantiated by meta-analyses. However, there is agreement that there is no evidence for differing outcomes for well versus moderately differentiated. Regarding therapy, grading is most relevant for early (pT1) non-poorly differentiated squamous cell carcinomas as these may prompt a primary surgical approach.

Neoplasms without usual morphologic evidence of squamous differentiation and which do not stain with squamous immunohistochemical markers (e.g. p63 or p40) should be designated as undifferentiated carcinoma.

Extent of Local Tumour Spread

Border: pushing/infiltrative.

Lymphocytic reaction: prominent/sparse.

Depth of spread: submucosa, muscularis propria of rectum or anal sphincters, extrarectal and extraanal tissue including ischiorectal fossae and pelvic structures. Clinical assessment is by MRI scan and endoanal ultrasound for local spread, and CT scan for distant disease.

At diagnosis a majority have spread through sphincteric muscle into adjacent soft tissues.

The TMN8 classification applies only to carcinomas (Figs. 8.2 and 8.3).

pTis	Carcinoma in situ, Bowen's disease, high-grade squamous intraepithelial lesion (HSIL) and intraepithelial neoplasia 2–3 (AIN2 and 3)
pT1	Tumour ≤2 cm in greatest dimension
pT2	Tumour >2 cm but ≤5 cm in greatest dimension
pT3	Tumour >5 cm in greatest dimension
pT4	Tumour of any size invading adjacent organ(s), e.g. vagina, urethra, bladder (Direct invasion of the rectal wall, perianal skin, subcutaneous tissue or the sphincter muscle(s) alone is not classified as T4)

Lymphovascular Invasion

Present/absent.

Intra-/extratumoural.

Perineural spread.

Distant metastases are present in 5–10% of cases at the time of diagnosis. Haematogenous spread is to liver, lung and skin.

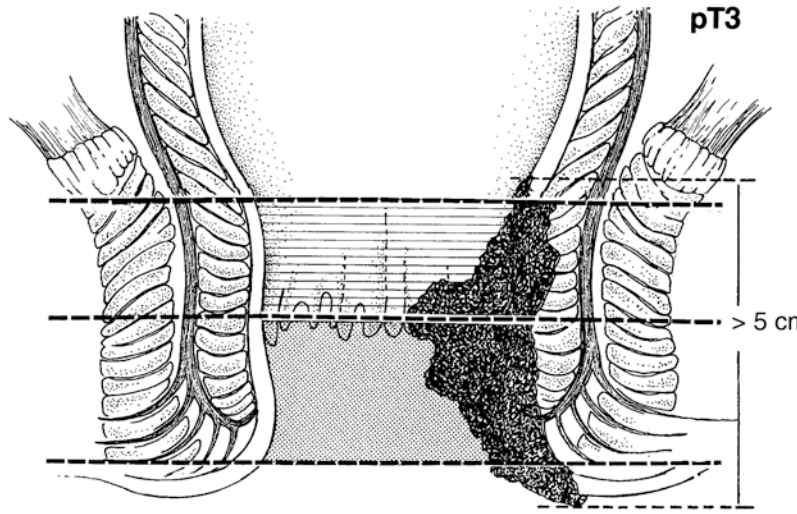

Fig. 8.2 Anal canal carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

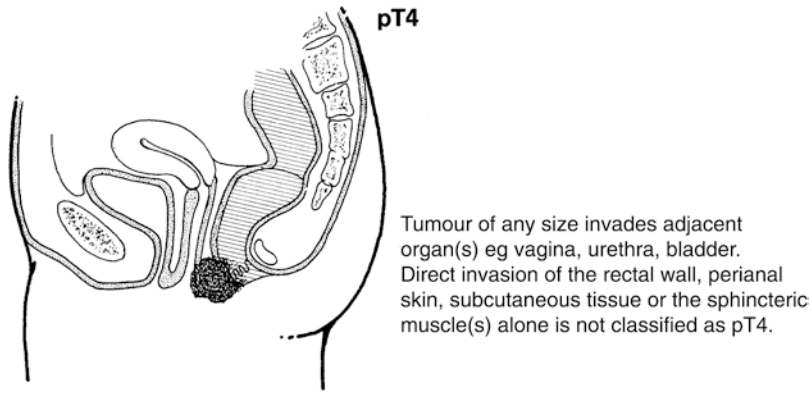

Fig. 8.3 Anal canal carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

Lymph Nodes

Site/number/size/number involved/limit node/extracapsular spread.

Regional nodes: perirectal, internal iliac, inguinal. Anal margin tumours go initially to inguinal nodes → iliac nodes. Anal canal tumours go initially to haemorrhoidal nodes → perirectal and inguinal nodes. A regional perirectal/pelvic lymphadenectomy will ordinarily include a minimum of 12 lymph nodes, an inguinal lymphadenectomy 6 or more lymph nodes.

NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in regional lymph node(s)
N1a	Metastases in inguinal, mesorectal and/or internal iliac nodes
N1b	Metastases in external iliac nodes
N1c	Metastases in external iliac and in inguinal, mesorectal and/or internal iliac nodes

Lymph node involvement is present in 10–50% of cases at presentation.

Excision Margins

Distances (mm) to the nearest longitudinal (rectal or perianal) resection limit and deep circumferential radial margin.

Other Pathology

Carcinoma of the anal canal: (F:M 3:2) is commoner (3:1) than carcinoma of the anal margin (M:F 4:1).

HPV infection: a common aetiological agent associated with a spectrum of anal viral lesions, preneoplasia and carcinoma. *Preneoplasia* is variably site designated as anal intraepithelial neoplasia (AIN) *or* perianal intraepithelial neoplasia (PAIN) depending on location. Preneoplasia may also be classified as a squamous intraepithelial lesion (SIL) with high grade (HSIL) or low grade (LSIL) morphology. HPV subtypes 16 and 18 are particularly neoplasia progressive. Infection with HIV and other sexually transmitted viruses also contributes.

Traditionally used clinical terms: that are no longer preferred include condyloma accuminatum, giant condyloma of Buschke-Löwenstein, and Bowen's disease of anal skin.

Multifocal anogenital neoplasia: there is potential for concurrent cervical intraepithelial neoplasia (CIN) and AIN associated with anal canal carcinoma. A premalignant phase or model of progression in AIN is not as well established as in CIN although cancer risk appears to be greatest for high-grade AIN 3. As with vulval intraepithelial neoplasia (VIN) *AIN is either classical or differentiated/simplex in character.* The

former is commoner, occurs in younger patients with HPV infection, shows Bowenoid morphology and is p16 positive/p53 negative. The latter is in older patients and p53 positive/p16 negative. Diagnosis of AIN can be facilitated by use of p16, p53 and Ki-67 positive immunohistochemistry. It can be treated by local excision if small (<50% of the anal circumference), ablation (laser, photodynamic) or immunomodulation therapy (Imiquimod).

Differential diagnoses: a significant proportion of anal canal carcinomas arise in the vicinity of the dentate line from the transitional/cloacal zone and spread preferentially upwards in the submucosal plane thereby presenting as ulcerating tumour of the lower rectum. Due to the differential options of primary neoadjuvant therapy versus primary resection, anal canal carcinoma must be distinguished by biopsy from both rectal adenocarcinoma superiorly and basal cell carcinoma or squamous cell carcinoma of the perianal margin/skin inferiorly.

Anal Paget's disease: must be distinguished from AIN/SIL and Pagetoid spread of malignant melanoma. Mucin stains and immunohistochemistry are necessary (mucicarmine, PAS ± diastase, cytokeratins, melanoma markers: pigment, S100, HMB-45, melan-A, SOX10). It may be associated with concurrent or subsequent low rectal adenocarcinoma with the Paget's cells showing intestinal type gland formation and CK20 positivity. More often it is a primary epithelial lesion lacking intestinal glandular differentiation and CK20 positivity but is CK7/GCDFP-15 positive. Other associations are with bladder and cervical carcinoma. It may progress to submucosal invasion with a tendency for local recurrence. However, a *majority remain as intraepithelial malignancy*. A further differential diagnosis is Pagetoid spread from a primary anorectal signet ring cell carcinoma.

Radiotherapy necrosis.

Leukoplakia: a clinical term and denotes a white plaque with or without AIN. Occasionally seen and requires biopsy to establish the presence or otherwise of dysplasia.

Immunohistochemistry: also important in the differential diagnosis of anal basaloid squamous cell carcinoma (CK5/6, p63, p16, EMA, CEA positive), malignant melanoma, malignant lymphoma (CD 45 positive), spindle cell carcinoma (cytokeratin positive), and leiomyosarcoma (desmin, h-caldesmon, smooth muscle actin positive).

Prognosis

Carcinoma of the anal margin/perianal skin is treated primarily by *surgery* ± *radiotherapy.* Well differentiated tumours <2 cm diameter or occupying <50% of the anal circumference can initially be locally excised and followed up surgically. An oncoplastic surgical procedure with flap rotation may be required to give skin coverage. *Anal canal squamous carcinoma* responds well to *primary radio−/chemotherapy* and abdominoperineal resection is reserved for locally extensive, recurrent or non-responsive tumours, or other lesions such as malignant melanoma and leiomyosarcoma. *Perianal carcinoma: 5 year survival 85%; anal canal carcinoma: 5 year survival 65–80%.* Adverse prognostic indicators are advanced stage or depth of spread, tumour in inguinal lymph nodes (10–50%) and post-treatment recurrence in the pelvic and perianal regions, e.g. pT1 carcinoma has a 5 year survival of 91%, pT3 16%. Histological grade is not a strong indicator but may be helpful in poorly differentiated squamous cell carcinoma of large cell type. Ductal differentiation in basaloid squamous cell carcinoma is an adverse factor. Recurrence in men is pelvic and perineal, in women pelvic and vaginal.

Other Malignancy

Malignant Lymphoma/Leukaemia

- Secondary to systemic/nodal disease.
- HIV: also Kaposi's sarcoma.

Leiomyosarcoma

- Desmin, h-caldesmon positive.

Presacral Tumours

- Teratoma, peripheral neuroectodermal tumours (including Ewing's sarcoma), multiple myeloma, metastatic carcinoma.

Rhabdomyosarcoma

- Childhood, embryonal (desmin/myo D1/myogenin positive).

Comments on Pelvic Exenteration

In general pelvic exenteration is considered for locally advanced or recurrent pelvic malignancy in the absence of extra-pelvic metastases.

- *Degree of disease spread:* assessed by
  - CT scan: pelvic and retroperitoneal lymphadenopathy, extra-pelvic metastases.
  - MRI scan: local cancer spread into adjacent tissues.
  - CT/PET scan: detects metabolic activity in malignant tumours and is useful in localising recurrent or distant metastatic disease, and distinguishing neoplasia from radiotherapy induced fibrosis.
- *Relevant malignancies:* cervical carcinoma, rectal carcinoma, anal carcinoma, soft tissue lesions (e.g. malignant fibrous histiocytoma, aggressive angiomyxoma), aggressive muscle invasive bladder cancer, and occasionally advanced high-grade endometrial, vaginal or vulval cancers. Exenteration is not recommended in ovarian malignancy as high stage disease shows peritoneal involvement outside the pelvis.
- *Contraindications*: significant comorbidity, distant metastases (except resectable liver metastases from a rectal carcinoma) and involvement of major pelvic vessels, nerves, pelvic side walls or sacrum, although the latter can be resected en-bloc in rectal cancer.
- *Preoperative adjuvant therapy:* may result in significant tumour regression so that it can be difficult to find residual disease and lymph nodes which shrink and hyalinise. Deep spread and margins fibrose making accurate assessment of pT stage and resection status potentially problematic.
- *Surgery:* may be with curative intent or palliative to obviate complex and debilitating pelvic symptoms due either to spread of malignancy or as a consequence of adjuvant therapy, e.g. pain, fistulae. The latter can produce unusual symptoms, e.g. pneumaturia or faecaluria.
- *Pelvic exenterations* (Fig. 8.4)
  - *Anterior:* bladder, lower ureters, reproductive organs, draining lymph nodes and pelvic peritoneum.
  - *Posterior:* rectum, distal colon, internal reproductive organs, draining lymph nodes and pelvic peritoneum.
  - *Total:* anterior and posterior.
- *Principles of specimen reporting*
  - Identify the specimen type and component organs.
  - Block longitudinal transection limits, i.e. ureters, urethra, vagina and proximal/distal bowel.
  - Paint circumferential radial fascial or soft tissue margins, comment on the uterine/bladder dome/colonic/upper anterior rectal peritoneum, and integrity of the mesorectum and its fascia.
  - *Sagittal hemisection* can be very useful in demonstrating the relationships between the tumour and the constituent organs. Fistulae can be cut along the line of an exploring probe. Also document the status of circumferential radial margins.
  - Report as per individual cancers noting in particular the degree of locoregional spread, margin status and effects of preoperative adjuvant therapy.
  - The diagnosis and organ of origin will have been previously established by clinical and radiological investigation and diagnostic biopsy. In an exenteration specimen good macroscopic description, orientation, fixation and blocking are paramount to establish that a *complete (R0) resection* has been

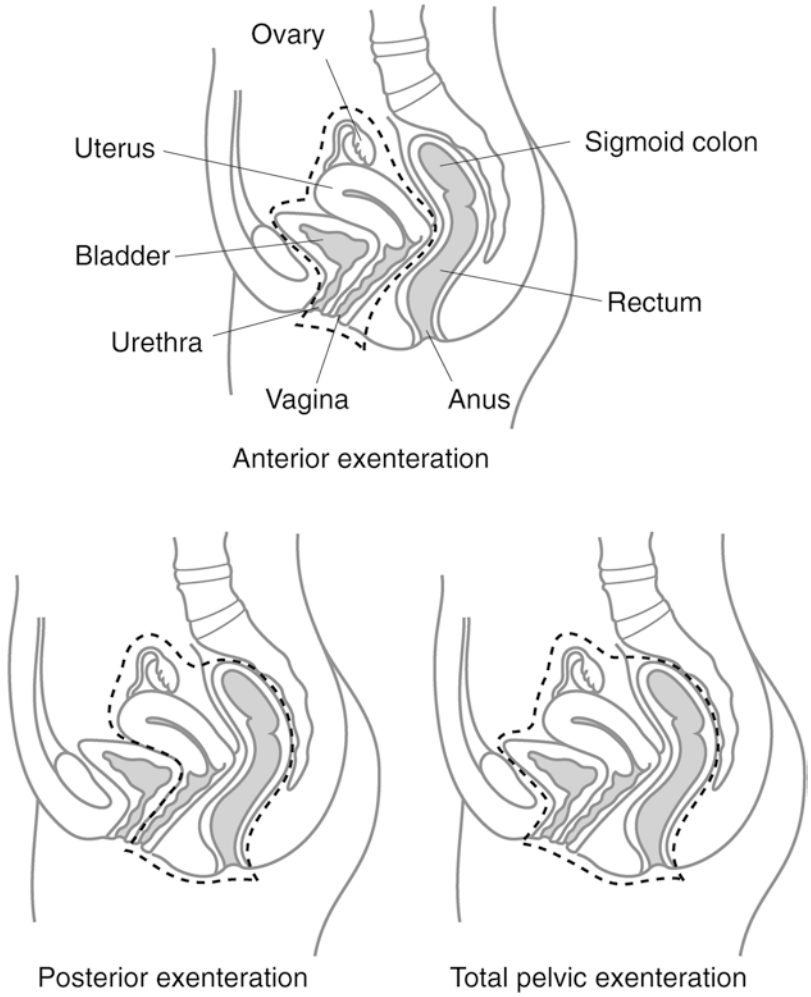

Fig. 8.4 Pelvic exenterations. Reproduced, with permission, from *Histopathology Reporting: Guidelines for Surgical Reporting*, *2nd ed.,* © 2006, Springer

achieved. Microscopy further identifies lymphovascular and microscopic margin involvement that determines adjuvant treatment. It also documents the effects of neoadjuvant therapy.

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Gall Bladder Carcinoma

9

Gerard McVeigh

The incidence of gallbladder cancer varies globally and this likely relates to differences in genetic predisposition and environmental exposures. Despite being the most common malignancy of the biliary tract, gallbladder cancer is rare. However, incidence appears to be rising in the UK, possibly reflecting an aging population. Cholelithiasis is found in the vast majority of gallbladder cancer cases. Despite being a common finding in benign cholecystectomy specimens, gallstone related chronic irritation represents a major risk factor for cancer through a metaplasia, dysplasia, carcinoma sequence. Conversely, very few (<0.2%) patients with cholelithiasis overall develop gallbladder cancer. Other risks include genetic predisposition (relating to propensity to form calculi), anomalous biliary system anatomy (abnormal choledochopancreatic junction), chronic infections (e.g. *Salmonella typhi*), primary sclerosing cholangitis (through chronic inflammation), obesity (possibility confounded by diabetes, itself a risk for calculi formation) and environmental exposures (oil/rubber/textile industries and tobacco).

Approximately 50% of gallbladder cancers are an incidental finding following routine cholecystectomy for presumed benign gallstone disease. Early stage gallbladder cancer is often asymptomatic, although more advanced tumours may also present with usual symptoms of cholecystitis/cholelithiasis. However, more advanced malignancy can cause weight loss and jaundice, the latter due to metastatic disease compressing bile ducts. Suspicion is raised when encountering porcelain (due to gallbladder wall calcification) gallbladders: partial, stippled and multifocal punctate mucosal calcification may be more likely to be associated with carcinoma than transmural calcification. The typically insidious nature of gallbladder cancer and its anatomical extension into the liver bed often results in advanced stage diagnosis and related poor prognosis.

Usual investigation for gallbladder disease includes liver function tests and abdominal ultrasound to assess calculi, luminal/mural lesions and cystic duct/extrahepatic large duct obstruction. This may be followed by CT/MRI scan, cholangiography and endoscopic ultrasound to demonstrate and stage any tumour mass. The latter two modalities can provide tissue for cytological or histological examination. The tumour marker CA19.9 may also be significantly elevated.

Only a small proportion of patients present with early-stage disease suitable for curative surgery. Ideally an elective resection should include cholecystectomy, resection of gallbladder hepatic bed and regional lymph node resection. Extended duct excision may be performed. A deeper tumour may require hepatic segmental resection. However, pT4 disease involving

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_9

main portal vein, hepatic artery or two or more extrahepatic organs/structures may be considered inoperable. If a gallbladder cancer is removed incidentally, second stage resection of gallbladder hepatic bed and surrounding liver with regional lymph node resection may be performed. Early stage (pTis and pT1a) cancers with clear margins may be managed by observational follow up, negating more radical treatment. Adjuvant chemotherapy and radiotherapy has a role to play in both operative and non-operative disease. With extensive locally infiltrative disease, palliation may involve stenting or bypass surgery of the biliary tree or other local structures, to relieve jaundice or gastric outlet obstruction.

Gross Description

Specimen

- Size (mm) and weight (g).
- Open/intact. Open through serosal surface to preserve cystic or hepatic margin.
- Contents: bile/calculi (number, size, shape, colour).
- Lymph nodes: site/size/number.
- Cystic duct length.

Tumour

Site
- Fundus (50%)/body/cystic duct.

Size
- Length × width × depth (mm) or maximum dimension (mm).

Appearance
- Grossly apparent/inapparent/ obvious invasion into adjacent liver or other organ/structure/distance to cystic duct and hepatic resection margin.
- Diffuse (65%)/polypoid (30%—including papillary)/ulcerated.

Edge
- Circumscribed/irregular.

Histological Type

More than 85% of gall bladder cancers are adenocarcinoma.

Adenocarcinoma

- *Biliary:* most common type and typically a well to moderately differentiated tubular pattern of low cuboidal to tall columnar cells with a biliary type appearance. Focal intestinal differentiation with goblet and Paneth cells may be present. Occasional neuroendocrine cells/cytotrophoblasts/syncytiotrophoblasts can also occur.
- *Papillary:* well differentiated and associated with better prognosis. An exophytic luminal papillary lesion with invasion often restricted to the superficial aspect of the mucosa. It can be difficult to distinguish between papillary adenoma (usually of intestinal phenotype).
- *Gastric foveolar/intestinal/mucinous/signet ring cell/cribriform/hepatoid/ clear cell*: all unusual. Distinguish from metastatic stomach or bowel cancer by adjacent mucosal dysplasia and also usually areas of conventional adenocarcinoma present. Mucinous/hepatoid adenocarcinomas require >50% of the tumour to be composed of this pattern. Differentiate from clear cell carcinoma of kidney with PAX8 and cribriform carcinoma of breast with ER/PR.

Adenosquamous Carcinoma

- Moderately differentiated with mucin secretion and keratin pearl formation.

Squamous Cell Carcinoma

- Pure squamous cell carcinoma is rare, accounting for 1% of gallbladder cancers. Thorough

sampling often shows areas of glandular differentiation in keeping with adenosquamous carcinoma. May arise from squamous metaplasia or high grade intra-epithelial neoplasia.

Neuroendocrine Tumour/Carcinoma

- Low-grade/high-grade: neuroendocrine tumours, e.g. well differentiated neuroendocrine (carcinoid) tumour, or, poorly differentiated/high-grade (small cell/large cell) neuroendocrine carcinomas including composite tumours (MANECs—mixed adenoneuroendocrine carcinomas: must have at least 30% of each component for this designation).
- Neuroendocrine carcinoma (small cell and large cell) is *aggressive* and has poorer outcome than usual adenocarcinoma. May respond to cisplatin based chemotherapy. Occurs more frequently in the gall bladder than at other gastrointestinal sites. Synaptophysin/CD56 positive, high Ki-67 index.
- About 50% of carcinoid tumours are confined to the gall bladder at diagnosis. Chromogranin/synaptophysin positive, CD56±, low Ki-67 index (≤2%).
- MANECs: behave similarly to conventional adenocarcinoma.

Spindle Cell Carcinoma/ Carcinosarcoma

- Biphasic carcinoma/sarcoma like components ± specific mesenchymal differentiation. Mesenchymal elements negative with epithelial markers.

Undifferentiated Carcinoma

- Medullary/spindle cell/giant cell/osteoclast-like giant cell/non-neuroendocrine small cell variants.

Metastatic Carcinoma/Melanoma

- Direct extension of tumours from colon, stomach, pancreaticobiliary system may occur. Metastases from virtually any site can occur but are uncommon. Metastatic melanoma accounts for more than 50% of all metastases to the gallbladder and biliary tract. Followed by carcinomas of the stomach, breast, kidney, lung.

Malignant Melanoma

- Primary melanoma much rarer than metastatic melanoma. Junctional activity and the absence of known primary melanoma elsewhere may be indicative.

Differentiation

Well/moderate/poor/undifferentiated,

- Usually well to moderately differentiated arising from mucosal dysplasia (BilIN: Biliary Intraepithelial Neoplasia), or mucosal intestinal metaplasia and dysplasia.
- Signet ring cell carcinoma is poorly differentiated. Small cell and undifferentiated carcinoma (no gland formation).

Extent of Local Tumour Spread

- Border: pushing/infiltrative.
- Lymphocytic reaction: prominent/sparse.
- Characteristic perineural spread.

The TNM8 classification applies only to carcinomas of the gall bladder and cystic duct:

pT1	Tumour invades lamina propria or muscular layer
pT1a	Tumour invades lamina propria
pT1b	Tumour invades muscular layer

pT2	Tumour invades perimuscular connective tissue; no extension beyond serosa or into liver
pT2a	Tumour invades perimuscular connective tissue on the peritoneal side with no extension to the serosa
pT2b	Tumour invades perimuscular connective tissue on the hepatic side with no extension into the liver
pT3	Tumour perforates the serosa (visceral peritoneum) and/or directly invades the liver and/or directly invades one adjacent organ or structure, such as stomach, duodenum, colon, pancreas, omentum, extrahepatic bile ducts.
pT4	Tumour invades main portal vein or hepatic artery or invades two or more extrahepatic organs or structures.

Note that carcinoma in situ and adenocarcinoma may extend into Rokitansky-Aschoff sinuses or gallbladder bed Lushka's ducts, and this must be distinguished from deeply invasive tumour which shows a lack of low power lobular organisation, deficient basement membrane and stromal desmoplasia (Figs. 9.1, 9.2 and 9.3).

Lymphovascular Invasion

- Present/absent.
- Intra-/extratumoural.

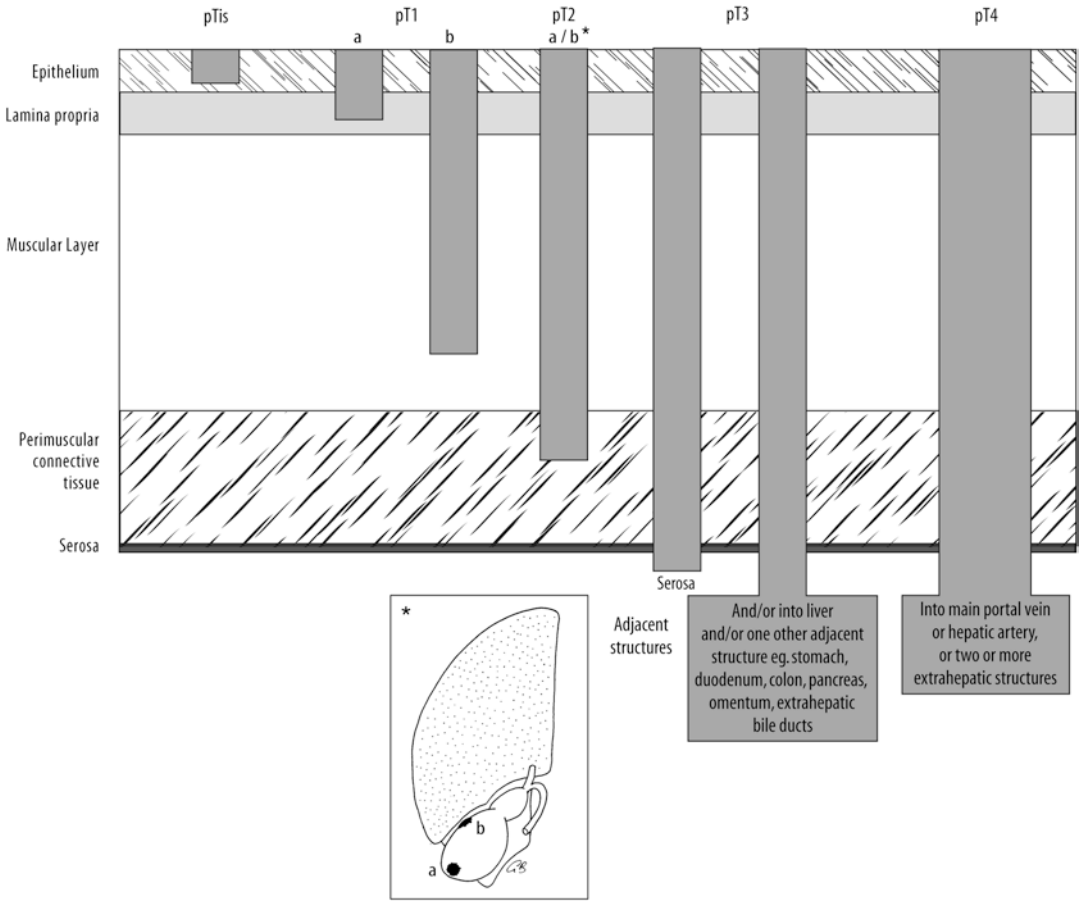

Fig. 9.1 Gall bladder carcinoma. Adapted from *Histopathology Reporting: Guidelines for Surgical Reporting*, *2nd ed.*, © 2006, Springer

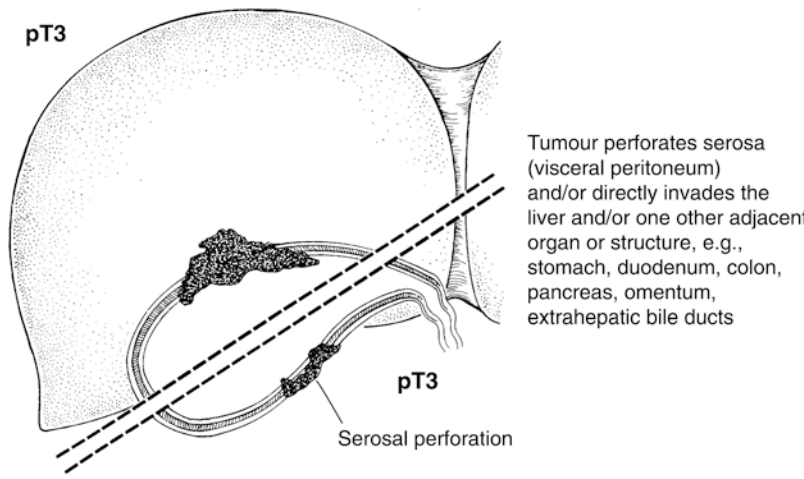

Fig. 9.2 Gall bladder carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

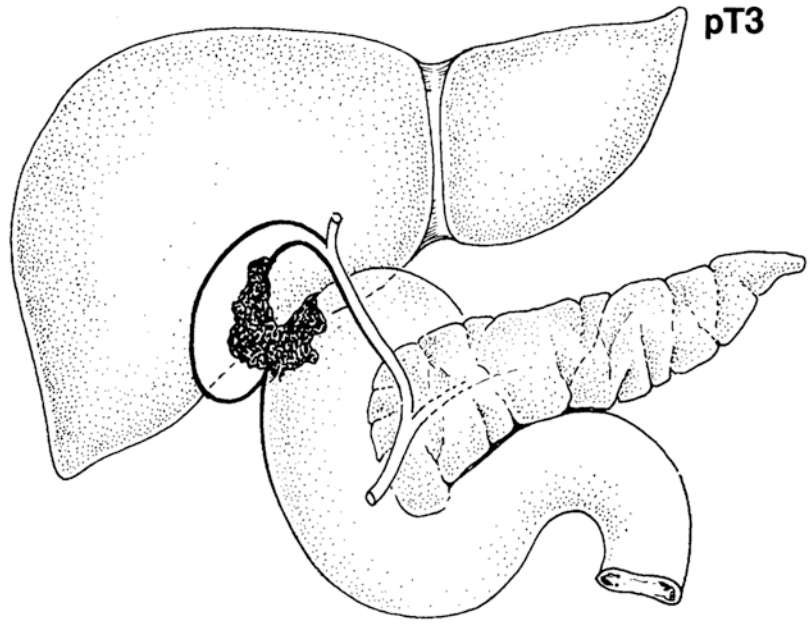

Fig. 9.3 Gall bladder carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

- Perineural invasion is a helpful diagnostic clue to adenocarcinoma but is also an adverse prognostic factor.

Lymph Nodes

Site/number/size/number involved/limit node/extracapsular spread.

Regional nodes: hepatic hilus nodes (including nodes along the common bile duct, hepatic artery, portal vein and cystic duct), coeliac and superior mesenteric artery nodes. Periduodenal, peripancreatic, periaortic and pericaval nodes are considered metastatic (pM1) disease. A regional lymphadenectomy will ordinarily include a minimum of 6 lymph nodes.

pN0	No regional lymph node metastases
pN1	Metastasis to 1–3 regional nodes
pN2	Metastasis to 4 or more regional nodes

The most commonly involved lymph nodes are the pericholedochal (Fig. 9.4).

Excision Margins

Record distances (mm) of tumour to the proximal limit of the cystic duct and adventitial/liver bed margin (or deep hepatic margin). Microscopic involvement (R1) is generally regarded as tumour clearance <1 mm.

Mucosal dysplasia (BilIN) in adjacent gall bladder mucosa, the cystic duct and its limit. Histological detection of mucosal dysplasia in a routine cholecystectomy block should prompt extra blocks to look for an occult invasive cancer.

Other Pathology

Adenoma: rare (occur as an incidental finding in 0.3–0.5% cholecystectomies) and can occur with increased frequency in Peutz-Jeghers syndrome and Gardner syndrome. As in the colorectum the risk of malignancy in an adenoma increases with size, villousity and degree of dysplasia. Histologically there are pyloric gland (commonest), intestinal, foveolar and biliary subtypes. Other precursor lesions include BilIN, intracystic papillary neoplasm and very rarely mucinous cystic neoplasm.

Immunophenotype

Gall bladder carcinoma is cytokeratin (CAM 5.2, AE1/AE3, CK7, CK19), CA19–9 and CEA positive. Additionally, intestinal type adenocarcinoma may be CK20 and CDX2 positive.

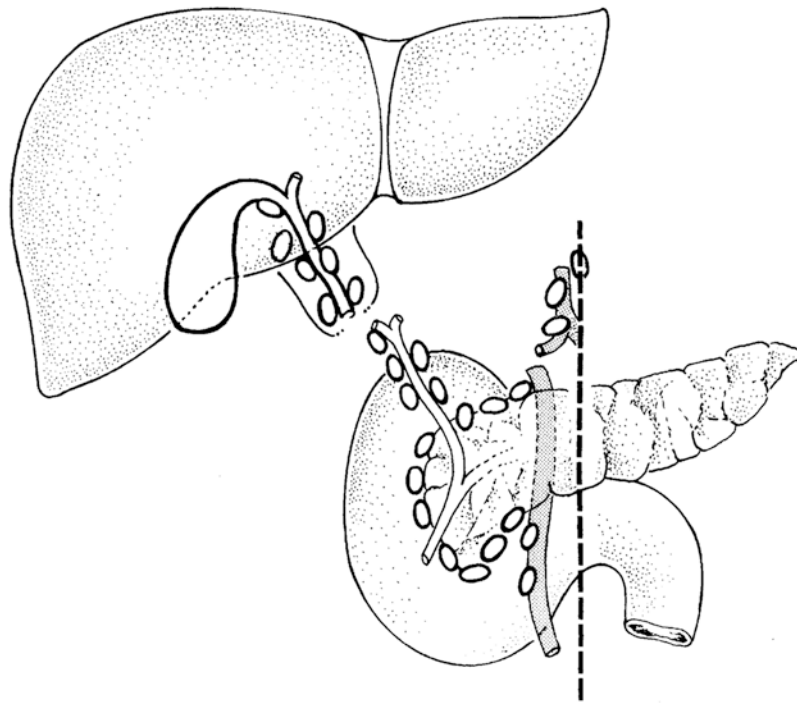

Fig. 9.4 Gall bladder: regional lymph nodes: hepatic hilus nodes (including nodes along the common bile duct, hepatic artery, portal vein and cystic duct), coeliac and superior mesenteric artery nodes. Periduodenal, peripancreatic, periaortic and pericaval nodes are considered metastatic (pM1) disease. Adapted from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

Prognosis

Prognosis is strongly related to *cancer type, grade and stage.* It is 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). *Full wall thickness infiltration has a 10 year survival rate of 30%. Spindle and giant cell subtypes of undifferentiated carcinoma have an extremely poor survival rate.* Whilst some carcinomas are grossly inapparent and a microscopic finding only, up to 50–70% present with regional lymph node metastases and involvement of the liver. In these patients 5 year survival rates are 5–10%.

Other Malignancy

Malignant Lymphoma/Leukaemia

- MALToma are more usually secondary to systemic nodal disease.

Sarcoma (Rare)

- Embryonal rhabdomyosarcoma (children: desmin/myo D1/myogenin positive), leiomyosarcoma (may be EBV associated), angiosarcoma.

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Perihilar and Distal Extrahepatic Bile Duct Carcinoma

10

Paul J. Kelly

Introduction

Predisposing conditions to extrahepatic bile duct carcinoma include chronic ulcerative colitis, primary sclerosing cholangitis, gallstones, Caroli's disease and choledochal cysts.

Extrahepatic bile duct cancer presents with obstructive jaundice, right upper quadrant pain, weight loss, malaise, pruritis and abnormal liver function tests. Pyrexia can result from ascending cholangitis and the gall bladder may be distended depending on the anatomical site of the tumour. Investigations include: serum CA19–9 levels, liver function tests, ultrasound scan and cholangiography (either percutaneous, MR, ERCP (endoscopic retrograde cholangiopancreatography)) to detect large duct obstruction and strictures. CT and MRI scans can be used for tumour staging of local and distant disease. Endoluminal ultrasound (ELUS) may be used in addition to cross sectional imaging to assess locoregional disease. ERCP is used as both a therapeutic tool (stent insertion and biliary drainage) and for obtaining bile duct brushings. ELUS can also be used to obtain fine needle aspiration or core biopsy specimens. Peroral cholangioscopy allows direct visualization of strictures and tissue biopsy. Diagnostic yields for malignancy are at best 30–40% and a presumptive working diagnosis may have to be based on clinical grounds.

Radical resection is usually for a distal bile duct or ampullary mass (Whipple's pancreaticoduodenectomy) causing obstructive jaundice. The diagnosis may or may not have been proven preoperatively by cytology or biopsy. Segmental resection for a mid bile duct tumour may be considered. Unfortunately, only a minority of patients have disease that is considered resectable at the time of presentation due to local infiltration, peritoneal involvement or distant metastasis. Proximal extension of tumour into the intrahepatic biliary tree determines resectability. For perihilar tumours, resection consists of excision of the hilum and (extended) hemihepatectomy, including the caudate lobe. This is accompanied by lymphadenectomy of the hepatoduodenal ligament and bilioenteric anastomosis. Before embarking on hemihepatectomy, patients require preoperative biliary drainage, either via percutaneous transhepatic biliary drainage (PTB) or ERCP. Intraoperative frozen section may be required for assessment of bile duct resection margins, enlarged suspicious lymph nodes or a subcapsular liver nodule. This can be augmented by prior diagnostic and staging laparoscopy with biopsy of suspected lymph nodes, and, peritoneal and subcapsular liver deposits that would preclude radical surgery. Liver transplantation is not considered routine standard of care in resectable hilar cholangiocarcinoma. In a number of highly

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_10

specialised centres worldwide a specific liver transplant program with careful patient selection (tumour <3 cm and no metastases) and rigorous neoadjuvant therapy can achieve acceptable outcomes. This has been popularized by the Mayo clinic protocol. This strategy is not part of the UK transplant program currently.

Gross Description

Specimen

- Cytological brushings, fine needle aspirate/biopsy and washings/biopsy/resection.
- Weight (g) and size/length (mm), number of fragments.

Tumour

Site

- Perihilar tumour/proximal third (50–60%: equally between the right/left/common hepatic, and upper common bile ducts), intermediate third (25%), distal third (10%), multifocal/diffuse (15%) (see Figs. 10.1 and 10.2).
- Tumours of the perihilar ducts and distal extrahepatic bile ducts are staged separately. Perihilar tumours occur proximal to the cystic duct origin, up to and including the second order branches of the left and right hepatic ducts. They often involve the confluence of the main right and left hepatic ducts in the hilar region (Klatskin tumour). Distal extrahepatic bile duct carcinomas (cholangiocarcinomas) arise distal to the

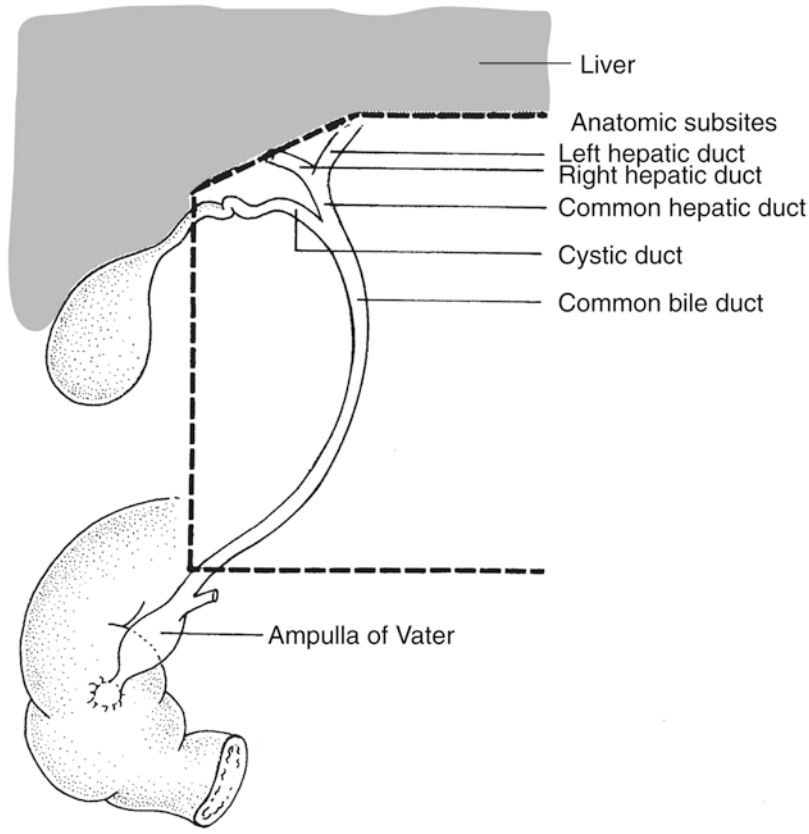

Fig. 10.1 Extrahepatic bile ducts. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

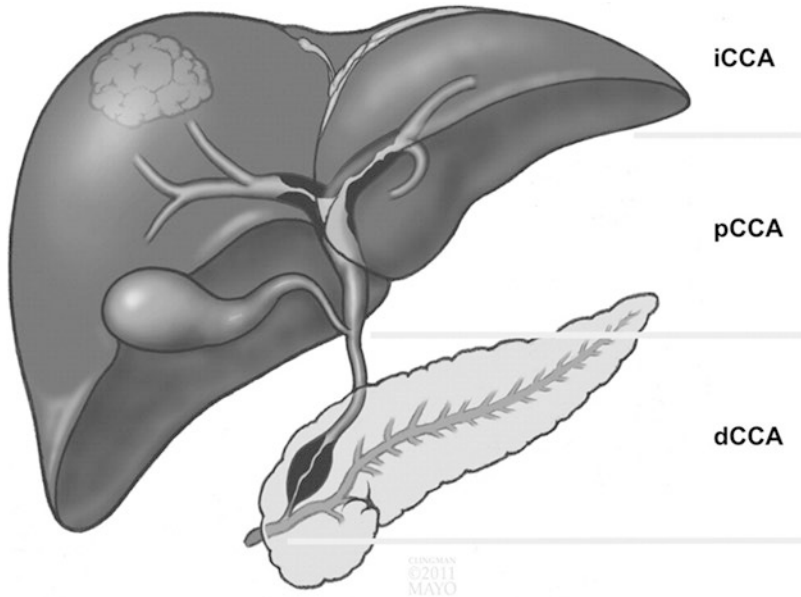

Fig. 10.2 Cholangiocarcinoma is a heterogeneous group of tumours and classification is based on anatomical location. Intrahepatic cholangiocarcinoma (ICCA), perihilar cholangiocarcinoma (pCCA) and distal extrahepatic cholangiocarcinoma (dCCA). (Reproduced with permission from Razumilava and Gores (2013), Elsevier)

insertion of the cystic duct and may involve the extra- or intrapancreatic portion of the common bile duct. The cystic duct is considered separately and grouped with gallbladder cancers.

Size

- Length × width × depth (mm) or maximum dimension (mm).
- Perihilar cancers often demonstrate periductal spread and involve the more proximal intrahepatic ducts, with or without liver invasion. This may impart the impression of duct thickening without formation of an obvious mass and lead to underestimation of the tumour size. There can also be extensive fibrosis due to cholangitis or stenting, compromising accurate estimation of tumour size. In contrast to this there can be microscopic infiltration of tumour beyond its macroscopic edge.
- Localised (a majority), the entire common bile duct or multifocal throughout the extrahepatic biliary system.

Appearance

- Papillary/polypoid/intraductal: distal third.
- Nodular: intermediate third.
- Ulcerated/sclerotic/scirrhous: proximal third.

The majority are nodular or sclerosing with deep penetration of the wall, although the macroscopic appearances overlap considerably. A small minority have a cystic component.

Edge

- Circumscribed/irregular.

Histological Type

Adenocarcinoma

- *Tubular/acinar:* usual type and a well to moderately differentiated biliary pattern of low cuboidal to tall columnar cells.

- *Papillary:* polypoid and well differentiated in the distal third with a better prognosis. It is of biliary or intestinal phenotype.
- *Intestinal:* well to moderate differentiation ± mucin secretion in a fibrous stroma.
- *Sclerosing:* perihilar tumour. Well to moderately differentiated tubular adenocarcinoma and rarely mucinous or signet ring cell. Fibrous nodule, short or long segmental stenosis with a periductal infiltrative pattern, or papillary. *Indolent growth* with potentially prolonged survival but late presentation and ultimately *death is from liver failure rather than tumour dissemination.* Includes the classical Klatskin tumour at the confluence of the right and left hepatic ducts and which also often shows intrahepatic extension. Arises in a field of mucosal dysplasia and resection limits should be checked for this.
- *Others:* mucinous carcinoma/signet ring cell carcinoma (>50% of the tumour area), clear cell carcinoma are unusual.
- *Cystadenocarcinoma:* a small number are the malignant counterpart of biliary mucinous cystic neoplasm (bile duct cystadenoma) with variable benign, borderline and focal malignant change. Middle aged females, resectable, good prognosis.

Adenosquamous Carcinoma

- Moderately differentiated with mucin secretion and keratin pearl formation.

Squamous Cell Carcinoma

- Most represent adenosquamous carcinoma with a minor glandular component.

Neuroendocrine Tumour/Carcinoma

- Neuroendocrine neoplasms, e.g. well differentiated/low-grade neuroendocrine tumour, poorly differentiated/high-grade (small cell/large cell) neuroendocrine carcinoma, or mixed neuroendocrine non-neuroendocrine neoplasms. Variably chromogranin, synaptophysin, CD56 positive. Ki67 immunohistochemistry is important for grading neuroendocrine neoplasms.

Carcinosarcoma/Spindle Cell Carcinoma

- Cytokeratin positive spindle cells and varying degrees of homologous or heterologous stromal mesenchymal differentiation.

Undifferentiated Carcinoma

- Nodular or solid/spindle cell/giant cell variants.

Malignant Melanoma

- Metastatic or primary (rare). Comprises up to 50% of metastatic cancers to the gall bladder and biliary tree.

Metastatic Carcinoma

- Stomach, breast (infiltrating lobular), colorectum, kidney.

Differentiation

Well/moderate/poor/undifferentiated, or, Grade 1/2/3/4 based on the percentage tumour gland formation (well/G1 > 95%: moderate/G2 50–95%: poor/G3 < 50%).

Signet ring cell carcinoma is grade 3, and undifferentiated carcinoma (no gland formation) grade 4—these are prognostically adverse cancers.

Extent of Local Tumour Spread

Border: pushing/infiltrative.

Lymphocytic reaction: prominent/sparse.

Perineural spread is a characteristic and may be *present beyond the resection line causing surgical failure.*

The TNM8 classification distinguishes between carcinomas of the extrahepatic bile ducts by their location. Either distal (distal to the insertion of the cystic duct) or perihilar (Klatskin tumour) (proximal to the cystic duct origin up to and including the second branches of the left and right hepatic ducts). Cystic duct carcinoma is included under gallbladder.

Distal

pTis	Carcinoma in situ
pT1	Tumour invades bile duct wall[a] to a depth less than 5 mm
pT2	Tumour invades bile duct wall to a depth of 5 mm up to 12 mm
pT3	Tumour invades bile duct wall of more than 12 mm
pT4	Tumour invades the coeliac axis, superior mesenteric artery and/or the common hepatic artery

[a]The wall of the bile duct comprises the subepithelial connective tissues and underlying fibromuscular layer. Depth of tumour invasion is the area of deepest infiltration from the mucosal surface (e.g. from the basal lamina of the adjacent normal epithelium to the deepest tumour focus). In cases containing carcinoma in situ at the periphery of the invasive tumour, the basal lamina of in situ disease is used

Perihilar

pT1	Tumour invades the bile duct wall, with extension up to the muscle layer or fibrous tissue
pT2a	Tumour invades beyond the wall of the bile duct to surrounding adipose tissue
pT2b	Tumour invades adjacent hepatic parenchyma
pT3	Tumour invades unilateral branches of portal vein or hepatic artery
pT4	Tumour invades main portal vein or its bilateral branches; or the common hepatic artery; or unilateral second order biliary radicals with contralateral portal vein or hepatic artery involvement,

Lymphovascular Invasion

Present/absent.

Intra-/extratumoural.

An adverse prognostic indicator.

Lymph Nodes

Site/number/size/number involved/limit node/extracapsular spread.

Regional nodes:

- Distal: along the common bile duct, hepatic artery, back towards the coeliac trunk, posterior and anterior pancreaticoduodenal nodes, and nodes along the superior mesenteric artery.
- Perihilar: hilar and pericholedochal nodes in the hepatoduodenal ligment.

A regional lymphadenectomy will ordinarily include a minimum of 12 and 15 lymph nodes for distal and perihilar lesions, respectively.

pN0	No regional lymph node metastasis
pN1	Metastasis to 1–3 regional nodes
pN2	Metastasis to 4 or more regional nodes

Lymph node metastases are usually present at the time of diagnosis with subsequent spread to local structures (liver, pancreas, gall bladder, duodenum), lungs and peritoneal cavity.

Excision Margins

Distances (mm) to the nearest longitudinal and circumferential resection margins of carcinoma and presence of mucosal dysplasia at the longitudinal limits.

Local recurrence usually relates to longitudinal or soft tissue radial margin involvement by adenocarcinoma and is commonest in proximal tumours. Microscopic involvement (R1) is generally regarded as tumour clearance <1 mm.

Other Pathology

Diagnostic presentation: 90% of patients (>60 years, F:M 1:1) present with jaundice and diagnosis is by cholangiography (retrograde endoscopic or percutaneous transhepatic) or cross sectional imaging supplemented by fine needle aspiration/brushings/washings cytology and/or biopsy.

Frozen section diagnosis: of bile duct carcinoma can be difficult due to the presence of ductulo-glandular structures in normal bile duct submucosa and the distortion that can occur in inflammatory strictures.

Dysplasia (BilIN): of adjacent bile duct mucosa can have a flat or micropapillary epithelial pattern and must be noted at the resection limits. Intraductal papillary neoplasia (previous referred to biliary papillomatosis) is a relatively uncommon precancerous lesion which can develop an associated invasive component.

Predisposing conditions: chronic inflammatory bowel disease (ulcerative colitis), primary sclerosing cholangitis, gall stones and choledochal cysts all show an increased incidence. Radiologically it can be difficult to distinguish between primary sclerosing cholangitis and a stenotic carcinoma.

Benign versus malignant biliary strictures: approximately 15% of suspicious biliary strictures are found to be benign postoperatively. IgG4 sclerosing cholangiopathy can mimic extrahepatic cholangiocarcinoma and may be associated with elevated IgG4 serum levels.

Immunophenotype

Markers (e.g. cytokeratins, CEA) may be helpful in identifying poorly differentiated single cell infiltration on biopsy. Other markers of bile duct carcinoma are cytokeratins 7 and 19, EMA and CA19–9. There is also over expression of p53 in contradistinction to normal duct structures. Perineural invasion can alert to a diagnosis of malignancy.

Prognosis

Prognosis is generally poor for extrahepatic cholangiocarcinoma. The 5-year survival rate at diagnosis for all stages combined is 10%. Surgical resection offers the only curative therapeutic option. Distal biliary cholangiocarcinoma resected by a Whipple's procedure has reported 5-year survival rates of 27–37%. Surgical strategies for perihilar cancer include resection or, less commonly, liver transplantation. Most series are burdened with mortality of 5–10% and 5-year survival rates for resected perihilar tumours range form 11–42%. Recurrence rates of 20% and up to 50% are observed with surgical resection and transplant respectively. Nodal involvement, tumour grade and resection status (R0 vs. R1) are major prognostic factors influencing outcomes after surgical resection. Adjuvant chemotherapy with capecitabine-based regimens is now recognised as an important facet of patient care. In the palliative setting patients may be offered locoregional therapies or palliative chemotherapy with median survival of approximately 7–12 months. Biliary drainage and stenting have an important role in the palliative or supportive care setting.

Other Malignancy

Neuroendocrine Neoplasms

- Well differentiated neuroendocrine tumours, poorly differentiated neuroendocrine carcinomas and mixed neuroendocrine non-neuroendocrine neoplasms.

Malignant Lymphoma/Leukaemia

- Secondary to systemic/nodal disease.

Mesenchymal

Benign

- Paraganglioma, granular cell tumour, ganglioneuroma, EBV-associated smooth muscle tumour

Malignant

- Embryonal (botryoid) rhabdomyosarcoma in children with direct invasion of abdominal structures, metastases to bone and lungs and poor prognosis. Desmin/myo D1/myogenin positive small cells, subepithelial cellular cambium layer, deeper myxoid zone.
- Leiomyosarcoma, angiosarcoma.

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Liver Carcinoma

11

Paul J. Kelly

Primary liver cancer is the fifth most common cancer and second most frequent cause of cancer related death worldwide. It's prevalence varies according to geographic region with primary liver cancers being much more common in sub-Saharan Africa and South East Asia. Hepatocellular carcinoma is the most frequent subtype (80–90%). Risk factors include chronic viral infection (hepatitis B (HBV), hepatitis C (HCV)), alcohol, tobacco, aflatoxin ingestion, and particularly in Western society, cirrhosis due to various causes, e.g. alcohol, non-alcoholic fatty liver disease, alpha-1-antitrypsin deficiency, and haemochromatosis. There is a rising incidence of hepatocellular carcinoma, particularly in Europe and the USA due to the increasing prevalence of chronic liver disease. Some 50–90% of cases arise in a background of cirrhosis, the presence of cirrhosis or advanced fibrosis being an indicator of an "unstable liver".

Intrahepatic cholangiocarcinoma forms a minority of primary liver cancers (approximately 10–20%) and risk factors include long standing ulcerative colitis, particularly when associated with primary sclerosing cholangitis, fibrocystic disease of the liver, parasitic infection, cirrhosis, drugs (Thorotrast), smoking and alcohol. Most patients with intrahepatic cholangiocarcinoma do not present with obvious risk factors and 20–40% are diagnosed incidentally. Intrahepatic cholangiocarinoma is less common than extrahepatic cholangiocarcinoma. From a biological perspective peripheral, typically mass forming, tumours appear to be different to central tumours, with more frequent association with chronic liver diseases, less common local recurrence and longer median survival times.

Liver disease can be asymptomatic until relatively late in the disease course. Otherwise presentation of hepatic malignancy may be with jaundice, weight loss, anaemia and anorexia. There can be right upper quadrant pain or a palpable mass and investigations include serum alpha-fetoprotein (AFP) and CA19–9, liver function tests and imaging studies. Diagnosis of hepatocellular carcinoma is usually based on a combination of elevated (>400 ng/mL) or continuously rising serum AFP levels and appropriate radiological features obviating the need for biopsy. Cholangiocarcinoma may be diagnosed by demonstration of obstruction and distortion of the intrahepatic bile duct system by magnetic resonance cholangiography (MRCP) or as a mass-forming lesion on CT or MRI. Suspected metastatic colorectal or pancreatic cancer may have an appropriate past history and raised serum CEA and CA19-9 tumour markers. CT/PET scan is of use in distinguishing metabolically active tumours from benign or necrotic mass lesions. Where metastases or a primary

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https://doi.org/10.1007/978-3-030-27828-1_11

hepatocellular carcinoma are potentially resectable, or transplant is considered, there is a reluctance to carry out fine needle aspiration (FNA)/needle biopsy for fear of upstaging the tumour, e.g. needle tract seeding (1–2% of cases). However in the absence of a significantly elevated serum AFP, cirrhosis, characteristic imaging findings or other obvious primary site, targeted needle biopsy (percutaneous or transjugular) under USS/CT scan guidance may be needed for a robust tissue diagnosis and to exclude other treatable tumours, e.g. malignant lymphoma. Tissue biopsy is also indicated when systemic therapy is being considered and is now part of the work up for many patients being enrolled in trials. Needle biopsy yields either a positive diagnosis or the changes adjacent to a mass lesion: i.e. liver plate atrophy, prominent sinusoids and focal inflammation.

Transjugular cores are very fine and require careful handling in the laboratory. However, they can produce useful morphological and immunohistochemical results if the tumour is in a suitably accessible location.

Hepatic resection for malignant disease is a well-established treatment modality for both primary and secondary liver tumours. In the absence of cirrhosis above Childs-Pugh A, extensive resections of primary liver cancer can occur provided there is an adequate liver remnant, and this may involve major venous reconstructions. For patients with cirrhosis, liver transplantation is the treatment of choice provided that the disease burden meets threshold Milan criteria (one lesion <5 cm or up to three lesions <3 cm). The most common indication for surgery in Western countries is metastatic colorectal cancer and surgical resection offers the only prospect of long-term cure with 5-year survival rates in excess of 40%. Over the past decade there has been a paradigim shift in the management of colorectal cancer with an expanding understanding of what is considered resectable. Long-term survival can now be achieved in patients with multiple sites of disease in both lobes of the liver. This has been facilitated by major developments in the chemotherapeutic manipulation of metastatic disease resulting in a subgroup of cases being converted from borderline or unresectable to resectable disease. Surgical strategies, including parenchymal preserving techniques, are now routinely performed and can facilitate multiple sites of resection provided that 30% of liver volume remains. This has also been complimented by techniques such as pre-operative portal vein embolisation, which can induce rapid hypertrophy in the liver remnant. Other techniques include radiofrequency or microwave ablation of tumours. Diagnostic and staging assessment of patients for liver resection of primary and metastatic cancer is performed by regional or network specialist teams.

Depending on the anatomical extent of disease as determined by imaging scans the resection can be major (partial hepatectomy, lobectomy) or segmental, the latter excised with its supplying lymphovascular pedicle. Note that the surgical definition of lobes and their constituent segments differs from the classical anatomical lobes. Small subcapsular metastases can be removed by open or laparoscopic non-anatomical wedge resection, or may be erroneously diagnosed as such at frozen section evaluation during radical cancer surgery due to mimicry by a bile duct adenoma or Von Meyenberg complex.

Gross Description

Specimen

- FNAC (fine needle aspiration cytology)/core biopsy/wedge excision/segmentectomy/partial hepatectomy/R/L lobectomy.
- Size (mm) and weight (g).

Tumour

Site

- Subcapsular/parenchymal/ductocentric/vasculocentric/lobe/multifocal (particularly when cirrhosis is present).

Size

- Length × width × depth (mm) or maximum dimension (mm).
- In a cirrhotic liver a solid irregular lesion >5 cm diameter is probably hepatocellular carcinoma.
- The diffuse, periductal pattern of cholangiocarcinoma often shows microscopic infiltration beyond its clinical macroscopic extent.

Appearance

- Hepatocellular carcinoma: solitary/nodular/diffuse/multifocal (particularly in cirrhosis)/bile stained/venous spread/pedunculated/encapsulated/background cirrhosis/haemochromatosis. The cancer often shows a mosaic of macroscopic patterns.
- Cholangiocarcinoma: mass-forming/papillary/intraductal/nodular/stenotic/scirrhous/ductocentric/multifocal.
- Metastatic carcinoma: single/multiple/necrotic/umbilicated/calcification/diffuse/mucoid/subcapsular.

Edge

- Circumscribed/irregular.

Histological Type

Hepatocellular Carcinoma

- *Trabecular, plate like, or sinusoidal.*
- *Pseudoglandular (acinar).*
- These are the *usual types* comprising hepatoid cells, bile cytoplasmic staining and canalicular plugging, eosinophilic intranuclear pseudoinclusions, and a sinusoidal vascular pattern with a CD34 positive endothelial lining (capillarisation).
- *Solid (compact):* inconspicuous sinusoids.
- *Scirrhous:* fibrotic. Distinguish from cholangiocarcinoma and post chemo−/radiotherapy changes.
- *Recognised variants*: pleomorphic, clear cell, spindle cell (sarcomatoid), undifferentiated, lymphoepithelial (EBV positive) or osteoclast like.
- Other histological subtypes include chromophobe-like, massive macrotrabecular, steatohepatitic, granulocyte colony stimulating factor-producing, diffuse cirrhosis-like/cirrhotomimetic
- *Variants with good prognosis:* fibrolamellar carcinoma (90% <25 years old); pedunculated carcinoma; minute, small or encapsulated carcinoma (see Section "Other pathology").

Intrahepatic Cholangiocarcinoma

- *10–20% % of primary liver cancers*. Usually *mass forming* (single/multifocal/peripheral), *central periductal infiltrative* or *intraductal* (papillary: rare in the West). *Mixed types* also occur.
- *Ductulo-acinar pattern* of heterogeneous cuboidal to columnar mucin secreting cells in a fibrous stroma with a hyalinised sclerotic hypocellular centre and more cellular periphery. Sometimes papillary.
- *Portal expansion/periportal sleeve like* and *parenchymal sinusoidal* distributions.
- Few survive longer than 2–3 years due to late presentation and limited resectability.
- *Rarely:* mucinous; signet ring cell; adenosquamous; squamous; clear cell; pleomorphic; mucoepidermoid; osteoclast like; spindle cell (sarcomatoid). These are prognostically adverse variants. EBV-associated lymphoepithelioma-like variant also recognised and reported to have a better prognosis.
- *Cholangiolocellular carcinoma.* May be a distinct form of primary liver malignancy but currently managed as cholangiocarcinoma. Confers a better prognosis.

Combined Hepatocellular/Cholangiocarcinoma

- Diagnosis requires morphological evidence of both hepatocellular and biliary/cholangiocytic differentiation, supported by immunohistochemistry. Foci of intermediate phenotype

may be seen. Once estimated to represent 1–5% primary liver cancers. Currently staged and managed as cholangiocarcinomas.

Hepatoblastoma

- 50–60% of childhood liver cancers, 90% are <5 years of age.
- Usually a large solitary mass (85% of cases), right lobe > > left lobe and raised serum AFP. Epithelial component of two cell types (fetal/embryonal hepatocytes or small cell anaplastic) and fibrous mesenchyme (25% of cases: osteoid or undifferentiated spindle cells). May have teratoid features. Treatment is neoadjuvant chemotherapy to shrink the tumour and then surgery with a 50–70% long term survival. Age < 1 year, large size and a significant small cell component are adverse factors.

Metastatic Carcinoma

- *In order of frequency:* secondary carcinoma (breast, colorectum, pancreas, stomach, lung), neuroendocrine tumour (pancreas) and malignant melanoma. Sarcoma (except metastatic GIST) is uncommon (6%). Hodgkin and non-Hodgkin lymphomas may involve the liver in up to 20% of patients at presentation. Lymphomas can form single or multiple mass lesions mimicking metastatic carcinoma.
- *Direct spread:* stomach, colorectum, pancreas, gall bladder and biliary tree.
- *Distant spread:* stomach, oesophagus, colorectum, lung, breast, malignant melanoma, kidney, urinary bladder, ovary, teratoma.

The *tumour distribution* and *appearance* may reflect its origin:

- Colorectum:
  - Multiple, large nodules with central necrosis and umbilication, ± mucin, ± calcification. May grow into and along bile ducts mimicking a primary tumour. As in renal cell carcinoma can be solitary and massive.
- Gall bladder:
  - The bulk of disease is centred on the gall bladder bed.
- Lung:
  - Medium sized nodules and fleshy appearance (small cell carcinoma).
- Breast, stomach:
  - Medium sized nodules or diffuse cirrhotic like pattern of sinusoidal infiltration.
- Malignant melanoma:
  - Pigmented.
- Angiosarcoma, choriocarcinoma, leiomyosarcoma, renal/thyroid carcinoma, gastrointestinal neuroendocrine tumours, GIST:
  - Haemorrhagic/cystic.

Note that *carcinoma rarely metastasises to a cirrhotic liver*: i.e. the tumour is more likely to be a primary liver cancer. Histologically there can be considerable difficulty distinguishing hepatocellular carcinoma and its variants from other metastases, e.g. neuroendocrine tumour, renal cell carcinoma, adrenal cortical carcinoma and malignant melanoma. Similarly cholangiocarcinoma (look for adjacent BilIN/mucosal dysplasia) from gastrointestinal secondaries. *Morphology allied to a panel of antibodies* should be used including:

Hepatocellular	Hep Par 1, Arginase 1, AFP, canalicular polyclonal CEA/CD 10, CD34 (capillarisation of sinusoids). Most (90%) are BerEP4/EpCam negative *cf* adenocarcinoma.
Adrenal	Inhibin, melan-A, vimentin, synaptophysin.
Renal	EMA, vimentin, RCCab, CD10, PAX8.
Colorectum	CK20, CDX-2, SATB2.
Lung adenocarcinoma	CK7, TTF-1, napsin A.
Malignant melanoma	S100, HMB-45, melan-A, SOX10.
GIST	DOG-1, CD117.
Neuroendocrine tumours	Chromogranin A, synaptophysin, CD56(±), Ki-67, CDX-2 (from gastrointestinal tract), TTF-1 (from lung). Note high grade neuroendocrine carcinomas may express TTF1 regardless of origin.

Resection of hepatic metastases: can be done to good effect, e.g. well differentiated neuroendocrine (carcinoid) tumour, colorectal carcinoma. Tumour macroscopic and microscopic appearances may be altered by preoperative ablative, embolisation (bland or with chemoembolization), selective internal radiation therapy (SIRT) or neoadjuvant therapy. Extensive sampling is required to establish response which can be assessed as complete, incomplete or absent. *Tumour regression* is often accompanied by a histiocytic and fibrous reaction with mucin lakes. The *number of metastatic deposits and clearance of metastasectomy surgical margins are prognostically significant* although equivocal margins are often obliterated by operative diathermy and haemostasis techniques. Other prognostic factors: vascular and lymphatic invasion; tumour growth pattern, presence/absence of tumour pseudocapsule.

Hepatic mass lesions at clinical follow up: a common issue at specialty multidisciplinary team meetings is the finding of a solitary (or several) liver lesion(s) on CT scan staging or post treatment follow up of a cancer originating from various primary sites. A circumscribed hypodense, or, characteristic appearance may allow the radiologist to designate simple cysts or specific entities such as haemangioma with confidence. *Indeterminate lesions can be monitored for size and contour change with time.* Irregularity of edge or content signal is more worrying for a metastatic deposit and *further characterisation* can be sought with *MRI* and *CT/PET scans.* This is correlated with *serum tumour marker levels,* e.g. CEA, CA125, CA19–9. Ultimately, if radiologically accessible, a *tissue diagnosis* may be desirable either by FNAC or core biopsy, as a basis for proceeding to further surgical or medical oncological treatment. Importantly *new pathology* can also be excluded, e.g. a patient with previously treated colorectal cancer developing metastatic pancreatic neuroendocrine tumour rather than colorectal metastases, or a mass forming lymphoma.

Differentiation/Grade

For hepatocellular carcinoma a 3-point grading system is now advocated: well, moderate or poor corresponding to Edmonson/Steiner Grades I/II (well), III (moderate) and IV (poor).

This is based on the degree of *resemblance to hepatic tissue.* Well differentiated (GI/II) hepatocellular carcinomas resemble hepatocytes raising the differential diagnosis of dysplastic nodule in cirrhosis, or an adenoma in non-cirrhotics. Poorly differentiated (GIV) cancers are not recognisably hepatocytic on morphology, requiring appropriate immunohistochemistry and close correlation with clinical parameters such as serum AFP +/− imaging. Well to moderately differentiated lesions show trabecular (plate like) or pseudoglandular patterns seen in tumours <2–3 cm diameter. Larger lesions (>3 cm) may only have a well differentiated periphery, with a less differentiated centre characterised by greater cytoarchitectural atypia and no discernable sinusoids. This nodule within nodule appearance is diagnostically useful and highlights the heterogeneity and active evolution of hepatocellular carcinoma. Tumour grade and morphological subtype in a preoperative biopsy can predict patient survival.

For cholangiocarcinoma: a well-defined scheme has not been proposed. One suggested scheme is based on the percentage tumour gland formation: well/G1 > 95%: moderate/G2 50–95%: poor/G3 < 50%: undifferentiated/G4 < 5% glands. An alternative scheme classifies as well, moderate or poorly differentiated based on morphology and gland formation: well—tubular adenocarcinoma with or without micropapillary features; moderate—moderately distorted tubular glands with cribriform formations and/or cord-like pattern; poor—distorted tubular or cord-like structures and marked pleomorphism.

It is recognised that hepatocellular carcinoma and cholangiocarcinoma form a morphological spectrum with origin from a common progenitor stem cell. Mixed cancers can therefore occur. An intermediate cell carcinoma has also been described as a monomorphic primary liver cell carcinoma composed of tumour cells that have

features intermediate between hepatocytes and cholangiocytes. Tumour cells may be arranged in trabeculae, cords, nests or strands and are set with a marked desmoplastic stroma.

Extent of Local Tumour Spread

Border: pushing/infiltrative.

Lymphocytic reaction: prominent/sparse.

Invasion through the hepatic (Glisson's) capsule.

The TNM8 classification distinguishes between hepatocellular carcinoma and intrahepatic cholangiocarcinoma.

Hepatocellular Carcinoma

pT1	Solitary tumour ≤2 cm, or > 2 cm without vascular invasion
pT1a	Solitary tumour ≤2 cm with or without vascular invasion
pT1b	Solitary tumour >2 cm without vascular invasion
pT2	Solitary tumour >2 cm with vascular invasion, or multiple tumours, none >5 cm
pT3	Multiple tumours, at least one of which is >5 cm
pT4	Single tumour or multiple tumours of any size involving a major branch of the portal vein or hepatic vein, or tumour(s) with direct invasion of adjacent organs other than the gall bladder *or* with perforation of visceral peritoneum

Vascular invasion is diagnosed by clinical imaging. The pathological classification includes gross and histological involvement. Criteria are: location within a portal tract appropriate to the site of a portal vein, an identifiable lumen and endothelial lining.

Multiple tumours includes multiple independent primaries or intrahepatic metastases from a single hepatic carcinoma. A *multicentric distribution* is associated with a *poor prognosis* (Figs. 11.1, 11.2, 11.3 and 11.4).

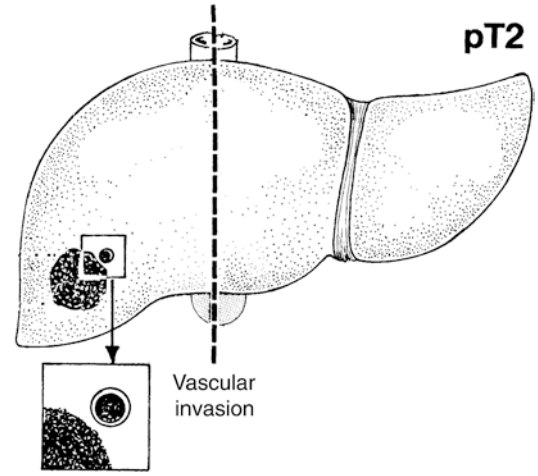

Fig. 11.1 Hepatocellular carcinoma. Stage pT2: solitary tumour >2 cm with vascular invasion, or multiple tumours, none >5 cm. Adapted from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

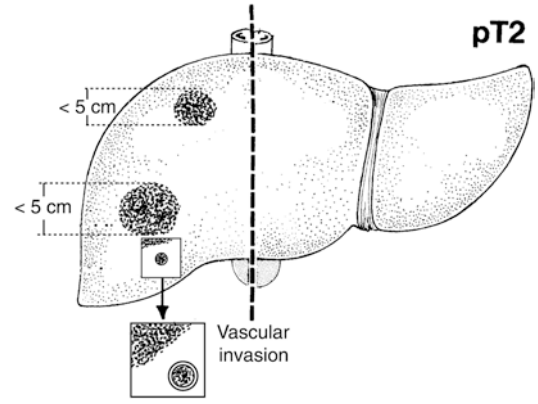

Fig. 11.2 Hepatocellular carcinoma. Stage pT2: solitary tumour >2 cm with vascular invasion, or multiple tumours, none >5 cm. Reproduced, with permission, and adapted from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

Intrahepatic Cholangiocarcinoma

pT1	Solitary tumour without vascular invasion
pT1a	Solitary tumour ≤5 cm without vascular invasion
pT1b	Solitary tumour >5 cm without vascular invasion

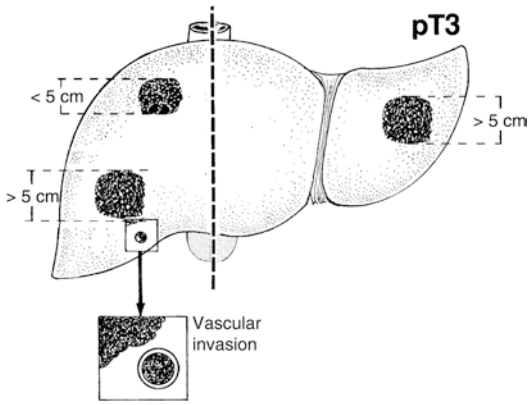

Fig. 11.3 Hepatocellular carcinoma. Stage pT3: multiple tumours, at least one of which is >5 cm. Adapted from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

pT2	Solitary tumour with intrahepatic vascular invasion or
	Multiple tumours with or without vascular invasion
pT3	Tumour perforates the visceral peritoneum
pT4	Tumour involving local extrahepatic structures by direct invasion

Cholangiocarcinoma can be *mass forming* directly invading the adjacent liver parenchyma, *periductal* infiltrating along portal pedicles, or, rarely shows *ductal intraluminal spread. Mixed patterns of invasion also occur.*

Lymphovascular Invasion

Present/absent.

Intra-/ extratumoural.

Note the particular propensity for *vascular invasion by hepatocellular carcinoma* to involve portal tract veins, major branches of portal and hepatic veins and inferior vena cava, ultimately with metastases to lung (47%), adrenal gland (12%) and bone (37%).

Cholangiocarcinoma typically shows *lymphovascular and perineural invasion* with spread to regional lymph nodes, lungs, bone, adrenal gland, kidney, pancreas and peritoneum.

Lymph Nodes

Site/number/size/number involved/limit node/extracapsular spread.

Regional nodes: for hepatocellular carcinoma: hilar, hepatoduodenal ligament, inferior phrenic and caval lymph nodes among which the most prominent are hepatic artery and portal vein nodes. Lymph node metastases are much more common with intrahepatic cholangiocarcinomas compared to hepatocellular carcinoma. Left side: inferior phrenic, hilar (common bile duct, hepatic artery, portal vein and cystic duct) and gastroduodenal nodes. Right side: hilar, periduodenal, peripancreatic lymph nodes. Periaortic, pericaval and coeliac lymph nodes are distant metastases for cholangiocarcinomas (pM1) (Fig. 11.5).

For primary liver cancers a regional lymphadenectomy will ordinarily include 3 or more lymph nodes. In cholangiocarcinoma lymph node metastases are often microscopic and subcapsular requiring careful scrutiny of serial slices of the lymph nodes.

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

Excision Margins

Distances (mm) to the limits of excision of parenchyma, bile ducts and veins. Microscopic involvement (R1) is generally regarded as tumour <1 mm from a surgical margin.

Mucosal dysplasia (BilIN) at the bile duct excision limits.

Other Pathology

Budd Chiari Syndrome

Veno-occlusion or small/large calibre hepatic venular obstruction secondary to malignant infiltration and thrombosis. It leads to hepatic ischaemia.

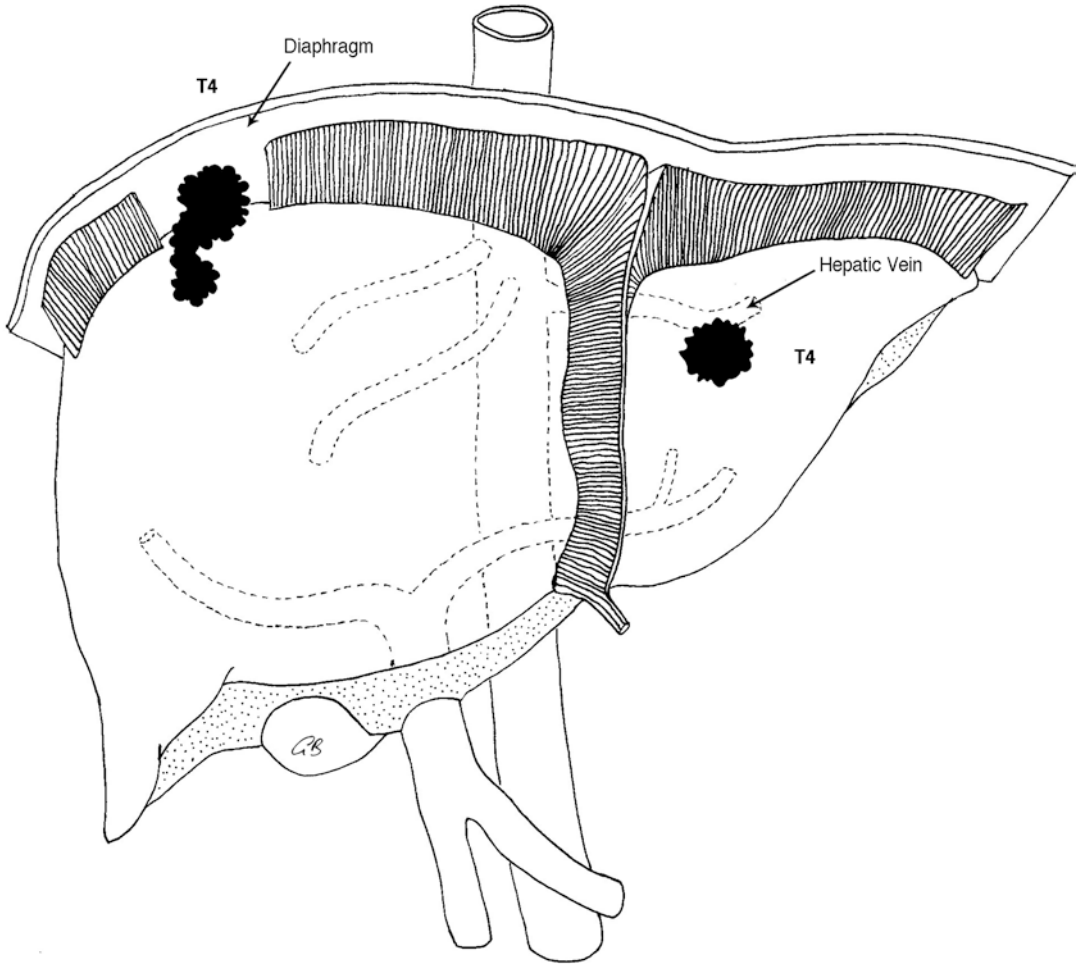

Fig. 11.4 Hepatocellular carcinoma. Stage pT4: single tumour or multiple tumours of any size involving a major branch of the portal vein or hepatic vein, or tumour(s) with direct invasion of adjacent organs other than the gall bladder *or* with perforation of visceral peritoneum

Hepatocellular Carcinoma

Risk factors: Hepatitis B and C (in 50–70% and 20–30% of cases respectively), world-wide. Seropositive hepatitis B patients have *x100 risk* of developing hepatocellular carcinoma.

Cirrhosis: its extent is assessed clinically by the Child-Pugh score and it is present in *60–80%* of cases in the West secondary to viral hepatitis, alcohol, congenital bile duct atresia, alpha-1-antitrypsin deficiency or haemochromatosis.

Small/large cell liver cell change or dysplasia: a microscopic finding and usually <1 mm, there is a strong association between large cell change and hepatitis B surface antigen. *Small cell change* (enlarged nucleus, decreased volume of cytoplasm, nuclear crowding) is regarded as being *premalignant* and a more important risk factor for the development of carcinoma. Liver nodules can be macroregenerative, focal nodular hyperplasia like, or dysplastic.

Dysplastic nodules: arise in a background of cirrhosis and range from 1 mm to 2–3 cm diameter (usually 5–15 mm), ± a fibrous rim, and variable cytoarchitectural atypia (plates 3 cells thick, irregular edges, loss of reticulin, ± cellular dysplasia, arterialisation, increased Ki-67 prolif-

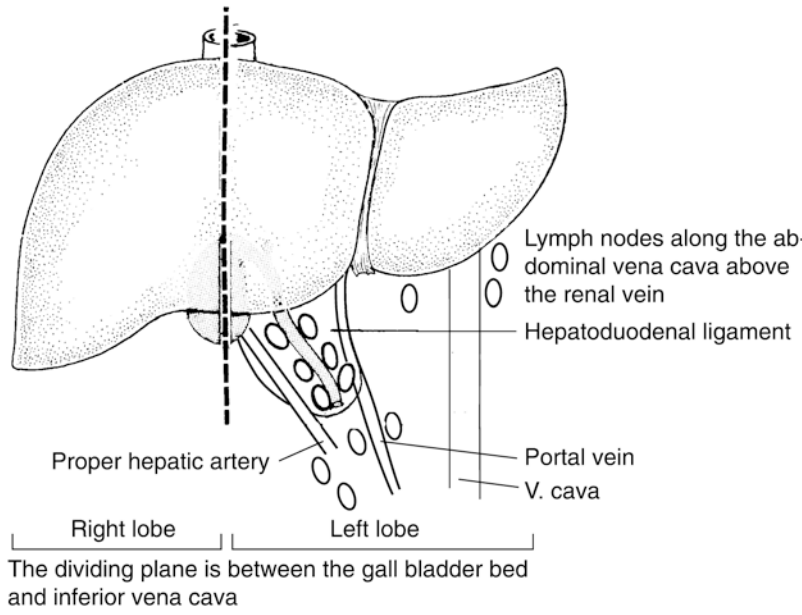

Fig. 11.5 Liver and regional lymph nodes. The regional lymph nodes are the hilar, hepatic (along the proper hepatic artery), periportal (along the portal vein), inferior phrenic, and caval nodes. Adapted from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

eration index). They show a spectrum of changes towards hepatocellular carcinoma with *high-grade dysplastic nodules* particularly significant. Increasingly sophisticated radiological imaging is leading to greater detection of these premalignant dysplastic nodules and small, early hepatocellular carcinomas. Nodules <1 cm require reassessment by 3 to 6 month interval ultrasound. Nodules 1 to 2 cm in size are treated as hepatocellular carcinoma if there is typical hypervascularity and portal venous phase wash out on dynamic imaging (CT/MRI scan or contrast enhanced US). If the radiological investigations are inconsistent, biopsy may be required. Nodules >2 cm in size with characteristic imaging findings do not require biopsy as a presumptive diagnosis of malignancy can be made.

Immunophenotype: hepatocellular carcinoma is positive for Hep Par 1 (86%), Arginase 1 (84%), AFP (37%: high specificity but low sensitivity) and polyclonal CEA/CD10 in bile canalicular pattern. Also CAM 5.2 (but not AE1/AE3), cytokeratins 8, 18 (but not 7, 19), ER/PR. PAS positive cytoplasmic glycogen, intracellular PAS positive globular inclusions, loss of pericellular reticulin. EMA, BerEP4 usually negative. Also positive for glypican 3 (moderate to poorly differentiated hepatocellular carcinoma), HSP70, glutamine synthetase one protein and nuclear Ki-67. Nuclear β catenin can be seen in a subset of hepatocellular carcinoma.

Cholangiocarcinoma

Risk factors: hepatolithiasis/primary sclerosing cholangitis/ulcerative colitis/liver fluke/biliary tree anomaly. Peripheral mass forming lesions also associated with chronic liver disease such as viral hepatitis and cirrhosis. Arises from flat mucosal dysplasia or BilIN—rarely from an intraductal papillary neoplasm (previously designated as biliary papillomatosis) and mucinous cystic neoplasm of liver (biliary cystadenoma). *Treatment is surgical* (partial/total hepatic resection ± liver transplantation) but often *palliative* with stenting or drainage to relieve obstructive jaundice as prognosis is poor and overall *mean survival is < 2 years*.

Immunophenotype: cytokeratins (7, 19), EMA, CEA, CA19–9, mucin positive. Also CAM 5.2 (low molecular weight cytokeratins, as for hepatocellular carcinoma) and AE1/AE3 (including high molecular weight cytokeratins, negative in hepatocellular carcinoma) .

Differential Diagnosis of Hepatic Mass Lesions

Focal nodular hyperplasia: the commonest benign liver nodule after cavernous haemangioma. It most commonly occurs in young to middle aged women, is usually solitary, <5 cm diameter and asymptomatic. It has a radiological and gross central scar devoid of bile ducts with thick walled vessels, marginal ductular proliferation, plates 2 or 3 cells thick, and is a cirrhosis like nodule with adjacent normal parenchyma. It is a vascular anomaly representing an area of hyperplastic liver due to hyperperfusion by an anomalous artery. Oestrogen and synthetic oestrogens exert a trophic effect on these lesions.

Hepatocellular adenoma: majority are solitary, occur in non-cirrhotic liver, 5–15 cm diameter. More common in females compared to males with strong association with oral contraceptive exposure, obesity, diabetes mellitus, androgenic steroid use (β catenin subtype). Well-defined border with background liver, liver cell plates ≥2 cells thick with retention of reticulin pattern and sinusoidal Kupffer cells (CD 68 positive). Minimal atypia, no native portal tracts or central veins, unaccompanied arteries may be seen. Normal bile ducts absent but may show ductules. Foci of haemorrhage, ischaemic change or necrosis may be seen.

Five main subtypes of hepatocellular adenoma recognized and these have specific histologic features:

1. *HNF1α mutated (~35%)*: steatosis.
2. *β catenin mutated (~10%; males > females)*: pseudoacinar architecture, cytological atypia, glutamine synthetase positive. Higher risk of malignant transformation in this subtype.
3. *Inflammatory (~35%)*: sinusoidal dilataion/peliosis, pseudoportal tracts with ductules, inflammatory infiltration.
4. *Sonic Hedgehog (~5%)*: no specific features but may be more likely to bleed.
5. *Hepatocellular adenoma, not otherwise specified (~7%)*: may have haemorrhage and necrosis.

Distinction of hepatocellular adenomas from carcinomas, and subtyping of adenomas is critical and determines clinical management.

Hepatocellular carcinoma: there is often evidence of risk factors, e.g. cirrhosis. Plates are >2–3 cells thick, show loss of reticulin pattern and Kupffer cells, sinusoid capillarisation (CD34), cellular atypia, and look for stromal, capsular and vascular invasion. Serum AFP is markedly elevated in 40% of cases. CT/MRI scan shows the location of the lesion, its extent of invasion and multicentric distribution. *Diagnostic features for hepatocellular carcinoma* include hepatoid cells (polygonal with central nucleus), nuclear/nucleolar enlargement, a trabecular pattern with sinusoidal capillarisation, nuclear pseudoinclusions, bile secretion and an absence of bile duct epithelial and inflammatory cells. Immunostaining may also be helpful e.g. HepPar1, Arginase 1, AFP/polyclonal CEA/CD10/CD34/glypican 3 positive, diffuse glutamine synthetase staining. CK7/19 usually negative in hepatocellular carcinoma. Focal positivity can be seen, may be associated with a worse prognosis and does not indicate a mixed tumour.

Poorly differentiated metastatic carcinoma: specific histological appearance (e.g. small cell carcinoma lung), immunogenicity (e.g. PSA positive) or histochemical feature (e.g. mucin positive—this cannot distinguish secondary adenocarcinoma from primary cholangiocarcinoma).

Problematic cases: sometimes the distinction between a regenerative nodule, adenoma and well-differentiated hepatocellular carcinoma can be difficult and judicious use of immunohistochemistry, careful interpretation and expert opinion is recommended. Rarely subsequent *clinical course* establishes the diagnosis. FNAC of hepatic mass lesions is useful for simple cysts, abscesses and some metastatic cancers (e.g. small cell lung carcinoma) and can be used to diagnose moderately differentiated hepatocellular carcinomas. In most cases core needle biopsy is preferable for distinguishing tumour types, particularly primary hepatocellular neoplasms and regenerative nodules for which assessment of architecture, background liver and patterns of staining,

e.g. glutamine sythnthetase, reticulin, is important. Secondary adenocarcinoma cannot always be distinguished from cholangiocarcinoma and previous history is important, e.g. resection for colorectal carcinoma. Differential cytokeratin expression may help: colorectal cancer (CK20+/CK7-), cholangiocarcinoma (CK7, 19+/CK20 ±).

Prognosis

Treatment of hepatocellular carcinoma depends on *surgical resection ± liver transplantation*, the latter treating the underlying causative disease. Surgical resection is most successful for tumours in non-cirrhotic liver or in cirrhotic liver with adequate reserve. Patients selected for transplant have a 5 year survival rate exceeding 70%. *Local resection of carefully selected patients has a 50% 5 year survival rate but a 70% recurrence rate.* Small tumours (<3 cm) may also be successfully treated by *high radiofrequency ablation (RFA). Adjuvant therapies* are not recommended for hepatocellular carcinoma after transplant, liver resection or local ablation. Patients with advanced disease or may be treated with VEGFR inhibitors such as sorafenib. Prognosis relates to tumour size (> 5 cm), cell type or differentiation, encapsulation, multifocality, high serum AFP levels (> 100 ng/mL at diagnosis), vascular invasion and the presence or absence of a background cirrhosis (an adverse indicator). However, resectabilty is the single most important prognostic factor. *Five year survival is at most 10–15% and more usually about 3%.* The majority die within several months of presentation with liver failure, haemorrhage and infection. Small tumours (<3–5 cm) and variants such as fibrolamellar and pedunculated carcinoma are potentially curable (see below). In cases that are resected, tumour stage and resection margin status are important prognostic indicators. Transarterial chemoembolization, and *radiofrequency ablation* also have roles to play with potential survival benefit and easing of pain in non-operable disease. These modalities can also be applicable to metastatic deposits in the liver, e.g. colorectal carcinoma.

Cholangiocarcinoma is an *aggressive disease* with early invasion, widespread metastases, late presentation and ineffectual treatment modalities. Radical surgery with lymphadenectomy combined with adjuvant or neoadjuvant therapies is the only potentially curative treatment. Intrahepatic cholangiocarcinoma resectabilty rates range from 20 to 70% with a 5-year survival rate after surgery of 20–40%. Mass forming tumours have a more favourable outlook than a periductal infiltrating type lesions. Patients with locally advanced or inoperable disease may be offered systemic chemotherapy or selective internal radiotherapy (SIRT). There is a 2% 5 year survival rate for patients with metastatic disease.

Fibrolamellar Carcinoma

- Non-cirrhotic liver, 1% of hepatocellular carcinoma, 80% occur between the ages of 10–35. Serum AFP not elevated. Molecular studies have identified that the majority of fibrolamellar carcinomas harbour a deletion in chromosome 19 that results in a novel DNAJB1-PRKACA kinase fusion.
- Large eosinophilic cells with prominent nucleoli and cytoplasmic inclusions (pale and hyaline bodies) in a fibrous stroma, microcalcifications. CK7 and CD68 positive, AFP negative. Lymph node metastasis in 65% of cases.
- Potentially resectable. 50% cure rate.

Pedunculated Carcinoma

- Inferoanterior aspect right lobe, up to 1 kg weight.

Minute, Small Encapsulated Carcinoma

- 2–5 cm, encapsulated by fibrous tissue.
- 90–100% 5 year survival if no angio-invasion.

Other Malignancy

Malignant Lymphoma/Leukaemia

- Secondary involvement by *Hodgkin's/non-Hodgkin's malignant lymphoma* (20% of cases at presentation or 55% at autopsy) or *leukaemia* (80% of CLL cases). *Malignant lymphoma is mainly portal* and *leukaemia sinusoidal* but mixed patterns of distribution are common. May form a mass.
- Primary malignant lymphoma is rare but of more favourable prognosis. Solitary/multiple masses or diffuse and *high-grade large B cell* in type. Associated with hepatitis C infection, HIV and primary biliary cirrhosis.

Angiosarcoma

- Cirrhosis, PVC, thorotrast exposure, the *commonest liver sarcoma.* Poor prognosis, most patients die within 1 year.
- Exclude peliosis (well-differentiated angiosarcoma) and primary and secondary carcinoma (poorly differentiated angiosarcoma).
- Growth is typically along vascular structures (sinusoids, vessels) and the liver cell plates. The endothelial cells are atypical and CD31/34 and ERG positive.

Epithelioid Haemangioendothelioma

- Multinodular fibrous masses with a zoned periphery of cords and tube like structures of spindle and epithelioid cells in myxoid stroma and a central hyalinised scar. *Paranuclear cytoplasmic vacuoles, CD31, CD34, ERG or Fli-1 positive*. May be positive for keratins (up to 15%).
- Associated with Budd Chiari syndrome.
- Of *low to intermediate-grade malignancy*: also seen in *skin, lung and bone.*

Kaposi's Sarcoma

- AIDS (15–20% of fatal cases).

Embryonal Sarcoma

- 15% 5 year survival in patients of 6–10 years of age.
- Spindle/stellate/pleomorphic/rounded cells.

Embryonal Rhabdomyosarcoma

- <5 years of age, poor prognosis although changing with emerging neoadjuvant therapies and aggressive surgery. Desmin/myo D1/myogenin positive small cells in a cellular subepithelial cambium layer.
- Arises from major bile ducts near the porta hepatis.

Leiomyosarcoma, Fibrosarcoma

- Rare. More often represents spread from a retroperitoneal primary.
- Exclude sarcomatoid liver carcinoma, and, more commonly secondary sarcoma, e.g. GIST.

Well Differentiated Neuroendocrine Tumour

- Usually represents metastases from gastrointestinal tract or pancreas. Associated with carcinoid syndrome. Detected clinically by octreotide, MRI or CT scans.

Mimics of Malignancy

- *Abscess* secondary to ascending cholangitis, portal pyaemia or septicaemia, *cavernous*

haemangioma, sclerosed haemangioma, inflammatory myofibroblastic or pseudotumour (spindle cells in a storiform pattern, plasma cells), *PEComa/angiomyolipoma* (fat, dystrophic vessels, HMB45 positive spindle cells, coexisting renal lesion(s)), *solitary fibrous tumour* (storiform spindle cells, CD34 positive) *primary hepatic leiomyoma (EBV positive)*.

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Part II

Head and Neck Cancer

- Lip and Oral Cavity Carcinomas
- Oropharyngeal Carcinoma (With Comments on Nasopharynx and Hypopharynx)
- Nasal Cavity and Paranasal Sinus Carcinomas
- Laryngeal Carcinoma
- Salivary Gland Tumours

General Comments

Basic rules are applied to carcinomas arising at various sites in the upper aerodigestive tract (lip, oral cavity, pharynx, nasal cavity, paranasal sinuses and larynx), 95% of which are squamous cell carcinoma.

The surgeon should mark clinically relevant resection margins in the primary specimen and lymph node territories in neck dissections.

Prognosis

Prognosis and prediction of response to adjuvant radiation and/or chemotherapy relate to carcinoma:

Type

- E.g. p16 positive HPV-associated squamous cell carcinoma versus non-HPV associated squamous cell carcinoma in the oropharynx, the former has a much more favourable prognosis to the latter, stage for stage.

Grade

- Not a useful predictor as the majority are moderately differentiated but identify well differentiated lesions that may not metastasise. Base the tumour grade on the most aggressive area (medium magnification field).

Size

- Maximum diameter (mm): macroscopic or microscopic, if the macroscopic assessment is flawed.

Depth

- Maximum depth of invasion (mm) below the luminal aspect of the surface measured from the reconstructed level of the adjacent mucosa. At least one block per cm diameter of the tumour is required and the whole lesion is submitted if less than 1 cm in maximum dimension. For large tumours an estimate of depth may be an approximation.

Invasive front

- A cohesive versus non-cohesive pattern of infiltration. The latter equates to single cells, small groups or multiple thin (<15 cells across) strands of cells at the deep aspect of the tumour. It suggests a greater likelihood of nodal metastasis than an invasive border comprising broad cohesive sheets of tumour cells.

Margins of Excision

>5 mm	Clear
1–5 mm	Close to; also a high risk of recurrence if the invasive edge is non-cohesive or shows vascular invasion
<1 mm	Involved

Incomplete excision is associated with a significantly increased risk of local recurrence. Note also the presence of severe dysplasia at the resection edge—if present to within 5 mm it can also predict likelihood of local recurrence.

Lymphovascular and Perineural Spread

- Vascular invasion is a relatively weak predictor of cervical lymph node metastases. Perineural invasion is an indicator of more aggressive disease, local recurrence and lymph node metastases especially in carcinoma of the vermilion border.

Bone Invasion

- Distinguish erosion of the cortex from infiltration of the medulla. Bone involvement is important for accurate staging.

Lymph Node Status

- The *number identified* and *number involved* at each anatomical level of the neck dissection. The number of involved nodes affects TNM staging—the pattern of involvement influences postoperative treatment. A typical radical neck dissection without previous radiotherapy should yield in excess of 20 lymph nodes. Isolated tumour nodules in the connective tissue are regarded as lymph node metastases unless within 10 mm of the main tumour where they may represent discontinuous extension.
- The *maximum dimension* of the largest nodal deposit is a determinant in TNM staging.
- *Extranodal extension (aka extracapsular spread):* if present prompts the use of adjuvant radiotherapy.
- The significance of *micrometastases* is uncertain but should be counted as involved.
- *Persistent cervical lymph node enlargement* in an older patient is commonly malignant due to either malignant lymphoma or metastases. The latter are generally due to head and neck tumours, particularly mucosal squamous cell carcinomas and thyroid gland carcinoma but cutaneous squamous cell carcinomas and malignant melanoma of skin may metastasise to cervical nodes, usually after some months of surgery to the primary. Occult primary squamous cell carcinoma may be located in the tonsil, base of tongue, pyriform fossa or nasopharynx. Panendoscopy of the upper aerodigestive tract is undertaken to establish and biopsy the primary site and to exclude the possibility (10% of cases) of a synchronous cancer e.g. bronchus or oesophagus. CT, MRI, and CT/PET scans and thyroid ultrasound are also used for investigation and staging. A small minority (10%) can be due to metastatic spread of non-head and neck cancers e.g. breast, bronchus, kidney, prostate, GI tract or testis, usually to Virchow's node in the left supraclavicular fossa. Positivity of the lymph node metastasis for p16 and HPV (by PCR or in situ hybridization) points to an oropharyngeal origin, and EBER positivity a nasopharyngeal primary (Fig. 1).

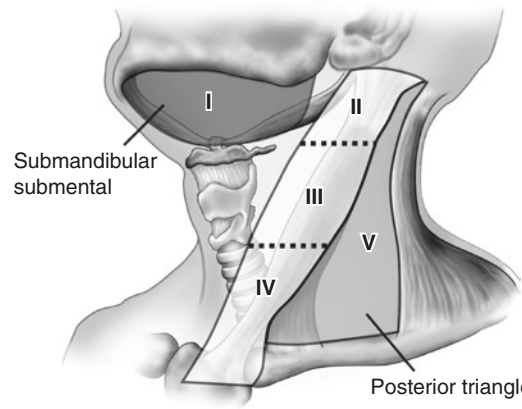

Fig. 1 Lymph node groups in block dissection of the neck. Reproduced, with permission, from *Histopathology Reporting: Guidelines for Surgical Reporting, 2nd ed.,* © 2006, Springer

- *Neck dissection* is either *therapeutic* (to remove known metastases) or *elective* in a clinically negative neck or it may follow tumour persistence after irradiation of the neck. Sentinel node biopsy is increasing available as a method to reduce the frequency of pN0 neck dissections in small intraoral cancers – if the sentinel node is negative for tumour, no further dissection of the neck is performed. Alternatively, a *'watch and wait' policy* is followed or elective *irradiation* of the neck. This decision will depend on the site, size, morphology of the primary tumour, age and fitness of the patient. *Extent of resection* relates to the tumour type, site and expected pattern of spread and is usually more limited (selective neck dissection e.g. Levels I–III or II– IV) in elective cases. Oral and oropharyngeal cancers tend to spread to Levels I - III lymph nodes and rarely to Level V; laryngeal and hypopharyngeal to Levels II and III but never Level I. Positive lymph nodes (≥2) and/or the presence of extranodal extension in the resection warrant *postoperative radiotherapy.* Head and neck cancer specimens should also be interpreted in the light of any previous radiotherapy or chemotherapy due to potential morphological alterations and tumour regression that may make accurate staging more difficult.

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Lip and Oral Cavity Carcinomas

12

Séamus Napier

Introduction

Oral cancers comprise 1–2% of malignancies and are increasing in incidence with ageing populations. Tobacco and alcohol use are multiplicative risk factors for intraoral squamous cell carcinoma; these cancers occur most frequently in patients between 40 and 60 years of age and in males marginally more often than females. Solar exposure is the main factor for carcinoma of the vermilion border and twice as many males as females are affected.

Pathological lesions present either as a lump, ulcer, or white/red mucosal patches that require biopsy to determine their nature. A wide variety of conditions may present as a white/red patch but all require assessment histologically for dysplasia. FNAC (fine needle aspiration cytology) may be of help for submucosal masses and to determine if cervical lymphadenopathy is due to metastatic disease. Preoperative investigation of a mass will include biopsy, plain X-ray, CT scans to assess local spread, bone destruction and for the presence of cervical lymph node metastases; MRI overcomes the amalgam induced scatter artefact that may impede visualization of small intraoral tumours.

Wide local excision is used for tumours of the lip, buccal mucosa, floor of mouth and lateral border of the tongue; wedge excisions of lip may include shave excision of the adjacent unstable vermilion mucosa; the sublingual gland is submitted with anterior floor of mouth lesions. Hemiglossectomy is indicated for deeply infiltrative tongue cancers and subtotal glossectomy for large tumours crossing the midline or involving the posterior one-third that have recurred following front line chemoradiotherapy. Overlying skin may be included with deeply invasive tumours of the buccal mucosa. Periosteum acts as a barrier to bone spread provided there has been no prior radiotherapy to the jaw so that superficial tumours involving gingiva may require mucosal excision only but where it is demonstrated radiologically local resection of the alveolar process (rim resection) or removal of a segment of mandible (hemimandibulectomy) is favoured. Adequate demonstration will require decalcification but only the smallest tumours require the overlying soft tissues to be left in place. Where there is proven or likely lymph node metastasis, neck dissection is performed. Thus treatment of the patient is determined by the tumour site, local spread, lymph node metastases, presence of any other synchronous primary lesion and patient fitness. Lip cancer can present early with relatively good prognosis but intraoral cancer is often late in its presentation with 5 year survival of 50%.

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_12

Gross Description

Specimen

- FNAC/diagnostic (punch/incisional) or wide local excision/ glossectomy (partial/hemi–/total)/wedge (upper/lower lip). May include rim of alveolar bone, cortical bone plate/segment of mandible (hemimandibulectomy) / maxillectomy/neck dissection. Local anatomy usually permits orientation in larger resections but orientation of smaller specimens by the surgeon is crucial in identifying resection margins when assessing clearance.
- Anatomical sites and tissues represented.
- Size (mm)

Tumour

Site

- Vermilion of lip:
  - External upper.
  - External lower.
  - Commissures.

When the skin is involved if >50% of the tumour is within the vermilion border the tumour is designated as lip in origin. Otherwise it is a cutaneous lesion.

- Oral cavity:
  - Buccal mucosa—mucosa of lips/cheek/retromolar areas/buccoalveolar sulci.
  - Upper alveolus and gingiva (upper gum).
  - Lower alveolus and gingiva (lower gum).
  - Hard palate.
  - Tongue—dorsal surface and lateral borders (anterior two thirds); inferior (ventral) surface.
  - Floor of mouth.

The *commonest sites* are, in order of decreasing frequency: lip (90% lower), lateral borders of tongue (35%), anterior floor of mouth (20%) and the soft palate complex (soft palate, anterior pillar of fauces and retromolar areas).

Multifocal lesions are not uncommon *(10%),* both synchronous and metachronous, also at other upper aerodigestive sites e.g. oesophagus. Upper aerodigestive tract panendoscopy is performed prior to surgery to identify occult second primary neoplasm.

Size

- Length × width × depth (mm).
- Depth of invasion; > 5 mm is predictive of cervical lymph node metastases for intraoral carcinomas.
- Measure with reference to reconstructed mucosa surface; estimate as best possible if ulcerated or fungating.

Appearance

- Polypoid/warty/sessile/plaque/ulcerated/fungating.

Histological Type

Squamous Cell Carcinoma

- *90% of cases*.
- Keratinising/non-keratinising.

Variants:

- *Verrucous:* elderly, tobacco usage, broad based exophytic with a pushing deep margin of cytologically bland bulbous processes. Locally invasive (75% 5 year survival).
- *Papillary:* >70% exophytic or papillary malignant epithelial fronds with focal invasion at the base (70% 5 year survival).
- *Spindle cell:* polypoid and pleomorphic, cytokeratin (AE1/3, CK5/6) and p63 not always positive. Surface in situ changes at base of polyp help distinguish from sarcoma, conventional invasive squamous component may be seen. Lymph node metastases as either or both epithelial and spindle cell component. Prognosis (80% 5 year survival) relates to the depth of invasion.
- *Basaloid:* poor prognosis, nests of palisaded basaloid cells with central comedonecrosis,

hyalinised stroma; usually high grade dysplasia on surface. NB: Must be negative for p16 by IHC, otherwise regard as p16 positive HPV-associated SCC.
- *Adenoid squamous:* usual prognosis, acantholytic (pseudoglandular) pattern.
- *Adenosquamous:* poor prognosis, distinct areas of squamous cell carcinoma and bone fide adenocarcinoma (either obvious glands or solid with mucin positive cells).
- *Undifferentiated carcinoma.*

Salivary Gland Tumours

- There is a higher frequency in the oral cavity (particularly tongue, floor of mouth, lower lip) of carcinoma of minor salivary gland origin but pleomorphic adenoma is still the commonest intraoral salivary tumour. Examples include polymorphous adenocarcinoma (previously polymorphous low-grade carcinoma), adenoid cystic carcinoma, mucoepidermoid carcinoma, acinic cell carcinoma.

Small Cell Carcinoma

- A poorly differentiated/high-grade neuroendocrine carcinoma; intraoral primary so rare more likely to be metastasis or component of HPV-associated SCC of oropharynx. *Aggressive*. Chromogranin± /synaptophysin / CD56 /TTF-1 /paranuclear dot CAM 5.2 positive, and a high Ki-67 index.

Malignant Melanoma

- Represents 25% of mucosal malignant melanomas. Japanese/Africans, palate and gingiva, ± adjacent junctional activity. *Prognosis is poor* (< 25% 5 year survival) with local recurrence, lymph node and distant metastases common. This aggressive behavior is recognised in TNM8 by designating all mucosal malignant melanoma as moderately advanced (pT3: mucosal) or very advanced (pT4: beyond the mucosa) disease.
- Sun-exposed lip: desmoplastic melanoma. Shows S100 positivity to distinguish it from fibrous tissue; ± neurotropism. Can be negative for HMB-45 and melan-A.

Metastatic Carcinoma

- *Direct spread*: nasal cavity/maxillary sinus/oropharynx.
- *Distant spread*: breast, bronchus, kidney, thyroid, prostate, gastrointestinal tract, testis, malignant melanoma.

Differentiation

Well/moderate/poor/undifferentiated.

- For conventional squamous cell carcinoma based on cellular atypia, keratinisation and intercellular bridges.
- Usually moderately differentiated. Where differentiation varies prognosis related to the worst area.
- Most *salivary gland tumours* are *graded according to type* e.g. acinic cell carcinoma and polymorphous low-grade adenocarcinoma are low-grade, mucoepidermoid carcinoma has a specific grading system.

Extent of Local Tumour Spread

Growth pattern of invasive front

- Cohesive/non-cohesive. Non-cohesive patterns (small islands or narrow strands with <15 cells) more predictive of regional lymph node metastasis. When mixture of growth patterns, relate to the worst area.

Perineural spread: when beyond the invasive front of the tumour, a predictor of *local recurrence* and more *aggressive disease.*

The TNM8 classification applies to carcinomas of the vermilion and intraoral mucosa of the lip and oral cavity including those of minor salivary glands.

pTis	Carcinoma in situ
pT1	Tumour ≤2 cm in greatest dimension and ≤5 mm in depth
pT2	Tumour ≤2 cm in greatest dimension and >5 mm but ≤10 mm in depth OR tumour >2 cm but ≤4 cm in greatest dimension and ≤ 10 mm in depth
pT3	Tumour >4 cm in greatest dimension OR >10 mm in depth
pT4a	Moderately advanced local disease
	Lip: tumour invades through cortical bone, inferior alveolar nerve, floor of mouth, or skin (of chin or nose).
	Oral cavity: tumour invades through cortical bone, into deep (extrinsic) muscle of tongue (i.e. genioglossus, hyoglossus, palatoglossus, styloglossus), maxillary sinus, skin of face
pT4b	Very advanced local disease
	Lip and oral cavity: tumour invades masticator space, pterygoid plates, or skull base, or encases internal carotid artery.

Superficial erosion alone of bone/tooth socket by gingival primary is not sufficient to classify as T4a. Distinguish tumour extending to or overlying bone from gross erosion or radiographic destruction of bone—the periosteum can act as a barrier to local invasion. (Figs. 12.1, 12.2, 12.3, 12.4 and 12.5).

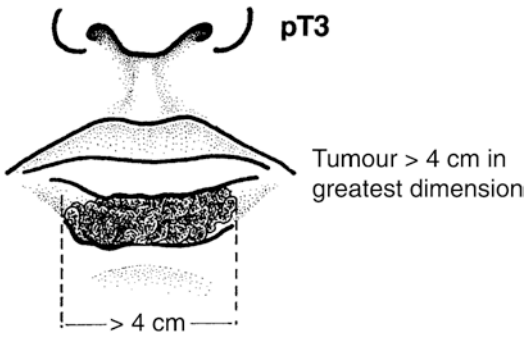

Fig. 12.1 Lip and oral cavity carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

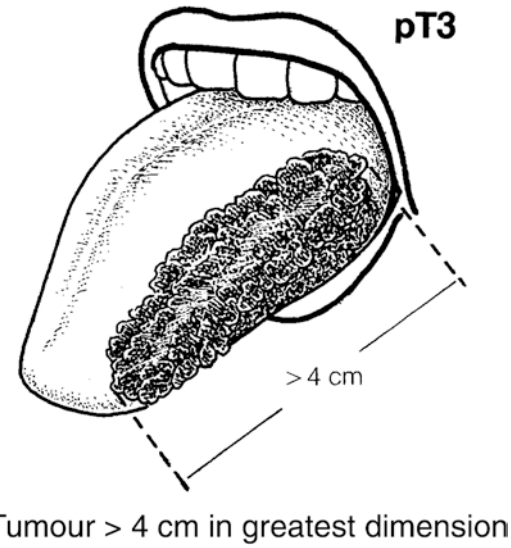

Fig. 12.2 Lip and oral cavity carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

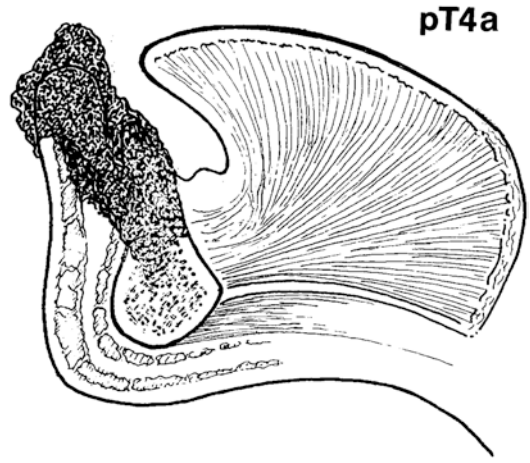

Fig. 12.3 Lip and oral cavity carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

Lymphovascular Invasion

Present/absent.

Vascular invasion is a relatively weak predictor of cervical lymph node metastases.

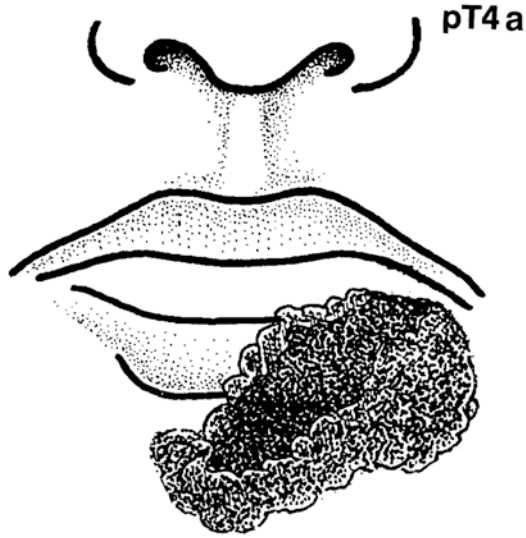

Fig. 12.4 Lip and oral cavity carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

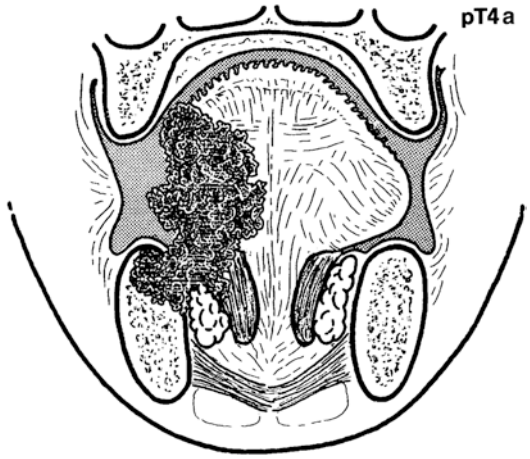

Fig. 12.5 Lip and oral cavity carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

Lymph Nodes

Metastases are mainly lymphatic with lip and floor of mouth tumours often metastasizing to Levels I or II and tongue cancers to Levels II or III. Lymph node metastases may also undergo cystic degeneration with viable cells at the tumour margin only. Residual paucicellular masses of keratin with a foreign body reaction may result from radiation therapy. These features should be borne in mind on FNAC of cervical lymph nodes. A common differential diagnosis of a neck mass in a younger to middle aged patient is branchial cyst.

Size/number involved/size of metastatic deposit/extranodal extension (extracapsular spread).

Regional nodes: cervical.

Level I:	Submental, submandibular
Level II:	Upper jugular
Level III:	Middle jugular
Level IV:	Lower jugular
Level V:	Posterior triangle

Radical and modified radical neck dissections are classed as comprehensive neck dissections. Radical neck dissections comprise Levels I-V and three non-lymphatic structures—the sternocleidomastoid muscle, internal jugular vein and spinal accessory nerve; modified radical neck dissections include Levels I-V but preserve one, two or all three non-lymphatic structures. Selective neck dissection specimens represent harvesting of specific groups of nodes, such as Levels I-III or II-IV. There is no agreed minimum number of lymph nodes that must be recovered but it is questionable whether a specimen that generates fewer than 8 nodes for examination can be classed as a comprehensive neck dissection.

pN0	No regional lymph node metastasis
pN1	Metastasis in a single ipsilateral node ≤3 cm without extranodal extension
pN2	Metastasis in: (a) Ipsilateral single node ≤3 cm with extranodal extension OR > 3 cm to 6 cm without extranodal extension (b) Ipsilateral multiple nodes ≤6 cm without extranodal extension (c) Bilateral, contralateral nodes ≤6 cm without extranodal extension

pN3	(a) Metastasis in a lymph node >6 cm without extranodal extension (b) Metastasis in a lymph node >3 cm with extranodal extension OR multiple ipsilateral or any contralateral or bilateral node(s) with extranodal extension

Extranodal extension (previously known as *extracapsular extension*) increases the risk of local recurrence and distant spread. Metastasis is usually to *ipsilateral lymph nodes* but lesions of the floor of mouth, the tip or posterior one-third of the tongue, or those that cross the midline can cause contralateral lymph node involvement. Up to *30% of patients* with excision of the tongue and floor of mouth will have *clinically occult cervical lymph node metastases*.

Excision Margins

Distances (mm) of tumour to the nearest painted excision margins.

Epithelial dysplasia/carcinoma in situ present at excision margins. Tumour or high grade mucosal dysplasia at or near (< 5 mm) a margin is a *predictor of local recurrence*. Proximity of the surgical margin is often determined by the site, size and pattern of spread of the carcinoma.

The ideal therapeutic margin is 10 mm but often resections only afford 2–3 mm which is compounded by a peritumoural zone of dysplastic or hyperplastic mucosa.

Other Pathology

Predisposing Factors

Gender (M:F, 2:1), smoking and alcohol are the main risk factors.

Clinical

Leukoplakia (i.e. a white plaque).

Classified as:

- Homogenous (i.e. thin, smooth, uniformly white)
- Non-homogeneous (i.e. thick, fissured, granular, speckled with red aka *erythroleukoplakia*)

Erythroplakia (i.e. a uniformly red plaque).

Leukoplakia and erythroplakia are clinical terms and should not be used in histopathology reports. The diagnosis requires exclusion of all other causes of white/red lesions, e.g. lichen planus/lichenoid reaction, chronic candidal infection, frictional keratosis. Risk factors for the increased likelihood of development of SCC in oral leukoplakia include: females, patients who have never used tobacco, lesions on the tongue, single large lesions, non-homogeneous lesions, the presence of dysplasia. Note that carcinoma can also arise from lesions with no dysplasia. Most clinical examples of leukoplakia do not show histological dysplasia.

Histological: Dysplasia

- Mild/moderate/severe OR low grade/high grade. The presence of dysplasia signifies an increased risk of development of SCC. There is a positive correlation of the degree of dysplasia with the likelihood of invasive SCC at presentation and also with the likelihood of the development of SCC in the future, but neither is a consistent one: the risk for all leukoplakia is estimated at 0.5% per year, cases with dysplasia is approximately 5% per year but some patients with high grade dysplasia never develop SCC.

Squamous cell carcinoma is positive for p63 and a range of cytokeratins including AE1/3, CK5/6, and 34βE12 but not CK20 and CAM 5.2. Routine p16 staining as a surrogate marker of HPV infection is not done in oral cavity and lip carcinoma.

Prognosis

Prognosis relates to tumour site, size, stage and histological grade. Histological type of squa-

mous cell carcinoma can also influence prognosis: *better* (verrucous, papillary), *usual* (spindle cell, adenoid squamous) and *worse* (basaloid, adenosquamous).

Lip	90% 5 year survival
Anterior tongue	60% 5 year survival (20% with large tumours and positive lymph nodes)
Floor of mouth	40% 5 year survival.

Treatment is by surgery and/or radiotherapy supplemented by chemotherapy depending on the site and stage of disease.

Other Malignancy

Malignant Lymphoma

- Waldeyer's ring is the commonest site of oropharyngeal *non-Hodgkin's malignant lymphoma (NHL)* but it can arise in gingiva, buccal mucosa and palate. Most are *B-cell and diffuse* although others e.g. T-cell NHL and anaplastic large cell lymphoma do occur. Some are MALT derived and associated with extranodal malignant lymphomas elsewhere e.g. stomach, whereas others are of nodal type e.g. mantle cell. Prognosis relates to histological type, grade and stage of disease. There is an increasing incidence with HIV.

Leukaemia

- Direct infiltration or ulceration with opportunistic infection e.g. herpes simplex virus, cytomegalovirus. Gingival involvement is seen in *4% of acute myeloid leukaemia.*

Plasmacytoma/Myeloma

κ, λ light chain restriction. Look for evidence of systemic disease e.g. serum immune paresis and monoclonal gammopathy, Bence-Jones proteinuria, radiological lytic bone lesions.

Odontogenic/Osseous Cancers by Direct Spread

Sarcoma

- Leiomyosarcoma: cheek, maxillary bone.
- Rhabdomyosarcoma: embryonal—children soft palate/infratemporal fossa, desmin/myo D1/myogenin positive.
- Synovial sarcoma: young adults, cheek, tongue, palate.

Granular Cell Tumour

- A benign nerve sheath tumour composed of S100 positive granular cells. Commonly, overlying pseudoepitheliomatous hyperplasia can mimic squamous cell carcinoma and a careful search for granular cells in the biopsy subepithelial connective tissues must be made.

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13 Oropharyngeal Carcinoma (with Comments on Nasopharynx and Hypopharynx)

Séamus Napier

Introduction

Human Papilloma Virus (HPV) infection has a major role in the aetiology of the majority of oropharyngeal carcinomas arising in tonsil and posterior third of tongue (aka base of tongue), particularly in never-smokers, usually males (4:1) aged 45–55 years. Tobacco and alcohol are the major risk factors in cancers of the soft palate and hypopharynx, male patients aged 40-70 years being the most at risk. Post cricoid carcinoma is associated with Plummer-Vinson syndrome in older females (iron deficiency anaemia, achlorhydria, upper oesophageal web). Nasopharyngeal carcinoma, particularly the non-keratinising (undifferentiated) squamous cell carcinoma, is strongly associated with Epstein-Barr Virus (EBV). It has a biphasic age presentation (15–25 years, 60–90 years), the keratinising squamous cell variant (not EBV related) occurring in the older age group.

Depending on the anatomical site of the lesion, patients present with dysphagia, deafness, cranial nerve palsy, hoarseness or cervical lymphadenopathy. Investigation is by endoscopy with biopsy, and cervical lymph node fine needle aspiration cytology (FNAC) with or without ultrasound guidance to obtain a diagnosis. When presenting as cervical lymph node metastasis, primary tumours may be small or deeply placed thwarting detection by superficial biopsies, especially in the posterior third of tongue. CT and MRI scans are used to assess local tumour spread and metastasis to the neck or elsewhere and have largely replaced chest X-ray to detect concurrent lung cancer. PET CT is reserved for cases where a mucosal primary lesion has not been detected (occult primary) or in patients with advanced locoregional disease who are deemed at high risk of distant metastasis.

Extent of resection depends on the tumour site, stage, lymph node spread, fitness of the patient and any concurrent tumour that is detected by endoscopy or cross-sectional imaging. Tonsillectomy and/or robotic mucosectomy of posterior third of tongue is submitted electively as a possible site of an occult primary in FNAC proven cervical lymph node metastasis. Occult carcinoma in the post nasal space may occasionally be detected by "blind biopsy". IgA or IgG antibody titres to Epstein-Barr virus (EBV) may help in assessing efficacy of treatment and subsequent local recurrence of nasopharyngeal carcinoma.

Gross Description

Specimen

- FNAC/biopsy (punch, incisional)/tonsillectomy/adenoidectomy/transoral mucosal resection/

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_13

pharyngectomy/laryngectomy/pharyngolaryngooesophagectomy ± neck dissection.
- Size (mm) and number of fragments.
- Size (mm) and weight (g) of tonsil.
- Length of larynx/pharyngooesophagectomy.
- Lymph node levels of neck dissection.

Tumour

Site

Oropharynx: lies between the soft palate and the epiglottis. Most tumours arise in the posterior third of tongue and the tonsil.

Components:

1. Anteriorly	Posterior third tongue, vallecula, oral aspect of epiglottis
2. Laterally	Tonsil, tonsillar fossa and pillars
3. Posteriorly	Posterior wall to the level of the hyoid bone
4. Superiorly	Inferior surface soft palate, uvula

Nasopharynx (post nasal space): superiorly from the skull base and delineated inferiorly by the superior surface of the soft palate; fossa of Rosenmüller is in the lateral wall.

Hypopharynx: comprises the pyriform sinuses lateral to the aryepiglottic folds of the larynx, postcricoid mucosa anteriorly covering the back of the larynx, the lateral and posterior walls from the level of the hyoid bone funneling into oesphagus. The majority (75%) of tumours arise in the pyriform fossa (Fig. 13.1).

Size

- Length × width × depth (mm) or maximum dimension (mm).

Tumours of the posterior tongue, tonsil, pyriform fossa and nasopharynx may be small when presenting with cervical lymph node metastasis.

Appearance

- HPV-associated cancers may exhibit global enlargement of the tonsil or base of tongue, ulceration of the mucosal surface or may be inconspicuous macroscopically.
- Non-HPV-associated oropharyngeal and hypopharyngeal cancers will resemble those of the lip and oral cavity: polypoid/warty/sessile/ulcerated/fungating.
- Nasopharyngeal cancers are not usually resected except as salvage.

Histological Type

Squamous Cell Carcinoma

- *85% of cases.*
- Divided into HPV-associated/non-HPV associated.
- NB: site-dependent: HPV-associated SCC arise in the reticulated cryptal epithelium of palatine and lingual tonsils.
- If non-HPV associated, usually keratinizing (see Chap. 12: Lip and Oral Cavity Carcinomas).
- *HPV associated cancers* are usually centred deep to the mucosal surface and often exhibit a basaloid morphology with little nuclear pleomorphism, abrupt keratinisation with minimal stromal reaction. Although histologically distinctive, demonstration of overexpression of the tumour suppressor gene protein product p16 and/or high-risk HPV by in-situ hybridization are important adjuncts to diagnosis. Large tumours of the soft palate, tongue or buccal mucosa may extend to include the posterior third of tongue or tonsil and raise the possibility of origin there—exploration of possible association with HPV may be required.

Variants of HPV-associated include papillary, adenosquamous, sarcomatoid, basaloid and even

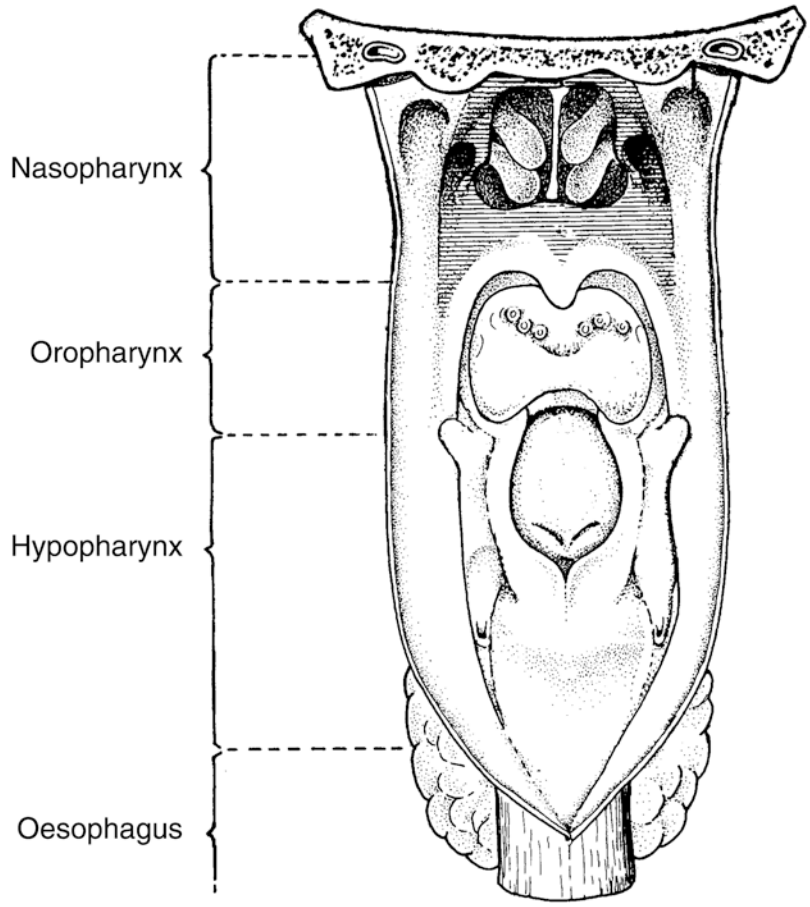

Fig. 13.1 Pharynx. Reproduced, with permission, from *TNM Atlas: Illu.strated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

small cell patterns but these tend to have similar clinical behavior and prognosis.

Undifferentiated Carcinoma

- Preferred term: non-keratinising (undifferentiated) squamous cell carcinoma, *15% of cases.*
- Usually *nasopharynx* where it is *EBV related* but also in the tonsil associated with HPV; prominent component lymphocytes percolating through the tumour (lymphoepithelial carcinoma). CK5/6, p63, EBER positive; S100 highlights numerous dendritic cells within the tumour nests.

Salivary Gland Tumours

- Adenoid cystic carcinoma is commonest but other patterns arise from the accessory glands, including pleomorphic adenoma, polymorphous adenocarcinoma (previously polymorphous low-grade carcinoma), mucoepidermoid carcinoma, acinic cell carcinoma.

Malignant Melanoma

- Primary (exceptionally rare) or secondary, *poor prognosis*. *Aggressive behaviour* is recognised in TNM8 by designating malignant

melanoma as moderately advanced (pT3: mucosal) or very advanced (pT4: beyond mucosa) disease.

Neuroendocrine Carcinoma

- Very rare.
- Likely to be HPV-associated especially if SCC component or invade directly from the supraglottic larynx.
- Usually poorly differentiated (small cell/large cell) carcinoma but also well/moderately differentiated neuroendocrine carcinoma (previously designated carcinoid/atypical carcinoid tumour) when extension from the larynx is more likely. Small cell and large cell patterns can co-exist and have relatively *poor prognosis* but nodal metastasis may also occur with the better differentiated forms. They are variably positive with chromogranin, synaptophysin and CD56.

Metastatic Carcinoma

- Breast, bronchus, kidney, thyroid, prostate, GI tract.

Differentiation

- Not relevant for HPV-associated squamous cell carcinoma that tends to exhibit lesser degrees of keratin production and pleomorphism
- For non-HPV associated squamous cell carcinoma, grading is based on cellular atypia, keratinisation and intercellular bridges. When differentiation varies relate to the worst area.
- Nasopharyngeal carcinoma is frequently of undifferentiated type (strongly associated with EBV) but may exhibit keratinizing or basaloid forms (less frequently EBER-positive).
- Most *salivary gland tumours* are *graded according to type* e.g. acinic cell carcinoma and polymorphous low-grade adenocarcinoma are low-grade. Mucoepidermoid carcinoma has a specific grading system.

Extent of Local Tumour Spread

Border: pushing/infiltrative.

- Not relevant for HPV-associated squamous cell carcinoma that tends to invade as broad strands.
- For non-HPV associated tumours, an infiltrative pattern of invasion at the deep aspect of the carcinoma is of adverse prognostic value.

Lymphocytic reaction: prominent/sparse.

The TNM8 classification of carcinomas divides cancers on their association with HPV, determined albeit incompletely by p16 positivity using immunohistochemistry.

Oropharynx, p16 Positive

pT1	Tumour ≤2 cm in greatest dimension
pT2	Tumour >2 cm but ≤4 cm in greatest dimension
pT3	Tumour >4 cm in greatest dimension or extension to lingual surface of epiglottis (except of lesions that arise in the posterior third of tongue)
pT4	Tumour invades any of: larynx, deep/extrinsic muscle of tongue (genioglossus, hyoglossus, palatoglossus, styloglossus), medial pterygoid, hard palate, mandible, lateral pterygoid muscle, pterygoid plates, lateral nasopharynx, skull base, or encases carotid artery.

Oropharynx if Negative or Untested for p16, and Hypopharynx

pT1	Tumour ≤2 cm in greatest dimension (hypopharynx—and/or limited to one subsite)
pT2	Tumour >2 cm but ≤4 cm in greatest dimension (hypopharynx—and more than one subsite or adjacent site, without fixation of hemilarynx)

pT3	Tumour >4 cm in greatest dimension or extension to lingual surface of epiglottis (hypopharynx—or with fixation[a] of hemilarynx and/or extension to oesophagus)
pT4a (oropharynx)	Moderately advanced local disease. Tumour invades any of: larynx, deep/extrinsic muscle of tongue (genioglossus, hyoglossus, palatoglossus, styloglossus), medial pterygoid, hard palate, and mandible
pT4b (oropharynx)	Very advanced local disease. Tumour invades any of: lateral pterygoid muscle, pterygoid plates, lateral nasopharynx, skull base, or encases carotid artery.
pT4a (hypopharynx)	Moderately advanced local disease. Tumour invades any of: thyroid/cricoid cartilage, hyoid bone, thyroid gland, oesophagus, central compartment soft tissue (including prelaryngeal strap muscles and subcutaneous fat)
pT4b (hypopharynx)	Very advanced local disease. Tumour invades any of: prevertebral fascia, encases carotid artery, or invades mediastinal structures.

[a]*Fixation of hemilarynx is diagnosed endoscopically by immobility of the arytenoid or vocal cord. pT4a and pT4b represent moderately advanced and very advanced local disease, respectively* (Figs. 13.2 and 13.3)

Nasopharynx[a]

pT1	Tumour confined to nasopharynx or extends to oropharynx and/or nasal cavity without parapharyngeal involvement
pT2	Tumour with extension to posterolateral parapharyngeal space OR infiltration of medial pterygoid/lateral pterygoid or paravertebral muscles
pT3	Tumour invades bony structures of skull base, cervical vertebra, pterygoids and/or nasal sinuses
pT4	Intracranial extension and/or into cranial nerves, hypopharynx, orbit, parotid gland and/or infiltration beyond the lateral surface of the lateral pterygoid muscle

[a]*Histological confirmation of nasopharyngeal carcinoma staging is often not possible as it is not usually treated with resection.* (Figs. 13.4 and 13.5)

Lymphovascular Invasion

Present/absent.

Perineural spread: predictor of *local recurrence* and more *aggressive disease*. Vascular invasion is a relatively weak indicator for cervical lymph node metastases.

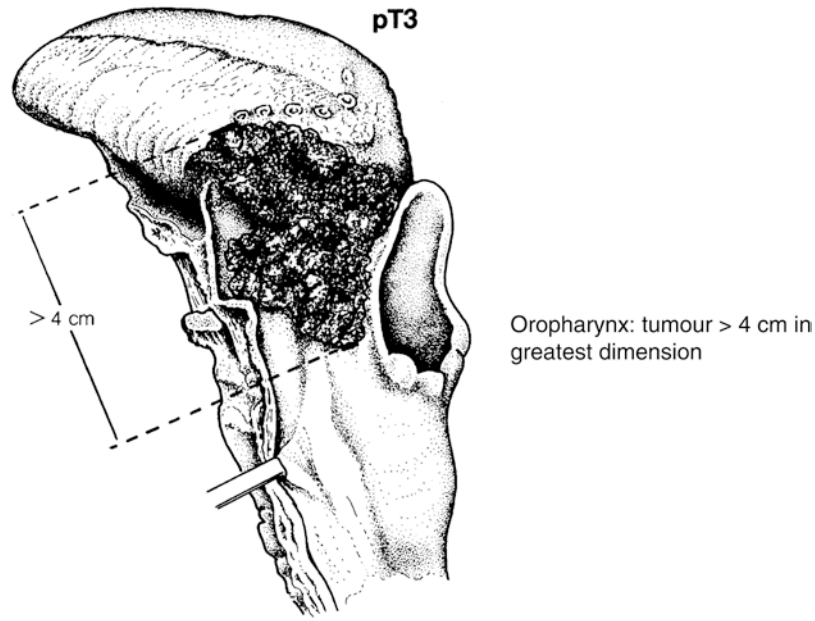

Fig. 13.2 Oropharyngeal carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

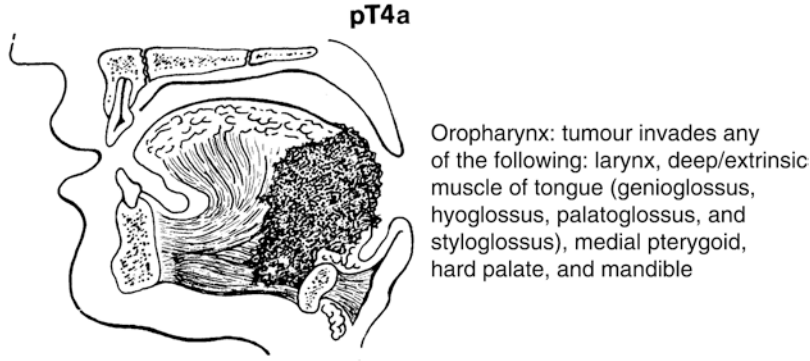

Fig. 13.3 Oropharyngeal carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

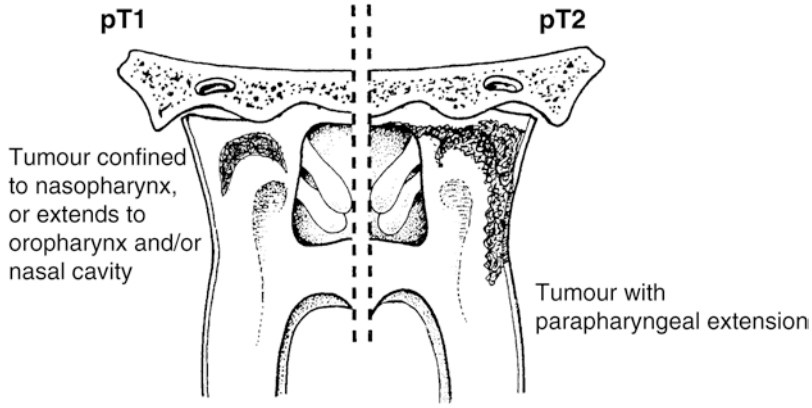

Fig. 13.4 Nasopharyngeal carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

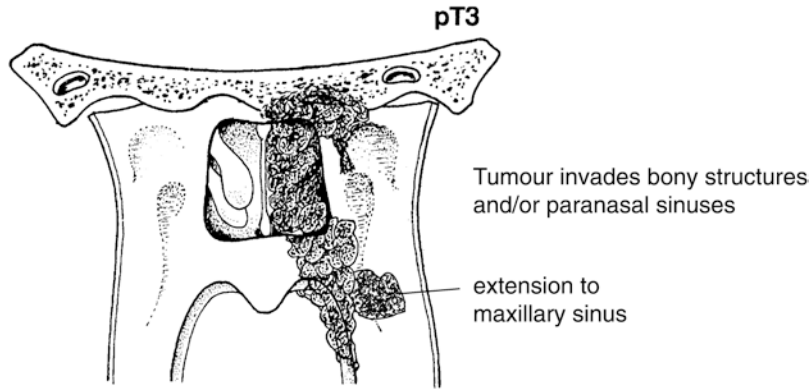

Fig. 13.5 Nasopharyngeal carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

Lymph Nodes

Metastases are mainly lymphatic. Lymph node metastases often undergo cystic degeneration and residual paucicellular masses of keratin with a foreign body reaction may follow chemoradiotherapy, changes that should be borne in mind on FNAC of cervical lymph nodes. Caution should be exercised when reporting a cystic neck mass in a middle aged

or older patient as branchial cyst until the possibility of a cystic metastasis from oropharynx can be excluded. Similarly, p16 positivity by immunohistochemistry is not exclusive to HPV-associated oropharyngeal squamous carcinoma and the possibility of origin in lung, oesophagus, skin and female genital tract merits consideration in core needle or excision biopsies of cervical lymph nodes with metastatic tumour.

Regional nodes: cervical; midline nodes are considered ipsilateral number involved/size of metastatic deposit/extranodal extension (extracapsular spread).

Level I:	Submental, submandibular (rarely involved)
Level II:	Upper jugular
Level III:	Middle jugular
Level IV:	Lower jugular
Level V:	Posterior triangle (not uncommon in nasopharyngeal tumours)

Radical and modified radical neck dissections are rarely performed for pharyngeal tumours as Level I nodes are almost never involved by metastasis. Selective neck dissection specimens represent harvesting of specific contiguous groups of nodes, such as Levels II-IV or III, IV & V. Retropharyngeal nodes are often involved and are usually not resectable. *Extranodal extension* (previously known as *extracapsular extension*) increases the risk of local recurrence and distant spread. Staging is subdivided based on positive p16 staining by immunohistochemistry.

Oropharynx, p16 Positive

pN0	No regional lymph node metastasis
pN1	Metastasis in a single unilateral node ≤6 cm
pN2	Metastasis in bilateral or contralateral node(s), all ≤6 cm
pN3	Metastasis in a lymph node(s) > 6 cm

Oropharynx if Negative or Untested for p16, and Hypopharynx

pN0	No regional lymph node metastasis
pN1	Metastasis in a single ipsilateral node ≤3 cm without extranodal extension
pN2	Metastasis in: (a) Ipsilateral single node ≤3 cm with extranodal extension OR > 3 cm to 6 cm without extranodal extension (b) Ipsilateral multiple nodes ≤6 cm without extranodal extension (c) Bilateral, contralateral nodes ≤6 cm without extranodal extension
pN3	(a) Metastasis in a lymph node >6 cm without extranodal extension (b) Metastasis in a lymph node >3 cm with extranodal extension OR multiple ipsilateral or any contralateral or bilateral node(s) with extranodal extension

Nasopharynx

pN0	No regional lymph node metastasis
pN1	Unilateral cervical nodal metastasis, and/or uni−/bilateral retropharyngeal nodal metastasis ≤6 cm, above the caudal border of the cricoid cartilage
pN2	Bilateral cervical nodal metastasis ≤6 cm, above the caudal border of the cricoid cartilage
pN3	Cervical metastasis in nodes >6 cm OR below the caudal border of the cricoid cartilage

Presentation in up to 10% of cases is with *upper cervical lymph node metastases* mimicking malignant lymphoma. Cervical metastases of nasopharyngeal carcinoma may also show a necrotising granulomatous nodal reaction. Carcinomas of the base of the tongue and oropharynx tend to metastasise to the retropharyngeal nodes and rarely (6%) the posterior triangle of neck.

Excision Margins

Distances (mm) to the nearest longitudinal and mucosal excision margins. Tumour or mucosal dysplasia at or near (< 5 mm) a lateral resection margin may predict local recurrence.

Due to anatomical limitations on resection, margins are usually only several millimetres.

Other Pathology

Concurrent carcinoma bronchus, mouth, larynx, oesophagus: 10–15% of cases. Endoscopic assessment may be required further to any indicators on staging scans.

Primary treatment of oropharyngeal and hypopharyngeal carcinoma: surgical ± neoadjuvant chemoradiotherapy. Small oropharyngeal lesions can be locally excised or treated with radiotherapy (80-90% 5 year survival) but larger lesions are treated by primary chemoradiation with salvage surgery reserved for recurrent disease. HPV related cases are particularly chemoradiation responsive.

Primary treatment of nasopharyngeal carcinoma is radiotherapy with concurrent chemotherapy for advanced disease: most nasopharyngeal carcinomas are of non-keratinising (undifferentiated) type comprising enlarged tumour cells with a prominent nucleolus and an accompanying lymphoid stroma characterized by prominent tumour infiltrating lymphocytes. The tumour is strongly associated with *EBV infection*, which can be shown by EBER (EBV encoded RNA) in-situ hybridisation techniques. *Serum EBV viral capsid antigen IgA levels* are also useful for *monitoring the effects of treatment* and in *detecting recurrence*. Markers are helpful in distinguishing carcinoma (cytokeratins, p63, EMA) from high-grade malignant lymphoma (CD45) and malignant melanoma (S100, HMB-45, Melan-A). Hypopharynx may also be submitted with a laryngectomy specimen due to local invasion from a laryngeal carcinoma.

Prognosis

Prognosis of pharyngeal carcinoma varies and relates to tumour site, stage and histological subtype. HPV-associated oropharyngeal squamous carcinoma has an 85-90% 5 year survival rate; patients with non-HPV associated lesions are twice as likely to die of tumour. Small lesions can be adequately treated by complete local excision but there are *20–40% 5 year survival rates* for cancers of the posterior tongue, tonsil and palate.

Stage is the most powerful prognostic indicator for *nasopharyngeal carcinoma*, especially volume of primary tumour: 5 year survival in 90% of cases at Stage I and II with chemoradiotherapy and 10 year survival rates of 40-70%. The keratinising squamous cell variant in the older age group is believed to be of worse prognosis—although less likely to involve nodes, it tends to be bulkier. Cancers with lower cervical rather than upper cervical lymph node metastases fare worse.

Primary non-surgical treatment is recommended for small *hypopharyngeal tumours* but bulky locally advanced disease usually responds best to surgery; extensive submucosal spread of tumour requires wide margins. Five-year survival is poor overall (30%) but small tumours have 60% 5 year survival.

Other Malignancy

Malignant Lymphoma

- Diffuse large B cell non-Hodgkin's malignant lymphoma.
- Mantle cell lymphoma: intermediate-grade and aggressive.
- Angiocentric T cell lymphoma: aggressive.

Plasmacytoma/Myeloma

κ, λ light chain restriction. Look for evidence of systemic disease e.g. serum immune paresis and monoclonal gammopathy, Bence-Jones proteinuria, radiological lytic bone lesions.

Sarcoma

- Children: embryonal rhabdomyosarcoma (subepithelial cellular cambium layer; deeper myxoid zone; desmin/myo D1/myogenin positive).
- Young adults: alveolar rhabdomyosarcoma; Ewing's sarcoma; pharynx, palate.

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Nasal Cavity and Paranasal Sinus Carcinomas

14

Séamus Napier

Introduction

Sinonasal cancer accounts for about 3% of head and neck malignancies and in general affects patients aged 55–65 years. About two thirds are of epithelial origin, with others including malignant melanoma and malignant lymphoma. Some also represent direct spread from adjacent local sites, e.g. primary tumours in the oral cavity or pituitary and meninges.

Clinical presentation is with nasal obstruction, rhinorrhoea, epistaxis, deafness, proptosis, epiphora or facial pain. Investigation is by endonasal endoscopy with biopsy. Plain X-ray, CT and MRI scans can demonstrate and stage a soft tissue mass and any bone destruction that is present. Spread intracranially or into the orbit and relationship to the cranial nerves and carotid artery can also be determined. Most cancers arise in the maxillary sinus.

Tumour can be removed piece-meal by functional endoscopic sinus surgery (FESS) or by formal resection. Nasal septal lesions are excised via a lateral rhinotomy. Medial maxillectomy is the commonest procedure for low-grade tumours of the lateral nasal cavity, or maxillary, ethmoid and frontal sinuses e.g. transitional papilloma. These specimens are usually received as multiple fragments 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. 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. 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 may necessitate exenteration of the orbit. Concomitant neck dissections are usually not indicated unless there is proven metastatic disease.

Gross Description

Specimen

- FNAC (fine needle aspirate cytology)/biopsy/resection e.g. piece-meal, rhinectomy, maxillectomy, craniofacial resection/neck dissection.
- Number of fragments, size (mm) of largest
- Components of maxillectomy or craniofacial resection (e.g., nasal floor, antral walls, zygomatic arch, nasal process), size (mm)
- Levels of neck dissection, if present.

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_14

Tumour

Site

Nasal cavity

- Septum
- Floor
- Lateral wall
- Vestibule

Maxillary sinus.
Ethmoid sinus.
Tumours of sphenoid sinus and frontal sinuses are rare; maxillary and ethmoid sinuses are the commonest tumour sites.

Size

- Length × width × depth (mm) or maximum dimension (mm).
- An indicator of disease volume, allows correlation with imaging studies. Not used for TNM staging.

Appearance

- Usually a non-descript fleshy mass, focally necrotic but may be papillary/mucoid/pigmented (Figs. 14.1 and 14.2).

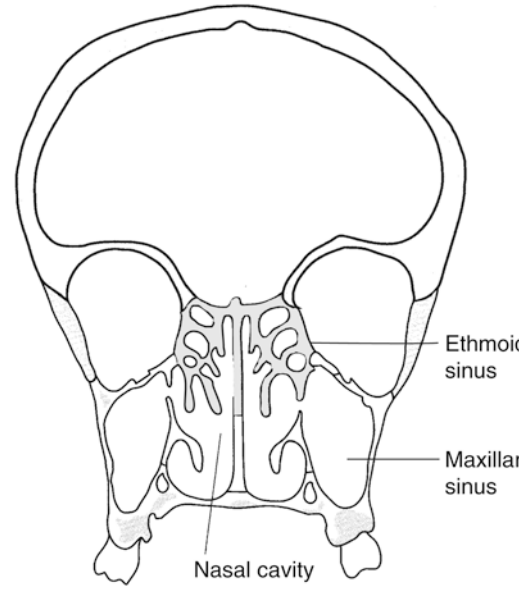

Fig. 14.1 Paranasal sinuses. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

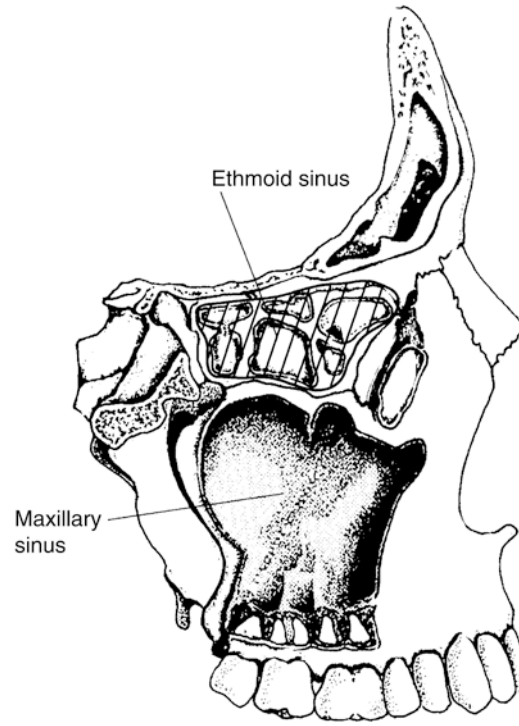

Fig. 14.2 Paranasal sinuses. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

Histological Type

Squamous Cell Carcinoma

- 85% of cases of sinonasal carcinoma.
- Usually non-keratinising SCC, commonest in the nasal cavity proper and the maxillary sinus; broad ribbons, pushing border, no keratinization, nuclear atypia variable, frequent mitoses and necrosis.
- Conventional squamous cell carcinoma NOS (known as keratinizing SCC), alone or in combination with non-keratinising type; may also include inverted type (Schneider) papilloma as presumed precursor

Rare variants: HPV-related carcinoma with adenoid cystic-like features (resembles solid variant adenoid cystic carcinoma, p16 positive, confirm presence of high-risk HPV with ancil-

lary techniques, surface dysplasia often present), spindle cell carcinoma (elderly males, polypoid mass, dysplasia), lymphoepithelial carcinoma (rare, high in nasal cavity, EBER positive, exclude extension from nasopharyngeal carcinoma).

NB: Lesions of the nasal vestibule resemble conventional squamous cell carcinoma NOS of cutaneous pattern.

Sinonasal Undifferentiated Carcinoma (SNUC)

- Clinically distinctive: rapid onset of symptoms of destructive nasal or ethmoid sinus mass; usually but not always older patients
- Nests, lobules and sheets of cytologically atypical cells with prominent apoptotic and mitotic activity
- Relatively uniform nuclei, varying amounts of cytoplasm
- Minimal neuroendocrine differentiation but absent squamous or glandular differentiation.
- Cytokeratin (CK7, 8, 18) positive but CK5/6 and p40 negative; p16 positive.

NUT Carcinoma

- Rare, highly *aggressive* carcinoma of the midline axis in young adults, often females.
- Undifferentiated basaloid cells with focal abrupt squamous cell differentiation; no association with HPV or EBV.
- Diagnosis by balanced translocation of NUT gene, commonly t(15;19) with BRD4 gene; nuclear positivity with NUT antibody, also p63, p40 and cytokeratins.

SMARCB1-Deficient Carcinomas

- A rare, incompletely described group of highly *aggressive* carcinoma of sinonasal regions (usually centred in the ethmoid sinus) with abnormalities in SWI/SNF pathways (important in chromatin integrity/transcription), middle aged adults
- Undifferentiated basaloid and/or rhabdoid cells with only minimal glandular, squamous or neuroendocrine differentiation; no association with HPV or EBV, biallelic loss/inactivation of genes associated with the SWI/SNF pathway, readily identifiable by negative staining on immunohistochemistry
- Diagnosis by IHC: negative for INI1 (SMARCB1) or BRM (SMARCA2).

Adenocarcinoma

- Salivary, intestinal or non-intestinal in type.
- *Salivary*: most forms of salivary gland tumour have been reported.
- Intestinal: colonic (commonest), papillary, solid or mucinous (rarest) patterns. Occurs with environmental exposure (males, leather/wood dust exposure) or without (females) in the ethmoid sinus or nasal cavity; locally aggressive and recurrent but nodal metastasis in 10%. Prognosis relates to the type (papillary 80% best, goblet cell pattern of mucinous worst <20% 5 years).
- Non-intestinal adenocarcinoma: low-grade (nasal cavity and ethmoid sinuses) unencapsulated glandular and/or papillary, bland cytology, recurrences in one-third, rare metastasis; or, high-grade (maxillary sinus) solid or sheets, much nuclear pleomorphism; metastasis in one third, 20% 3 year survival. Recently described renal cell-like carcinoma with large polygonal clear cells—very good prognosis.

Neuroendocrine Carcinoma

- Rare, usually a high-grade neuroendocrine carcinoma, characteristic morphology of small cell or large cell types, of ethmoid sinuses or nasal cavity in middle aged or elderly men
- Differential diagnosis includes metastasis and olfactory neuroblastoma
- Paranuclear dot CAM5.2/synaptophysin/CD56 positive, TTF-1 variable and a high Ki-67 index

Metastatic Carcinoma

- Breast, bronchus, kidney, thyroid, prostate, GI tract carcinomas (NB: primary intestinal type adenocarcinoma is the main differential)

Differentiation

Non-keratinising SCC is not graded

- Keratinising squamous cell carcinoma based on cellular atypia, keratinisation and intercellular bridges
- Adenocarcinoma based on the architectural pattern, cellular constituents and nuclear pleomorphism.

Extent of Local Tumour Spread

Border: pushing/infiltrative. The microscopic pattern of invasion does not have a consistent prognostic value; high grade adenocarcinomas infiltrate submucosa and bone.

Lymphocytic reaction: prominent/sparse.

Desmoplastic stromal response; minimal/marked.

The TNM8 classification applies only to carcinomas.

Maxillary Sinus (Figs. 14.3, 14.4, and 14.5)

pT1	Tumour limited to the antral mucosa
pT2	Tumour causes erosion or destruction of bone (including extension into hard palate and/or middle nasal meatus), except posterior antral wall and pterygoid plates
pT3	Tumour invades any of: posterior wall maxillary sinus, subcutaneous tissues, floor/medial wall orbit, pterygoid fossa, ethmoid sinuses
pT4	Tumour invades any of:
pT4a	Anterior orbital contents, skin of cheek, pterygoid plates, infratemporal fossa, cribriform plate, sphenoid or frontal sinuses.
pT4b	Orbital apex, dura, brain, middle cranial fossa, cranial nerves (other than maxillary division trigeminal nerve V2), nasopharynx, clivus.

Fig. 14.3 Maxillary sinus carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

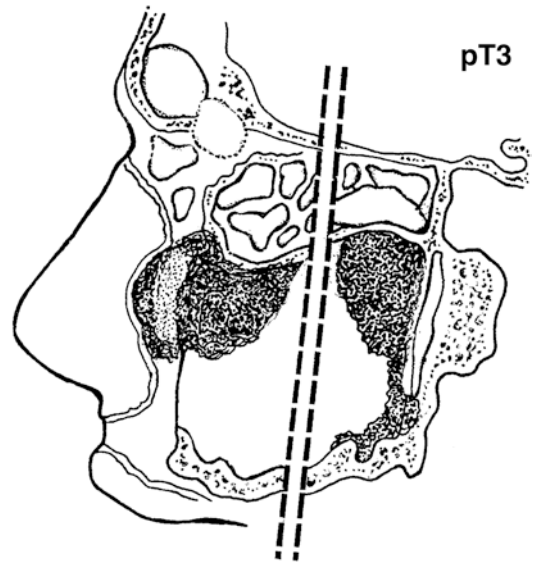

Fig. 14.4 Maxillary sinus carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

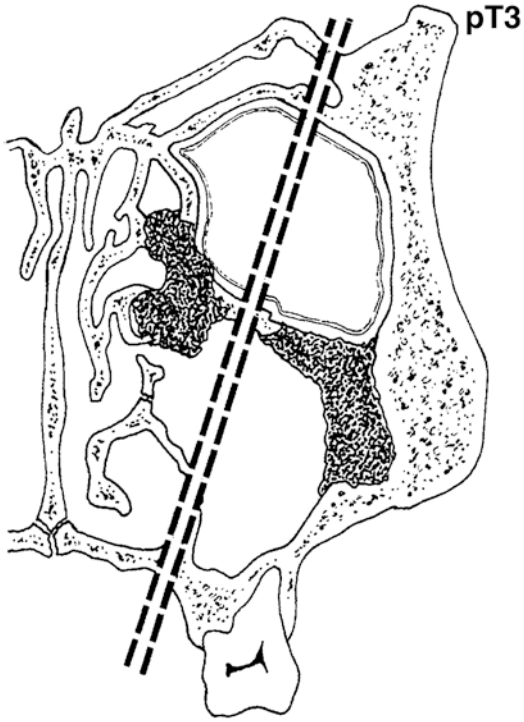

Fig. 14.5 Maxillary sinus carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

Nasal Cavity and Ethmoid Sinus

pT1	Tumour confined to one subsite of nasal cavity or ethmoid ± bone invasion
pT2	Tumour involves two subsites in a single site or extends to involve an adjacent site within the nasoethmoidal complex ± bone invasion.
pT3	Tumour extends to medial wall or floor of orbit, maxillary sinus, palate, or cribriform plate.
pT4	Tumour invades any of:
pT4a	Anterior orbital contents, skin of nose or cheek, minimal extension to anterior cranial fossa, pterygoid plates, sphenoid or frontal sinuses
pT4b	Orbital apex, dura, brain, middle cranial fossa, cranial nerves other than V2, nasopharynx, clivus.

Invasion of bone includes only involvement of the spongiosa, not the cortex.

Presentation is not infrequently late with bone destruction already present (Figs. 14.6 and 14.7).

Lymphovascular Invasion

Present/absent.

Vascular invasion is a relatively weak indicator for cervical lymph node metastases but may point to an increased tendency to local recurrence. *Perineural spread*: when beyond the invasive front of the tumour, a predictor of *local recurrence* and more *aggressive disease.*

Lymph Nodes

Metastases are mainly lymphatic usually metastasizing to Levels II or III.

Number involved/size of metastatic deposit/extranodal extension (previously known as extracapsular spread).

Regional nodes: cervical. The external nose and anterior nasal cavity usually drain to the Level I cervical lymph nodes, the rest of the nasal cavity and paranasal sinuses to the Level II and III lymph nodes.

Level I:	Submental, submandibular
Level II:	Upper jugular
Level III:	Middle jugular
Level IV:	Lower jugular
Level V:	Posterior triangle

Selective neck dissection specimens represent harvesting of specific groups of nodes, such as Levels I-III or II-IV. There is no agreed minimum number of lymph nodes that must be recovered but it is questionable whether a specimen that generates fewer than 8 nodes for examination can be classed as a neck dissection (as opposed to lymph node sampling).

pN0	No regional lymph node metastasis
pN1	Metastasis in a single ipsilateral node ≤3 cm without extranodal extension

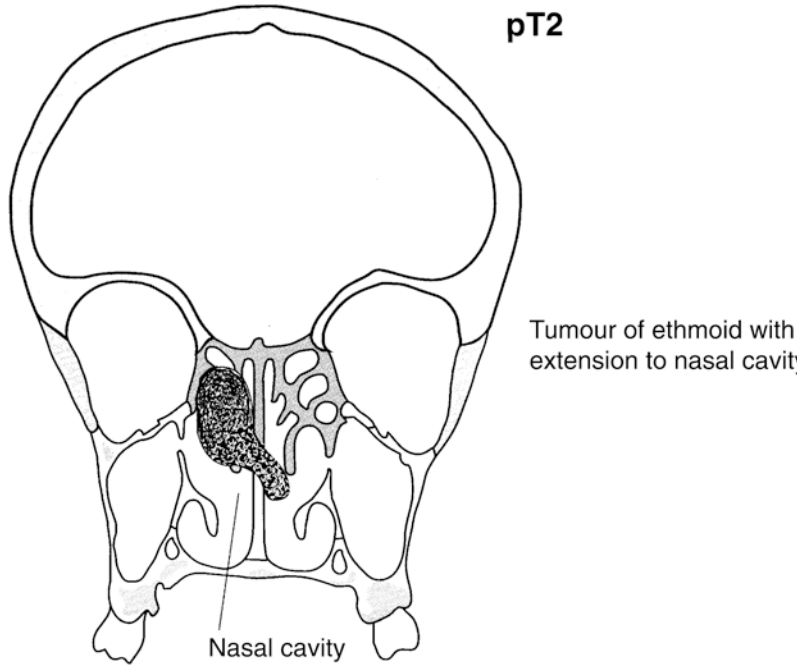

Fig. 14.6 Ethmoid sinus carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

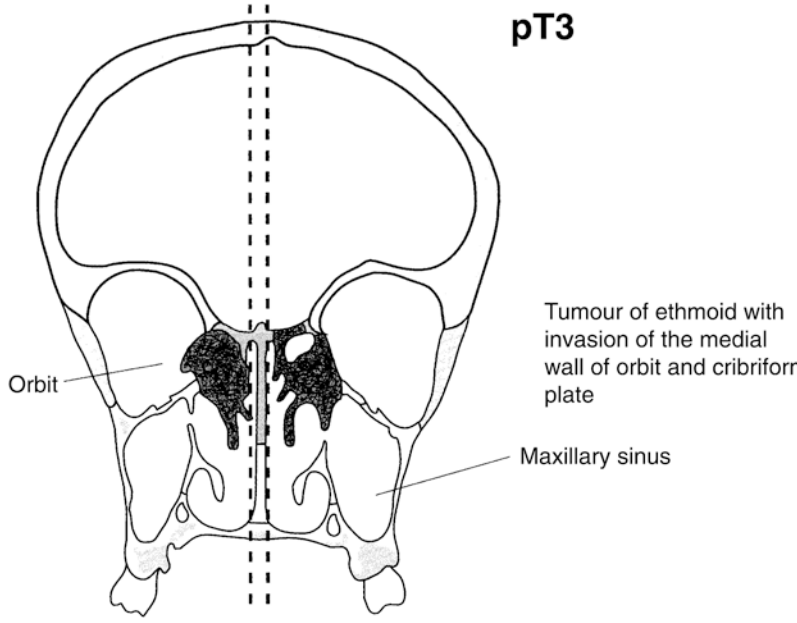

Fig. 14.7 Ethmoid sinus carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

pN2	Metastasis in: (a) Ipsilateral single node ≤3 cm with extranodal extension OR > 3 cm to 6 cm without extranodal extension (b) Ipsilateral multiple nodes ≤6 cm without extranodal extension (c) Bilateral, contralateral nodes ≤6 cm without extranodal extension

pN3	(a) Metastasis in a lymph node >6 cm without extranodal extension (b) Metastasis in a lymph node >3 cm with extranodal extension OR multiple ipsilateral or any contralateral or bilateral node(s) with extranodal extension.

Extranodal extension (previously known as *extracapsular spread*) increases the risk of local recurrence and distant spread.

Excision Margins

Distance (mm) to the nearest painted excision margin.

Tumour at or close to (< 5 mm) a margin is a predictor of *local recurrence* and may necessitate adjuvant therapy. Dysplasia is uncommon except as part of the mucosa adjacent to an invasive carcinoma.

Due to anatomical limitations on resection, margins are usually only several millimetres.

Other Pathology

Relative incidence: malignant tumours are more common than benign in the paranasal sinuses with the reverse being the case in the nasal cavity. Equivalent nasal cavity tumours have a better prognosis. About 55% of sinonasal malignancies occur in the maxillary sinus, 35% in the nasal cavity and 9% in the ethmoid sinus. Most lesions (85%) are squamous cell carcinoma and its variants with adenocarcinoma representing only 5–10% of cases. Most cases are *locally advanced at presentation* and *local recurrence is common* despite surgery and radiotherapy. About 10% of patients present with lymph node metastases. Immunohistochemical markers are essential in differentiating the more poorly differentiated carcinomas from malignant melanoma and malignant lymphoma.

Primary sinonasal undifferentiated neoplasms comprise a broad range of epithelial and non-epithelial lesions. The *epithelial group* includes: poorly differentiated squamous cell carcinoma, nasopharyngeal type undifferentiated carcinoma, SNUC, NUT carcinoma, SMARCB1-deficient carcinoma, neuoendocrine carcinoma and high grade adenocarcinomas. The *non-epithelial group* includes: malignant melanoma, neuroectodermal tumours (olfactory neuroblastoma, Ewing's sarcoma/PNET), haematopoietic tumours (malignant lymphoma, plasmacytoma/myeloma), sarcomas (rhabdomyosarcoma, synovial sarcoma), teratocarcinosarcoma, germ cell tumours. Small crushed biopsies may render diagnosis challenging.

Prognosis

Prognosis is strongly related to tumour type and thereafter to stage.

Other Malignancy

Malignant Lymphoma

- *Diffuse large B cell lymphoma* is commonest.
- *Extranodal NK/T cell lymphoma* (nasal type):
  - Destructive nasal/midline tumour with large areas of zonal necrosis and vasculocentric/angiodestructive patterns. It comprises polymorphic tumour cells (CD3/CD2/CD56) which may be hard to recognise amongst the inflammatory infiltrate. EBV associated (EBER by in-situ hybridization); usually of poor prognosis but indolent presentations recognised. It can show pseudoepitheliomatous hyperplasia mimicking squamous cell carcinoma. Differential includes granulomatous polyangiitis (previously known as Wegener's granulomatosis: neutrophil microabscesses, giant cells with smudgy nuclei, focal collagen necrosis more common than vasculitis or granulomas) .

Plasmacytoma/Myeloma

- κ, λ light chain restriction and clinical evidence of myeloma e.g. elevated ESR, immune paresis, monoclonal gammopathy, Bence-Jones proteinuria, radiological lytic bone lesions.

Sarcomas

Rhabdomyosarcoma (embryonal, alveolar), angiosarcoma, fibrosarcoma, undifferentiated pleomorphic sarcoma (previously known

as malignant fibrous histiocytoma), leiomyosarcoma, malignant peripheral nerve sheath tumour, chondrosarcoma, synovial sarcoma, biphenotypic sinonasal sarcoma, sinonasal glomangiopericytoma, ameloblastoma

- About 40% of rhabdomyosarcomas occur in the head and neck and 20% in the nasal cavity and nasal sinuses. It is the commonest sarcoma in childhood and of embryonal type with a better prognosis than the alveolar type (overall 40–50% 5 year survival). May present as a fleshy polyp; desmin, myogenin, myoD1 positive; confirm with molecular testing.

Olfactory Neuroblastoma, Ewing's Sarcoma/Primitive Neuroectodermal Tumour (PNET)

- *Olfactory neuroblastoma*: an uncommon neuroectodermal malignancy arising in a broad age range from the olfactory membrane of the upper nasal cavity. Small round blue cell tumour aggregates (lobules/nests ± rosettes) in a vascular stroma with or without calcification, positive for neuroendocrine markers chromogranin, synaptophysin, NSE, neurofilament, GFAP; sustentacular cells are positive for S100. *Hyams' grade* (Grade 1- Grade 4) is of *prognostic significance* and is based on the degree of lobular architecture, mitotic activity, cellular pleomorphism, neurofibrillary stroma and necrosis. Kadish staging: tumours confined to the nasal cavity (Kadish A) have a reasonable prognosis while those in the nasal cavity and paranasal sinuses (Kadish B) an intermediate outlook. Extranasal/paranasal extension (Kadish C) and visceral lesions are of poor prognosis. Treatment is by a combination of *surgery* and *radiotherapy*. Overall *5 year survival is 50–60% with a tendency for late recurrences*. Its immunophenotype aids in distinction from the differential diagnoses of malignant melanoma, malignant lymphoma, plasmacytoma/myeloma and embryonal/alveolar rhabdomyosarcoma.
- *Ewing's sarcoma/PNET*: very rare, small densely packed immature-looking cells in sheeted pattern and geographic necrosis, FLI-1 positive and variably neuroendocrine marker positive (NSE, PGP 9.5, neurofilament); confirm with molecular techniques. Potentially responsive to *high-dose irradiation*, *multi-drug chemotherapy and surgery (60–70% 5 year survival)* but very large tumours and those that do not respond to chemotherapy unfavourable.

Chordoma

- A locally destructive midline low-grade malignancy located in the clivus extending into the nasopharynx or nasal cavity; derived from notochordal remnants with characteristic vacuolated physaliphorous cells positive for S100, cytokeratins, EMA. Outcome depends on complete removal.

Pituitary neoplasms
Meningiomatous neoplasms
Malignant teratoma and other germ-cell type neoplasms

- Very rare, young adult men; distinguish embryonal carcinoma, yolk sac tumour and choriocarcinoma from seminoma and teratocarcinosarcoma (older patients; combination of carcinoma, sarcoma and immature neuroepithelial components without malignant germ cell elements)

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Laryngeal Carcinoma

15

Séamus Napier

Introduction

Laryngeal squamous cell carcinoma comprises 1–2% of malignancies but is slowly decreasing in incidence with cessation of tobacco use. Tobacco and alcohol use are synergistic risk factors; these cancers occur most frequently in patients between 50 and 60 years of age and in males at least twice as often than in females. Persistent hoarseness is an early finding in glottic cancers but a late finding in supraglottic and subglottic forms; occasionally with dysphagia, dyspnea, stridor or cervical lymphadenopathy. Investigation is by fibre-optic endoscopy passed through an anesthetized nose or indirectly visualized using a laryngeal mirror held against the soft palate. Biopsies can be readily obtained under general anaesthesia using a laryngoscope and operating microscope. Endoscopy of the upper aerodigestive tract is performed for malignant disease for staging purposes, to identify occult second primary neoplasms, and assess surgical resectability. CT and MRI scans are used to stage the tumour and cervical lymph node enlargement can be followed by ultrasound guided fine needle aspiration cytology (FNAC) to establish if there are metastases. Tumour stage and fitness of the patient determine the appropriate choice of treatment i.e. radiotherapy, laser resection, local excision, laryngectomy, or neck dissection. Laryngectomy may also accompany a pharyngectomy for cancer of the hypopharynx.

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Gross Description

Specimen

- Biopsy/transoral laser resection/hemi-/partial or total laryngectomy/neck dissection. Local anatomy usually permits orientation in larger resections but orientation of smaller specimens by the surgeon is crucial in identifying resection margins when assessing clearance.
- Size (mm) of largest, if fragments
- Anatomical sites and tissues represented
- Length of larynx (from base of cricoid to tip of epiglottis)
- Other tissues, e.g., lobe of thyroid; levels of neck dissection, tracheostomy skin.

Tumour

Site

Supraglottis: from the tip of the epiglottis, both surfaces of the epiglottis, laryngeal aspect of aryepiglottic folds, arytenoid region, false vocal cords and ventricles.

D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_15

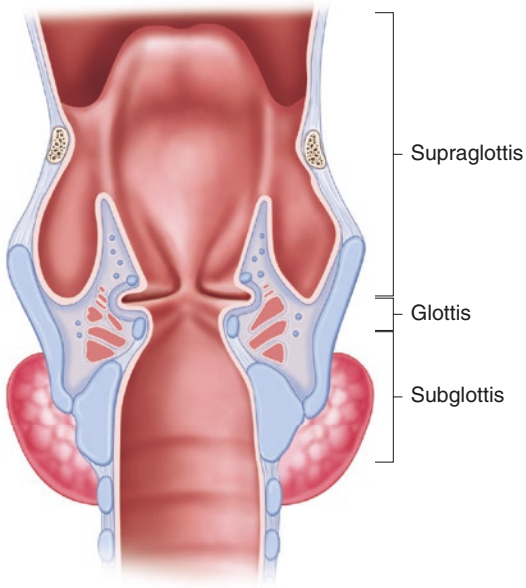

Fig. 15.1 Larynx. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

Glottis: both true vocal cords including anterior and posterior commissures.

Subglottis: from the lower border of the true cords to the first tracheal cartilage (Fig. 15.1).

Glottis and supraglottic commonest sites but marked geographic variations: continental Europe: 60% supraglottic, 25% glottic;

British Isles, North America and Scandinavia: 60% glottic, 25% supraglottic.

Subglottis the least common site (10%).

Transglottic tumours span all three regions of the larynx (5%).

Size

- Length × width × depth (mm) or maximum dimension (mm).
- Tumour size is the main contributor to pathological stage as it is an indicator of disease extent. The maximum depth of invasion is subordinate to the nature of the tissue planes involved. Invasion of or through thyroid or cricoid cartilages or the elastotic membranes that join them is an important staging criterion.

Appearance

- Polypoid/warty/plaque-like/ulcerated/fungating

Histological Type

Squamous Cell Carcinoma

- *90% of cases.*
- Keratinising/non-keratinising.
- Prognosis is *better* (verrucous/papillary), *usual* (spindle cell/adenoid squamous) or *worse* (basaloid/adenosquamous)

Variants:

- *Verrucous:* broad based exophytic and "church-spire" hyperkeratosis with a pushing deep margin of cytologically bland bulbous processes arising in the glottis. Locally invasive, rarely metastatic; 70% 5 year survival. If contains a focus of conventional squamous cell carcinoma, manage as conventional SCC.
- *Papillary:* >70% papillary or exophytic fronds, covered by malignant type epithelium with focal invasion at the base. Better prognosis (70% 5 year survival).
- *Spindle cell:* polypoid, conventional squamous cell element may be present; immunohistochemical staining for cytokeratin not always positive. Distinguish from sarcoma and bizarre post-irradiation granulation tissue.
- *Basaloid:* poor prognosis, nests of basaloid cells with peripheral palisading and central comedonecrosis. Presents with more extensive disease but is more radiosensitive than other squamous cell carcinoma subtypes.
- *Adenoid squamous:* usual prognosis, acantholytic (pseudoglandular) pattern.
- *Adenosquamous:* poor prognosis, mixed differentiation squamous cell carcinoma and adenocarcinoma (either obvious glands or solid with mucin positive cells).
- *Lymphoepithelial carcinoma:* very rare, absence of squamous cell or glandular differ-

entiation; not usually associated with EBV, propensity for cervical lymph node and distant metastases.

Neuroendocrine Carcinomas

- Usually supraglottic larynx; middle aged and older men in particular.
- Distinctive morphological patterns with positive staining for CD56 and at least one of chromogranin/synaptophysin; TTF1 variable. Ki-67 index is not used in grading.
- Well differentiated: previously known as *carcinoid tumour*. Low mitotic count (<2/10 hpfs).
- Moderately differentiated: previously known as *atypical carcinoid tumour* with characteristic spread to locoregional lymph nodes.
- Poorly differentiated: *small cell/large cell carcinoma* 60-90% of which present with distant metastases. High Ki-67 index and mitotic count.

Moderately differentiated and poorly differentiated large cell neuroendocrine carcinomas are commoner in the larynx than well differentiated neuroendocrine carcinomas and they present with advanced disease and have *high recurrence and mortality (50-70%)*.

Adenocarcinoma

- *Salivary type:* most types of salivary gland tumour but adenoid cystic carcinoma is the commonest form.
- *Adenocarcinoma of no special type*
- *Amphicrine carcinoma* with both neuroendocrine and exocrine differentiation patterns

Metastatic Carcinoma

- *Direct spread:* oropharynx/hypopharynx; thyroid.
- *Distant spread:* breast, bronchus, kidney, thyroid, prostate, GI tract, malignant melanoma. Usually associated with disseminated disease.

Differentiation

Well/moderate/poor

- For conventional squamous cell carcinoma based on cellular atypia, keratinisation and intercellular bridges.
- Usually moderately differentiated.
- Most *salivary gland tumours* are *graded according to type*

Extent of Local Tumour Spread

Growth pattern of invasive front

- Degree of keratinisation, nuclear pleomorphism, pattern of growth and peritumour inflammatory response: the higher the "invasive front grading" score, the poorer the outcome

Perineural spread: when beyond the invasive front of the tumour, a predictor of *local recurrence, nodal metastasis* and *poorer survival.*

Glottic and subglottic tumour is best demonstrated by horizontal slices to demonstrate its anatomical relationships, tumour in the supraglottis is better seen with radially orientated slices in the vertical plane.

The TNM8 classification applies only to carcinomas.

Supraglottis

pT1	One subsite, no fixation of larynx (i.e., normal vocal cord mobility)
pT2	Mucosa of more than one adjacent subsite of supraglottis or glottis or adjacent region outside the supraglottis (e.g., medial wall of pyriform fossa); without fixation
pT3	Tumour limited to larynx with vocal cord fixation and/or invasion of post cricoid area, pre-epiglottic tissues, paraglottic space, and/or erosion of inner cortex of thyroid cartilage
pT4a	Through thyroid cartilage and/or into tissues beyond larynx, e.g., trachea, soft tissues of neck including deep/extrinsic muscle of tongue (genioglossus, hyoglossus, palatoglossus, styloglossus), strap muscles, thyroid, oesophagus
pT4b	Invades prevertebral space, mediastinal structures, encases carotid artery

Glottis

pT1	Limited to vocal cord(s), normal cord mobility (a) One cord only (b) Both cords
pT2	Into supraglottis and/or subglottis and/or with impaired cord mobility
pT3	Limited to larynx with vocal cord fixation and/or invasion into paraglottic space and/or erosion of inner cortex of thyroid cartilage
pT4a	Through thyroid cartilage and/or into tissues beyond larynx, e.g., trachea, soft tissues of neck including deep/extrinsic muscle of tongue (genioglossus, hyoglossus, palatoglossus, styloglossus), strap muscles, thyroid, oesophagus
pT4b	Invades prevertebral space, mediastinal structures, encases carotid artery

Subglottis

pT1	Limited to subglottis
pT2	Extends to vocal cord(s) with normal/impaired mobility.
pT3	Limited to larynx with vocal cord fixation
pT4a	Through thyroid cartilage and/or into tissues beyond larynx, e.g., trachea, soft tissues of neck including deep/extrinsic muscle of tongue (genioglossus, hyoglossus, palatoglossus, styloglossus), strap muscles, thyroid, oesophagus
pT4b	Invades prevertebral space, mediastinal structures, encases carotid artery (Fig. 15.2)

Lymphovascular Invasion

Present/absent.

Vascular invasion is associated with a high probability for cervical lymph node and/or distant metastasis, peristomal recurrence and poor survival.

Perineural invasion indicates *more aggressive disease* with likelihood of local recurrence, cervical nodal metastasis and the need for adjuvant therapy.

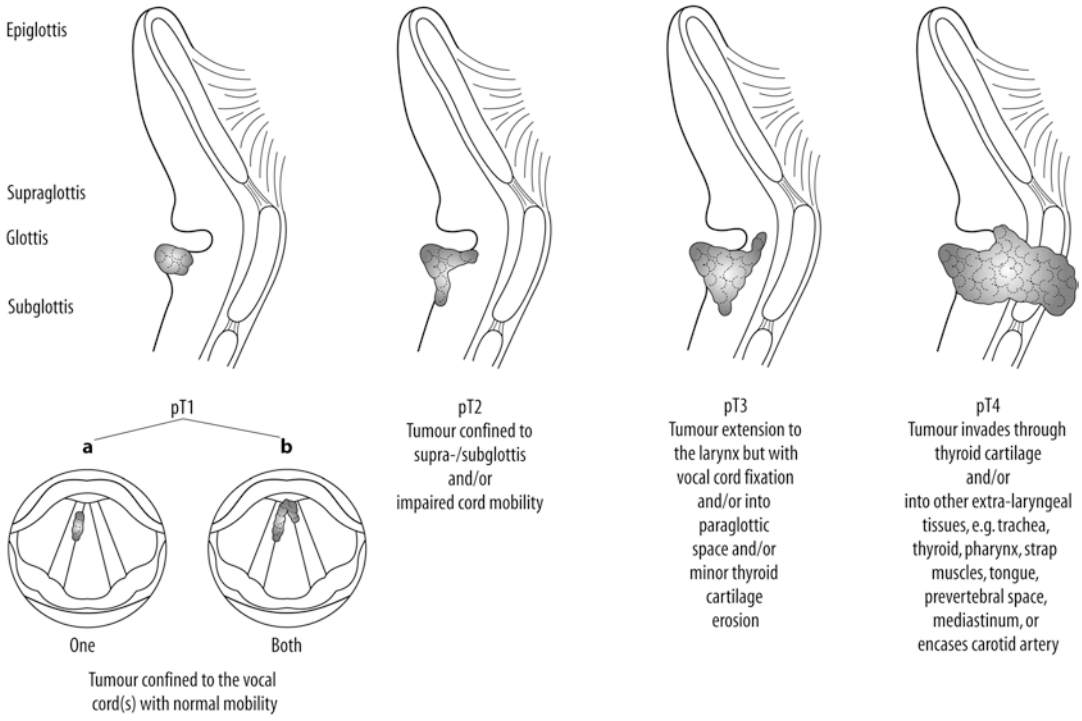

Fig. 15.2 Laryngeal carcinoma: glottis. Reproduced, with permission, from *Histopathology Reporting: Guidelines for Surgical Reporting*, *2nd ed.,* © 2006, Springer

Lymph Nodes

The *incidence of lymph node metastases at presentation* varies according to the site of the primary tumour from glottic (<10%) to supra-/subglottic (30–50%). Well differentiated carcinomas are less likely to metastasise than poorly differentiated cancers.

Size/number involved/size of metastatic deposit/extranodal extension (extracapsular spread).

Regional nodes: cervical.

Level I:	Submental, submandibular (rarely if ever involved)
Level II:	Upper jugular
Level III:	Middle jugular
Level IV:	Lower jugular
Level V:	Posterior triangle

Radical and modified radical neck dissections are not performed as Level I nodes are rarely involved. Selective neck dissection specimens represent harvesting of specific groups of nodes, such as Levels II-IV or II-V. There is no agreed minimum number of lymph nodes that must be recovered but it is questionable whether a specimen that generates fewer than 8 nodes for examination can be classed as a "neck dissection".

pN0	No regional lymph node metastasis
pN1	Metastasis in a single ipsilateral node ≤3 cm without extranodal extension
pN2	Metastasis in:
	a. Ipsilateral single node ≤3 cm with extranodal extension OR > 3 cm to 6 cm without extranodal extension
	b. Ipsilateral multiple nodes ≤6 cm without extranodal extension
	c. Bilateral, contralateral nodes ≤6 cm without extranodal extension
pN3	a. Metastasis in a lymph node >6 cm without extranodal extension
	b. Metastasis in a lymph node >3 cm with extranodal extension OR multiple ipsilateral or any contralateral or bilateral node(s) with extranodal extension.

Extranodal extension (previously known as *extracapsular extension*) increases the risk of local recurrence and distant spread. Metastasis is usually to *ipsilateral lymph nodes* but bilateral or contralateral lymph node involvement is common, especially in supraglottic, subglottic and transglottic tumours.

Excision Margins

Distances (mm) to the tracheal limit, aryepiglottic fold and pre-laryngeal anterior fascia of infiltrating carcinoma and any mucosal dysplasia. Tumour or mucosal dysplasia at or near (<5 mm) a margin indicates a *greater likelihood of local recurrence* and requires consideration of adjuvant therapy. Intraoperative frozen section assessment of surgical margins usually not required.

Other Pathology

Laryngeal precursor lesions: twice as often in males as females, usually 50–60 years of age; rarely in those who have never smoked; high-risk HPV occasionally implicated (<10%). Present with hoarseness or chronic cough; lesions may appear white, red or both, small or large, localized or diffuse on laryngoscopy; other sites in upper aerodigestive tract also at risk; Candida may be present. Several different grading systems but all agree the greater the degree of disturbance of epithelial maturation, the greater the likelihood of invasive tumour (at presentation and with the passage of time). Proportions of patients with precursor lesions developing infiltrative squamous cell carcinoma: 1–2% if no dysplasia, 10–12% with low-grade lesions, 40% with high grade lesions); average time to SCC 4–5 years.

Radionecrosis: post-radiotherapy laryngeal dysfunction due to confluent necrosis which may lead to local airway compromise and aspiration ("crippled larynx"). Mucosal ulceration with exposure and infection of cartilages; necessitates laryngectomy.

Concurrent carcinoma bronchus/oral cavity/pharynx/oesophagus: 10–15% of cases. Can become apparent on CT staging scan of

the primary laryngeal cancer or on upper aerodigestive tract endoscopy; usually requires biopsy.

Verrucous squamous cell carcinoma: must be distinguished from benign squamous epithelial papilloma and hyperplasia by its pushing deep margin but not always seen in small biopsies. It can also co-exist with squamous cell carcinoma of usual type. Beware the large and/or recurrent "squamous papilloma" in a smoker. Also granular cell tumour with overlying pseudoepitheliomatous hyperplasia—the granular cells (Schwann cell origin) are S100 protein positive.

Juvenile laryngeal papillomatosis: multiple HPV related squamous cell papillomas of the upper respiratory tract and a rare cause of squamous cell carcinoma, usually in association with smoking or radiotherapy. These papillomata often require *repeated endoscopic laser or microdebrider debulking* to avoid airway obstruction. A minority persist and can spread to trachea and bronchi.

Prognosis

Prognosis relates to tumour site, stage and certain histological factors: grade/invasive front, vascular invasion and, where relevant, *resection margins.* Early (pT1, pT2) glottic and supraglottic carcinomas may be treated by voice sparing *local excision, laser* or *radiotherapy. Partial laryngectomy* (supraglottic or vertical hemilaryngectomy) is a rare procedure but may be carried out for small volume T2 cancers. Total laryngectomy is the operation of choice in cases of radiotherapy failure, bulky T3 and T4 lesions, subglottic tumours, and in post-radiation "crippled larynx".

Site-related 5 year survival:

Glottic	80%
Supraglottic	65%
Transglottic	50%
Subglottic	40%

Stage-related 5 year survival:

Glottic	I	90%
	II	85%
	III	60%
	IV	<5%

Other Malignancy

Malignant Lymphoma/Leukaemia

- Primary MALToma or more commonly secondary to lymph node/systemic disease.
- Sinonasal (angiocentric) T/NK cell lymphoma.

Plasmacytoma/Myeloma

- Initially localised but generally becomes part of disseminated myeloma. Look for κ, λ light chain restriction and evidence of systemic disease (elevated ESR, immune paresis and monoclonal gammopathy, Bence-Jones proteinuria, radiological lytic bone lesions).

Sarcoma

- Particularly low-grade chondrosarcoma (mostly the cricoid or thyroid cartilages), inflammatory myofibroblastic tumour (especially ALK1-negative ones) and rhabdomyosarcoma (embryonal—childhood; alveolar—young adults).
- NB: A malignant-looking spindle cell lesion in the larynx of a smoker is most likely to be a spindle cell carcinoma.

Malignant Melanoma

- Primary (rare) or secondary (most likely): S100, HMB-45, Melan-A positive.

- *Aggressive (20% 5 year survival)* usually present with bulky local disease and involved nodes; TNM8 designates as moderately advanced (pT3: mucosal) or very advanced (pT4: beyond mucosa) disease.

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Salivary Gland Tumours

16

Séamus Napier

Introduction

Salivary gland swelling has diverse causes including outflow obstruction, infection secondary to duct obstruction or as a primary viral infection, global enlargement (sialosis) associated with systemic diseases e.g. diabetes and alcohol abuse, immunologically mediated disease such as Sjögren's syndrome, IgG4-related disease or HIV, and primary or secondary neoplasms. Presentation can mimic cervical lymphadenopathy, and investigation is undertaken to determine whether the swelling is focal or diffuse, cystic or solid, salivary or non-salivary, affects one gland or many, and to determine the cellular changes.

Comprising about 6% of head and neck cancers, salivary gland neoplasia presents as a persistent and usually painless unilateral enlargement. The majority of primary salivary gland tumours arise in the parotid gland, of which 80% are benign. Tumours in the sublingual gland are rare but are almost always malignant (90%), those in the submandibular gland are twice as often malignant as benign. Minor salivary gland tumours are just as likely to be malignant as benign but this varies according to intraoral location: usually benign in the palate and upper lip but malignant if sited in the tongue, floor of mouth and retromolar region. Tenderness is a worrying feature and suggestive of carcinoma. Other symptoms that suggest malignancy are a rapid increase in size of a pre-existing mass, ulceration, induration or fixation of overlying mucosa or skin. A history of previous skin squamous cell cancer or malignant melanoma, intercurrent autoimmune disease or radiation to the head and neck area are relevant clues.

Investigations include plain X-ray (for calculus), ultrasound, MRI and CT scans. Precise definitive pre-operative diagnosis by FNAC is difficult and prone to benign-malignant inversion. Triaging into broad categories—in conjunction with the clinical and imaging findings—provides more useful guidance to the surgeon: if high-grade malignancy (including metastasis) and lymphoid proliferations that might represent lymphoma can be excluded, the low-grade cancers and benign tumours that make up the remaining category of "primary salivary neoplasm" are all treated similarly by complete but conservative excision. Needle core biopsy provides more tissue than FNAC and can refine a differential diagnosis but also suffers from significant sampling error. "Open" incisional biopsy is avoided due to the risk of nerve damage, salivary fistula and compromising complete local surgical clearance although diagnostic biopsy of large minor salivary gland lesions may assist in planning radical surgery.

Surgical treatment in the parotid is either by "extracapsular dissection" (small lesions distant

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_16

to the main branches of the facial nerve), or by partial or total excision of the gland to include the tumour mass. Most tumours in the parotid gland lie superficial to the facial nerve hence most specimens comprise a superficial parotidectomy, i.e. removing the entire lesion with all the glandular tissue superficial to the nerve. For tumours in the deep lobe, the superficial lobe is removed to facilitate preservation of the facial nerve before the tumour in the deep lobe is excised. The facial nerve may be sacrificed if infiltrated by tumour; similarly for skin of the side of the face and upper neck. Submandibular and sublingual glands are removed in their entirety. Occult or clinically evident cervical lymph node metastases may require neck dissection but most low-grade tumours do not involve lymph nodes at presentation. Post operative radiotherapy is determined by the tumour type, grade and status of the surgical resection margins.

The most important prognostic features are tumour type (with grade influential in some malignancies), stage and adequacy of local excision.

Gross Description

Specimen

- Parotid/submandibular/sublingual/minor (not always intraoral).
- Incisional or excisional biopsy/surgical excision: extracapsular dissection/superficial parotidectomy/conservative subtotal/radical parotidectomy, submandibulectomy/excision of oral tumour (sublingual glands, or minor salivary glands of mucosal origin), neck dissection.
- Size (mm) and weight (g)
- Size of oral mucosa or facial skin (mm)/levels of neck dissection.

Tumour

Site

- Salivary gland/intrasalivary lymph node based.
- Parotid gland: superficial or deep lobe (subdivided by the plane of the facial nerve) or both
- Submandibular gland: tumour located within gland (metastatic deposits in nodes tend to displace rather than invade the gland)
- Sublingual gland: almost always over-run by tumour
- Bilateral: Warthin's tumour

Size

- Length × width × depth (mm) or maximum dimension (mm).
- Size is part of TNM8 staging for carcinoma and a major factor in treatment outcome and survival.
- The distance the malignant component invades beyond the capsule of an old pleomorphic adenoma is felt to be more useful than size alone in carcinoma ex pleomorphic adenoma.

Appearance

- Solid/cystic.
- Gelatinous/firm/necrotic/fleshy/scirrhous.
- Central elastotic scar representing a pre-existing pleomorphic adenoma.
- Single/multiple (multiple lesions tend to represent recurrence with the exception of Warthin's tumours).

Edge

- Circumscribed/encapsulated/infiltrative: presence of macroscopic extraglandular extension.

Gland

- Atrophic/fibrotic/intrasalivary lymph nodes.

Histological Type

Adenomas

- Pleomorphic adenoma; 70% of salivary gland tumours, 80% in the parotid gland.
- Warthin's tumour (aka adenolymphoma/papillary cystadenoma lymphomatosum); 15% of salivary gland tumours, almost never in glands other than the parotid, can mimic cystic metastatic squamous cell carcinoma on imaging and FNAC.

- Other recognized adenomas, e.g. myoepithelioma, basal cell adenoma, canalicular adenoma (may be multifocal), oncocytoma, lymphadenoma (sebaceous and non-sebaceous forms), cystadenoma (papillary or oncocytic), sialadenoma papilliferum (usually minor glands), ductal papillomas (include intraductal papilloma and inverted duct papilloma), sebaceous adenoma.

Carcinomas

Common forms include:

- Mucoepidermoid carcinoma (common distinctive mixture of squamoid cells, mucous-producing cells and intermediate clear cells; keratinisation exceptionally rare).
- Carcinoma ex pleomorphic adenoma (CA ex PA; usually high-grade carcinoma NOS or may demonstrate combinations of different patterns of salivary carcinoma; intracapsular forms are confined to the capsule of the old PA).
- Salivary duct carcinoma (resembles infiltrating ductal carcinoma of breast; commonly arises ex pleomorphic adenoma).
- Adenoid cystic carcinoma: cribriform/tubular/solid patterns (rare for complete excision due to insidious infiltrative pattern, tends to spread by haematogenous rather than lymphatic routes, patients usually survive 10 years but are very rarely cured).
- Acinic cell carcinoma (often accompanied by a florid lymphoid reaction).
- Polymorphous carcinoma (previously known as polymorphous low-grade carcinoma but not always low grade, minor salivary glands only).
- Wide variety of other uncommon entities, e.g., clear cell carcinoma (previously hyalinising clear cell carcinoma), basal cell adenocarcinoma, intraductal carcinoma, myoepithelial carcinoma, epithelial-myoepithelial, secretory carcinoma (previously mammary analogue secretory carcinoma), sebaceous adenocarcinoma, "poorly differentiated" carcinoma (includes undifferentiated, large cell neuroendocrine and small cell neuroendocrine forms), lymphoepithelial carcinoma, oncocytic carcinoma, squamous cell carcinoma (more likely to be metastasis); adenocarcinoma NOS includes mucinous cystadenocarcinoma, papillary cystadenocarcinoma but must exclude other recognized types; carcinosarcoma (immunohistochemistry usually required to demonstrate true mesenchymal differentiation to distinguish it from metaplastic carcinomas), sialoblastoma.

Malignant Lymphoma

- Extranodal lymphoma of salivary gland (MALToma).
- Nodal lymphoma in salivary gland lymph nodes.

Metastatic Tumour

Lymphatic spread

- Squamous cell carcinoma or malignant melanoma from ear, scalp or facial skin
- Occasionally squamous cell carcinoma from the upper aerodigestive tract may metastasise to a high Level II node and mimic a primary salivary neoplasm in the tail of the parotid gland

Haematogenous spread

- Breast, bronchus, kidney, thyroid, prostate, GI tract.

Differentiation (i.e. Grading)

Grade is related to the *risk of local recurrence, regional* and *distant metastasis* but is less important than stage. Most salivary gland carcinomas are low-grade but *elderly patients* not infrequently present with high-grade tumours.

Salivary gland carcinomas are usually not graded independent of type except for:

Mucoepidermoid carcinoma

- Low grade: cystic, mucous cells predominate
- High grade: solid, pleomorphism, necrosis, mitotic figures; extensive invasion
- The rest are intermediate grade

Adenoid cystic carcinoma

- Lesions with a solid component (homogenous cellular nodules with only occasional peripherally located ducts, nuclear pleomorphism and/or necrosis) that constitutes more than one third of the tumour volume more likely to metastasise to lymph nodes and are considered high grade
- Cribriform and tubular patterns tend to have a less aggressive clinical course

Adenocarcinoma, NOS

- According to the degree of cytological atypia; high grade lesions tend to be more solid; lower grade lesions tend to consist of ductal or cystic structures

Usually high-grade cancers

- CA ex PA, salivary duct carcinoma, "poorly differentiated carcinoma", carcinosarcoma

Usually low-grade

- Acinic cell carcinoma, polymorphous carcinoma, clear cell carcinoma, basal cell adenocarcinoma, intraductal carcinoma, myoepithelial carcinoma, epithelial-myoepithelial, secretory carcinoma, sebaceous adenocarcinoma, lymphoepithelial carcinoma, oncocytic carcinoma, squamous cell carcinoma; adenocarcinoma NOS including mucinous and papillary cystadenocarcinoma.
- Low grade carcinomas not infrequently show a range of grade with *progression to high-grade or dedifferentiation*; thorough sampling is important.

Extent of Local Tumour Spread

Border: minimally or widely infiltrative.

Infiltrative margins are a useful diagnostic feature of malignancy in low-grade lesions e.g. polymorphous adenocarcinoma and adenoid cystic carcinoma but a trap for the unwary in pleomorphic adenoma

- Distance of infiltration beyond the capsule of the pre-existing pleomorphic adenoma has prognostic value in CA ex PA (metastasis unlikely if no more than 1.5 mm)
- *Macroscopic extraparenchymal extension* of carcinoma to involve adjacent structures is a predictor of local recurrence and cervical lymph node metastasis for parotid carcinoma.

Perineural involvement is a common finding in adenoid cystic carcinoma but is not seen in all cases. Its importance is probably exaggerated by anecdote but the possibility of an adenoid cystic carcinoma in a site where a tumour mass might be difficult to detect, such as the deep lobe of the parotid, merits consideration in patients with intractable facial pain or unusual neurological signs, particularly since this tumour tends to be ill-defined and soft. Perineural invasion is also *diagnostically useful* in adenoid cystic and polymorphous low-grade adenocarcinoma that may otherwise resemble pleomorphic adenoma.

The TNM8 classification applies to major salivary glands: parotid, submandibular and sublingual. Minor gland tumours (i.e. from the mucosa of the upper aerodigestive tract) are classified according to anatomical site, e.g. minor salivary gland tumours of the lip are staged as if they were an intraoral carcinoma.

pT1	Tumour ≤2 cm, without extraparenchymal extension[a]
pT2	Tumour >2 cm but ≤4 cm in greatest dimension without extraparenchymal extension[a]
pT3	Tumour >4 cm in greatest dimension and/or with extraparenchymal extension
pT4	Tumour invades (a) Skin, mandible, ear canal, and/or facial nerve. (b) Base of skull, and/or pterygoid plates and/or encases carotid artery.

[a]*Extraparenchymal extension is clinical or macroscopic evidence of invasion of soft tissues or nerve, except as listed under pT4a and b; microscopic evidence alone is not sufficient* (Figs. 16.1, 16.2, 16.3, and 16.4)

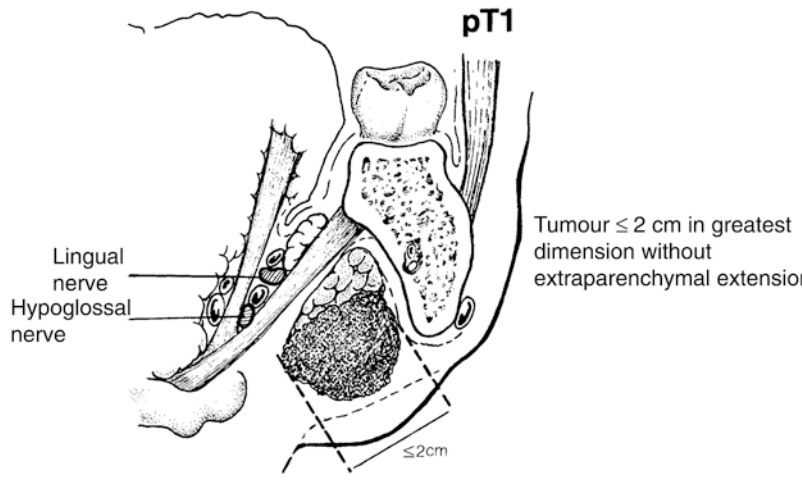

Fig. 16.1 Salivary gland carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

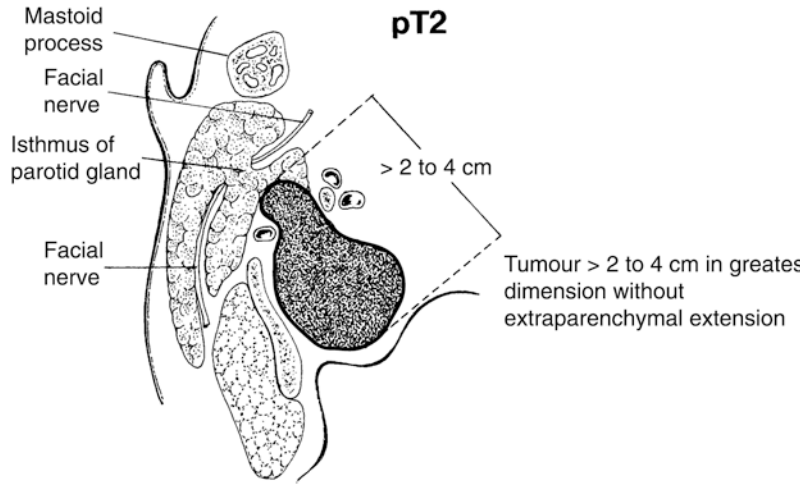

Fig. 16.2 Salivary gland carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

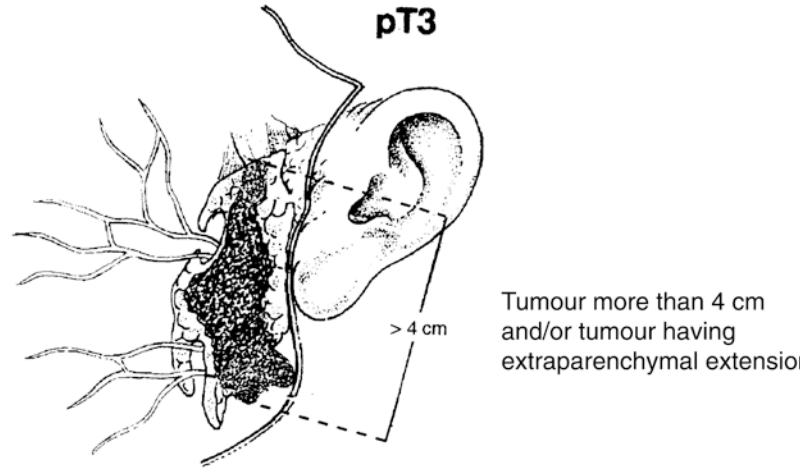

Fig. 16.3 Salivary gland carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

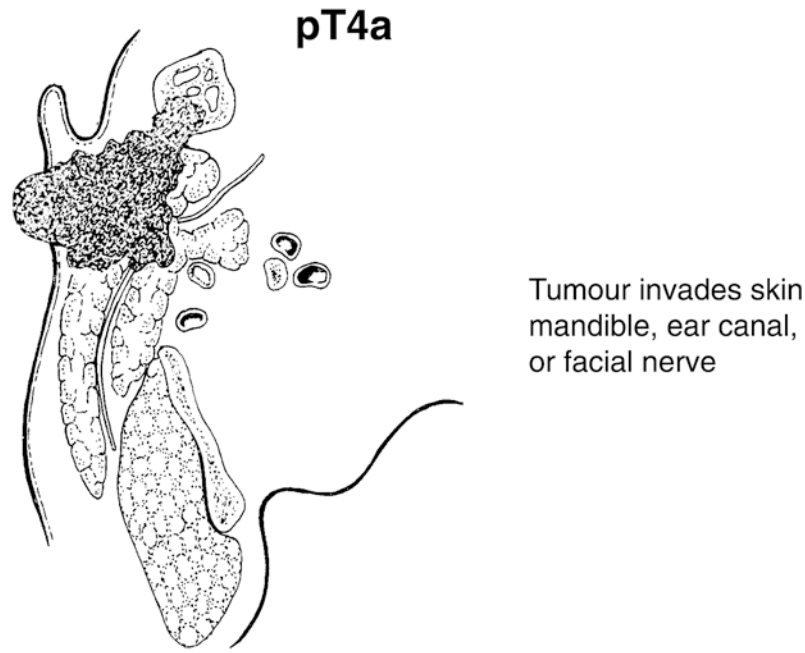

Fig. 16.4 Salivary gland carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

Lymphovascular Invasion

Present/absent.

Predictive for local recurrence.

Lymph Nodes

Intra-/extraglandular: the parotid gland can contain up to 20 intraglandular lymph nodes.

Site/number/size of tumour deposit/number involved/extranodal extension (extracapsular spread).

Regional nodes: intraparotid nodes and cervical; midline nodes are considered ipsilateral.

Level I:	Submental, submandibular
Level II:	Upper jugular
Level III:	Middle jugular
Level IV:	Lower jugular
Level V:	Posterior triangle

pN0	No regional lymph node metastasis
pN1	Metastasis in a single ipsilateral node ≤3 cm without extranodal extension
pN2	Metastasis in: (a) Single ipsilateral node >3 but ≤6 cm without extranodal extension (b) Ipsilateral multiple nodes ≤6 cm without extranodal extension (c) Bilateral, contralateral nodes ≤6 cm without extranodal extension
pN3	(a) Metastasis in a lymph node >6 cm without extranodal extension (b) Metastasis in single or multiple lymph nodes >3 cm with extranodal extension

The *cervical regional lymph nodes* are the commonest site of *metastasis* followed by *lungs* and *bone.* The parotid gland drains to Level II and III lymph nodes, the submandibular and sublingual glands to the Level I and II lymph nodes. *Extracapsular lymph node spread* is an indicator of more *aggressive disease* and is likely to prompt consideration of postoperative radiotherapy.

Excision Margins

Distance (mm) to the nearest painted excision margin. Tumour at or close to (<5 mm) the nearest excision margin is an important factor in *local control of disease* and *survival.*

Other Pathology

Necrotising sialometaplasia: usually minor salivary glands in the palate, can mimic carcinoma e.g. mucoepidermoid carcinoma or adenoid cystic carcinoma. It presents as a clinically malignant-looking ulcerating lesion in middle

aged smokers. Similar changes can be seen in operative sites that are re-biopsied soon after the initial procedure.

Benign soft tissue tumours such as schwannoma (neurilemmoma) can occur in the major salivary glands, especially the parotid, and can be mistaken on limited samples such as core needle biopsies for cellular or spindle cell variants of pleomorphic adenoma.

Salivary gland tumours are highly variable morphologically, demonstrating a wide variety of architectural patterns and cell types often overlapping between tumour of different types but also within tumours of the same type. Many tissue blocks should be sampled, not only to establish the diagnosis but also to identify areas of high-grade change.

Salivary tumours with clear cells: tend to be *malignant* and must also be distinguished from secondary renal cell carcinoma. A wide range of salivary tumours can show clear cell differentiation: acinic cell, mucoepidermoid, epithelial-myoepithelial, sebaceous, clear cell carcinomas and malignant myoepithelioma. Abdominal CT scan may be necessary to exclude metastatic renal cell carcinoma; extended panels of immunohistochemical stains to exclude clear cell malignant melanoma and clear cell thyroid carcinoma. Primary clear cell carcinoma arises in minor salivary glands, has uniform clear cells in a dense hyalinising stroma and is locally infiltrative.

Adenoid cystic carcinoma: Perhaps the commonest salivary cancer, about 25% of salivary carcinomas, shows cribriform, hyaline and tubular patterns. It is *slow growing, relentless* and *locally recurrent*; *10 year survival* is common but cure is rare. Infiltrates widely in the tissues beyond the mass, usually evoking little stromal response hence rare for complete excision and local recurrence is common; tends to spread by hematogenous route to lungs, bone and liver rather than to lymph nodes. Prognosis relates adversely to a solid growth pattern (when solid component represents more than one-third of tumour volume, also nodal involvement more likely), stage and incomplete primary excision, with *radical surgery* being the treatment of choice.

Mucoepidermoid carcinoma: the second commonest malignant salivary gland tumour (20% of cases); commonest salivary cancer in children. It shows a spectrum of squamoid (i.e. epidermoid cells) and mucous cells in varying proportions (mucin stains may be necessary) but never forms keratin. Low grade lesions are largely cystic with only mural tumour. High grade lesions are more solid, squamoid and infiltrative. There is often an accompanying fibro-inflammatory reaction. *Tumour grade* and *complete excision* dictate prognosis with multiple local recurrences dominating and lymph node metastases (10% of cases) late.

Acinic cell carcinomas: 5% of salivary tumours, mostly parotid. Have deceptively bland granular, clear or eosinophilic cells with variably solid, papillary, follicular or microcystic pattern; often accompanied by a florid lymphoid reaction. More aggressive pleomorphic so-called "dedifferentiated" variants occur. Overall recurrence can be seen in 10–30% but 20 year survival is the norm. Peak incidence is in the third decade of life, second commonest salivary neoplasm in childhood and teenage years. Gross invasion, cellular pleomorphism, lymph node metastasis at presentation and incomplete primary excision are adverse indicators.

Polymorphous adenocarcinoma: Previously *polymorphous low-grade carcinoma;* the term "low grade" can be used on a case-by-case basis as not all tumours exhibit low-grade behaviour. Second commonest intraoral salivary carcinoma (25%), is characterised by cellular uniformity and architectural diversity (solid/cribriform/single cell/cords/tubular/ductal/papillary) with hyalinised stroma; oncocytic and mucous cells may help distinguish from adenoid cystic carcinoma (but not pleomorphic adenoma). Minor glands only. An infiltrative margin and perineural invasion can be helpful in making the diagnosis. A related tumour is the low-grade papillary adenocarcinoma, commonly located in the posterior third of tongue. Metastasis to lymph nodes and beyond in 10%.

Carcinoma ex pleomorphic adenoma (CA ex PA): 5% of cases manifesting as symptomatic regrowth or facial pain in an existing lesion

present for 10 years or longer; 70% parotid gland, 15% in minor glands. The diagnosis may be apparent at macroscopic examination, when abundant calcification or the elastic core of the old PA might be noted. The malignant component is usually adenocarcinoma NOS or salivary duct carcinoma but many other patterns exist and a combination of different malignant tumours is common, e.g. carcinoma NOS, adenoid cystic carcinoma, squamous cell carcinoma and/or myoepithelial carcinoma. Prognosis of CA ex PA relates to local excision and nodal involvement, which are often related to the *subtype of the carcinoma in the tumour* (e.g., low grade forms such as myoepithelial carcinoma are more favourable) and the *distance of extracapsular extension* of the malignancy beyond that of the original PA (minimally invasive CA ex PA infiltrating no more than 1.5 mm beyond capsule have a favourable outcome, 80% at 5 years; if the carcinoma is restricted to the capsule—known as intracapsular CA ex PA—recurrence and metastasis are unlikely).

Salivary duct carcinoma: resembles high-grade ductal carcinoma in situ of the breast. It shows aggressive behaviour with poor prognosis and 70% die within 5 years. Patients are >50 years of age with parotid being the main site. Sarcomatoid, mucin-rich, invasive micropapillary and oncocytic variants. Androgen receptor and HER2 are often positive and offer therapeutic targets but ER and PR are negative. The previously described low-grade variant is now listed separately as "intraduct carcinoma".

Myoepithelial carcinoma: is a deceptive tumour of rounded uniform cells in either a myxoid or hyalinised matrix; keratin nests are common. Mitotic activity, invasion and occasionally necrosis are the give-away, despite the absence of nuclear atypia. Unpredictable—one third fare well with local resection but one third suffer metastasis (usually to nodes and lung).

Epithelial-myoepithelial carcinoma: is a low-grade malignancy mainly of the major glands and recurs in one third of cases, comprising darkly staining luminal (ductal) cells and clear abluminal (myoepithelial) cells in a distinctive lobular arrangement. Nuclear atypia in >20% of cells is an adverse prognostic factor. Often the clear cell abluminal (myoepithelial) element predominates, especially in recurrences.

Basal cell adenocarcinoma: is the malignant counterpart of basal cell adenoma (closely packed solid nests surrounded by hyaline basal lamina material) except that it contains mitotic figures and shows infiltration, perineural and vascular invasion. Metastasis and death from tumour are rare.

Primary squamous cell carcinoma: mostly in the parotid gland but uncommon. Metastases to the parotid gland from other sites must be excluded, particularly from skin of the head and neck. Some represent squamous cell carcinoma ex PA.

Immunohistochemistry: diagnosis of salivary gland tumours is mainly morphological. Most benign tumours and many of the low-grade tumours of salivary gland consist of combinations of luminal (i.e. duct-like) and abluminal (i.e. myoepithelial-like) cells, echoing the relationship in the normal gland and giving rise to the broad spectrum of morphological patterns and the wide variety of cell types that can be seen in these tumours. As a result, there are relatively few diagnostic combinations of immunohistochemical stains that might resolve a differential diagnosis although immunohistochemistry can help to highlight the presence and proportions of the different cellular populations: abluminal or myoepithelial cells (CK14, CK5/6, p63, p40, calponin, caldesmon, S100) and luminal or ductal epithelial cells (Cam5.2, CK7, EMA, BerEP4). Luminal or ductal epithelial cells in adenoid cystic carcinoma are positive for CD117 (c-kit) although so are other basaloid tumours as well as polymorphous carcinoma. Pleomorphic adenoma is GFAP positive as can be polymorphous carcinoma. Ki-67 index is a not a reliable gauge of prognosis but can help highlight areas of increased proliferation or anaplasia. A few useful exceptions are listed below:

- Acinic cell carcinoma: negative for S100 and mammaglobin; positive for DOG1 and SOX10.
- Salivary duct carcinoma: positive for androgen receptor, p53, occasionally for HER2 but negative for ER and PR.

- Secretory carcinoma (previously mammary analogue secretory carcinoma): positive for S100 and mammaglobin; negative for DOG1.

Prognosis

Prognosis relates to anatomical location, tumour histological type, grade, stage and adequacy of excision. Most carcinomas are of intermediate grade—risk of local recurrence if incompletely excised; approximately 10% risk of lymph node metastasis—but many of the low-grade tumours are unpredictable. The best outcome for low-grade tumours such as acinic cell carcinoma or mucoepidermoid carcinoma is provided by complete excision, i.e. without exposing the tumour in the wound, with preservation of facial nerve function. However, the constraints of local anatomy often require compromise when the tumour lies close to the nerve, prompting consideration of adjuvant radiotherapy. High grade lesions such as CA ex PA or salivary duct carcinoma are usually managed with wide local excision and neck node sampling for staging followed by radiotherapy; occasionally the expression of androgen receptor or HER2 in salivary duct carcinoma can be used for treatment of disseminated metastasis.

Other Malignancy

Rhabdomyosarcoma

- Children and adolescents. Desmin/myo D1/myogenin positive. Note that mucoepidermoid and acinic cell carcinomas can also typically occur in childhood and young adults.

Malignant Fibrous Histiocytoma, Fibrosarcoma, Malignant Peripheral Nerve Sheath Tumour

- Adults

Malignant Lymphoma

- *2–5% of salivary gland neoplasms*.
- 20% have *Sjögren's syndrome* or *LESA* (lymphoepithelial or myoepithelial sialadenitis).
- One third are *diffuse large B cell lymphoma*, of lymph node or parenchymal origin.
- One third are *follicular lymphoma*, usually of lymph node origin.
- One third are *MALTomas* i.e. originating in mucosa associated lymphoid tissue. LESA has x40 increased risk of developing low-grade malignant lymphoma and 15–20% do so over a variable period of 5–20 years. MALToma is characterised by lymphoepithelial lesions surrounded by broad haloes or sheets of centrocyte like (marginal zone/monocytoid) B cells. Other features include monotypic plasma cells and follicular colonisation. High grade transformation to large cell lymphoma can occur. PCR demonstration of clonality does not always reliably predict those lymphoid lesions that will progress to clinical malignant lymphoma.

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Part III

Respiratory and Mediastinal Cancer

- Lung carcinoma
- Malignant mesothelioma
- Mediastinal cancer

Lung Carcinoma

17

Neil Anderson

Introduction

The commonest worldwide cancer, over 35,000 people die from lung cancer each year with 85–90% attributed to tobacco smoking. The incidence has decreased in males but increased in females reflecting changes in smoking habits and lifestyle.

Lung cancer may present with persistent *cough, haemoptysis* secondary to ulceration of the tumour, *obstructive effects* (pneumonia), *local infiltration* (pleural effusion, chest wall pain/mass, hoarseness, Horner's syndrome due to apical Pancoast's tumour, superior vena cava syndrome), *systemic effects* (finger clubbing, paraneoplastic syndromes, weight loss), or as an *incidental finding* on radiology for other reasons. Urgent referral to a member of the lung cancer multidisciplinary team, initially a chest physician is required.

Investigation is by chest X-ray and staging by CT scan/PET CT and endobronchial ultrasound to assess spread to locoregional lymph nodes, liver, adrenal glands and brain. MRI scan can detect invasion into the axilla, chest, vertebral column and spinal cord. High resolution CT (HRCT) can demonstrate lymphangitis carcinomatosa.

Tissue diagnosis is obtained in a high percentage (>90%) of cases by a variety and combination of techniques depending on the tumour site, local infiltration and type. These include sputum cytology, bronchial brushings/washings/biopsy, transbronchial or percutaneous image guided (endobronchial ultrasound (EBUS) or CT) FNAC (fine needle aspirate cytology)/needle core biopsy, open lung wedge or mediastinoscopic/thoracoscopic biopsy. Thoracoscopic sampling of mediastinal lymph nodes may be required for staging purposes although the requirement for this has declined due to increased reliance on PET CT and EBUS sampling. In bronchogenic carcinoma diagnostic yield increases with the number of biopsy fragments. Transthoracic FNAC/needle biopsy is of particular use for peripheral lesions, and, transbronchial biopsy for lymphangitis carcinomatosa and cancers causing bronchostenotic extrinsic compression. Where a firm preoperative diagnosis of a peripheral lesion has not been obtained intraoperative frozen section is indicated as a prequel to opting for either a more radical cancer resection operation or a lung sparing wedge resection. Tracheal lesions may require assessment by rigid bronchoscopy. In the presence of extensive disease pleural fluid cytology or peripheral lymph node FNAC may provide a more accessible site for a positive diagnosis.

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_17

Accurate typing of the tumour in addition to staging information and assessment of the patient's fitness are crucial in selection of appropriate non-surgical versus surgical primary treatment. Clinical staging determines suitability for surgical resection, neoadjuvant or palliative chemoradiotherapy. Pathological staging of the surgical specimen points to those patients that might benefit from postoperative adjuvant treatment. Subtyping of non-small cell carcinoma by a combination of routine morphology, immunohistochemistry and genetic analysis can indicate a potential role for targeted drug therapy in patients with advanced, recurrent or metastatic lung adenocarcinoma.

Peripheral wedge or segmental resection can be by either open surgery, or a closed video assisted technique. Sleeve resections (bronchial or lobectomy) are lung sparing aimed at removal of a proximal endobronchial lesion, at or adjacent to the carina, with reanastomosis of the proximal major airway to the distal bronchial tree. Lobectomy resects one or more lobes with the draining hilar lymph nodes. Pneumonectomy comprises 20% of lung resections and is indicated when there is tumour involvement of hilar structures or the oblique fissure is traversed. Segmentectomy, sleeve resections, lobar resections and pneumonectomy can all be extended to include en bloc excision of involved contiguous chest wall or thoracic structures. Extrapleural pneumonectomy encompasses removal of visceral and parietal pleurae, lung, ipsilateral hemidiaphragm and pericardium.

The usual surgical approach is posterolateral thoracotomy with lobectomy and regional (hilar, mediastinal) lymphadenectomy. Patients require full clinical assessment of their fitness.

Patients with endobronchial obstruction may be managed by a variety of interventional techniques: brachytherapy, electrocautery, cryotherapy, thermal laser ablation, photodynamic therapy and airway stents. This can also be a prequel to further elective planned treatment.

Gross Description

Specimen

- Exfoliative cytology/aspiration cytology or needle biopsy (percutaneous/transbronchial/US or CT guided)/bronchial biopsy/thoracoscopic or mediastinoscopic biopsy/wedge resection/sleeve resection/segmentectomy/(bi-)lobectomy/pneumonectomy (standard/extrapleural/extra-/intrapericardial) ± en bloc resection (may include mediastinal pleura, pericardium, great vessels, atrial wall).
- Resection can be either open or thoracoscopic (VATS: video assisted thoracoscopic surgery).
- Size (mm) and weight (g)/number of fragments.

Tumour

Site

- Central (main/segmental bronchus): <2 cm or ≥2 cm from the carina; RUL/RML/RLL/LUL/LLL.
- Hilar: at the hilum but not within a specific lobe.
- Peripheral (parenchymal/pleural).

Size

- Length × width × depth (mm) or maximum dimension (mm).
- Size is a TNM staging criterion.

Squamous cell carcinomas can attain a large size and remain localized, whereas small cell carcinomas can be small primary lesions but with extensive local mediastinal lymphadenopathy and distant spread and clinical symptoms reflecting disseminated disease.

Appearance

- Necrosis/haemorrhage/mucoid/cavitation.
- Polypoid/nodular/ulcerated/stenotic.
- Endobronchial/bronchial/extrabronchial.

Squamous cell carcinoma frequently cavitates, central carcinoid tumour is polypoid or nodular, small cell carcinoma is submucosal and bronchostenotic or shows extrinsic compression.

Edge

- Circumscribed/irregular.

Pulmonary Changes

- Scar: peripheral adenocarcinoma.
- Fibrosis/asbestosis.
- Partial and hilar, or total atelectasis/obstructive pneumonitis the extent of which helps determine the pT stage. It is probably more accurately described radiologically but can be mapped in extent by sampling blocks of lung away from the tumour mass.

Histological Type

Crucial therapeutic distinction is made between *the major types of lung cancer—small cell carcinoma, squamous cell carcinoma, adenocarcinoma, large cell neuroendocrine carcinoma and large cell carcinoma, NOS.* Typing is primarily *morphological* on routine H&E sections. An increasing proportion of cases require supplementary *immunohistochemistry* for designation of non-small cell carcinoma subtype (adenocarcinoma versus squamous cell carcinoma), neuroendocrine tumours/carcinomas (well differentiated/low-grade (carcinoid) tumour, or poorly differentiated/high-grade small cell/large cell carcinoma), and primary versus secondary carcinoma.

Squamous Cell Carcinoma

- *30–45% of cases.* It requires nuclear stratification, intercellular bridges, ± keratinisation.
- Large cell/small cell.
- Keratinising/non-keratinising.

Variants:

- *Spindle cell (see carcinosarcoma):* cytokeratin positive ± vimentin positive.
- *Basaloid:* poor prognosis, nests of palisaded basaloid cells with central comedonecrosis, hyalinised stroma. More radiosensitive than other squamous cell carcinoma subtypes.
- *Papillary:* >70% exophytic or papillary malignant epithelial fronds with focal invasion at the base and better prognosis (70% 5 year survival).
- *Adenoidsquamous:* usual prognosis, acantholytic (pseudoglandular) pattern.
- *Adenosquamous:* mixed differentiation with the minor component at least 10% of the tumour. It is of worse prognosis. The adenocarcinoma element may have obvious glands or is solid with mucin positive cells.
- *Clear cell.*

Small Cell Carcinoma

- *25% of cases*. A poorly differentiated/high-grade neuroendocrine carcinoma. Small round/fusiform nuclei (x2–3 lymphocyte size) with granular chromatin, moulding and an inconspicuous nucleolus. There is also DNA crush and vessel artifact (Azzopardi phenomenon), fir tree hyaline stroma, prominent apoptosis, necrosis and mitoses. The nuclear features are the diagnostic characteristic of small cell carcinoma. Note that there can be a scattered population of large bizarre (polyploid) cells.
- *Oat cell:* usual type.
- *Intermediate cell:* larger nucleus/more cytoplasm and most likely represents better preserved/fixed oat cell, i.e. the same tumour.
- *Combined:* + non-small cell component (at least 10%), e.g. squamous or adenocarcinoma.

Adenocarcinoma

- *15–25% of cases* (50% of lung cancer in females and also in non-smokers) and showing a *progressive increase in incidence* with more accurate histological subtyping. 40% are endobronchial, 60% peripheral, and may involve the pleura at presentation and unusually can give an encasing pseudomesotheliomatous picture. There is commonly a central scar in peripheral lesions. Adenocarcinoma is more often suitable for resection than squamous cell carcinoma.

- Pulmonary adenocarcinoma is classified as either *non-mucinous* (acinar, papillary, micropapillary, solid, lepidic) or *mucinous* in type, although up to 85% show *heterogeneity* with more than one, often multiple cell patterns.

Non-mucinous

- *Acinar:* gland forming.
- *Papillary:* frond forming with stromal cores.
- *Micropapillary:* papillary nodules without stromal cores. A papillary or micropapillary pattern indicates *more aggressive disease*, with a high incidence of lymph node and pleural metastases and venous invasion.
- *Solid:* with mucus formation (>5 PAS/AB-diastase positive cells in at least two high power fields).
- *Lepidic:* formerly bronchioloalveolar adenocarcinoma (BAC). Peripheral, single/multiple or a pneumonic infiltrate with lepidic spread along alveolar walls and no stromal, vascular or pleural invasion. Lepidic denotes use of the alveolar walls as a scaffold for growth giving a honeycomb appearance on microscopy.
- Prognosis of non-mucinous lung adenocarcinoma decreases in the following order: lepidic, acinar, solid, (micro)papillary.

Mucinous

- Colloid, goblet cell, columnar cell, signet ring cell, clear cell types.

For further discussion of lepidic/BAC and atypical adenomatous hyperplasia see Section "Other Pathology".

Large Cell Carcinoma

- *5–10% of cases*.
- Shows no evidence of squamous cell or glandular differentiation although they probably represent undifferentiated forms of these. Immunostains for squamous differentiation or adenocarcinomatous differentiation are negative.
- Variants: giant cell, rhabdoid, clear cell, lymphoepithelioma like, basaloid and neuroendocrine.

In primary clear cell carcinoma (rare) exclude: clear cell change in squamous cell or adenocarcinoma, secondary thyroid, salivary or renal cell carcinoma, malignant melanoma, and benign clear cell (sugar) tumour of lung (HMB-45 positive)—a perivascular epithelioid cell tumour (PEComa).

Miscellaneous

- *Pulmonary endodermal tumour or adenocarcinoma of fetal type:* young patients with a solitary mass, endometrioid type glands and better prognosis.
- *Pulmonary blastoma:* adults, peripheral, solitary and large. Well differentiated fetal type tubular glands in a cellular embryonal stroma and poor prognosis.
- *Carcinosarcoma:* forms a pulmonary or polypoid bronchial mass. Squamous cell or large cell/adenocarcinoma with fibrosarcoma like spindle cells (diffuse or focal cytokeratin (AE1/AE3) ± TTF-1 positive) representing spindle cell carcinoma with or without heterologous mesenchymal differentiation, e.g. cartilage, bone, striated muscle. It has a *poor prognosis* and overlaps with pleomorphic (spindle/giant cell) carcinoma. Metastases can be epithelial, sarcomatoid or both.

Note that only about 40% of primary lung carcinomas are of homogeneous histological type and *mixed differentiation and patterns* are reasonably common, e.g. squamous cell and small cell components, acinar and papillary adenocarcinoma. The second component must comprise at least 10% of the tumour volume to be regarded as a mixed tumour.

Neuroendocrine Tumours/ Carcinomas

- *5% of primary pulmonary neoplasms*. See also *small cell carcinoma* (above).
- Chromogranin, synaptophysin, CD56 (NCAM), TTF-1 positive. Well differentiated lesions stain more strongly with chromogranin than CD56, and poorly differentiated lesions the converse of this.
- A spectrum of tumours with *low-grade* well (carcinoid), moderately (atypical carcinoid) and *high-grade/*poorly differentiated (small cell/large cell) forms.
- *Carcinoid tumour:* in younger patients and polypoid/dumbbell shaped, either central or peripheral. Central cases present with haemoptysis and are dome shaped at bronchoscopy. Lymph node metastasis in 5–15% and *70–90% 10 year survival*.
- *Atypical carcinoid tumour:* central/peripheral and spindle cells with cellular atypia, necrosis (usually punctate) and mitoses >2–10/10 high power fields. Lymph node metastasis in 40–60% with *60% 5 year survival*.
- *Large cell neuroendocrine carcinoma: 34% 5 year survival*. Significant numbers of endocrine cells present rather than just a non-small cell carcinoma with focal endocrine differentiation. Shows solid sheets/nests/peripheral palisading/moderate cytoplasm.
- High resolution CT scan has led to an increasing awareness of *diffuse idiopathic neuroendocrine cell hyperplasia* as both a reactive phenomenon in chronic inflammatory lung diseases, e.g. bronchiectasis, and, as a possible indolent precursor to extraluminal carcinoid tumourlets/tumour. Carcinoid tumourlet is defined as <5 mm maximum dimension.

Salivary Gland Type Adenocarcinoma

- Bronchial mucosal gland origin.
- *Adenoid cystic carcinoma:* indolent growth but prognosis is poor with late metastases to nodes and lung parenchyma common. Along with squamous cell carcinoma it is the *commonest primary tracheal tumour*.
- *Mucoepidermoid carcinoma:* prognosis is determined by the histological grade.

Metastatic Carcinoma

Lung can be the *sole site of metastatic spread in 15–25% of patients* with cancer. Tumours commonly metastasising to lung in order of frequency are: breast, colon, stomach, pancreas, kidney, malignant melanoma, prostate, thyroid and genital tract origin. Up to 40% can be oligometastatic, in particular colorectum or kidney, *mimicking a primary lung lesion.*

Various *patterns of spread* are encountered

- Multiple/bilateral/well defined/rapid growth/nodular or mass lesions: breast, gastrointestinal tract, kidney, sarcoma, malignant melanoma, ovary, germ cell tumour.
- Lymphangitis carcinomatosis: stomach, breast, pancreas, prostate, lung.
- Cavitation in a mass lesion: squamous cell carcinoma, gastrointestinal tract, leiomyosarcoma.
- Endobronchial polypoid mass: breast, kidney, gastrointestinal tract, sarcoma.
- Vasculoembolic: breast, stomach, liver, choriocarcinoma.
- Lepidic/bronchioloalveolar pattern of spread: gastrointestinal tract, pancreas (metastases are more pleomorphic and necrotic than lepidic carcinoma), prostate.

Tissue Diagnosis

In limited biopsy material a positive diagnostic yield is increased by *multiple biopsies (5 or 6 minimum)* examined histologically through at least three levels, the aim being to *designate basic neoplastic categories,* e.g. primary versus secondary cancer, small cell versus non-small cell carcinoma, other neuroendocrine (carcinoid)

lesions and malignant lymphoma. This is due to *limited material, tumour heterogeneity and poor observer agreement* (50% at best) at subclassifying moderately to poorly differentiated cancers of non-small cell type. Non-small cell carcinoma is a category of exclusion in that the tumour lacks distinctive morphological or immunohistochemical features of small cell, squamous cell or glandular differentiation. With use of immunohistochemistry, this group comprises 5–10% of lung cancers. This gives good biopsy to resection correlation despite some overlap in expression of these markers. Designation of small cell carcinoma is reasonably robust and it must be distinguished from carcinoid tumour (Ki-67 < 2%), malignant lymphoma (CAM5.2, CD56, CD45) and the small cell variant of squamous cell carcinoma. In this context the relatively *robust preservation of immunophenotypical expression* despite extensive biopsy crush artifact is a diagnostically useful tool.

Bronchial squamous cell carcinoma and basaloid carcinoma are preceded by a *squamous cell metaplasia—dysplasia—carcinoma in situ sequence*, often as a multifocal mucosal field change in the lower respiratory tract. The presence of carcinoma in situ and a lack of demonstrable invasion is not unusual in biopsies and must be *correlated with the clinical findings*. It may be representative of the lesion if derived from a segment of thickened, irregular mucosa. However, in the presence of an obvious *bronchoscopic abnormality* and *radiological mass lesion* it usually represents the edge of an invasive carcinoma. Squamous metaplasia may be entirely non-specific, associated with a carcinoma or overlying a lesion such as carcinoid tumour or small cell carcinoma when it can be atypical and suggest an erroneous cytological diagnosis of non-small cell carcinoma in brushings material. Sometimes the main biopsy fragments are negative but dyscohesive clusters of cytologically malignant cells lie in mucus separate from the epithelial surface.

Close correlation with cytology preparations, e.g. bronchial brushings and washings and transbronchial fine needle aspirates increases diagnostic yield and accuracy with *agreement rates of 70–90%* for small cell carcinoma, well differentiated squamous cell carcinoma and adenocarcinomas. Cytology is particularly helpful where there is biopsy sampling error, extensive biopsy crush artifact, e.g. small cell carcinoma, and extrabronchial or peripheral cancers. Cell yield and preservation can be high when biopsy fragments are negative or uninterpretable. Conversely the tissue pattern and capability for immunohistochemistry in a positive biopsy can be helpful in specific situations, e.g. primary versus secondary adenocarcinoma, small cell carcinoma versus malignant lymphoma. Immunochemistry can now also be reliably applied to liquid based cytology preparations: however preparation of a cell block wherever possible is now recommended for potential molecular testing. Thoracoscopic biopsy is used for patients suspected of having malignancy but in whom bronchial biopsy and cytology are negative and in suspected bilateral disseminated disease. Other sources of a positive diagnosis are radiologically guided FNAC or core biopsy of peripheral lung tumours, pleural fluid cytology, and FNAC or biopsy of cervical or supraclavicular lymph nodes. These approaches should be considered when the tumour is difficult to diagnose by conventional means, the patient is unfit for bronchoscopy or there is suspected disseminated disease. Thus a *tissue diagnosis* is obtained providing *staging information, a basis for adjuvant therapy,* and *exclusion of unrelated non-respiratory malignancy,* e.g. malignant lymphoma.

The pulmonary nodule: a relatively common issue emerging at specialty multidisciplinary team meetings is the *significance of a solitary pulmonary nodule* detected at CT scan staging or follow up of a primary cancer originating at various organ sites. The nodule may be *benign*, e.g. healed tuberculosis, abscess, rheumatoid nodule, aspergilloma, pulmonary hamartoma or, *malignant*, e.g. a primary lung cancer, solitary metastasis or well differentiated neuroendocrine (carcinoid) tumour. A range of *clinical and radiological features* help determine the *probability of malignancy* such as a history of haemoptysis, a

previous high stage cancer with lymphovascular invasion, older age and smoking habit. *CT scan clues* include spiculated edges, size (2.1–3 cm: malignancy likelihood ratio of 3.67), and change in size over a period of time. *PET scan* metabolic activity has a high sensitivity and specificity for malignancy although lesions <1 cm are usually below its resolution. Indeterminate small lesions can be monitored by serial CT scans. Serum tumour markers may be of help. If suspected of being malignant, transbronchial or transthoracic biopsy may be required as the outcomes for the patient can differ significantly. The nodule could represent a new primary lung cancer amenable to resection, a potentially resectable solitary metastasis from a previously completely excised primary (e.g. colorectal cancer), or, result in exclusion from further radical treatment due to it being non-regional metastatic (pM) disease with an adverse outlook e.g. oesophagogastric carcinoma.

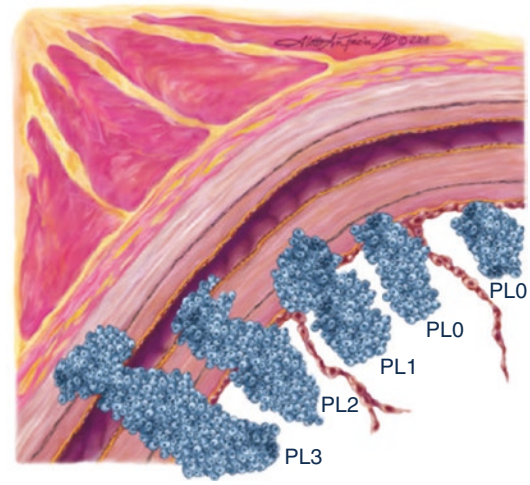

Fig. 17.1 Substaging of pleural invasion. (Reproduced, with permission, from Travis WD, Brambilla E, Rami-Porta R, Vallières E, Tsuboi M, Rusch V, Goldstraw P. Visceral Pleural Invasion: Pathologic Criteria and Use of Elastic Stains: Proposal for the 7th Edition of the TNM Classification for Lung Cancer. J Thorac Oncol. 2008; 3(12): 1384–90. Reproduced, with permission, from Elsevier)

Differentiation

- For squamous carcinoma based on cellular atypia, keratinisation and intercellular bridges.
- For adenocarcinoma based on percentage tumour gland formation (well/G1 > 95%; moderate/G2 50–95%; poor/G3 < 50%).

Small cell carcinoma and large cell carcinoma are by definition undifferentiated (grade 4) and have poor prognosis.

Extent of Local Tumour Spread

Border: pushing/infiltrative.
Lymphocytic reaction: prominent/sparse.
Distance to the proximal bronchial limit (mm).
Distance to the mediastinal limit (mm).
Distance to the pleura (mm).

- Visceral pleural invasion is recognised by direct perforation of the mesothelium and also infiltration of the inner elastin layer in the submesothelial plane. Note that the pleura can be distorted without actual true invasion and use of an elastin stain is helpful. Under TNM8 pleural invasion can be substaged as none (PL0), visceral pleura (PL1), surface of visceral pleura (PL2) or parietal pleura (PL3) (Fig. 17.1).

Distance to the pericardium (mm).
Mucosa, cartilage plates, parenchyma.
Tumour necrosis.

The TNM8 classification applies to non-small cell carcinomas, small cell carcinomas, and bronchopulmonary carcinoid tumours. It does not apply to sarcomas and other rare tumours.

pT1	Tumour 30 mm or less in greatest dimension, surrounded by lung or visceral pleura, without bronchoscopic evidence of invasion more proximal that the lobar bronchus
T1mi	Minimally invasive adenocarcinoma
T1a	Tumour 10 mm or less in greatest dimension
T1b	Tumour more than 10 mm but not more than 20 mm in greatest dimension
T1c	Tumour more than 20 mm but not more than 30 mm in greatest dimension

T2	Tumours more than 30 mm but not more than 50 mm in greatest dimension; or tumours with any of the following features (T2 tumours with these features are classified as T2a if 40 mm or less, or cannot be determined, or T2b if more than 40 mm but not more than 50 mm): • Involves the main bronchus • Invades visceral pleura • Associated with atelectasis or obstructive pneumonitis that extends to the hilar region, either involving part of the lung or the whole lung
T2a	Tumour more than 30 mm but not more than 40 mm in greatest dimension
T2b	Tumour more than 40 mm but not more than 50 mm in greatest dimension
T3	Tumour more than 50 mm but not more than 70 mm in greatest dimension, or one that directly invades one of the following: parietal pleura (PL3), chest wall (including superior sulcus tumours), phrenic nerve, parietal pericardium; or associated separate tumour nodule(s) (intra-pulmonary metastases) in the same lobe as the primary
T4	Tumour more than 70 mm in greatest dimension or one that directly invades one of the following: diaphragm, mediastinum, heart, great vessels, recurrent laryngeal nerve, carina, trachea, oesophagus, vertebra; or separate tumour nodule(s) (intra-pulmonary metastases) in different ipsilateral lobe to that of the primary

Note that for accurate postsurgical staging *clinical details are required,* e.g. proximal tumours in the main bronchus may require bronchoscopic information to distinguish between pT2 and pT4, if tumour involves the carina. Relevant clinical information may be supplied on the bronchoscopy specimen request form, or it may only be finalised after discussion at the multidisciplinary team meeting.

Involvement of parietal pericardium, rib and phrenic nerve are pT3.

Vocal cord paralysis, superior vena cava syndrome, compression of trachea or oesophagus, or involvement of visceral pericardium are classified as pT4 (Figs. 17.2, 17.3, 17.4 and 17.5).

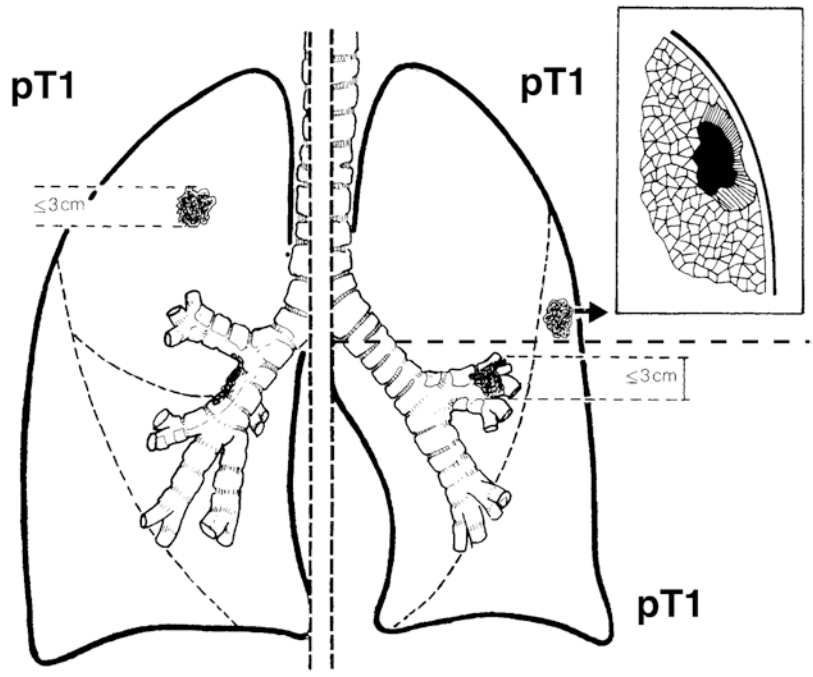

Fig. 17.2 Lung carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag.

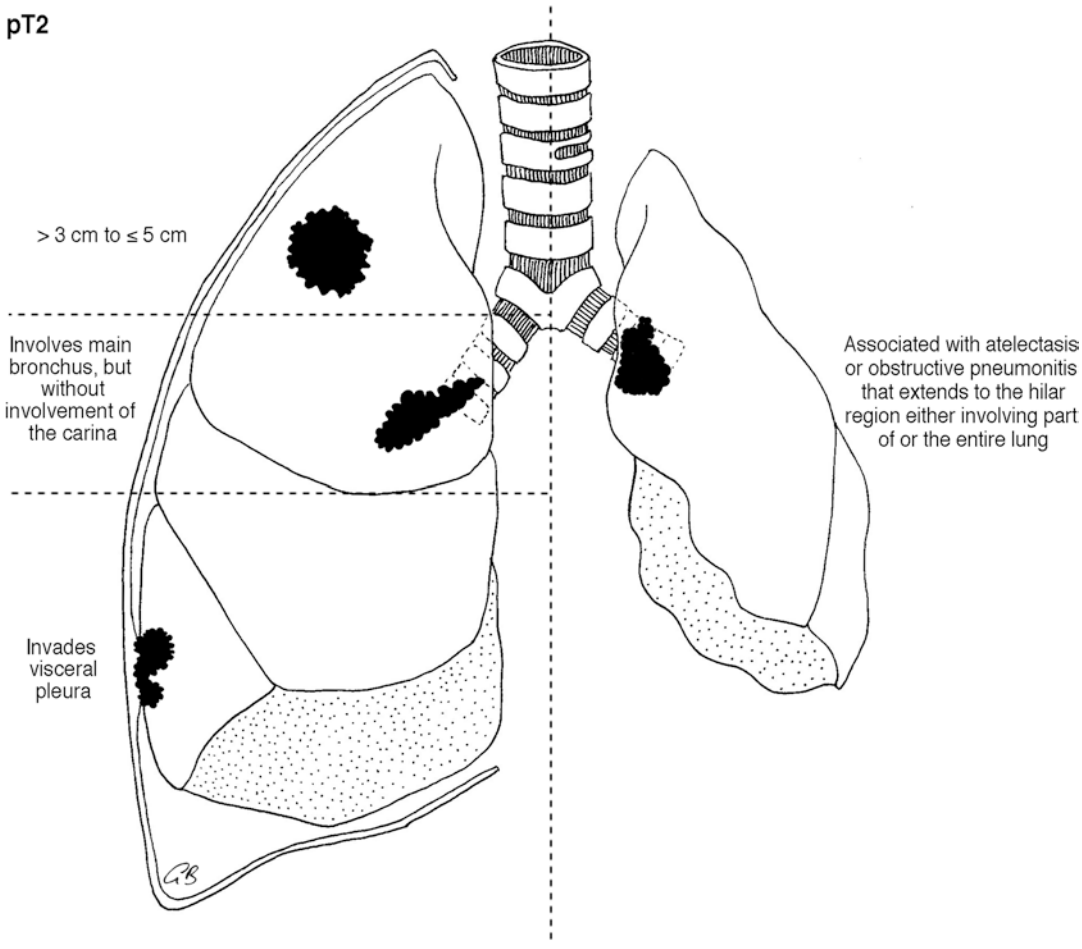

Fig. 17.3 Lung carcinoma

Some *60–75% of lung cancers are incurable at presentation* due to extensive local or distant spread with symptoms developing late in the disease course. Spread is by endobronchial polypoid growth, direct extension along the bronchus (proximally and distally) either with in situ or penetrating submucosal invasion, or direct into the lung parenchyma, and to the mediastinum and pleura when diaphragm and chest wall may be involved. Distant metastases are commonly seen in the liver, lung elsewhere (by lymphovascular or aerogenous spread), adrenals, bone, skin, kidney and central nervous system (particularly adenocarcinoma). A *majority of small cell carcinomas have extensive metastatic spread at the time of diagnosis* and they are regarded clinically as being either limited (confined to one hemithorax: use TNM8) or extensive disease for non-resectable cases.

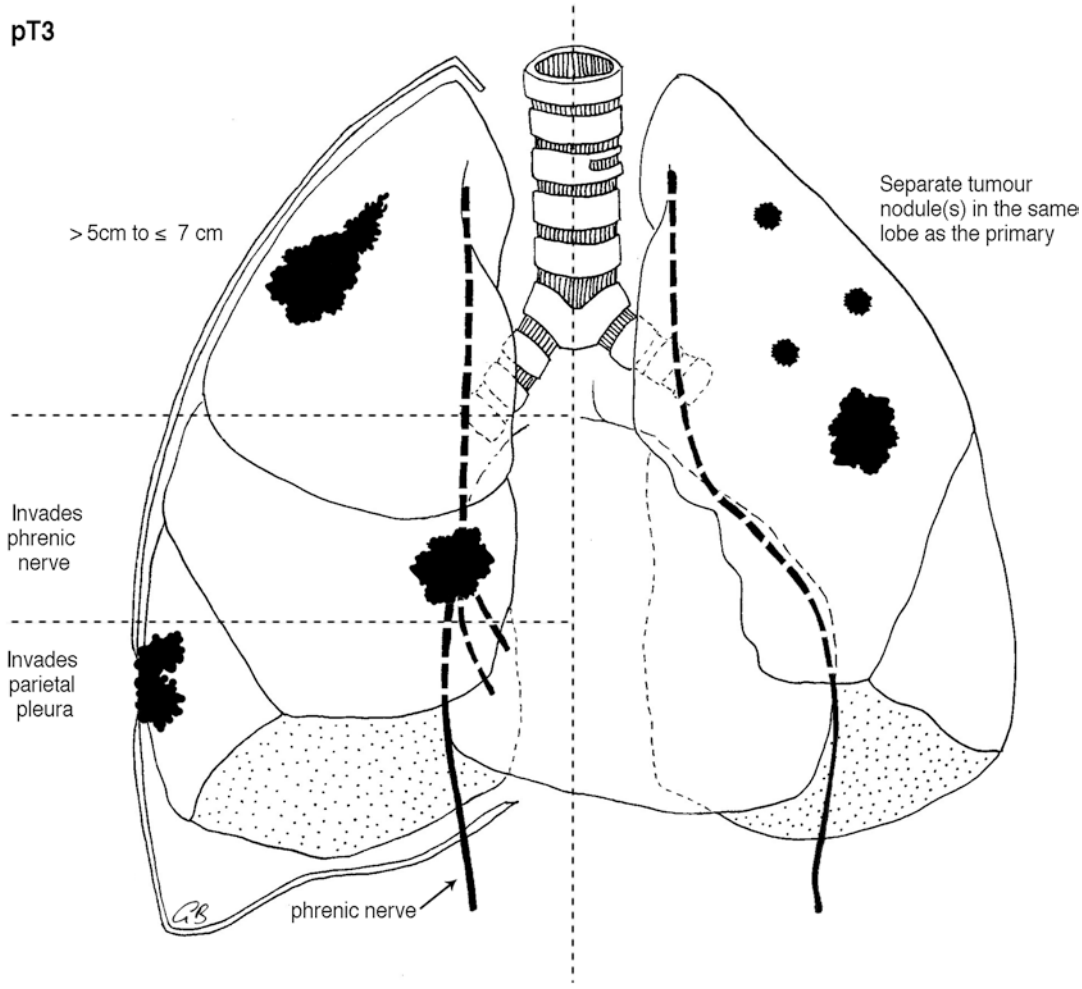

Fig. 17.4 Lung carcinoma

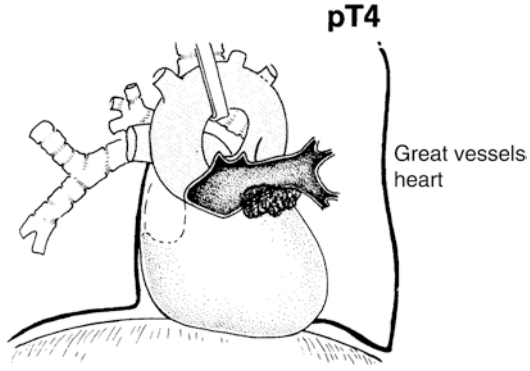

Fig. 17.5 Lung carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

Lymphovascular Invasion

Present/absent.

Intra-/extratumoural.

Common (80%) in lung cancer and along with lymph node metastases is an *adverse prognostic indicator.*

Lymph Nodes

Site/number/size/number involved/limit node/extracapsular spread.

Regional nodes: intrathoracic, scalene, supraclavicular.

A regional lymphadenectomy will ordinarily include a minimum of six lymph nodes. The surgeon will often submit separately dissected and labelled lymph node stations. Three of these lymph nodes/stations should be mediastinal, including the subcarinal nodes and three from N1 nodes/stations. Lymph node metastases are unusual for primary tumours <2 cm in diameter.

pN0	No regional lymph node metastasis
pN1	Metastasis in ipsilateral peribronchial/hilar/ intrapulmonary nodes including involvement by direct extension (node stations 10–14)
pN2	Metastasis in ipsilateral mediastinal/subcarinal nodes (node stations 1–9)
pN3	Metastasis in contralateral mediastinal, contralateral hilar, ipsi-/contralateral scalene or supraclavicular nodes.

M1 is distant metastasis and includes: (M1a) separate tumour nodule(s) in a contralateral lobe, or tumour with pleural nodules or malignant pleural or pericardial effusion. M1b is single extrathoracic metastasis in a single organ and involvement of a single distant (non-regional) lymph node. M1c is multiple extrathoracic metastases in one or several organs.

Cervical, scalene or mediastinal lymph node FNAC or biopsy is sometimes used to establish a diagnosis of carcinoma in patients suspected of having a malignancy but in whom bronchial biopsy and cytology are negative, when it represents recurrent disease, or who are medically unfit for invasive procedures (Figs. 17.6, 17.7 and 17.8).

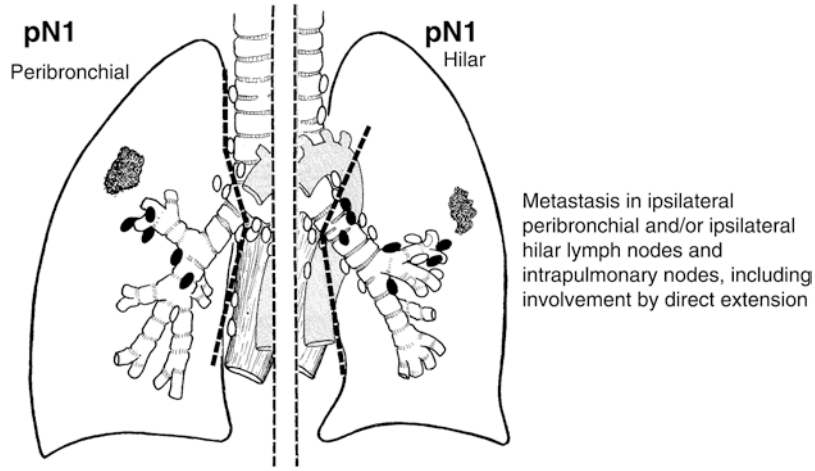

Fig. 17.6 Lung carcinoma: regional lymph nodes. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

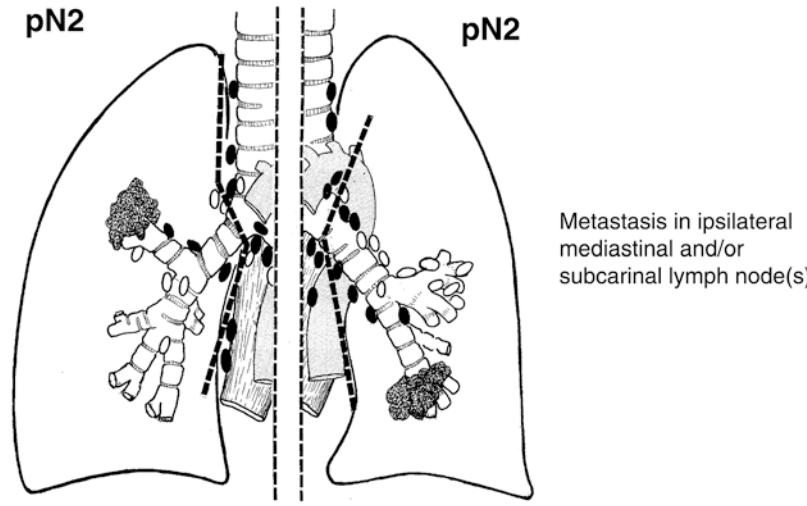

Fig. 17.7 Lung carcinoma: regional lymph nodes. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

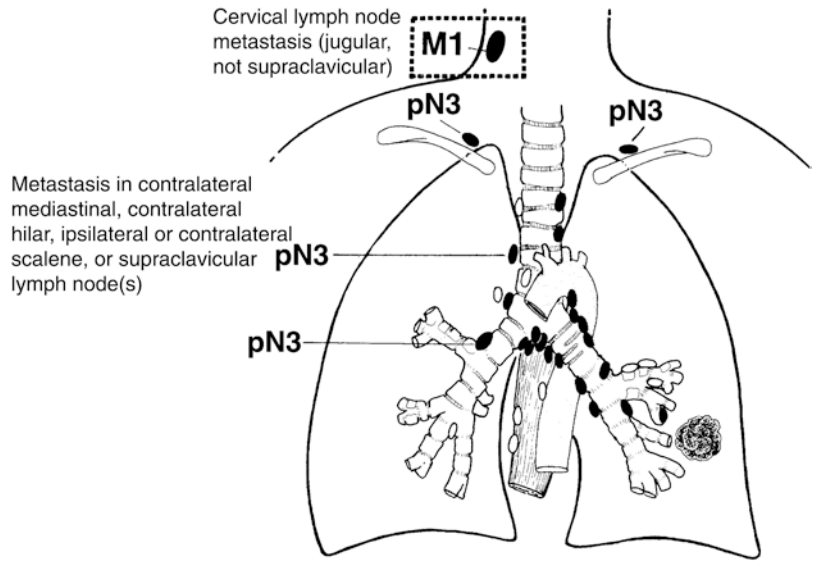

Fig. 17.8 Lung carcinoma: regional lymph nodes. M1 disease includes involvement of a single non-regional node. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

Excision Margins

Distances (mm) to the proximal bronchial, vascular, mediastinal soft tissue and chest wall limits and pleura. *Completeness of surgical resection and lymph node status* are the most important determinants of prognosis.

The presence of significant dysplasia at the bronchial mucosal limit can be a marker of potential local recurrence. Carcinoma in situ at the proximal bronchial margin is considered to represent residual disease.

Other Pathology

Lymphangitis carcinomatosa: can be diagnosed on transbronchial biopsy and is characterised by involvement of peribronchial and perivascular lymphatics.

Asbestos bodies/asbestosis/malignant mesothelioma: 5% of lung cancer deaths. Asbestos exposure can also be a co-factor for development of bronchogenic carcinoma.

Scar/fibrosis: in lung periphery can occur within, predispose to, harbor or arise secondary to pulmonary adenocarcinoma.

Synchronous or metachronous lung cancers: in up to 5% of cases and associated with independent cancers of the head and neck region in 10–20% of cases. Upper aerodigestive tract panendoscopy may be required, often guided by relevant findings on CT scan.

Metastatic carcinoma: distribution varies greatly and may be single or multifocal, diffuse or nodular, endobronchial, parenchymal, lymphovascular, vasculoembolic or pleural. *Knowledge of the clinical history and direct comparison of morphology with the previous primary tumour are important.* This can be supplemented by cytokeratin profile expression (CK7/20) and specific immune markers, e.g. thyroglobulin (thyroid), PSA/PSAP (prostate), CA 125/WT1 (ovary), ER/GCDFP-15/GATA3 (breast), CK20/CDX-2 (colorectal), TTF-1/Napsin A (lung adenocarcinoma) and CA19-9 (pancreatic and upper gastrointestinal cancers). Surgical resection of germ cell tumour and carcinomatous (e.g. colorectum) pulmonary metastases is not infrequent, the appearances of which can be greatly altered by adjuvant therapy, i.e. tumour necrosis, inflammation, fibrosis and tissue maturation. Similarly chemotherapy of metastatic osteosarcoma can result in pulmonary nodules of mature bone with no residual malignant tissue.

Atypical adenomatous hyperplasia: usually <5 mm diameter and well demarcated from adjacent lung parenchyma, it is regarded as *a*

precursor of malignancy with a *proposed AAH-lepidic/BAC-invasive adenocarcinoma sequence.* It is seen in 10–40% (often multifocal) of resected lung carcinomas especially peripheral or *lepidic/bronchioloalveolar carcinoma (BAC).* BAC (usually >1 cm) is either of type II pneumocyte or peripheral Clara cell origin and is generally *non-mucinous* in character (solitary, hob nail cells, good prognosis, 60–75% of cases). *Mucinous adenocarcinoma* (multifocal, bilateral, worse prognosis) is characterised as either *in situ (mucinous BAC), minimally invasive,* or *invasive.* The latter is characterized by size >3 cm, extent of invasion >0.5 cm, multiple nodules, an irregular border and miliary spread into adjacent lung parenchyma. A purely lepidic pattern tumour (BAC) has a better prognosis than other lung cancers, particularly when <3 cm diameter when it is regarded as adenocarcinoma in situ. It is being increasingly detected by CT/PET/FNAC investigations. Thorough sampling is necessary to detect any central prognostically adverse fibrous scar that may harbour more usual type invasive adenocarcinoma, or a micropapillary pattern. Their presence decreases 5 year survival rates from 78–100% down to 53–67%. CK7/TTF-1 and napsin A positivity are useful in distinguishing pulmonary adenocarcinoma from secondary carcinoma, e.g. large bowel and pancreas.

Extrapulmonary effects: inappropriate or ectopic hormone secretion, e.g. small cell carcinoma and Cushings syndrome (ACTH), carcinoid syndrome, diabetes insipidus (ADH), gynaecomastia, hypercalcaemia.

Immunophenotype

- *Small cell carcinoma*: chromogranin ±, positive for synaptophysin, CD56 (NCAM), CAM 5.2 (paranuclear dot reactivity), TTF-1, Ki67 usually >90%.
- *Squamous cell carcinoma*: cytokeratins (AE1/AE3, CK5/6, CK14, 34βE12), p40/p63 positive, ±EMA, vimentin, CEA, CK7, CAM 5.2.
- *Spindle cell carcinoma*: cytokeratin (focal AE1/AE3, CK5/6, 34βE12) positive, vimentin positive, ±CEA, TTF-1.
- *Adenocarcinoma*: CAM 5.2, CK7, TTF-1, Napsin A, EMA, CEA, vimentin, CD15, MOC-31, BerEP4 positive.
- *Lepidic/bronchioloalveolar carcinoma*: TTF-1, Napsin A, cytokeratin (CAM 5.2) positive. Non-mucinous is CK7 +/CK20-, mucinous CK7 +/CK20 +/TTF-1 ± .
- *Well differentiated neuroendocrine (carcinoid) tumour*: chromogranin, TTF-1, synaptophysin, CAM5.2, Ki67 ≤ 2%.
- *Specificity:* note that no single immunomarker is distinctive and that there can be considerable overlap between various non-small cell carcinoma subtypes and expression for cytokeratins, TTF-1, p40 and p63. Some squamous cell carcinomas can be CK7/TTF-1 positive and up to 20% of lung adenocarcinomas can be TTF-1 negative.

Prognosis

Prognosis in lung cancer (overall 13% 5 year survival) relates to weight loss, performance status, cell type (small cell carcinoma has 2% long-term survival and 90% present with locally advanced or systemic disease), cell differentiation (well differentiated is better than poorly differentiated), completeness of surgical resection and tumour stage, in particular nodal status. *Only 10–25% of non-small cell carcinomas are potentially curable by resection with a majority treated non-surgically.* An important therapeutic distinction must be made between *small cell carcinoma* and *non-small cell carcinoma.* A general *guide to suitability for surgical resection* is non-small cell cancer >2 cm from the carina and without mediastinal lymph node involvement. Operability may be induced by neoadjuvant chemotherapy or chemoradiotherapy.

Adjuvant or first line radical radiotherapy ± chemotherapy may be offered to patients with a poor performance status. *Palliative chemo−/radiotherapy* can be given to patients with high stage disease, or *local ablation* treatment if there is impending endoluminal airway obstruction. An important development in *pulmonary adenocarcinoma* is the determination of *epidermal*

growth factor receptor (EGFR) mutation status. 5–15% of cases, in particular females and never smokers, are positive by *genetic mutation analysis* and a proportion of these patients with locally advanced, recurrent or metastatic disease respond to *targeted oral erlotinib or gefitinib therapy*—an EGFR tyrosine kinase inhibitor. The role of the pathologist is to ascertain a firm diagnosis of pulmonary adenocarcinoma and to select an appropriate block rich in tumour for genetic mutation analysis. Conversely anti-endothelial growth factor therapy (bevacizumab) is contraindicated in squamous cell carcinoma due to the potential risk of haemorrhage. Other potential drug targets, mutation status (EGFR, ALK, KRAS) in non-small cell lung carcinoma and the effects on the differential tumour response to various chemotherapeutic agents are being explored. Surgery is only considered for small cell carcinoma if it is *limited in extent*, i.e. ≤3 cm diameter without lymph node metastases—otherwise *chemoradiation* is indicated. The significance of occult bone marrow micrometastases detected by immunohistochemistry is uncertain. Prognosis improves if the carcinoma is "early" or "occult" with positive cytology but negative chest radiology: preoperative chemotherapy may also be beneficial.

Operable (localised)	5 year survival
Squamous/large cell adenocarcinoma	35%
Well differentiated	75%
Poorly differentiated	35%
Overall	50%
Small cell	10%

Non-operable (extensive)	5 year survival
Squamous cell	6%
Small cell	2%

Other Malignancy

Leukaemia

- 50–60% of acute leukaemias.
- 15–40% of chronic leukaemias.
- Pleural, peribronchial and perivascular lymphangitic spread.
- Rarely, there is a granulocytic sarcoma mass lesion; CD34/CD43/CD68/CD117/chloroacetate esterase/myeloperoxidase positive.

Malignant Lymphoma

- Primary *MALToma* or *diffuse large B cell lymphoma (5–20% of cases*) or secondary to nodal/systemic disease. Pulmonary involvement is present in up to 38% of patients with lymph node based malignant lymphoma. Designation depends on the constituent cell type and clinicopathological staging of the extent of disease. There may also be a previous history of nodal lymphoma.

MALT Lymphoma

- *The commonest primary lung lymphoma.*
- Sixth/seventh decade.
- ± Sjögren's syndrome or rheumatoid arthritis.
- Central mass with peripheral tracking along septa, bronchovascular bundles and pleura.
- Solitary or multifocal ± spread to other MALT sites.
- Limited resection ± chemo−/radiotherapy.
- 5 year survival is 80–90%, most are low-grade but can transform to high-grade (40–60% 5 year survival).
- Some large B cell (high-grade) pulmonary lymphomas probably originate in low-grade MALToma.

High-grade lesions are easily assessed as malignant but must be distinguished from other tumours, e.g. non-small cell carcinoma, using immunohistochemistry. *Low-grade lesions* are characterised by a dense monomorphic interstitial population of centrocyte like cells, absence or colonisation of reactive follicles, lymphoepithelial lesions and local invasion. B cell predominance, light chain restriction and *immunoglobulin heavy/light chain monoclonal gene rearrangements* are confirmatory in distinction from a lymphoid interstitial pneumonitis

or follicular bronchiolitis. Mass lesions previously designated pseudolymphoma are now considered to be low-grade MALTomas.

Primary or Secondary Hodgkin's Lymphoma

- Usually secondary to spread from mediastinal disease.
- Parenchymal nodules, endobronchial plaque/nodules or miliary interstitial spread.

T Cell Rich Large B Cell Lymphoproliferative Disorder/ Malignant Lymphoma (EBV Related)

- On a spectrum with B cell lymphomatoid granulomatosis/angiocentric immunoproliferative lesion and associated with EBV.
- Polymorphous (lymphocytes, plasma cells, histiocytes) angiocentric/destructive infiltrate containing atypical lymphoid cells.
- Clonal immunoglobulin heavy chain gene rearrangements are present in 50% of cases.
- *Prognosis (poor*) is dictated by the *histological grade* (number of atypical cells) and extrapulmonary lesions are common (kidneys, liver, brain, spleen).

Intravascular Malignant Lymphoma

- Malignant angioendotheliomatosis or *angiocentric large B cell lymphoma*: skin, central nervous system and adrenal gland involvement with poor prognosis.

Post Transplant Lymphoproliferative Disorder (PTLD)

- An EBV associated spectrum of lymphoproliferation (immortalised B cells) following (first 2 years) immunosuppression for solid organ or bone marrow transplantation. It occurs in the native or transplant lung affecting 2% of patients.
- Can respond to *reduction of immunosuppression* and antiviral therapy.
- *Early* (plasma cell hyperplasia), *polymorphous* (infectious mononucleosis-like) or *monomorphic* (as in large B cell lymphoma) stages. Also shows angioinvasion and necrosis.
- *Monomorphic/monoclonal lesions* are worse prognosis than *polymorphic/polyclonal lesions.*
- Diagnosis by chest X-ray, CT scan, transthoracic needle biopsy, morphology, immunophenotype, clonality (PCR: 50% of monomorphic cases are monoclonal) and EBV status (in situ hybridisation).

Other Haematopoietic Malignancy

- *Anaplastic large cell lymphoma:* CD30/ALK positive. Null phenotype but T cell receptor gene rearrangements in 90% of cases.
- *Extramedullary plasmacytoma:* peri−/intrabronchial mass, amyloid, light chain restriction, 40% 5 year survival.

Epithelioid Haemangioendothelioma (Intravascular Sclerosing Bronchioloalveolar Tumour (IVSBAT))

- A vascular tumour of intermediate-grade malignancy (CD31/CD34 positive) in young adult females. Slow progression and association with liver and skin lesions.

Kaposi's Sarcoma

- HIV.

Angiosarcoma

- Primary or secondary.

Malignant Melanoma

- Usually secondary, intraparenchymal or endobronchial.

Sarcomas Including Synovial Sarcoma, Leiomyosarcoma, Rhabdomyosarcoma (Embryonal Children, Pleomorphic Adults)

In any lung sarcoma it is important to exclude the more common possibilities of either metastatic disease from a primary elsewhere, a lung carcinoma with sarcoma like morphology, or a spindle cell malignant mesothelioma. Endobronchial sarcoma presents earlier with better prognosis than intrapulmonary sarcoma. Sarcoma may also arise as an intraluminal mass within the pulmonary artery. Synovial sarcoma can be lung or pleura based, leiomyosarcoma, angiosarcoma and intimal sarcoma centred on major vessels, while chondrosarcoma, osteosarcoma and malignant small blue cell tumours can arise from rib or chest wall and invade underlying lung.

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Malignant Mesothelioma

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Neil Anderson

Introduction

Malignant mesothelioma is a major asbestos related worldwide health problem with a peak of some 250,000 deaths predicted in Europe over the next decade.

Pleural disease can be asymptomatic or present with pain, breathlessness or general systemic effects, e.g. weight loss. Pleural plaques, fibrotic thickening, calcification and pleural effusion are demonstrated by chest X-ray and CT scan. Pleural thickening >1 cm, nodularity and extension on to the mediastinal surfaces make malignancy more likely.

Ultrasound localised thoracentesis or pleural fluid aspiration can be diagnostic or therapeutic for symptomatic relief. Percutaneous closed needle biopsy is insufficient in up to 30% of cases and only diagnostic in a minority (16–40%) of patients. It may need to be supplemented by CT guided or video assisted thoracoscopic (VATS) biopsy or open pleural biopsy. The latter may be allied to decortication or stripping of the constricting visceral peel carried out for the dual purpose of making a diagnosis and to permit expansion of the underlying lung. Specimen size is important with a positive diagnosis in a majority of specimens >10 mm. Histological subtype also affects diagnostic yield with paucicellular desmoplastic and sarcomatoid mesotheliomas providing the greatest diagnostic challenges. Chest wall biopsy site seeding is a potential problem for which preventative radiotherapy is used.

Pleurectomy attempts to debulk the malignant mesothelioma providing multiple strips of pleural membrane. Extrapleural pneumonectomy is en bloc resection of the pleurae, lung, ipsilateral hemidiaphragm and pericardium. Peritoneal disease may present with ascites and a tissue diagnosis is obtained by peritoneal fluid cytology, laparoscopic or open biopsy.

Gross Description

Specimen

- Pleural, peritoneal or laparoscopic aspiration cytology or biopsy/ thoracoscopic or open biopsy/pleurectomy/extrapleural pneumonectomy/omentectomy.
- Size (mm) and weight (g).

Tumour

Site

- Pleural (visceral/parietal)/pericardial/peritoneal.

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_18

- Pleura (>80%) is the commonest site then peritoneum (10–15%).

Size

- Length × width × depth (mm) or maximum dimension (mm).

Appearance

- Localised (solitary)/diffuse/nodular/plaque/infiltrative/cystic change.

Edge

- Circumscribed/irregular.

Histological Type

Multilocular Peritoneal Inclusion Cysts (Multicystic Peritoneal Mesothelioma)

- On the surfaces of the uterus, ovary, bladder, rectum and pouch of Douglas it is *potentially locally recurrent*, and rarely, invasive into retroperitoneum, bowel mesentery and wall. Differential diagnoses include unilocular peritoneal inclusion cysts, cystic adenomatoid tumour and malignant mesothelioma. About 50% recur over many years and some have a previous history of surgery, endometriosis or pelvic inflammatory disease.

Well Differentiated Papillary Peritoneal Mesothelioma

- In middle aged women, it is rare, with most being an incidental finding at hysterectomy. Localised and benign but can be extensive and diffuse nodular serosal/omental disease with ultimately progression and ascites.

Diffuse Malignant Mesothelioma

- The main variants are *epithelial (epithelioid), sarcomatoid* and *biphasic*. Rarer types are desmoplastic, small cell, lymphohistiocytoid, deciduoid and undifferentiated or anaplastic.

– Epithelial (60%):	Tubulopapillary
	Microglandular
	Solid (epithelioid)
	Small cell 6%
	Pleomorphic (large cell)
	Lymphohistiocytoid 1%: aggressive
	Deciduoid
	Rhabdoid
	Clear cell
	Signet ring cell
– Sarcomatoid (15%):	Fibrosarcomatous like/cellular storiform patterns
	Fibrous (desmoplastic) 5–10%
	Angiomatoid
	Chondroid/osteoblastic/rhabdomyoblastic/leiomyoid
– Mixed (biphasic) (25%)	

Metastatic Carcinoma

- *The commonest malignant tumour affecting the pleura.*
- Lung/breast/stomach/ovary/prostate/kidney carcinomas, malignant melanoma, soft tissue sarcoma and germ cell tumours can all *mimic malignant mesothelioma* on histology, and even gross distribution of disease resulting in an encasing pseudomesotheliomatous picture. Knowledge of a *relevant previous history* and close clinicopathological correlation with *comparison of tumour morphology* and *immunohistochemistry* are needed.
- Metastatic malignant melanoma occasionally involves the pleura. It can be spindle cell in type mimicking lung cancer and malignant mesothelioma. Standard malignant melanoma markers (S100, melan-A, HMB-45, SOX-10) may be only variably positive. A *previous clinical history* is important to think of the diagnosis and *comparison of the initial malignant melanoma histology* is helpful in confirmation.

Differentiation

- *No formal grading system* is currently used, and probably best regarded as a minority of well differentiated lesions (e.g. papillary and

multicystic peritoneal variants), and others which are not graded. *Sarcomatoid lesions* are considered *poorly differentiated*, *epithelial lesions* as *well to moderately differentiated*. A high mitotic rate is an adverse indicator.

Extent of Local Tumour Spread

Border: pushing/infiltrative.

Lymphocytic reaction: prominent/sparse.

The TNM8 classification applies to pleural malignant mesothelioma only.

pT1	Tumour limited to the ipsilateral parietal with or without involvement of the visceral, mediastinal or diaphragmatic pleura
pT2	Tumour involving each of the ipsilateral pleural surfaces (parietal, mediastinal, diaphragmatic and visceral pleura) with at least one of the following features: – Involvement of diaphragmatic muscle – Extension of tumour from visceral pleura into the underlying pulmonary parenchyma.
pT3[a]	Tumour involves ipsilateral pleural surfaces with also any of: invasion of endothoracic fascia, mediastinal fat, solitary chest wall soft tissue focus, non-transmural pericardial involvement.
pT4[b]	Tumour involves ipsilateral pleural surfaces with also any of: contralateral pleura, peritoneum, rib, extensive chest wall or mediastinal invasion, myocardium, brachial plexus, spine, transmural pericardium, malignant pericardial effusion.

[a]Locally advanced but potentially resectable tumour
[b] Locally advanced but technically non-resectable tumour (see Figs. 18.1 and 18.2)

Spread is typically pleural, encasing the lung with extension along fissures and septa and into subpleural lung parenchyma. *Lymph node spread* and *distant metastases* (up to 30% of cases) occur *late in the disease course*.

Contiguous spread through the diaphragm with involvement of abdominal organs is not infrequent.

Peritoneal disease is usually secondary to pleural tumour but can also be primary and asbestos related. *Pericardial disease* usually represents spread from pleural tumour.

Flat or granular pleura adjacent to tumour nodules may show cytological atypia constituting *"mesothelioma in situ"* and although unusual in pleural biopsies this can be a useful indicator of potential for progression to invasion or the presence of concurrent malignant mesothelioma not sampled by the biopsy needle.

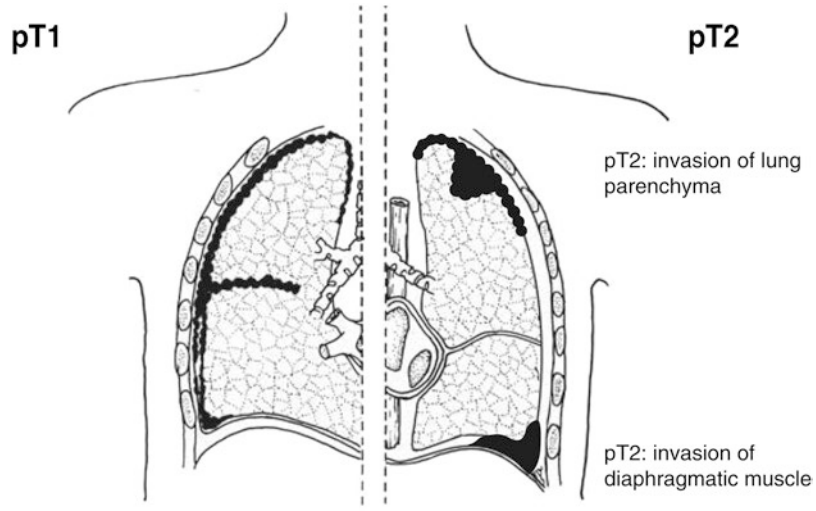

Fig. 18.1 Pleural malignant mesothelioma. Adapted from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

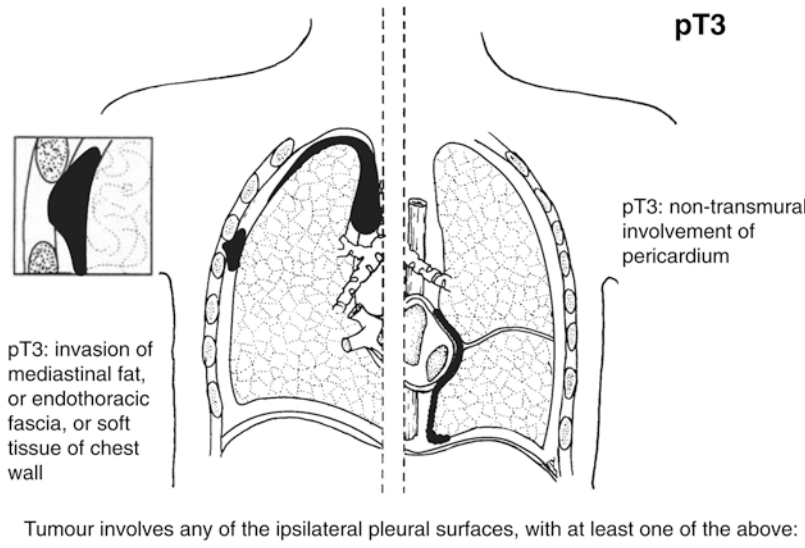

Fig. 18.2 Pleural malignant mesothelioma. Adapted from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

Lymphovascular Invasion

- Present/absent.
- Intra-/extratumoural.

Lymph Nodes

Site/number/size/number involved/limit node/extracapsular spread.

Regional nodes: intrathoracic, internal mammary, scalene, supraclavicular.

pN0	No regional lymph node metastasis
pN1	Metastases in the ipsilateral bronchopulmonary, hilar, or mediastinal lymph nodes (including the internal mammary, peridiaphragmatic, pericardial fat pad, or intercostal lymph nodes)
pN2	Metastases in the contralateral bronchopulmonary, hilar, or mediastinal lymph nodes or ipsilateral or contralateral supraclavicular lymph nodes

Excision Margins

Distance (mm) to the nearest painted excision margin of local resection for limited disease.

Other Pathology

Adenomatoid Tumour

- Benign: a circumscribed pale nodule in the epididymis, fallopian tube or uterine myometrium with or without a serosal connection. It usually comprises a microcystic pattern of mesothelial cell proliferation with prominent intervening smooth muscle.

Localised Solitary Fibrous Tumour

- Rare/solitary/visceral pleura, circumscribed/smooth or bossellated.
- "Patternless" fibroblasts and vessels with bland cytology, 90% are benign, and CD 34/bcl2/CD99 positive.
- Now regarded as arising from subserosal fibroblasts/mesenchymal cells rather than from mesothelial cells, and is encountered in other organs.

Pleural plaques, subpleural fibrosis, asbestosis, bronchogenic carcinoma:

About 2–3% of people with exposure to significant amounts of asbestos develop malignant

mesothelioma. The consequences of asbestos exposure depend on the *fibre type* (amphiboles, e.g. crocidolite are particularly pathogenic), and can be both *dose related* and *idiosyncratic*. Some individuals require less exposure to develop asbestos-related disease while others with extensive fibre burden do not. Classically there is a *long lag period* of 20–50 years until illness develops. *Exposure can also be second-hand,* e.g. washing a partner's contaminated clothing. Fibre burden can be assessed by incineration of lung tissue and quantitation by scanning electron microscopy. Identification of ferruginous asbestos bodies by light microscopy correlates with a significant fibre load. Exposure is usually *occupation related*. A typical clinical history of malignant mesothelioma is a unilateral opaque chest radiograph with necessity for multiple, repeated pleural taps. Note that the tumour may infiltrate chest wall through the biopsy needle track.

Metastatic malignancy: a number of *malignant tumours metastatic to the pleura can mimic malignant mesothelioma.* These include, amongst others, breast carcinoma, adenocarcinoma of lung, spindle cell carcinoma/carcinosarcoma of lung, renal cell carcinoma, malignant melanoma, malignant lymphoma and leukaemia, ovarian carcinoma, thymoma and sarcoma. There is no one specific marker for malignant mesothelioma and diagnosis often relies on exclusion of metastatic carcinoma and sarcoma by *clinical history, examination, radiology, comparative morphology and immunohistochemistry* of cytology or biopsy material. *Radiology* is very useful in demonstrating the distribution of disease, e.g. diffuse pleural thickening of malignant mesothelioma versus an intrapulmonary/hilar mass with pleural thickening (lung carcinoma), or multiple discrete lung nodules with pleural thickening (metastatic carcinoma).

Diagnostic immunohistochemistry: positivity with one or more of CEA, BerEP4, MOC-31, TTF-1, E-cadherin, NapsinA (Table 18.1), indicating *pulmonary adenocarcinoma* and excluding

Table 18.1 Immunoexpression in mesothelial cell proliferations

Antibody	Epithelial mesothelioma	Reactive mesothelial cells	Pulmonary adenocarcinoma
CAM5.2	+	+	+
Vimentin	+	±	±
EMA	+	−	+
CK 5/6	+	+	−
HBME-1	±	+	−
Thrombomodulin	±	−	−
WT-1	+	+	−
CEA	−	−	+
Leu M1 (CD15)	−	−	+
Ber EP4	−	−	+
TTF-1	−	−	+
E-cadherin	−		+
N-cadherin	+		−
Podoplanin (D2–40)	+		−

Other markers include:
- BAP-1: loss of BAP-1 expression is seen in a significant number of malignant mesotheliomas
- S100, HMB-45, melan-A (malignant melanoma)
- CD 45, CD 20, CD 3 (B/T cell malignant lymphoma)
- Thyroglobulin, CK 19, TTF-1, RET (thyroid papillary carcinoma)
- CA125, CK 7, WT-1 (ovarian serous carcinoma)
- EMA, vimentin, CD10, abdominal ultrasound/CT scan (renal cell carcinoma)
- PSA, PSAP (prostate carcinoma)
- βHCG, AFP, CD30, PLAP, CD117, OCT3/4, SALL4 (germ cell tumour)
- ER/PR, GCDFP-15, CK 7 (breast carcinoma)
- CEA, CK 20, CDX-2 (gut carcinomas)
- CA 19–9, CK 7, CK 20, CA125 (pancreatic carcinoma)

malignant mesothelioma (although some cases can be BerEP4 positive). Putatively positive *mesothelioma markers* that are negative in lung adenocarcinoma are CK 5/6, calretinin, thrombomodulin, HBME-1, N-cadherin, D2–40, mesothelin and WT-1. EMA usually shows strong membranous positivity in epithelial malignant mesothelioma. *A practical panel for diagnostic use is CEA, BerEP4, TTF-1, EMA, CK5/6, calretinin* and *WT-1.* Note that immunoreactivity for various of these markers can be reduced in poorly differentiated epithelioid and spindle cell malignant mesotheliomas. Adenocarcinoma may also be mucicarmine positive/PAS-diastase resistant for mucin (60% of cases). Distinction of malignant mesothelioma from non-small cell lung cancers is usually by morphology as there can be overlap in cytokeratin profiles, e.g. squamous cell carcinoma is CK5/6 positive although it should be calretinin/WT-1 negative.

Sarcomatoid mesothelioma: may co-express cytokeratins, vimentin and muscle-specific actin. Differential diagnosis is spindle cell lung carcinoma and primary or secondary sarcoma with similar immunophenotypic co-expression, e.g. epithelioid vascular tumours, leiomyosarcoma or synovial sarcoma. *Desmoplastic mesothelioma* (>50% of the tumour is poorly cellular fibrous tissue) must be distinguished from *fibrous pleurisy* (inflammatory and reactive looking) and pleural plaque (acellular basket weave pattern of collagen) neither of which show parenchymal or chest wall infiltration, or strongly cytokeratin positive spindle cells distributed diffusely throughout the full depth of the fibrous tissue. *Solitary fibrous tumour* (CD34 positive, cytokeratin negative) should also be considered.

Mesothelial malignancy versus reactive mesothelial hyperplasia: morphological markers are *cytological atypia* and *cellularity, necrosis* and *invasion* of subpleural connective tissue, i.e. *the extent, degree of atypia,* and *invasiveness of the mesothelial cell population* in an adequate specimen. Malignant mesothelial cells also may express p53 and EMA but unlike reactive mesothelial cells are negative for desmin. Staining with BAP1 and assessment of p16 ISH may help in the distinction of malignancy versus a reactive proliferation. However *ancillary studies may be of limited diagnostic use in making this distinction* and the emphasis should be on morphology and correlation with imaging findings. In a significant minority of cases diagnosis may not be able to be made at first presentation, but the possibility raised from a constellation of atypical features in the pleural fluid cytology and biopsies over a series of medical admissions with attendant investigations.

Reactive mesothelial hyperplasia and dense inflammatory fibrosis do not clinically progress unlike malignant mesothelioma with *repeated clinical presentations* and *the need for symptomatic relief by paracentesis.* Some well differentiated lesions pursue such a biological course over a span of several years but normally the *clinical progression is relatively rapid* indicating a malignant diagnosis. Reactive mesothelial hyperplasia may be seen in pulmonary infarction, tuberculous pleuritis, rheumatoid arthritis, systemic lupus erythematosus, and overlying primary or secondary neoplasms.

Prognosis

Prognosis of diffuse malignant mesothelioma relates to stage but is generally poor with the majority of patients dead from their disease within 1–3 years: 4–12 months for pleural mesothelioma, less than 18 months for peritoneal mesothelioma. Spindle cell tumours are more aggressive than epithelioid variants. Malignant mesothelioma is variably sensitive to *chemoradiation*. *Adjuvant chemotherapy* combined with *resection of limited disease* (pleural decortication or pleuropneumonectomy) can occasionally result in prolonged remission with 15–30% 3 year survival rates, but only a small minority of patients are considered eligible for this treatment. *Symptomatic relief* may be gained by multiple paracenteses of malignant pleural or peritoneal fluid supplemented by *intracavitary chemotherapy*. This may act either directly on the tumour cells reducing secretions or produce a loculated, sclerosant effect. *Pallative radiotherapy* and *chemical (talc) pleurodesis* also have roles to play. The former can induce bizarre

multinucleated tumour cell forms and the latter a pseudosarcomatous biphasic pattern. Iatrogenic *wound site implantation* metastases can also occur in 15–20% of cases. Well-differentiated multicystic and papillary peritoneal mesotheliomas are regarded as being of borderline or low malignant potential.

Other Malignancy

- Epithelioid haemangioendothelioma, angiosarcoma, synovial sarcoma.
- Thymoma.
- Malignant small cell tumour of thoracopulmonary region (Askin tumour).
- Desmoplastic small cell tumour.
- Malignant lymphoma: secondary to a lymphoproliferative disorder (e.g. CLL), nodal or systemic disease, or, primary, e.g. the rare pyothorax, or, effusion associated malignant lymphomas (large B cell lymphomas: EBV and HHV 8/immunodeficiency associated, respectively).

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Mediastinal Cancer

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Neil Anderson

Introduction

Mediastinal neoplasms are often asymptomatic (50% of cases) particularly if benign. Malignant tumours are more often associated with both local and systemic symptoms.

Mediastinal neoplasms can present in a number of ways:

1. During staging or follow up (chest X-ray, CT or MRI scans) of a patient with a known cancer elsewhere e.g. lung carcinoma, colorectal carcinoma, non-Hodgkin's/Hodgkin's malignant lymphoma, or testicular/ovarian germ cell tumour.
2. As an incidental finding on chest X-ray in a patient who may or may not have ill-defined symptoms e.g. dyspnoea, cough, dysphagia.
3. As a finding in the investigation of a patient with other presenting features, e.g. pneumonia or pleural effusion.
4. As superior vena cava (SVC) syndrome due to malignant infiltration or compression of local structures, e.g. lung carcinoma (primary or secondary), malignant lymphoma.
5. As a paraneoplastic syndrome, e.g. myasthenia gravis, Cushings disease (ACTH: adrenocorticotrophic hormone) or secretion of inappropriate antidiuretic hormone (SIADH).

Therefore, knowledge of a relevant past medical history is fundamental in determining the nature of the underlying abnormality. Further clinical investigations are viewed in light of this and can include: CT/MRI scans to determine local invasion and solid/cystic features, angiography, blood tests[1] (AFP, HCG, LDH, parathormone, alkaline phosphatase, acetylcholine receptor antibodies), electromyography and tensilon test, oesophagoscopy and bronchoscopy. Age is also indicative in that thymomas are the commonest thymic tumours in adults followed by malignant lymphoma, whereas the converse applies to thymic based tumours in children. Tissue diagnosis is often obtained by thoracoscopic, CT guided percutaneous/transbronchial FNAC (fine needle aspiration cytology) or needle core biopsy. Material is provided not only for routine morphology, but, importantly, ancillary techniques such as immunohistochemistry to aid in the distinction between diagnoses such as metastatic carcinoma, lymphoblastic or large cell

[1] AFP: α-fetoprotein, HCG: β subunit human chorionic gonadotrophin, LDH: lactate dehydrogenase.

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_19

malignant lymphoma and thymoma. However, in some cases, particularly where malignant lymphoma is suspected, due to the limitations of these sampling techniques, invasive mediastinal incisional biopsy may be required via cervical mediastinoscopy or anterior mediastinotomy. This can improve diagnostic yields from 60% to over 80%. Prior to this it should be determined whether a more convenient source of tissue for diagnosis is available, e.g. palpable supraclavicular or cervical lymphadenopathy, or pleural fluid cytology. Needle core biopsy of a thymoma is also contentious due to disruption of the tumour capsule and the possibility of seeding into the operative site.

Tumour site within the mediastinum is an important clue to tumour type, e.g. middle mediastinal disease is most likely to be metastatic carcinoma or malignant lymphoma, whereas anterosuperior mediastinal lesions are more likely to be thymus based cancers, e.g. thymoma, germ cell tumours and malignant lymphoma. Thus *a diagnostic short list is created based on clinical presentation, patient age, gender and tumour location.*

Gross Description

Specimen

- Percutaneous or mediastinoscopic/thoracoscopic FNAC/(needle core) biopsy/resection (cervical thymectomy or thoracotomy).
- Number of fragments and their length (mm).
- Size (mm) and weight (g).

Tumour

Site

Mediastinal boundaries:	
Lateral	Pleural cavities
Anterior	Sternum
Posterior	Spine
Superior	Thoracic inlet
Inferior	Diaphragm

- Superior:
  - Thymoma and thymic cysts.
  - Malignant lymphoma.
  - Retrosternal nodular goitre thyroid.
  - Ectopic parathyroid lesions.

- Anterior:
  - Thymoma (75% of cases) and thymic cysts.
  - Well differentiated neuroendocrine (carcinoid) tumours.
  - Malignant lymphoma.
  - Germ cell tumours.
  - Metastatic carcinoma.
  - Thyroid/parathyroid lesions.
  - Mesenchymal lesions—lipoma, lymphangioma, haemangioma.

- Middle:
  - Metastatic carcinoma.
  - Malignant lymphoma.
  - Pericardial/bronchogenic cysts.
  - Primary cardiac tumours.

- Posterior:
  - Neural tumours—neurilemmoma, neurofibroma, ganglioneuroma, ganglioneuroblastoma, malignant schwannoma, neuroblastoma, paraganglioma, and, gastroenteric cysts.

Size

- Length × width × depth (mm) or maximum tumour dimension (mm).
- Size of thymoma can be a prognostic indicator.

Appearance

- Circumscribed/encapsulated/infiltrative/fleshy/pale/pigmented/cystic/ necrotic/haemorrhagic, e.g. thymoma can be encapsulated or infiltrative, solid/cystic or multiloculated, whereas malignant lymphoma is fleshy and pale ± necrosis and sclerosis. Teratoma can be cystic, solid, necrotic or haemorrhagic. Neurilemmoma is encapsulated ± cystic degeneration.

Edge

- Circumscribed/irregular.

Histological Type

Metastatic Carcinoma

- The *commonest malignant mediastinal tumour* (particularly in the middle mediastinum). It can mimic a primary thymic tumour both clinically and radiologically, e.g. small cell carcinoma lung can have a small primary lesion with extensive direct or lymph node spread to the mediastinum.
- *Direct spread:* lung, oesophagus, pleura, chest wall, vertebra, trachea.
- *Distant spread:* breast, thyroid, nasopharynx, larynx, kidney, prostate, testicular (or ovarian) germ cell tumour, malignant melanoma.

Identify a residual rim of lymphoid tissue at the tumour edge to indicate a lymph node metastasis.

Malignant Lymphoma

- *10–15% of mediastinal masses* in the adult and occurs in decreasing order of frequency in the anterior, superior and middle mediastinum. Thymic or lymph node based. It is the *commonest primary neoplasm of the middle mediastinum.*

Specific thymic/mediastinal features are:

Hodgkin's lymphoma: young females. Nodular sclerosis in type and fibrotic/lobulated ± thymic epithelial cysts and granulomas with lacunar cells (CD15/30 positive). Treatment is radiotherapy with or without chemotherapy and prognosis depends on the stage of disease.

Lymphoblastic lymphoma: acute dyspnoea in adolescent males due to compression of large airways by bulky disease, and sometimes SVC syndrome. Mediastinal plus cervical/supraclavicular and axillary disease; ± Hassall's corpuscles and can therefore mimic a thymoma. Small to medium sized lymphoid cells, apoptosis, TdT positive—usually T cell (CD3: 80% of cases) and a high Ki-67 index (>95%). Bone marrow, peripheral blood and central nervous system involvement are common. It is treated with aggressive intent.

Mediastinal large B cell lymphoma: young females presenting with SVC syndrome, airway obstruction, pleural/pericardial effusion or systemic symptoms. There is sclerosis/fibrosis—banded and pericellular. Immunophenotype is CD45, CD20 positive, Ki-67 positive in >70% of cells; also CD30, bcl-6 and CD10 the latter suggesting a follicle centre cell origin. Spread to pericardium, pleura, lung, sternum and chest wall are common. An initial response to chemotherapy is a good prognostic indicator.

Extranodal marginal zone lymphoma (*MALToma*)*/anaplastic large cell lymphoma:* occasionally.

Germ Cell Tumours

- *Up to 20% of mediastinal tumours/cysts* and commoner than in other extragenital sites, e.g. retroperitoneum or the sacrococcygeal region.
- Thymus based with a primary origin in extragonadal germ cells. *Type I neoplasms* ((immature) teratoma/yolk sac tumours) occur in neonates and young children, and *type II neoplasms* (non-seminomatous germ cell tumours) in young adults.
- Exclude metastases from a clinical or occult testicular/ovarian germ cell tumour, particularly if there is associated retroperitoneal disease.
- *Mature cystic teratoma:* the *commonest mediastinal germ cell tumour* and similar to that in the ovary.
- *Immature teratoma:* rare; immature epithelium, mesenchyme (cartilage) or neural elements.
- Mature and immature teratoma generally have a *benign course if completely resected* although adult immature teratoma is more aggressive and mature teratoma in the postpubertal patient is potentially malignant.

- *Mature teratoma ± yolk sac tumour:* encountered in infancy and early childhood the former is generally resectable and benign, the latter achieves 80% 5 year survival with appropriate treatment.
- *Embryonal carcinoma, yolk sac tumour, choriocarcinoma* (third decade, gynaecomastia): all require *chemotherapy* and are less responsive with a higher relapse rate and lower survival than equivalent testicular lesions. There is also a higher rate of *somatic malignant transformation,* e.g. adenocarcinoma, angiosarcoma, rhabdomyosarcoma and *myeloid malignancies* than in gonadal germ cell tumours. Serum HCG and AFP levels are raised in >90% of non-seminomatous germ cell tumours and high levels are an adverse prognostic indicator.
- *Seminoma:* PLAP, CD117, OCT3/4, SALL4 positive, cytokeratin negative and 69% 10 year survival. The seminoma cells can be obscured by granulomatous inflammation, reactive lymphoid follicular hyperplasia or thymic epithelial cysts. Immunohistochemical markers are helpful.
- *Chemotherapy:* of germ cell tumour results in necrosis. Residual tumour can regrow and follow up radiology, serum tumours markers and surgical excision or additional chemotherapy are carried out. As with retroperitoneal disease from gonadal germ cell tumours, this can be further malignant germ cell tumour, or, tissue maturation and growing teratoma syndrome with pressure effects on adjacent organs.
- *Metastases:* are seen overall in 20% of mediastinal germ cell tumours; seminoma 41% and non-seminomatous germ cell tumours 85–95%. Blood borne spread is to lung, bone, liver, brain and retroperitoneum.

Neurogenic Tumours

- Posterior mediastinum.
- *Children:* tumours derived from the sympathetic nervous system; neuroblastoma, ganglioneuroblastoma, ganglioneuroma.
- *Adults:* tumours derived from the peripheral nervous system; neurilemmoma, neurofibroma ± cystic degeneration. Malignant peripheral nerve sheath tumour: de novo or in von Recklinghausen's disease, ± enteric glands, ± rhabdomyoblasts (malignant Triton tumour). Poor prognosis with pleural and pulmonary spread.

Sarcoma

- Rarely primary: *liposarcoma, synovial sarcoma.*
- *Rhabdomyosarcoma* especially the alveolar subtype (desmin/myo D1/myogenin positive).

Thymoma

- *Anterosuperior mediastinum (80% of cases)* but can occur in other anatomical compartments, e.g. neck, thyroid, lung and pleura.
- Solid, yellow/grey, lobulated ± cystic change: *80% are encapsulated and easily excised, 20% are infiltrative.* It comprises a dual population of cytokeratin positive epithelial cells and T marker (CDs 1, 3, 4, 8, 99, 1a, TdT) positive lymphocytes of variable maturity. Classification which can reflect invasiveness and prognosis relies on:
  - The character of the epithelial cells and lymphocytes
  - The relative proportion of these cells
  - Their cellular atypia, and,
  - The organoid architecture: lobulated corticomedullary differentiation; epithelial lined glands and cysts; Hassall's-like corpuscles; perivascular spaces.

Individual tumours can show some heterogeneity in these features. Further refinement of diagnostic criteria (WHO 2015) stipulates obligatory and optional features of thymic tumours (see Table 19.1).

Table 19.1 Thymoma classification

Thymoma subtype	Obligatory criteria	Optional criteria	Additional information
Type A (medullary)	Bland, spindle shaped epithelial cells (at least focally); paucity or absence of immature (TdT+) T cells throughout the tumour	Polygonal epithelial cells CD20+ epithelial cells	Thick capsule; excellent prognosis
Atypical type A variant	Similar to type A but also: comedonecrosis; increased mitotic count (>4/2mm^2); nuclear crowding	Polygonal epithelial cells CD20+ epithelial cells	May be more aggressive variant
Type AB (mixed)	Bland, spindle shaped epithelial cells (at least focally); abundance of immature (TdT+) T cells focally or throughout the tumour	Polygonal epithelial cells CD20 + epithelial cells	Elderly patients; thick capsule; excellent prognosis
Type B1 (predominantly cortical)	Thymus-like architecture and cytology: abundance of immature T cells, areas of medullary differentiation (medullary islands); paucity of polygonal or dendritic epithelia cells without clustering (i.e. < 3 contiguous epithelial cells)	Hassall's corpuscles; perivascular spaces	Expansile growth; slightly more aggressive but good prognosis
Type B2 (cortical)	Increased numbers of single or clustered polygonal or dendritic epithelial cells intermingled with abundant immature T cells	Medullary islands; Hassall's corpuscles; perivascular spaces	Middle aged; locally invasive; long term survival despite recurrences
Type B3 (well-differentiated thymic carcinoma)	Sheets of polygonal slightly to moderately atypical epithelial cells; absent or rare intercellular bridges; paucity or absence of intermingled TdT+ T cells	Hassall's corpuscles; perivascular spaces	Lobulated, sclerotic, invasive; frequent recurrences. More aggressive than Type B2

Thymoma subtypes in parentheses state former nomenclature

Adapted from Marx A, Chan JK, Coindre JM, Detterbeck F, Girard N, Harris NL, Jaffe ES, Kurrer MO, Marom EM, Moreira AL, Mukai K, Orazi A, Ströbel P. The 2015 World Health Organization Classification of Tumors of the Thymus: Continuity and Changes. J Thorac Oncol. 2015 Oct;10(10):1383–95

Thymic Carcinoma

- Clear cut cytological features of malignancy.
- Exclude metastasis from lung or elsewhere.
- 90% are squamous cell carcinoma (±keratinisation): also lymphoepithelioma like carcinoma (similar to that of nasopharyngeal carcinoma).
- May be difficult to distinguish from type B3 thymoma. Distinction is based primarily on histomorphology and should not rely upon immunochemistry.
- Previous referred to as "type C" to highlight thymic origin. Term no longer used.
- *Others:*
  - Sarcomatoid carcinoma (spindle cell carcinoma/carcinosarcoma).
  - Clear cell carcinoma.
  - Basaloid carcinoma.
  - Mucoepidermoid carcinoma.
  - Adenocarcinoma (papillary; adenoid cystic carcinoma like features; mucinous)
  - NUT carcinoma
  - Small cell carcinoma (grade 3 neuroendocrine thymic tumour).
  - Undifferentiated and neuroendocrine large cell carcinoma.
  - Carcinoid tumour (classic/spindle cell/pigmented/with amyloid, or, atypical:
  - Grade 1, or, grade 2 neuroendocrine thymic tumours).

Differentiation/Grade

Metastatic Carcinoma

- Well/moderate/poor/undifferentiated.

Malignant Lymphoma

- Low-grade: extranodal marginal zone lymphoma.
- High-grade: diffuse large B cell lymphoma; lymphoblastic lymphoma.

Germ Cell Tumours

- Seminoma.
- Non-seminomatous: mature/immature teratoma with or without somatic malignancy, embryonal carcinoma, yolk sac tumour, choriocarcinoma.
- Mixed germ cell tumour (35% of cases).

Neurogenic Tumours

- Small round blue cell: neuroblastoma component.
- Low-grade/high-grade: sarcoma.

Thymoma

- See above.

Extent of Local Tumour Spread

- Border: pushing/infiltrative.
- Lymphocytic reaction: prominent/sparse.
- For all tumours:

- Confined to the mediastinal nodes.
- Confined to the thymus.
- Into the mediastinal connective tissues.
- Into other organs e.g. pleura, lung, pericardium, main vessels.

Thymoma (After Masaoka-Koga Staging System)

I	Completely encapsulated macroscopically and microscopically. Can include invasion into but not through the capsule, and no invasion into surrounding tissues.
II	Minimally invasive: with either a. microscopic transcapsular invasion <3 mm in extent into surrounding tissues, or, b. macroscopic adhesion, or, invasion into surrounding fatty tissues but not breaking through mediastinal pleura or pericardium.
III	Widely invasive and/or implants: into neighbouring organs, e.g. pleura, pericardium, great vessels and lung
IV	Metastatic: with either (a) widespread discontinuous pleural or pericardial dissemination, or, (b) lymphogenous or haematogenous disease.

The Masaoka-Koga staging system has been widely used and shown to be a reliable indicator of prognosis. However, more recently a TNM staging system has been proposed and may become more widely adopted. The TNM8 classification applies to epithelial tumours of the thymus including thymomas, thymic carcinomas and neuroendocrine tumours. Sarcomas, lymphomas and other rare tumours are excluded.

pT0	No evidence of primary tumour
pT1	Tumour encapsulated or extending into the mediastinal fat, may involve the mediastinal pleura.
pT1a	No mediastinal pleural involvement
pT1b	Direct invasion of the mediastinal pleura
pT2	Tumour with direct involvement of the pericardium (partial or full thickness).
pT3	Tumour with direct invasion into any of the following; lung, brachiocephalic vein, superior vena cava, phrenic nerve, chest wall, or extrapericardial pulmonary artery or vein.
pT4	Tumour with direct invasion into any of the following; aorta (ascending, arch or descending), arch vessels, intrapericardial pulmonary artery, myocardium, trachea, or oesophagus

Lymphovascular Invasion

Present/absent.
Intra-/extratumoural.

Lymph Nodes

Site/number/size/number involved/limit node/extracapsular spread.

Regional nodes: anterior (perithymic) nodes, the deep intrathoracic nodes and the cervical nodes.

pN0	No regional lymph nodes involved
pN1	Metastasis to anterior (perithymic) nodes
pN2	Metastasis to deep intrathoracic or cervical nodes

Excision Margins

Distances (mm) to the nearest painted margins of excision.

Other Pathology

Associations

About 30–45% of thymoma patients have *myasthenia gravis*—muscular fatigability of the proximal limbs and head and neck including extraocular, masticatory, speech and facial expression muscles. Acetylcholine receptor antibody is positive. These patients can also have a range of other *haematological, dermatological* and *systemic autoimmune diseases* which can either precede or follow surgical resection. Other *paraneoplastic syndromes* relate to the mediastinal cancer type, e.g. small cell lung carcinoma with secretion of ACTH or IADH.

Thymic carcinoid tumour is associated with *carcinoid tumour* at other sites, e.g. bronchus, ileum and type I multiple endocrine neoplasia (MEN I) syndrome. Typically ribbons, rosettes, and balls of cells with central necrosis and calcification.

Immunophenotype

- *Metastatic carcinoma*: cytokeratins, CEA, EMA, BerEP4, TTF-1, CA125, CA19–9, ER.
- *Hodgkin's lymphoma*: CD 15, CD 30.
- *Non-Hodgkin's lymphoma*: CD 45, CD 20, CD 3, CD 30, ALK1, κ/λ light chain restriction, molecular gene rearrangements.
- *Lymphoblastic lymphoma*: tdt, CD 10, CD 99, CD 3, Ki-67 > 95%.
- *Seminoma:* PLAP, CD117, OCT3/4, SALL4 (cytokeratin negative).
- *Embryonal carcinoma/yolk sac tumour*: cytokeratins, HCG, AFP, CD 30, CD117 and OCT3/4 (negative in yolk sac tumour), SALL4 (± PLAP—in embryonal carcinoma).
- *Thymoma*: cytokeratins, CK19, p63, p40, EMA, CEA, S100 positive interdigitating reticulum cells; usually CK20 negative; variably mature T lymphocytes CDs 1, 3, 4, 8, 99, 1a, TdT. Thymic carcinoma retains CD 5 positivity—also CD117 positive.
- *Carcinoid*: chromogranin, synaptophysin, CD56± CAM 5.2. Ki-67 index <2%.

Cystic Change

- *Unilocular thymic cysts*: developmental and thin non-inflamed wall with cubocolumnar epithelial lining.
- *Multilocular thymic cysts*: multilocular and adherent to mediastinal structures due to inflammation and fibrosis mimicking an invasive thymic tumour. There is a cubocolumnar or squamous epithelial lining.
- 50% of thymic *nodular sclerosing Hodgkin's lymphomas*.
- *Thymic seminoma* or *non-seminomatous germ cell tumour*.
- Cystic change can lead to a non-representative sample on needle core biopsy.

Prognosis

Prognosis relates to the nature of the underlying pathological abnormality and as to whether it represents primary or secondary disease. Cancer subtype also determines the choice of therapy, e.g. surgery, chemotherapy or radiotherapy. Note that prebiopsy or presurgical radiotherapy and chemotherapy can induce tumour apoptosis,

necrosis and hyalinisation which can lead to difficulties in accurate classification of disease.

In *thymoma several rules apply:*

1. Tumours with predominantly bland *spindle/oval cells* are usually encapsulated and of excellent prognosis.
2. Tumours with predominantly *round/polygonal epithelial cells* have a course related to the relative predominance of epithelial cells over lymphocytes and any cellular atypia that is present.
3. The *encapsulation of the tumour,* or its lack of encapsulation and any *signs of invasion at surgery* along with *completeness of excision* are, irrespective of the histological subtype, the best markers of future clinical behaviour. Macroscopic adherence in the mediastinum may be the only sign of capsular penetration—the surgeon should mark it and refrain from incising the capsule to allow full histological assessment.

Type A (medullary) and type AB (mixed) thymoma tend to present at stages I or II, type B1 (predominantly cortical) or type B2 (cortical) more often stages III or IV. However, *prognosis is usually 90–100% 5 year survival*, patients with myasthenia gravis doing worse than those without. *Treatment is surgical* with small localised lesions removed via a *transcervical route.* The usual surgical approach is *median sternotomy.* This is supplemented by *radiotherapy* if there is any possibility of residual tumour or local recurrence (2–10% of cases, more usually type B). *Distant metastases* may need *chemotherapy.* Type B3 thymoma has an 80% 5 year survival. Thymic carcinoma may require a combination of *surgery, radiotherapy and chemotherapy* for bulky local disease or distant spread. Disease course relates to tumour type being either aggressive (death in 6 months to 2 years in non-keratinising carcinoma, sarcomatoid/clear cell/undifferentiated carcinomas), intermediate (squamous cell carcinoma, carcinoid tumour) or indolent (mucoepidermoid/basaloid carcinomas).

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Part IV

Skin Cancer

- Non-melanocytic skin cancers
- Malignant melanoma

Non-Melanocytic Skin Cancers

20

David P. Boyle

(with clinical comments by Olivia Dolan)

Introduction

In the UK and Ireland non-melanoma skin cancer (NMSC) is the most common cancer group accounting for 20% of all new malignancies and 90% of all registered skin cancers. The incidence is increasing rapidly and is higher in males. Squamous cell carcinoma and basal cell carcinoma (previously referred to as epithelioma) are the commonest solar induced non-melanocytic cutaneous lesions. Other skin malignancies are relatively rare. Actinic keratosis and squamous cell carcinoma *in situ* (Bowen's disease) are extremely common non-invasive lesions. All the aforementioned lesions may present as red, scaly patches or non-healing sore nodular lesions on the sun exposed head and neck areas and hands of fair skinned people, who have a 10–20 fold increased risk over people with dark skin. Approximately 75% of NMSCs are basal cell carcinomas with the remaining proportion mostly squamous cell carcinomas. A mixed group of rare skin cancers of which Merkel cell carcinoma is the commonest, account for <1% of cases. The majority of these arise on the head and neck areas with exposure to ultraviolet irradiation a key aetiological factor. Almost 50% of new cases are in people aged over 75. A minority are associated with genetic disorders, or areas of chronic scarring and ulceration, e.g. Marjolin's ulcer of the leg. Patients who are immunocompromised due to long term immunosuppressive therapy, e.g. following solid organ transplantation, are at a much higher risk of developing NMSC. Delay in presentation of cutaneous carcinoma is associated with increased tumour growth with potential for local tissue destruction (e.g. spread to the orbit requiring exenteration), and, with squamous cell carcinoma potential for lymphovascular metastases.

Regarding the nature of specimen type, curettage, shave, and punch biopsies are often small, processed intact and embedded *in toto*. Curettage removes the lesion in fragments and is followed by electrothermal cautery to its base (C&C). Diathermy excision distorts the tissue, potentially rendering accurate histological assessment problematic. Shave biopsy is often used to excise benign surface lesions. Punch biopsy material can be either diagnostic (incisional) or therapeutic (excisional) in intent, and the submitting clinician should indicate this clearly on the request form. They can also be deep if it is necessary to visualise the subcutaneous fat. Slightly larger shave or punch biopsies may be bisected and similarly all processed. In general, margins are not marked when the intent is purely diagnostic. Excisional biopsies attempt to remove the lesion with clear margins of normal skin. Assessment, in these cases, is aided by painting the deep and

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_20

peripheral limits and use of serial transverse slices or quadrant (cruciform) blocks tailored to local protocols. Attached orientation sutures aid assessment of specific anatomical margins: orientation and inking during gross examination should be recorded in the final report. This is particularly useful when it is only feasible to achieve minimal clearance, e.g. on the face, ear, lip, nasal or periorbital areas. Thus, cases are handled individually according to the specimen size, type of lesion and its size and position within the specimen. If initial histological sections fail to reveal a tumour when an experienced clinician strongly suspects that one is present, the pathologist must always be prepared to take extra blocks or carry out many further levels for examination: a day's delay is preferable to a missed diagnosis. This most often occurs in small punch biopsies submitted intact that contain a centrally placed or off centre lesion not revealed in initial sections. Additional histological clues can be evident e.g. epidermal dysplasia, dermal inflammation/hyalinisation or retraction artefact that could suggest the possibility of an adjacent carcinoma. This is particularly so for recurrences which can be small and difficult to demonstrate.

Tumours arising in facial anatomic sites and in particular eyelids, medial canthus, nasal tip and ala, preauricular area, ears, and lips (H-zone) are more difficult to treat with a tendency for wider subclinical spread and a higher incidence of local recurrence and metastasis. A more specialised dermatological surgical technique may be required combining serial excisions with intraoperative frozen section checking of the entire circumferential surgical margin (Mohs micrographic surgery). The wound is then repaired after histologic confirmation of tumour clearance. Sometimes primary or secondary excision specimens are submitted with a central circular deficiency to aid excision or due to prior sampling for diagnosis or research by the clinician. Care must be taken in orientation of the specimen as accurate assessment of tumour diameter and margin distances can be somewhat problematic. In secondary excision for scar recurrence the tumour may be small or macroscopically difficult to define and eccentrically located within the specimen. Serial slices and total processing of the tissue may be required.

In general, treatment of cutaneous carcinoma is by wide local excision, or if indicated Mohs micrographic surgery. Mohs micrographic surgery is reserved for high risk primary and recurrent facial lesions with poorly defined margins, usually but not exclusively basal cell carcinoma. Other modalities are: radiotherapy, photodynamic therapy, topical imiquimod (an immune response modifier mediating cell death), curettage and cautery, cryotherapy and laser. Choice of therapy is determined by patient age and preference, comorbidities, tumour type, site, size, and whether it is a primary or recurrent lesion. Depending on local practice and knowledge or familiarity with referring clinicians, it is occasionally necessary to prompt the clinician to refer a case for local multidisciplinary team discussion, e.g. squamous cell carcinoma excised by general practitioner.

Gross description

Specimen

- Cytology/curettage/shave biopsy/punch biopsy/incision biopsy/excisional biopsy which may be immediately preceded by curettage of the lesion/re-excision/Mohs' surgery.
- Size: length x width x depth (mm).

Tumour

Site

- Anatomical site: limbs/trunk/head/neck/perineum/epidermal/dermal/subcutaneous.
- Sun exposed areas of the head and neck, scalp and back of hands are the most frequent sites for the common cancers. The central face, periorbital areas, lips and ears are clinical high risk anatomical sites prone to local recurrence due to difficulties in obtaining adequate primary margins.

Size

- Length x width x depth (mm) or maximum dimension (mm).
- Tumour size ≤2 cm or >2 cm is a pT1/pT2 stage determinant for many skin cancers (including squamous cell carcinoma, basal cell carcinoma, adnexal carcinomas and Merkel cell carcinoma). This is the clinical dimension but the macroscopic pathologic dimension can be used in lieu of an absent clinical measurement. Deep invasion defined as tumour thickness of >6 mm, invasion of an anatomically named nerve (or a nerve ≥0.1 mm diameter, or extradermal nerve), or minor bone erosion upstages pT1 and pT2 tumours to pT3. Prognosis of Merkel cell carcinoma >5 mm in thickness is poor. Cutaneous adnexal carcinoma >2 mm in thickness is a high risk feature.

Appearance

- Verrucous/warty/nodular/exophytic/sessile/ulcerated/invaginated/cystic/plaque/ haemorrhagic/necrotic/pigmented.

Edge

- Circumscribed/irregular.

Histological Type

Squamous Cell Carcinoma

- Forehead, face, ears, scalp, lip, neck, back of hands.
- Keratinising/non-keratinising.
- *80% are well to moderately differentiated and keratinising*.
- Although tumour location, stage, depth of invasion, differentiation and immunosuppression may be more important in terms of prognosis, particular histologic subtypes are associated with more aggressive behaviour: variants with adverse prognosis and higher rates of local recurrence and/or metastasis:
  - *Desmoplastic:* >30% of the tumour area is desmoplastic.
  - *Small cell* or *basaloid: associated with in situ disease and must be distinguished from basal cell carcinoma.*
  - *Spindle cell (sarcomatoid):* accounts for over 30% of metastatic squamous cell carcinomas. Those cases associated with radiation run an aggressive course while de novo examples may be no more aggressive than conventional squamous cell carcinoma.
  - *Post traumatic (de novo)*: not related to sun exposure and occur in the setting of chronic injury (often burns, i.e. Marjolin's ulcer). Paradoxically, may be moderate to well differentiated but not associated with actinic keratosis or dermal damage.
  - *Squamous cell carcinoma associated with adjacent in situ change (Bowen's disease).* Incidentally, Bowen's disease terminology was originally applied to *in situ* neoplasia in sun-protected skin but is now used interchangeably with *squamous cell carcinoma in situ* at both sun exposed and non-sun exposed areas.
  - *Acantholytic (pseudoglandular or adenoid):* there is conflicting evidence regarding risk and this subtype may be more indolent than previously reported when usual histologic factors are compared. It occurs more frequently in immunosuppressed patients.
  - *Adenosquamous* (mixed differentiation) arising from pluripotential acrosyringium cells.
- Others:
  - *Verrucous:* locally destructive but with little potential for metastases, this subtype may recur particularly on the foot and at the anal margin. Association with HPV infection. Superficial samples may resemble keratoacanthoma and verruca vulgaris. Ki67 and p53 expression in the lower third of epidermis in contrast to confluent epidermal expression in squamous cell carcinoma may help in diagnosis: distinguishing these may be important given the higher likelihood of nodal metastasis and

variation in treatment in squamous cell carcinoma.

- *Clear cell* (elderly, head and neck): differential diagnoses include metastatic renal cell carcinoma, clear cell acanthoma, sebaceous neoplasms and trichilemmoma. Many tumour types have a clear cell variant.
- *Lymphoepithelial* (elderly, head and neck): squamous differentiation may not be morphologically apparent.
- *Keratoacanthoma:* rapid clinical growth over a few months with potential for involution and even complete regression with variable histology depending on sample timing. It is crateriform with a central keratin plug and collarette of hyperplastic squamous epithelium. It may be difficult to distinguish from a well differentiated squamous cell carcinoma and in such cases may be best considered as the latter: the term invasive squamous cell carcinoma of keratoacanthomatous type has been proposed. A designation of keratoacanthoma is excluded by adjacent in situ change, desmoplasia, significant cellular pleomorphism, an inappropriate clinical history and should be avoided in immunocompromised patients.
- *Follicular variant of squamous cell carcinoma*: there is increasing recognition of a variant of squamous cell carcinoma with both in situ and invasive counterparts. Lesions are defined histologically by abrupt connections of the tumour with the epidermis at the site of follicular infundibula, presence of infundibular and/or tricholemmal differentiation, malignant cytological and/or architectural features and absence of bowenoid dysplasia or features of keratoacanthoma. Most lesions show relatively mild cellular atypia and clear cell change may occur. Additionally, there may be subtle peripheral cellular palisading as well as central acantholytic spaces containing mucin but in contrast to basal cell carcinoma, there is generally no mucin in stromal retraction artefact spaces. Recognition of the invasive form may have implications on prognosis and follow-up: in particular depth of invasion measurement may be problematic as it may not be appropriate to measure from granular cell layer of the surface epidermis in a tumour arising from a hair follicle as this could overestimate risk.

Basal Cell Carcinoma

- *The commonest non-melanocytic cutaneous carcinoma* comprising a proliferation of basaloid/germinative cells and characterised by slow growth and *local tissue infiltration* and *destruction*. Metastases are exceedingly rare and morbidity results from local tissue invasion and destruction, particularly on the face, head and neck. The morphoeic, infiltrative, micronodular and basosquamous types are associated with the highest risk of recurrence as they may be more pervasive microscopically than apparent clinically and more likely to exhibit perineural invasion. In contrast, circumscribed, local or expansile tumours and particularly those with a superficial or nodular growth pattern have a lower risk of local recurrence. More than one growth pattern may be apparent in any given lesion and the level of clinical risk is determined by the highest risk growth pattern present. Morphologic subtyping is an important facet in determining therapy options as some lower risk lesions may be treated by non-surgical means such as imiquimod or photodynamic therapy.
- *Nodular*: the commonest subtype (60–80% of cases, often head and neck area) with nodules of varying size, ± tumour necrosis and cystic spaces (nodulocystic), peripheral palisading, mitoses and dermal retraction artifact. Includes adenoid, (trabecular/ribbons of cells), keratotic (horn cysts, squamous metaplasia), pigmented, fibroepithelial (Pinkus tumour) variants, and, those with adnexal (follicular, sebaceous or eccrine) differentiation. Care should be exercised not to interpret squamous

metaplastic foci or keratocystic areas as representing basosquamous differentiation.

- *Superficial*: 10–20% of cases. Sometimes called multifocal given its apparent discontinuous nature when viewed on histologic sections, it comprises multifocal nests of tumour budding off the epidermal or hair follicle basal layer. Buds should be confined to papillary dermis and less than 1 mm thickness. It remains unclear whether this represents in situ or truly invasive disease. Recurrence is due to inadequate primary excision of peripheral margins. Often occur on the trunk.
- *Infiltrative/morphoeic*: small, irregular infiltrating groups in a fibrous, scirrhous or hyaline stroma in a poorly circumscribed lesion. The pattern resembles a tree trunk with spreading roots which can be deeply infiltrative, more often reaching subcutis and compromising the deep margin. Usually occurs on the upper trunk or face.
- *Micronodular*: multiple discrete small round nests less than 0.15 mm diameter (or approximately 25 cells in diameter), with an asymmetrical, infiltrative growth pattern. It is the infiltrative growth that confers high risk status to this subtype and can result in margin compromise that is not clinically apparent. Tumours comprising small nodules but with a well circumscribed edge, best appreciated on low power examination, and with no other high risk attributes are more appropriately categorised as nodular.
- *Basosquamous carcinoma*: tumours with moderately to severely atypical or malignant squamous cell differentiation. Tumours may have focal squamous areas or squamoid cells scattered throughout with transition areas containing intermediate cells. This is an intermediate tumour between basal cell and squamous cell carcinomas of usual type with potential for local recurrence (4.5% rate) and metastatic spread (nodal metastases in 5%). It is currently categorised a high risk variant of basal cell rather than squamous cell carcinoma. Basosquamous is the variant most likely associated with vascular invasion and has the greatest metastatic potential. Perineural invasion is found in approximately 10% of cases. It is recommended that the poorly defined term "metatypical" be avoided as it is probably a variant of basosquamous carcinoma with no robust discriminating histologic characteristics or appreciable differences in clinical outcome.
- Metastasis in basal cell carcinoma is extremely rare. *Low risk primary tumours* may be treated in *primary care* by suitably trained personnel, whereas *high risk tumours* are dealt with in *secondary care.* Features of low risk basal cell carcinoma include sites below the clavicle and size <20 mm. High risk lesions prone to local recurrence are characterised by: *anatomical site* (face, eyes, nose, lips, ears), *growth pattern* (infiltrative/morphoeic/micronodular), *cellular differentiation* (basosquamous), the presence of *perineural or lymphovascular invasion*, *Clark level V invasion,* and *stage ≥ pT2.*

Adnexal Carcinoma

- Adnexal tumours are relatively infrequently encountered in general practice. These comprise a diverse group with morphologic differentiation towards one or mixtures of adnexal epithelium: follicular epithelium, sebaceous, apocrine and eccrine glands. Whilst the majority will be benign, the rare malignant counterparts have potential for locally aggressive growth, nodal involvement and distant metastases with poor outcome. Correspondingly, these *tumours* are best dealt with by a pathologist with dermatopathological expertise in the context of a multidisciplinary meeting.

Diagnostic clues to malignant behaviour include asymmetry, cellular atypia, necrosis, mitoses, perineural or lymphovascular invasion and an unusually deep and infiltrative margin. Low power patterns are solid-cystic, papillary-tubular or sclerosing. Low-grade carcinomas show considerable cellular differentiation, uniform cell size with infrequent mitoses and little pleomorphism: they do well if small and

completely excised. Examples are microcystic adnexal carcinoma, syringoid eccrine carcinoma, adenoid cystic carcinoma and mucinous eccrine carcinoma. High-grade cancers show poor differentiation, necrosis and mitoses, and they can metastasise widely. Examples are malignant hidradenoma, malignant spiradenoma/cylindroma, aggressive digital papillary adenocarcinoma and porocarcinoma.

- Hair follicle differentiation: follicular differentiation is recognised by basaloid germinative cells, peripheral nuclear palisading and papillary mesenchymal cells. Ghosted epithelium and attachment to normal follicular structures are additional features. Examples include tricholemmal carcinoma, malignant pilomatrixoma and malignant proliferating trichilemmal cyst.
- Sebaceous differentiation: these tumours may have bubbly vacuolated cytoplasm and jagged irregular nuclei. Examples include sebaceous carcinoma and basal cell carcinoma with sebaceous differentiation.
- Ductal differentiation: this represents the most diverse group and includes apocrine and eccrine tumours—including syringoid carcinoma, microcystic adnexal carcinoma, malignant chondroid syringoma, hidradenocarcinoma, porocarcinoma, mucinous carcinoma, aggressive digital papillary adenocarcinoma and adenoid cystic carcinoma. EMA and CEA immunochemistry can be helpful in highlighting ductular structures. In some cases a precise diagnosis may not be possible and a management label of malignant cutaneous sweat gland tumour may suffice. Consideration should be given to cutaneous metastatic disease with more common mimics including carcinoma from thyroid, breast, salivary gland and kidney: clinical history and judicious immunochemistry may be beneficial.

Paget's Disease

- Extramammary sites include apocrine rich areas of vulva, perineum, scrotum, axillae. This may be associated with occult visceral malignancy (in about 15% of cases) and should be accurately differentiated from pagetoid squamous cell carcinoma in situ and melanoma in situ. A useful immunopanel to differentiate these includes CK7, CK20, CEA, EMA, CAM5.2 (expressed in extra-mammary paget's disease), p63 (expressed in squamous cell carcinoma in situ) and S100, SOX10 and melan-A (expressed in melanoma in situ).

Neuroendocrine Carcinoma: Merkel Cell or Small Cell Neuroendocrine Carcinoma of Skin

- On the head/neck, extremities, in the elderly, it is a poorly differentiated/high-grade neuroendocrine carcinoma comprising small blue cells with increased apoptosis and a high mitotic rate.
- Chromogranin/synaptophysin/CD56 and paranuclear or perineuclear dot CAM 5.2, AE1/3, CK20 positive. Ki67, CD117, CD99 and FLI1 positive, but TTF-1 and CD45 negative.
- May be associated overlying basal or squamous cell carcinoma (in situ or invasive) in >30% of cases although this may not necessarily confer a worse prognosis. Rarely the tumour can also show components of squamous cell or eccrine differentiation. Chronic lymphocytic leukaemia is the most common second extracutaneous malignancy.
- *Clinically exclude secondary small cell carcinoma of lung* (CK 20 negative/TTF-1 positive). *Histologically exclude malignant lymphoma* (CD45 positive) and *small cell variant of malignant melanoma* (S100, melanA positive). Polyoma virus is positive in 80% (molecular and immunochemical techniques available). Positive cases tend to have a better prognosis while negative cases may have greater cellular atypia and a greater association with UV light exposure. Cases with a second malignancy also tend to be negative.
- Treatment is *primary excision with wide margins and consideration for adjuvant radiotherapy. Adverse indicators* are: size >20 mm,

thickness >5 mm, mitoses >10 per high power field or Ki-67 >50%, an infiltrative border, subcutaneous fat invasion, lymphovascular or lymph node involvement or positive primary excision margins. Approximately 10% of Merkel cell carcinomas present as metastatic disease of unknown origin possibly related to primary tumour regression.

Metastatic Carcinoma

Skin metastases occur in up to *9% of malignancies* and can *predate, occur synchronously, or after the primary lesion*. *Clinical presentation* with a skin metastasis (2% of cases) is seen particularly with lung, kidney and ovarian cancer. *Late metastases* (at 10 years or more) come from breast carcinoma, malignant melanoma, kidney, bladder, colon and ovarian cancers. Some metastases are by *direct local extension* to overlying skin, e.g. breast carcinoma, others by *distant vascular spread,* e.g. kidney carcinoma and malignant melanoma. Although melanoma has a higher propensity to metastasise to skin, breast cancer is the most frequently encountered type as it is much more common. Other common sources of skin metastases in females are colon, ovaries and lung. In males: lung, colon and the oral cavity.

- Single/multiple nodule(s) commonly on the trunk and head and neck regions (especially umbilicus and scalp), sometimes *in the vicinity of the primary lesion*.
- Some metastases can be epidermotropic and *simulate a primary lesion,* e.g. malignant melanoma. Most are dermal and show nodular, interstitial and intravascular patterns necessitating distinction from primary adnexal carcinoma. *Previous clinical history, comparative morphology* and relevant *immunohistochemical profiles* are important in this respect.
- Immunochemistry should be judiciously applied. Primary adnexal tumours may commonly mimic skin metastases and in the case of breast, since this is a modified apocrine gland, immunochemical markers may be unhelpful: both show expression with markers such as GCDFP-15, GATA3, ER and PR. Adnexal tumours and metastatic lung adenocarcinomas are both usually CK7+/CK20 − .
- Secondary small cell carcinoma of lung or gastrointestinal tract can mimic Merkel cell carcinoma although both are CK20 negative.

Differentiation

Squamous cell carcinoma differentiation has been based on Broders' 4 tier classification but in practice this is difficult to apply. A modified 3 grade classification which assimilates additional cytologic and mitotic features is favoured with grading based on the worst area regardless of proportion involved.

Well differentiated tumours are easily recognised as squamous with abundant keratinisation, intercellular bridging and relatively infrequent mitoses usually basally located. At the other end of the spectrum, *poorly differentiated* tumours may only have extremely focal intercellular bridging or keratin formation and an overlying dysplastic epidermis may hint at the correct diagnosis. Such tumours may require immunochemistry to reveal their true nature. *Moderately differentiated* tumours will have an intermediate degree of differentiation. They exhibit greater architectural and cytonuclear atypia than well differentiated tumours but are still relatively readily recognised as squamous. Tumours showing no keratinization or intercellular bridging are regarded *anaplastic* and require immunochemistry for diagnosis. As a general rule of thumb, in the absence of morphologic prompts and if immunochemistry is required for the diagnosis then differentiation is likely to be at least poor.

Grading is not formally applied to basal cell carcinomas. These are subtyped according to low or high risk growth patterns with comment made on any unusual cellular differentiation features e.g. basosquamous carcinoma.

Grading of adnexal tumours broadly utilises the same approach to squamous cell carcinoma: well-differentiated tumours readily reveal their adnexal subtype with few mitoses and minimal cytonuclear atypia. The adnexal lineage is difficult to recognise in poorly differentiated tumours and there is

significant cytonuclear atypia with numerous atypical mitotic figures. Moderately differentiated tumours will have an intermediate degree of differentiation. Again, it is recommended that grading is based on the worst rather than predominant morphology although it may be useful to comment on relative proportions if the worst area is very focal.

Primary Tumour Staging

The following TNM8 classification applies to any skin carcinoma (except Merkel cell carcinoma) and to any site except eyelid, vulva, penis, non-hair bearing lip or non-hair bearing perianal skin (within 5 cm of the perianal margin). Staging (of all skin carcinomas including Merkel cell carcinoma) is based on the clinical measurement provided but the macroscopic pathologic measurement can be used if the former is unavailable.

pTis	Carcinoma in situ
pT1	Tumour ≤20 mm or less in maximum dimension
pT2	Tumour >20 mm to ≤40 mm in maximum dimension
pT3	Tumour >40 mm in maximum dimension
pT4a	Tumour with gross cortical/marrow invasion
pT4b	Tumour with axial skeleton/skull base/foraminal invasion

pT1 and pT2 tumours can be upstaged to pT3 by one or more high-risk clinical or pathological features including deep invasion (tumour thickness > 6 mm), bone erosion that does not fulfil pT4 criteria or perineural invasion defined as follow: invasion of an anatomically named nerve detected clinically or radiologically or involved nerves at least 0.1 mm diameter and any extradermal nerve involvement.

Clinical high risk factors for skin carcinomas include ear and hair bearing lip sites, recurrent or persistent lesions, reduced immunity, origin in non-exposed sites (e.g. sole of foot, perineum) and arising in areas of chronic injury. This information may be unavailable to the pathologist at the time of reporting.

Pathologic high risk factors for **squamous cell carcinoma** include high risk morphologic subtypes, poor differentiation, perineural invasion, thickness > 4 mm (squamous carcinomas of this size have a significantly increased metastatic potential, those >10 mm are very high risk with potentially high mortality), extradermal invasion, >pT1 stage and close margins (usually <1 mm). For **basal cell carcinoma** high risk factors are similar although >6 mm thickness is required and lymphovascular invasion is most important in the basosquamous type.

Tumour thickness (perpendicular distance from granular cell layer to deepest point of tumour invasion) may be difficult to measure in exophytic and ulcerating/endophytic or cup shaped lesions. The measurement is essentially similar to Breslow as used for melanoma with the acknowledgement that the granular cell layer may be lost in squamous cell carcinomas: therefore is may be advisable to take the measurement in relation to the granular cell layer of adjacent normal epidermis. This avoids overestimating risk in very exophytic lesions confined to superficial portions of the skin and, conversely, recognises the higher risk status of deeply ulcerating tumours that may appear rather thin if measuring from base of ulceration to base of tumour. In the case of a very exophytic tumour where the entire invasive portion is above the granular cell layer of adjacent normal epidermis and in the absence of other high risk factors, it may be appropriate to record thickness as <2 mm rather than stating a somewhat confusing negative value (Fig. 20.1).

The greater the number of clinical and pathologic risk factors, the higher risk the lesion is for local recurrence, nodal metastases and poorer outcome and survival. Overall assessment may only be possible by the referring clinician or through MDT discussion. A pragmatic approach to tumour thickness measurement should be taken and placed in the context of the overall lesion: any difficulties can be expanded in reports or discussed through MDT.

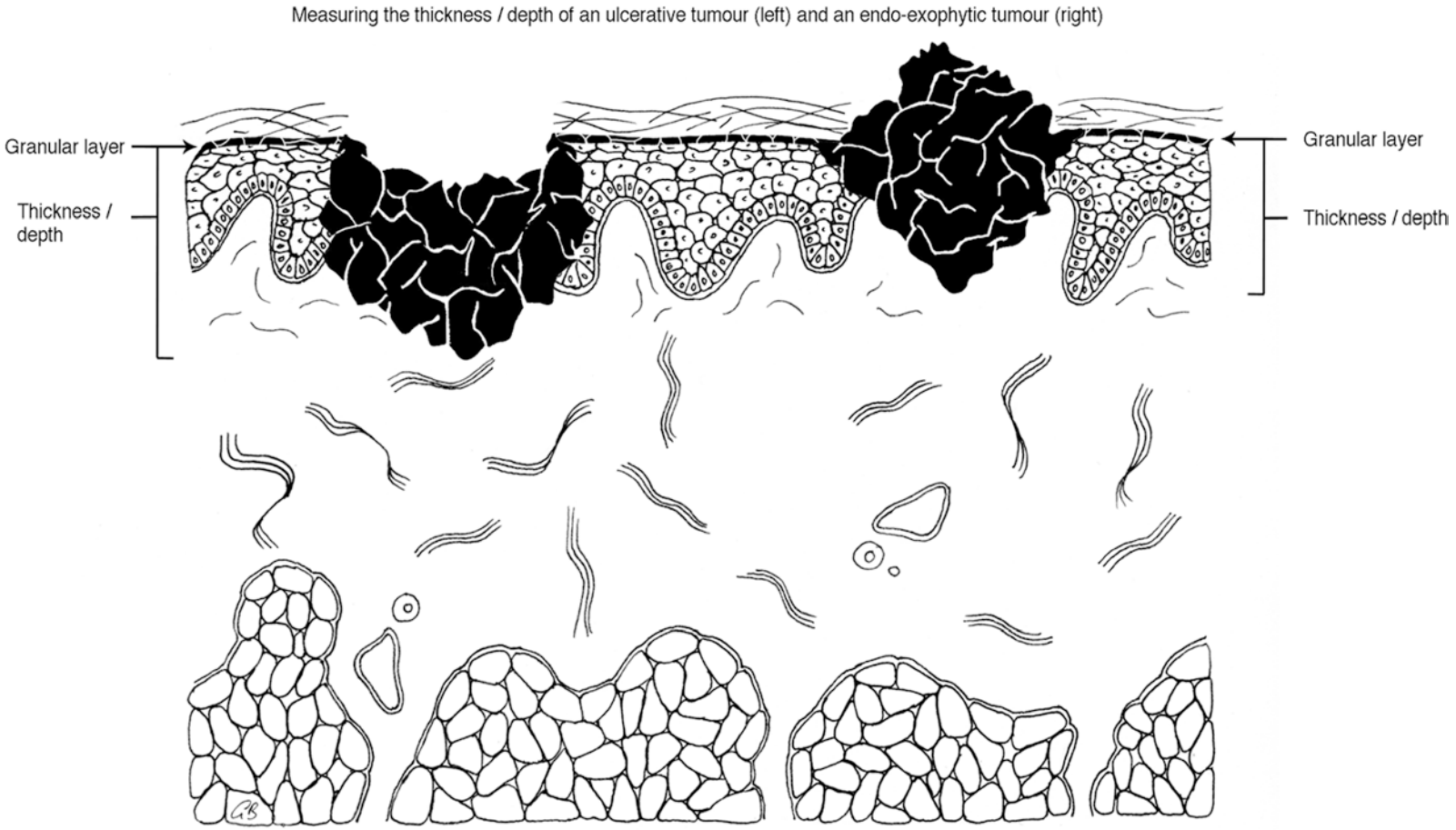

Fig. 20.1 Measuring tumour thickness in ulcerating and exophytic tumours. A modified and pragmatic approach is required in tumours from sites where adjacent skin is not flat or the tumour is cup shaped and not ulcerated

Merkel Cell Carcinoma

The staging of Merkel cell carcinoma includes all sites except the eyelid, non hair-bearing lip and non hair-bearing perianal skin:

pT0	No evidence of primary tumour (e.g. nodal/metastatic presentation without associated primary)
pTis	In situ primary tumour
pT1	≤20 mm maximum clinical dimension of tumour
pT2	>20 mm to ≤50 mm maximum clinical dimension of tumour
pT3	>50 mm maximum clinical dimension of tumour
pT4	Primary tumour invades fascia, muscle, bone or cartilage (i.e. beyond subcutaneous fat)

Lymphovascular Invasion

Perineural and lymphovascular invasion are not commonly seen (sometimes in squamous cell carcinoma, rarely in high risk basal cell carcinoma) but when present correlate with higher rates of local recurrence and metastases. This correlates most strongly with sentinel lymph node positivity and poor outcome in Merkel cell carcinoma and must be extratumoural. Lymphovascular invasion can be highlighted with D2–40 immunostaining.

Lymph Nodes

Site/number/size/number involved/extranodal extension.

Regional nodes are those appropriate to the site of the primary tumour:

Head and neck (preauricular, submandibular, cervical and supraclavicular).

Thorax and upper limb (axillae).

Abdomen, loins, buttocks, lower limbs, perianal/anal margin (all inguinal nodes: lower limbs also includes popliteal).

The following nodal staging refers to all carcinomas (except Merkel cell carcinoma) with ipsilateral nodes (contralateral nodal involvement represents distant metastatic spread: M1) and applies to all sites except eyelid, head and neck, perianal, vulval, and penis. In this case a regional lymphadenectomy should include at least 6 lymph nodes.

pN0	No regional lymph node metastasis
pN1	Metastasis in a single regional lymph node ≤30 mm in greatest dimension
pN2	Metastasis in a single regional lymph node >30 mm but <60 mm, or multiple ipsilateral nodes all ≤60 mm
pN3	Metastasis in a lymph node >60 mm in greatest dimension

Head and neck cutaneous carcinomas (excluding the eyelid and again excluding Merkel cell carcinoma) utilise a different nodal staging system. This will ordinarily include at least 10 lymph nodes in selective neck dissection and at least 15 in radical or modified neck dissection specimens.

pN0	No regional lymph node metastasis
pN1	Metastasis in a single ipsilateral lymph node ≤30 mm in greatest dimension, without extranodal extension.
pN2a	Metastasis in a single ipsilateral lymph node, more than 30 mm but <60 mm in greatest dimension, without extranodal extension.
pN2b	Metastasis in multiple ipsilateral lymph nodes, all ≤60 mm in greatest dimension, without extranodal extension.
pN2c	Metastasis in bilateral or contralateral lymph nodes, all ≤60 mm in greatest dimension, without extranodal extension
pN3a	Metastasis in a lymph node, more than 60 mm in greatest dimension, without extranodal extension
pN3b	Metastasis in a lymph node with extranodal extension

Extranodal extension may be reported pathologically as the presence of skin involvement or soft tissue invasion with deep fixation or tethering to underlying muscle or adjacent structures or clinical signs of nerve involvement as clinical extranodal extension.

Merkel Cell Carcinoma

pN0	No regional lymph node metastasis
pN1a	Microscopic (occult) involvement (sentinel or node dissection)
pN1b	Macroscopic involvement (clinically apparent)
pN2	In-transit metastasis without nodal involvement
pN3	In-transit metastasis with nodal involvement

In-transit metastasis refers to discontinuous tumour distinct from the primary lesion and located distal to the primary or between the primary and draining regional lymph nodes. In practice this is taken to mean discontinuous nests >0.05 mm diameter and separated by completely normal dermis from the main tumour by ≥1 mm.

Distant metastasis is: pM1 beyond regional nodes, pM1a distant skin, subcutaneous tissue or non-regional nodes, pM1b lung and pM1c other visceral sites.

Excision Margins

Distances (mm) to the nearest painted deep and peripheral excision margins, either of serial transverse slices (bread sliced) or quadrant blocks according to local protocols with pragmatic modifications based on closest grossly assessed edges. Comment should also be made on the presence of other benign lesions and of dysplasia or in situ change at the margins.

Adequate treatment is based on successful *complete primary excision* or, if there is initial margin involvement, on *secondary re-excision*. Clinically intended margins may not correspond to pathologic measurements due to specimen handling, tissue fixation and on occasion unexpected pathology. Pathologic involvement is tumour at (=0 mm) a margin; clear but close to is <1 mm; clear of is >1 mm. Quantification of margins to 1 decimal place is recommended particularly for lesions with "close" margins and may aid in subsequent treatment decisions. This information is often requested at MDM if a margin is quoted as "close" or <1 mm. Providing this degree of detail precludes any misunderstanding of the subjective term close which may vary depending on the tumour type. For example: with respect to clinical margins a 95% clearance rate may be expected with a 4 mm clinical margin for a low risk basal cell carcinoma. However, the same degree of confidence of clearance for a morphoeic basal cell carcinoma may necessitate a 13–15 mm clinical margin. It may be helpful to provide a further qualitative assessment of involved margins in certain instances: transection of tumour at a margin implies a large residue of disease contrasting to very focal involvement or if an apparently rounded lesion appears to have been 'scooped out'. Additionally superficial basal cell carcinoma at an edge may be treated more conservatively if a main, more clinically apparent tumour of another subtype is entirely resected. Cutaneous adnexal carcinomas can have ill-defined infiltrative lateral and deep margins compromising complete primary excision e.g. microcystic adnexal carcinoma.

Other Pathology

Squamous cell carcinoma: more prone to lymph node metastases particularly if >2 cm diameter or > 2 mm in thickness, poorly differentiated or with perineural spread. General *prognostic indicators* for squamous cell carcinoma are stage, level of dermal invasion and tumour thickness. *Recurrent tumours* tend to be ≥4 mm thick with involvement of the deep dermis, and *very high risk tumours* at least 1 cm thick with extension into subcutaneous fat. Tumours arising in non-sun exposed sites (e.g. trunk, perineum, penis) and areas of trauma (e.g. burns, Marjolin's ulcer) have a higher risk of metastasis.

Predisposing lesions to cutaneous carcinoma are:

- Actinic keratosis/solar elastosis: sun exposure.
- Psoralen plus ultraviolet A (PUVA) treatment for psoriasis.
- Varicose ulcers (Marjolin's), lichen planus, hidradenitis suppurativa.
- Immunosuppression post transplant or chemotherapy, HIV.
- Squamous cell carcinoma *in situ*: indolent progression to carcinoma.
- Condyloma accuminatum, Bowenoid papulosis, HPV infection: perineum/ perianal margin squamous carcinoma.
- Epidermodysplasia verruciformis, xeroderma pigmentosum.
- Naevus sebaceous of Jadassohn.
- Naevoid basal cell carcinoma (Gorlin) syndrome.

Double pathology: may be encountered e.g. basal or squamous cell carcinoma overlying Merkel cell carcinoma, basal cell carcinoma and syringocystadenoma papilliferum in naevus sebaceous of Jadassohn, basal cell carcinoma and dermatofibroma.

Pseudoepitheliomatous (pseudocarcinomatous) hyperplasia: may be seen in association with: chronic venous stasis, ulceration, chronic inflammation e.g. pyoderma gangrenosum, and overlying neoplasms e.g. granular cell tumour.

Actinic keratosis, carcinoma in situ and invasive squamous carcinoma: distinction can be difficult and some of these lesions may be designated "best regarded as squamous cell carcinoma". Treatment (primary surgical excision) is the same for both. Invasive squamous cell carcinoma may be recognized by jagged islands with eosinophilic keratinization of dermal invasive foci (multiple tissue levels may be required for demonstration).

Sebaceous carcinoma: both eyelid (also known as Meibomian gland carcinoma) and extraocular sites have a 30–40% risk of local recurrence, a 20–25% of distant metastasis and a 10–30% mortality risk. A minority of patients have Muir-Torre syndrome (a subset of the hereditary non-polyposis colorectal carcinoma (HNPCC) syndrome).

Immunophenotype

Most cutaneous carcinomas are reported without immunochemical stains. However, there are a number of scenarios where a particular immunophenotype may help secure the correct diagnosis.

Squamous cell carcinoma: AE1/3, 34BetaE12, CK5/6, EMA, p63, CEA positive; BerEP4 negative.

Basal cell carcinoma: BerEP4 positive; EMA, CEA negative.

A combination of EMA and BerEP4 is often helpful in distinguishing between squamous cell carcinoma and basal cell carcinoma.

Adnexal carcinomas: usually EMA and CEA positive, and differential molecular weight cytokeratin expression according to their differentiation. In general they are CK7, CAM 5.2 (low molecular weight), AE1/3, HMFG1 and GCDFP-15 positive.

Other Malignancy

Leukaemia

- Leukaemia cutis describes any cutaneous presentation of leukaemia. Most cases represent widespread systemic or recurrent disease but in around 5% skin involvement may be the first manifestation. Tumour cell immunophenotype (together with clinical information, bone marrow and peripheral blood findings) is vital for diagnosis. Specialist haematopathology input is essential.
- Children: acute lymphoblastic leukaemia (most common childhood leukaemia). CD79a, CD34, CD10 positive ± tdt, Ki-67 >90%.
- Adults:
  - Chronic lymphocytic leukaemia (CLL)—CD5, CD20, CD23, CD43 positive and CD10 negative
  - Chronic myeloid leukaemia (granulocytic sarcoma, chloroma)—CD34, CD43, CD117, myeloperoxidase

Primary Cutaneous Lymphoma

- Heterogenous group of T and B cell lymphomas of the skin in the absence of extracutaneous disease at diagnosis. Staging is required to exclude secondary cutaneous involvement by systemic lymphoma
- In western countries: 75–80% are T cell; 20–25% are B cell.

Immunohistochemistry (for cell lineage and light chain restriction) and *molecular studies (T cell receptor gene and immunoglobulin heavy chain gene rearrangements)* are also of use in cutaneous malignant lymphoma. *T cell malignant lymphomas* show *epidermotropism* while *B cell malignant lymphomas* often have a *dermal*

grenz zone and a *"bottom-heavy" infiltrate extending into the subcutaneous fat*. Note that the latter can also show reactive germinal centres and a polymorphous reactive cellular infiltrate. Low-grade T cell malignant lymphomas have a horizontal band like dermal growth pattern while high-grade lesions and B cell malignant lymphomas are sharply demarcated with a nodular, vertical and three-dimensional growth. Molecular studies may be helpful in inflammatory conditions simulating cutaneous malignant lymphoma (e.g. lymphocytoma cutis, lupus erythematosus profundus, and lymphomatoid reactions to drugs and insect bites) but may provide inconclusive results. Designation of malignant lymphoma can sometimes be difficult and should be clinicopathological in the context of a multidisciplinary meeting. In some cases the subsequent clinical progression or lack thereof is the final arbiter.

Some malignant lymphomas present in the skin and never as a primary lesion in lymph node or extracutaneous site, e.g. mycosis fungoides. Others can resemble their lymph node counterparts but show a different clinical behaviour.

T Cell, Indolent Behaviour

Mycosis fungoides (MF): the most common cutaneous T cell lymphoma and comprises nearly 50% of cutaneous T cell malignant lymphomas. MF has overlapping patch, plaque, tumour, erythrodermic and poikilodermic stages with late lymph node, blood and extracutaneous involvement. Cells are small to medium sized with cerebriform nuclei. There is epidermotropism (CD3+cells with increased CD4/CD8 ratio of positivity). This immunochemical profile may help in differentiating potential mimics such as spongiotic dermatitis and lichenoid keratosis. Multiple biopsies may be required before a histologic diagnosis is made.

Folliculotropic MF, pagetoid reticulosis and granulomatous slack skin are distinct variants of MF:

- *Pagetoid reticulosis:* intraepidermal (pagetoid) T cell infiltrate.
- *Granulomatous slack skin disease:* T cell infiltrate with giant cell elastophagocytosis in the major skin folds. Malignant lymphoma may occur years later.
- *Folliculotropic MF:* perifollicular infiltrate with variable infiltration of follicular epithelium. Cases may or may not show mucinous degeneration of hair follicles.

Primary cutaneous CD30+ T cell lymphoproliferative disorders (second most common group of cutaneous T cell lymphomas—30%) and comprises lymphomatoid papulosis and primary cutaneous anaplastic large cell lymphoma (C-ALCL).

Lymphomatoid papulosis: recurring self-healing papulonodular eruption of uncertain malignant potential with variable polymorphous/monomorphous pictures including CD30 positive large cells. A wedge shaped infiltrate at low power. Several subtypes exist (without treatment or prognostic implications), their importance in recognition relating to avoiding misdiagnosis. Most cases follow an indolent course but some may be at risk of developing another form of cutaneous or nodal lymphoma and therefore long term follow-up is recommended.

C-ALCL: usually cohesive sheets of anaplastic cells and >75% of these are CD30 positive. Usually T cell, sometimes null, 40% spontaneously regress and a 10 year survival of 90%. If clinical evidence of MF then diagnosis more likely to be MF with large cell transformation. Exclude systemic anaplastic large cell lymphoma with cutaneous involvement (this is EMA and ALK positive in contrast to C-ALCL).

T Cell, Aggressive Behaviour

Sézary syndrome: erythroderma, generalized lymphadenopathy and neoplastic T cells with cerebriform nuclei (Sézary cells). May appear histologically similar to MF. 5 year overall survival of 10–30%.

Adult T cell lymphoma: human T cell leukaemia virus type 1 linked. Various degrees of tumor cell infiltration in skin biopsy from epidermis to subcutis. May resemble MF. Some cases run a

protracted course, particularly if limited to skin but more extensive disease associated with greater disease aggression.

Cutaneous γδ T cell lymphoma: 1% of all cutaneous T cell lymphomas; 12 month median survival. May be epidermotropic, dermal and subcutaneous.

Subcutaneous panniculitis like T cell lymphoma: Rare. Multiple subcutaneous lumps in the extremities of young adults. Honeycomb panniculitic infiltrate of the subcutaneous fat with sparing of dermis/epidermis. CD8 positive, αβ T cell phenotype.

Extranodal NK/T cell lymphoma, nasal type: angiocentric/destructive, may involve dermis diffusely and extend into subcutis. CD56 positive, occasionally T cell positive. 25% 5 year survival especially if multiple lesions.

Primary Cutaneous B Cell, Indolent Behaviour (>95% 5 Year Survival)

Follicle centre lymphoma: 50% of all primary cutaneous B cell lymphomas. Trunk and scalp. Must rule out systemic disease. CD20/CD10 (negative in diffuse morphology)/bcl-6 positive but bcl-2 negative. Widely spaced follicles in the deep dermis and subcutaneous fat.

Marginal zone lymphoma: good prognosis—a minority have *Borrelia burgdorferi* organism as a chronic antigenic stimulus. Nodular perivascular/periadenexal, or diffuse dermal infiltrate of centrocytoid/monocytoid cells including reactive germinal centres. Neoplastic cells are bcl2 positive; CD10/bcl6 negative (reactive follicles are CD10/bcl6 positive; bcl2 negative). Preferentially located on trunk and arms.

B Cell, Intermediate Behaviour

Mantle cell lymphoma: rare but skin involved in 1–12% of cases; exclude spread from systemic disease.

Primary cutaneous diffuse large B cell lymphoma (DLBCL), leg type: typically elderly females. 10% occur at other sites. Grenz zone, with a dermal/subcutaneous, perivascular/periadnexal infiltrate. It is CD20/CD10/bcl2/MUM1/bcl-6 positive. Variable 50—95% 5 year survival. Multiplicity of lesions is adverse. Exclude secondary involvement by systemic DLBCL.

B Cell, Aggressive Behaviour

Intravascular large B cell lymphoma.

Primary cutaneous—DLBCL.

Lymphoblastic lymphoma in children and adults: tdt positive. Aggressive but can be cured particularly in children.

Lymphomatoid granulomatosis (EBV positive large B cells, with reactive small T cells). Skin is most common extrapulmonary site involved (EBV positive B cells rare to absent in skin lesions). Poor prognosis in most patients with mortality related to lung involvement.

Maligant Lymphoma: Secondary

- Secondary to nodal/systemic disease.

For details on the classification, immunophenotyping and staging of malignant lymphoma refer to Chap. 35.

Other

Langerhans cell histiocytosis: S100/CD1a positive, epidermotropic dendritic cell proliferation. Considered an inflammatory myeloid neoplasm. Can be single system but also potentially in bone, lung and lymph nodes. Single system disease has a good prognosis in contrast to multisystem disease.

Cutaneous mastocytosis: abnormal accumulation of clonal mast cells. Either solitary mastocytoma, maculopapular cutaneous mastocytosis (urticaria pigmentosa), or diffuse cutaneous mastocytosis (whole body). Skin involvement may be part of a systemic presentation (often bone mar-

row). A clonal mast cell infiltrate with a "fried egg" appearance (can also be spindle shaped), tryptase/toluidine blue/CD117 positive. Aberrant expression of CD25 and/or CD30 helps confirm a clonal mast cell origin.

Sarcoma

Soft tissue sarcomas are outnumbered 100 to 1 by benign soft tissue tumours. There are multiple different histological types often with more than one subtype. They vary in their behaviour from indolent to aggressive but *cutaneous sarcomas are more favourable* than their deep sited fascial counterparts. In general *5 year survival is 65–75%* with *complete local excision being the most important determinant.* A majority are locally infiltrative and a minority potentially metastatic. The latter depends on tumour type, grade, size and depth from the skin surface. They usually arise in older patients in the extremities (particularly thigh), trunk, head and neck and retroperitoneum. Cutaneous sarcomas form an elevated plaque or nodule that can ulcerate when malignant. Superficial lesions smaller than 2 to 5 cm can be *excised in their entirety.* Larger, deeper tumours may require initial diagnostic sampling by *needle core* or *deep punch biopsy* prior to *radical extirpative surgery. Molecular genetic analysis* has a *diagnostic* and *prognostic role* to play in select cases and to determine any indication for *neoadjuvant therapy.*

Dermal and subcutaneous soft tissue tumours may have classical clinical features, e.g. angiosarcoma of the scalp in the elderly and Kaposi's sarcoma in HIV. However, they are classified according to their cell of origin and malignancy is assessed by cellularity, cellular atypia, mitotic activity, necrosis and infiltrative margins. *Immunohistochemistry* is often very useful in determining histogenesis e.g. desmin, h-caldesmon, SMA, myogenin (muscular), S100 (neural, melanocytic (also HMB45, melanA, SOX10), chondroid, adipose), CD31, CD34, FLI1, ERG, factor VIII (vascular) and CD68 ((fibro-)histiocytic). Examples are: cutaneous leiomyosarcoma (SMA, desmin), dermatofibrosarcoma protuberans (DFSP) (CD34), angiosarcoma (CD31, CD34, FLI1, ERG—epithelioid variant may expression cytokeratins), epithelioid haemangioendothelioma, malignant nerve sheath tumour and pleomorphic liposarcoma (S100) and Ewing sarcoma/PNET (CD99). See Chap. 36 for further details.

Immunohistochemistry is also important in the differential diagnosis of *cutaneous spindle cell lesions, e.g. spindle cell squamous carcinoma versus malignant melanoma, leiomyosarcoma and atypical fibroxanthoma (AFX).* A working panel is CAM5.2, MNF116, AE1/3, CK5/6, p63, S100, melanA, SOX10, desmin, h-caldesmon, smooth muscle actin and CD68. Other morphological clues are dysplasia of the surface squamous epithelium (carcinoma), junctional activity and melanin pigmentation (melanoma), Touton-like giant cells (AFX) and eosinophilic fusiform spindle cells (leiomyosarcoma). *Clinical history* is vital to exclude a metastatic spindle cell carcinoma or malignant melanoma. Hence AFX is a diagnosis of clinical, morphological and immunohistochemical exclusion. It arises on the sun exposed head and neck area of elderly patients, is a *low-grade malignant tumour* regarded by some as a spindle cell squamous carcinoma, but behaves in a benign fashion if completely excised. It can show false positive melanA staining in the pleomorphic cells as well as SMA. There is also a monomorphic spindle cell variant. Temper a diagnosis of AFX in small biopsies when the base of the lesion is not seen. AFX is excluded by the following: perineural, lymphovascular or extensive subcutaneous invasion, or, necrosis (reported metastatic examples should be considered with extreme skepticism). If any of these are present it is designated *pleomorphic dermal sarcoma* with 30% recurrence rate and 10% metastatic rate with resultant high mortality.

Kaposi's sarcoma is found in the elderly (solitary) or young (HIV, multiple). The earlier patch and plaque phases are subtle, characterised by linear vascular slit-like spaces in the dermal collagen orientated parallel to the epidermis: promontory sign may be present (small vessel

protruding into an abnormal vascular space). Later there is a sieve-like pattern with extravasation of red blood cells and a spindle cell proliferation in the nodular phase (differential diagnoses in this phase include angiosarcoma, DFSP, leiomyosarcoma and spindle cell amelanotic melanoma). The causative agent is human herpes virus 8 (HHV8) and lesions express HHV8 immunochemistry (as well as CD31, CD34 and D2–40).

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Malignant Melanoma

21

David P. Boyle

(with clinical comments by Olivia Dolan)

Introduction

In the last 25 years the incidence of melanoma has increased dramatically in all age groups over 25 years old. Although the prevalence of melanoma is much less than that of non-melanoma skin cancer, it is a major cause of death from skin cancer and incidence is increasing. Mortality is related to the propensity for lymphatic and haematogenous metastases if left untreated. The main factors predisposing to development appear to be intermittent sun exposure, fair complexion, a history of blistering sunburn particularly in childhood and family history of melanoma. Melanoma may arise in a pre-existing mole but the majority of melanomas appear to arise de novo. Referable signs and symptoms include progressive change in the shape, size or colour (particularly three or more different colours) of a mole as well as ulceration, bleeding, or change in sensation. Patients with suspicious pigmented lesions are referred urgently for specialist consultation.

Incisional samples and incomplete excisions are avoided in cases of suspected melanoma as sampling error may result not only in erroneous diagnosis but also endanger accurate collection of histologic staging features. Surgical excision with adequate margins based on the clinical stage forms the management of both in situ and invasive melanoma. Occasionally, topical imiquimod may be applied to in situ disease where an adequate margin cannot be achieved. Excision samples are orientated, photographed and inked prior to sampling to facilitate diagnosis and margin examination. Re-excision samples should contain a fibrotic scarred area and a comment made whether the scar is excised. Although thoroughness of sampling in re-excisions may be influenced by margin closeness in the original specimen, re-excisions may contain micrometastatic deposits.

Additional treatment options are based on staging information as appropriate including molecular analysis, sentinel lymph node biopsy, nodal clearance and radiological investigations. Sentinel lymph node biopsy provides prognostic information but may also indicate suitability for adjuvant therapy. Primary tumour breslow depth >1 mm is used as a criterion for offering sentinel lymph node sampling. Patients with a positive sentinel lymph node are at a higher risk of recurrence. Recent advances in adjuvant therapy including checkpoint inhibitors and targeted agents may prove beneficial in lowering the risk of recurrence.

Known disseminated disease and prior wide local excision are contraindications to sentinel node assessment: in the former case lymph nodes completely replaced by tumour may be missed

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_21

due to altered lymphatic drainage; in the latter, the lymphatic drainage from the primary tumour site may be considerably altered potentially resulting in an erroneous result. Patients with BRAF V600 mutation positive unresectable or metastatic melanoma may be offered targeted therapy: 50–60% of advanced melanomas have a mutation in the BRAF gene with V600E the most common mutation. BRAF status is usually assessed in the secondary tissue. If there is no obvious primary lesion on the skin, or clinical history of excision of a melanocytic lesion, ocular or mucosal malignant melanoma (e.g. anorectal, oesophageal, vulval or penile) must be considered as a potential source for the locoregional or disseminated disease.

Gross Description

Specimen

- Curettage/shave biopsy/punch biopsy/incision biopsy/excision biopsy/re-excision
- Size: length × width × depth (mm).

Tumour

Site

- Anatomical location—clinical site as stated, multifocal (1–5%).
- Epidermal/dermal/subcutaneous.
- Commonly face, ear, head and neck with trunk (and particularly the back) in males (35%) and legs in females (50%).

Size

- Length × width × depth (mm) or maximum dimension (mm).

Appearance

- Verrucous/nodular/sessile/pigmented or non-pigmented/halo/satellite lesions/scarring. Atypical features are recorded and include asymmetry/irregular border/variegated appearance/ulceration

Histological Type

Melanomas can be essentially divided into those that remain within the epidermis and have no metastatic potential (melanoma in situ) and those that invade the dermis (malignant melanoma). However, there are different types of both in situ and malignant (invasive) melanoma reflecting different natural histories. Melanoma nomenclature can seem confusing at first and has changed since Clark's original descriptions. It appears that different classes and subtypes of melanoma have distinctive clinical and genetic features: broadly, there are lesions with a high degree of cumulative sun damage (CSD) (older patients; de novo lesions, e.g. lentigo maligna melanoma) and lesions with a low degree of CSD (younger patients; naevi and dysplastic naevi precursors, e.g. superficial spreading malignant melanoma). The degree of CSD can be histologically assessed by evaluating dermal solar elastosis. It is the author's practice to firstly refer to these lesions as melanoma in situ to imply no metastatic potential or malignant melanoma when dermal invasion is present. Lesions can then be further subtyped. The more common types are described here.

Melanoma In Situ

- Intraepidermal: spread can be lentiginous (continuous basal layer) as in lentigo maligna or upward (single cells, nests, "buck-shot" or Pagetoid) as in superficial spreading melanoma in situ. These lesions are non-invasive and have a radial growth phase.

Malignant Melanoma

Lentigo Maligna Melanoma

- Develop in pigmented macules called lentigo maligna or Hutchinson's melanotic freckle on the face of elderly patients: a lentiginous single cell and nested proliferation of melanocytes along the surface epidermal and append-

ageal basal layer. They show cytological atypia (enlarged, hyperchromatic angular nuclei and cytoplasmic vacuolation) ± architectural atypia (expanded junctional nests) on a background of dermal solar elastosis and epidermal atrophy reflecting chronic sun exposure. Expansion and spindling of junctional nests and any clinically nodular areas should raise suspicion of invasion. The clinical term lentigo maligna encompasses any degree of proliferation that is confined to the epidermis while lentigo maligna **melanoma** heralds the presence of dermal invasion (at least Clark level II). In general it has a favourable prognosis if completely excised.

Superficial Spreading Malignant Melanoma

- This subtype is often quoted as the most common and more frequently occurs in younger patients and can occur at any body site. It has a radial or vertical (see Section "Vertical and Radial Growth Phase") phase of spread.

Usually an asymmetrical lateral border of atypical junctional cell nests with a central segment of epidermis showing upward (pagetoid) melanocytic spread (single cells/nests/"buckshot" patterns). Moderate dusty pigmentation ± a dermal component related to the growth phase i.e. lateral growth before vertical invasion. Often on intermittently sun exposed sites of young patients. It is often quoted that the intraepidermal portion should extend >3 rete ridges beyond the dermal component otherwise the lesion has nodular morphology.

Nodular Malignant Melanoma

- Vertical Phase of Spread.

Often exophytic/nodular/ulcerated and thick ± pigmentation, with ≤3 rete pegs showing atypical junctional nests at the lateral border of the lesion (dermal and junctional components are usually coterminous). This is the most aggressive form of malignant melanoma, seen in older patients and exclusively in a vertical growth phase.

Acral (Lentiginous) Malignant Melanoma

- Sole of foot, palm of hand, nail bed. Occur more frequently in dark skinned and Asian populations. Features are often a combination of lentigo maligna and superficial spreading patterns ± a nodular, vertical growth phase component. Prognosis is poor due to delay in diagnosis (either not noticed given the site or clinically misdiagnosed) with increased thickness at presentation.

Mucosal Melanoma

- Represents approximately 1% of all melanomas. Occurs in non-skin surface epithelia: genitalia, distal urinary tract, anorectum, oral, nasal and paranasal cavities. Non-UV related. Poor prognosis probably relates to late detection. Aside from head and neck lesions which are at least pT3, no clear staging exists.

In general, these main subtypes show prognostic differences and there is correlation with recurrence and metastases e.g. nodular malignant melanoma is often thick (with a significant Breslow depth) and ulcerated. Knowledge of the various clinicopathological subtypes aids in their diagnostic recognition.

Others

- e.g. desmoplastic, neurotropic, verrucous, balloon cell, signet ring cell, small cell, myxoid, minimal deviation, metastatic malignant melanoma, malignant blue naevi.

Vertical and Radial Growth Phase

Vertical growth phase (VGP) tumour often comprises expansive nests, nodules or plaques of cytologically atypical melanoma cells in the dermis; it is always invasive and therefore implies a biological potential for metastatic spread. VGP has been minimally and somewhat loosely defined as a cluster of dermal melanocytes that is larger than the largest intraepidermal cluster (although epidermal nesting may not necessarily

be present in a VGP tumour). Only one dermal nest of sufficient size (and having malignant cytonuclear features) is required to label as VGP. Most agree that the dermal nest should be ≥10 cells diameter. A tumour with a single nest size smaller than this would be considered growing in a 'malignant' radial phase. Dermal malignant melanocytes with ≥1 mitosis are always VGP (identifiable mitotic activity is not a prerequisite for vertical growth). VGP melanomas are often at least Clark level III and thicker than 1 mm with an inconstant relationship between the width and depth of the lesion.

A completely radial growth phase in theory has no metastatic potential. However, given the definition of VGP (above) then radial growth phase includes melanoma in situ ± microinvasion of the papillary dermis. Therefore in reality the survival rate is 95–100%. The dermal component is usually <1 mm thick, i.e. the lesion is wider than it is deep and can have morphologically bland cell nests (usually <10 cells across) of uniform size and cytological appearance. Mitotic figures are absent. This may be accompanied by signs of regression with a brisk lymphocytic response. The radial phase potentially progresses by clonal expansion to the vertical phase.

Breslow Depth or Thickness (mm)

- Ocular micrometer, Vernier scale, eyepiece graticule and digital measurements of *tumour maximum vertical diameter* to one decimal place, from the top of the granular layer (or base of ulceration if no remaining epidermis) to the deepest point of invasion (see Fig. 21.1). Whichever method of measurement is used,

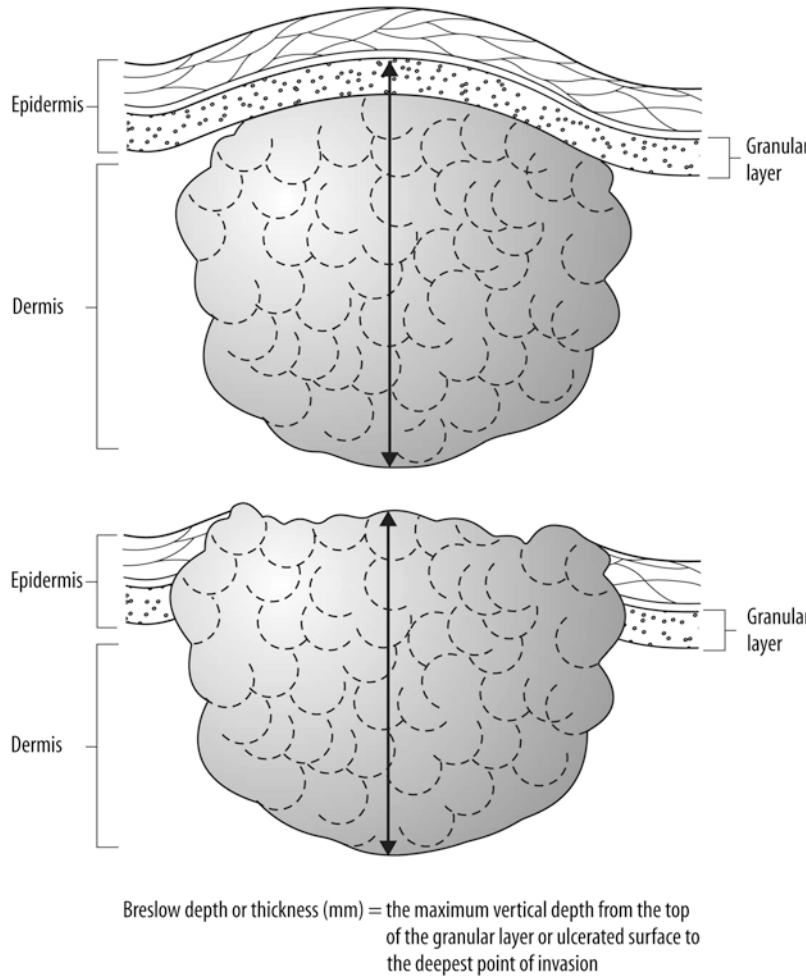

Fig. 21.1 Malignant melanoma. Reproduced, with permission, from Histopathology Reporting: Guidelines for Surgical Reporting, 2nd ed., © 2006, Springer

accurate calibration is required and the distance measured should be perpendicular to the skin surface. Malignant cells can occasionally track deeply along skin adnexal structures: these areas should not be used to define Breslow depth. Furthermore, areas of regression extending to a greater depth than measureable tumour should not be used in Breslow assessment but may be useful to record. Judicious use of immunochemical stains (consider applying a suitable different colour if heavily pigmented) can help highlight malignant cells in the dermis that might otherwise be obscured by a heavy lymphocytic infiltrate at the base of the lesion. SOX10 is preferentially used in this scenario as it provides a very legible nuclear expression pattern in melanocytes.

Anatomical Clark Level

- This is not required for TNM8 and may be less prognostically informative than tumour thickness measurement. Clark levels vary depending on anatomical location: two tumours at different sites may have identical Clark levels but differing tumour thicknesses. There is also interobserver variability in defining Clark levels. Increasing Clark levels of invasion are associated with decreased survival although Breslow thickness is a stronger prognostic indicator.

I	Intraepidermal
II	Papillary dermis or periadnexal connective tissue sheath but not expanding or filling the papillary dermis
III	Papillary-reticular interface: papillary dermis filled and expanded down to an interface marked by the position of the superficial vascular plexus. The thick reticular dermal fibres should be discernible beneath.
IV	Reticular dermis
V	Subcutaneous fat. Where fat is absent e.g. lip, this includes extension into other structures e.g. striated muscle.

TNM8 Staging Fig. 21.2

pTis	Melanoma in situ
pT1	Tumour ≤1 mm in thickness
	(a) <0.8 mm without ulceration
	(b) 0.8–1.0 mm without ulceration or ≤1 mm with ulceration
pT2	>1.0–2.0 mm
	(a) Without ulceration
	(b) With ulceration
pT3	>2.0–4.0 mm
	(a) Without ulceration
	(b) With ulceration
pT4	>4.0 mm
	(a) Without ulceration
	(b) With ulceration

At staging interfaces it is recommended to round to the nearest one decimal place when assessing tumour thickness: e.g. a tumour measured as 1.02 mm is not stage pT2 but should be recorded as 1.0 mm and stage pT1. One must resist the temptation to get a tumour "over the line", for example to influence sentinel node assessment.

Tumour related ulceration: (particularly if >5 mm or 70% of the lesion) is an *independent adverse prognostic factor*, with 50% 10 year survival versus 78% if non-ulcerated. It can also result in understaging of Breslow thickness although there is a strong correlation between them. It is an index of rapid tumour growth. Tumour ulceration is strictly defined as a full thickness epidermal loss with an inflammatory fibrinous response. It is not partial erosion or traumatic loss.

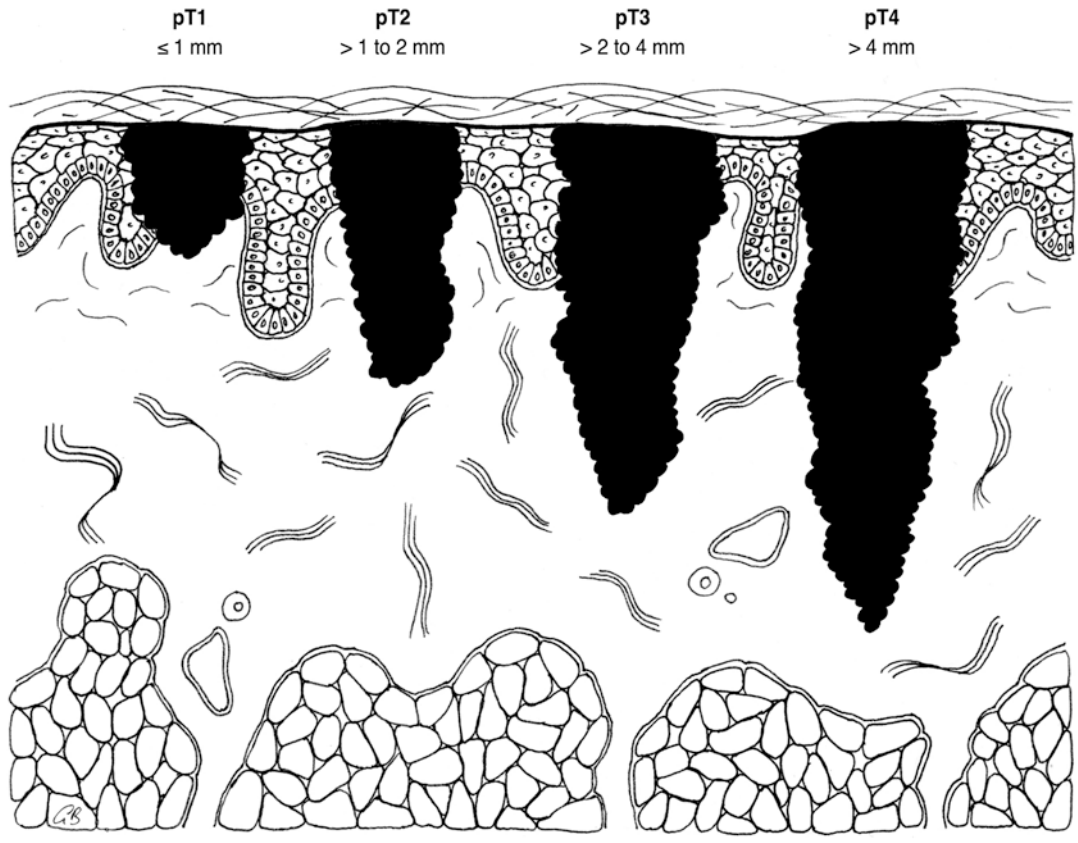

Fig. 21.2 Malignant melanoma pathologic staging based on depth of invasion as measured from the granular cell layer and subdivided on the presence of ulceration. pT1 tumours are subdivided into (**a**) <0.8 mm without ulceration and (**b**) 0.8–1.0 mm without ulceration or ≤1 mm with ulceration. pT2 to pT4 tumours are subdivided into (**a**) without ulceration and (**b**) with ulceration

Lymphovascular Invasion

Present/absent.

Intra-/extratumoural.

Rarely seen, but perineural or intraneural invasion is commonly present in neurotropic desmoplastic malignant melanomas with a subsequent high recurrence rate necessitating wide primary or secondary excision. Lymphovascular invasion and angiotropism (malignant melanoma cells cuffing small vessels) are adverse prognostic indicators.

Lymph Nodes

Sentinel lymph nodes.

Size of specimen/number of nodes (more than one may be present)/presence of dye/visible metastasis/size of metastasis/single or multiple.

Classification based on sentinel node biopsy alone is designated (sn) e.g. pN0 (sn).

Regional nodes are those appropriate to the site of the primary tumour. A regional lymphadenectomy will ordinarily include a minimum of 6 lymph nodes.

Site/number/size/number involved/apical node/extracapsular spread.

There is no agreed minimum cellular threshold to define nodal positivity: as such, isolated tumour cells are designated pN1.

pN0	No regional lymph node metastasis
pN1	Metastasis in **1** regional lymph node or regional intralymphatic metastasis with no regional involved node
	1a. Micrometastasis (clinically occult)
	1b. Macrometastasis (clinically apparent)
	1c. Satellite or in-transit metastasis without regional nodal metastasis
pN2	**2 or 3** regional histologically involved nodes or regional intralymphatic metastasis with regional involved node
	2a. Microscopic nodal metastasis (2 or 3)
	2b. Macroscopic[a] nodal metastasis (2 or 3)
	2c. Satellite or in-transit metastasis with 1 regional nodal metastasis
pN3	**4** or more regional histologically involved nodes or any number of matted nodes or regional intralymphatic metastasis with 2 or more involved nodes
	3a. Microscopic nodal metastasis (≥4)
	3b. Macroscopic[a] nodal metastasis (≥4)
	3c. Satellite or in-transit metastasis with ≥2 regional nodal metastases

[a]Although not specifically stated, it is generally agreed that pN2b and pN3b only require one clinically detected node from the total of positive nodes

Satellites: are tumour nests or nodules within 2 cm of the primary tumour (closely orbiting the tumour) but not contiguous with it (at least 0.3 mm away) and are *prognostically adverse*. Satellites are grossly visible. Microsatellites are only visible microscopically and are >0.05 mm dimension. They must be separated from the main tumour by uninflamed, non-fibrotic normal dermis (they do not have to be demonstrated to reside within endothelial lined channels). Levels are recommended to demonstrate lack of continuity.

In-transit metastases: involve skin or subcutaneous tissue more than 2 cm from the primary tumour but not beyond the regional lymph nodes.

Satellites, microsatellites and in-transit metastases have identical effects upon pN staging. Precise discrimination based on aforementioned somewhat arbitrary distances from primary tumour may therefore not be critical. Regardless, their presence portends a poorer prognosis.

Spread of malignant melanoma: is to regional lymph nodes, skin (satellite nodules and in-transit metastases), liver, lungs, gastrointestinal tract, bone and central nervous system. Distant metastases can be *late (>10–25 years)* possibly representing regrowth of "dormant" seedlings.

Excision Margins

Distances (mm) to the nearest painted deep and peripheral excision margins either of serial transverse slices or quadrant blocks according to local protocols—of the vertical and radial disease phases and any in situ change.

Recommended margins of clearance: these vary according to the tumour Breslow depth of invasion. Actual margins taken may vary based on tumour site, requirement for reconstructive surgery, and after discussion with the patient. All melanoma cases should be discussed at a local or specialist MDM. Pathologic margins may be less than clinically expected due to tissue shrinkage.

Adequate treatment is based on successful *complete primary excision* or, if there is initial margin involvement, *secondary re-excision* to avoid any possibility of locally persistent malignant melanoma or scar related local metastasis. This is supplemented by *regional lymph node dissection* and *systemic therapy* for metastatic disease. *Sentinel lymph node biopsy* should be considered in patients with clinically negative regional lymph nodes and a vertical growth phase malignant melanoma >1 mm thick as a prognostic indicator and opportunity for consideration of adjuvant therapy and for potential inclusion in research and trials. It involves 2 mm serial transverse slices of the lymph node with preparation of multiple haematoxylin and eosin (H&E) and matching immunohistochemical stains for S100 (being most sensitive) and an additional melanocytic marker (SOX10 or HMB45 preferred). The European Organisation for the Research and Treatment in Cancer (EORTC) trial protocol is also recommended and involves bivalving the node (slicing through convex capsule through

hilum in the long axis to reveal 2 cut surfaces). Differential diagnoses include sinus histiocytes, capsular naevus cell nests (should be HMB45 negative) and benign ductal epithelial inclusions. If the sentinel node is positive then options include close observation of the nodal basin or in higher risk scenarios a completion lymphadenectomy may be considered.

Other Parameters to Report

- *Pigmentation:* none/light/moderate/heavy. Often patchy in distribution due to regression or clonal growth of pigmented melanocytic cells. Amelanotic malignant melanoma is more frequent in the face and head and neck areas.
- *Mitotic index:* number/mm^2 counted in a mitotic 'hot spot' if present. Mitotic rate remains a recordable prognostic indicator despite its absence in TNM8 staging.
- *Solar elastosis*: present/absent. This is an indicator of cumulative sun damage.
- *Regression*: present/absent—inflammation/fibrosis/telangiectasia/melanophages. Seemingly paradoxically, in thin melanomas >75% regression is an adverse prognostic factor. However, this may be due to potential understaging of a previously thicker melanoma: appearing thinner at the time of sampling. Complete regression may be an explanation for widespread metastatic disease in the absence of an identifiable primary cutaneous lesion.
- *Pre-existing lesion*: present/absent and less common than de-novo malignant melanoma (60–70% of cases) although this proportion may shift as screening yields more early lesions.
- *Satellite lesions*: present/absent; distance (mm) from the primary.

Primary versus secondary/recurrence: secondary tumour tends to be nodular and dermal/subcutaneous ± vascular invasion with no epidermal component: often lacks any significant inflammatory response and tends not to involve adnexal structures. Occasionally, secondary melanoma may exhibit epidermotropism (5%) but usually the dermal disease is more extensive in width: clinical history is obviously vitally important. Some malignant melanomas can develop multiple locoregional cutaneous recurrences over many years and do not develop lymph node or distant metastatic disease, although such satellite nodule and in-transit metastases are an indicator of potential systemic dissemination. They are regarded as tumour emboli arrested in lymphatics which then grow to form a tumour mass. They are seen in about 5% of malignant melanomas >1 mm thick. They are surgically excised to achieve local control.

Dysplastic naevus: variable reports of sporadic and familial predisposition to malignant melanoma. A range of *benign naevi with active junctional components can mimic dysplastic naevus or malignant melanoma (pseudomelanoma).* These include: junctional/Pagetoid Spitz naevus, pigmented spindle cell naevus of Reed, halo naevus, traumatised and partially excised or recurrent naevi, irradiated naevus, mitotically active, desmoplastic, clonal and deep penetrating naevi, cellular blue naevus, acral and genital naevi. Age, anatomical site, lesion type and clinical history e.g. a previous malignant melanoma or change (pigment/profile/margin) in a naevus must all be considered along with the morphology.

- Single or multiple (dysplastic naevus syndrome).
- Often >4 mm with variable pigmentation and irregular borders.
- Nested and lentiginous melanocytic proliferation.
- Architectural and cytological atypia.
- Elongation/lateral fusion of rete pegs.
- Dermal lamellar fibroplasia with vascularisation and chronic inflammation in the dermis.

Morphological clues to a diagnosis of malignant melanoma: lesion *asymmetry, extension of atypical melanocytes* up into the epidermis and lateral to the lesion, *melanocytic atypia* and *lack of dermal maturation* (maturation is characterized by diminution in nest and cell size with

dermal depth), *deeply placed mitoses* (deeper than the superficial dermis), and a *dermal lymphocytic infiltrate*. Melanocytic cell nests also vary in size, shape and cytological atypia within a lesion. Melanocytic lesions in general can present as challenging histologic cases. A combination of the aforementioned features helps to decide whether a lesion is benign or malignant. There should be a low threshold to obtaining additional intra or extra-departmental opinions in difficult cases.

Mimics: there are also a number of *pigmented non-melanocytic tumours* that can mimic melanocytic neoplasms clinically and histologically and that may be considered in the differential diagnosis. These can be:

Epithelial: pigmented seborrhoeic keratosis, basal cell carcinoma, skin appendage tumours, Bowen's and Paget's disease, melanotic non-cutaneous carcinomas (e.g. renal, anorectum).

Mesenchymal: dermatofibroma, atypical fibroxanthoma, dermatofibrosarcoma protuberans, angiosarcoma.

Neural: schwannoma (neurilemmoma), neurofibroma, neurothekoma,

Neuroendocrine: Merkel cell carcinoma, medullary carcinoma thyroid.

Immunophenotype

- Useful in the distinction between *malignant melanoma* and *non-melanocytic tumours* but not reliable for separating benign and malignant melanocytic lesions. In general there are no immunohistochemical or molecular biomarkers with sufficient sensitivity or specificity to clearly separate diagnostically ambiguous melanocytic lesions into benign and malignant categories. Note also that some malignant melanomas are cytokeratin (CAM5.2), CEA and EMA positive.
- S100 protein, HMB45, SOX10 and melanA (MART1). Used in combination as other tumours can show positivity for these individual markers and conversely melanoma may not demonstrate panexpression. HMB45 may diminish in intensity in the dermis in benign lesions but is not a reliable single discriminator with respect to benign versus malignant: cyclinD1 may also show a similar staining pattern. S100 stains, amongst other things, cutaneous dendritic cells which may result in overestimation of melanocyte number. A non-brown immunostain can be applied in very pigmented lesions.
- Masson Fontana for pigment.
- Ki-67 proliferation index and p53: increased (>5%) in malignant melanocytic lesions. Ki-67 index is regarded as a more accurate prognostic indicator in established malignant melanoma than mitotic count.
- CyclinD1, Ki-67 and p53 may be more strongly expressed in metastatic melanoma and nodular melanoma than primary dermal melanoma.

Prognosis

Prognosis: of malignant melanoma is unpredictable but relates strongly to the *vertical component or thickness/depth of invasion* and *adequacy of excision* with the width of margins tailored accordingly. Estimated overall 5 year survival rates are about 80–90% with *tumour stage/thickness* and metastatic disease amongst the most powerful prognostic determinants reflecting clinical staging (localized melanoma—stages I and II; regional metastasis—stage III; distant metastasis—stage IV). Correspondingly, overall 5 year survival reduces from 85 to 99% (for stage I), 40–85% (for stage II), 41–65% (for stage III) and 9–20% (for stage IV). Wide quoted variation is partly reflective of changing patient demographics, treatment modalities and staging alterations. A small subset of stage I lesions are associated with a survival approaching 100%. These lesions are very thin, lack mitoses, are non-ulcerated and have a radial growth. Therefore they lack strong diagnostic criteria to distinguish them from precursor lesions and some may constitute overdiagnosis.

Recurrence rates: vary from 10 to 30% for stage I and II malignant melanomas, and more than 50% for stage III disease. The rate for lower

stage disease may be related to previously undetected regional disease before sentinel node assessment.

Other adverse indicators: patient age (>50 years), sex (male), histological regression in thin melanomas, vascular invasion, vertical growth phase, satellitosis, necrosis, ulceration, mitotic activity and anatomical site (hands, sole of foot, head, neck, trunk and back are worse). Apart from purely desmoplastic melanoma (more favourable outcome for comparable stages but only if >90% pure morphology, though tends to be diagnosed later), histologic subtypes alone appear to have no definite prognostic implications. Occasionally malignant melanoma may *present as metastatic disease* e.g. axillary nodes due to complete regression of a cutaneous lesion leaving no obvious primary tumour on examination. Other possible occult sources are the eye and mucosal surfaces of the upper aerodigestive tract (including oesophagus), vagina and anal canal. These mucosal, acral lentiginous and subungal malignant melanomas have poor prognosis due to late presentation. This is recognized in the upper aerodigestive tract by TNM regarding them as aggressive tumours and omitting T1 and T2 as options: these are either mucosal based (pT3) or very advanced (pT4). In general, factors such as age, pregnancy, lesion diameter, histological type and inflammatory infiltrate are outweighed by *tumour thickness* and *stage*. However, even in thick (>4 mm) melanomas there is a subset of patients with more favorable survival. Their tumours tend to be of spindle cell type with a lack of ulceration, mitoses, nodal involvement and vascular invasion. Conversely occasional thin melanomas can metastasise, possibly reflecting regression in some cases. Patients with metastatic disease may be treated with biological therapies such as immunotherapy or with targeted therapy (*tumour cell BRAF mutation* (50–60% of advanced cases) is predictive of response to vemurafenib targeted therapy). These treatments have been shown to be more effective than chemotherapy in controlling metastatic disease.

Desmoplastic variant arises mostly on the head and neck of elderly patients (particularly the lip) and shows a high incidence of local recurrence, and potentially metastases. It shows a propensity to neurotropism (30% of cases) and may occasionally form neural type structures. Diagnosis requires an index of suspicion to distinguish from a dermal scar, recognition of a tumour infiltrate in the deep dermis and accompanying clues in the form of an epidermal component. Less pure morphology (<90%) tends not to have the more favourable outcome associated with desmoplastic melanoma (although it is often diagnosed late). Immunohistochemistry (S100) is important in confirmation although it may be negative whilst HMB45 and melanA are often negative. Early benign fibrous scar may show spindle cell positivity with S100 and SOX10 together with inflammatory cells and mitotic activity.

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Part V

Breast Cancer

- Breast Carcinoma

Breast Carcinoma

22

Clinton Boyd

Introduction

Breast cancer accounts for 25% of all cancers in females. The risk of developing breast cancer increases with age. It is associated with hormonal and reproductive factors (early menarche, late menopause, late first childbirth, oral contraceptive use). The risk is increased in those with a positive family history. BRCA1/BRCA2 mutations confer a very high risk. More general risk factors include obesity, smoking and alcohol consumption.

Involvement of regional lymph nodes is the strongest prognostic indicator of locoregional and systemic disease relapse and overall survival. Lymph node status along with determination of predictive markers such as oestrogen receptor (ER) and HER2 status are instrumental in selection of hormonal or systemic chemotherapy for patients with localised, recurrent or advanced disease. Based on gene expression profiling, breast cancer can be classified into four main intrinsic molecular subtypes:

Cancer subtype	IHC expression	Additional information
Luminal A	ER+, PR+, HER2-, Ki-67 low	Low grade tumours; excellent prognosis
Luminal B	ER+, PR±, HER2±, Ki-67 high	Higher grade with a slightly worse prognosis
HER2 enriched	HER2+, ER-	Tumours respond to HER2 targeted therapies such as trastuzumab
Triple negative/ basal-like	ER-,PR-,HER2-	Often more aggressive and have poorer prognosis

Male breast carcinoma (about 1% of cases) occurs in older men, presents late and has a poor prognosis. It shows the same range of morphological characteristics as female breast cancer.

Symptomatic breast cancer usually presents with a palpable lump, skin tethering, or nipple abnormalities such as rash (Paget's disease), retraction or discharge (due to an intraductal epithelial proliferation). Asymptomatic in situ or invasive lesions are detected at two-view mammography (80–90% sensitivity) as either linear branching microcalcifications, a discrete mass or an area of stromal distortion and spiculate density. Mammographic screening takes place at 3 yearly intervals for patients aged 50–70 years in the United Kingdom. It detects a higher proportion of smaller invasive cancers and "earlier" lesions with a greater proportion of in situ carcinoma than in the symptomatic population.

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_22

Diagnosis of breast disease is based on a triple approach which involves clinical examination, radiological examination (usually an initial combination of mammography and ultrasound scanning), and cytological or histological examination of a biopsy specimen. In some centres, rapid cytological assessment of either an FNA specimen or needle core imprint is provided, allowing appropriate counselling of the patient at a one stop clinic. Therapeutic decision making is usually based on core biopsy histology.

Therapeutic surgery involves complete local excision of the primary lesion with or without an axillary lymph node procedure. This is either sentinel lymph node excision biopsy or sampling for a preoperatively node negative axilla, or clearance lymphadenectomy for a preoperatively node positive axilla.

A proportion of diagnostic needle core biopsies will identify a lesion of uncertain malignant potential, e.g. a radial scar or intraduct papilloma. In the UK these are referred to as B3 lesions (see SECTION "Other Malignancy" for classification information). Although some of these cases will require an open surgical biopsy for diagnosis, this procedure is gradually being replaced by wide bore (usually 7 G) vacuum-assisted excision techniques.

Screening detected impalpable lesions and areas of microcalcification require radiological localisation to ensure adequate excision. MRI scan is sometimes indicated where there is discrepancy between clinical and imaging disease extent, or if breast density, particularly in the younger patient, precludes accurate mammographic assessment. It can play a role in mapping the extent of ductal carcinoma in situ (DCIS) that is present, detecting satellite lesions in infiltrating lobular carcinoma, and assessing response to neoadjuvant treatment in patients who may be suitable for breast conservation. Full radiological staging (CT/PET scans, radioisotope bone scan) can be undertaken when there is significant lymph node disease (N2: $\geq$4 nodes involved), or for other indications such as bone pain or altered liver function tests.

Localisation biopsies are usually small (<20 g) and may or may not have been orientated. Therapeutic breast conserving surgery (usually wide local excision) aims to remove the tumour with a rim of normal tissue. The specimen should be a cylinder-like piece of tissue extending from the most superficial to the deepest aspect of the breast. Therapeutic specimens should be orientated according to an agreed local protocol, allowing differential painting of surgical margins. Mastectomy removes the breast tissue and overlying skin including the nipple with the chest wall left intact. A subcutaneous mastectomy leaves the skin intact for reconstructive procedures but removes the breast tissue and nipple-areolar complex (NAC). The axillary fat and contents may be submitted in continuity or separately as either a sentinel node(s), axillary sampling, or a clearance procedure. Sentinel lymph nodes (1–4 in number) are demonstrated in vivo by injection of a vital blue dye and/or radiocolloid around the area of the tumour prior to surgery. A small axillary incision with both direct visual inspection and use of a gamma probe allows identification of stained lymphatics leading to a "hot blue" sentinel lymph node(s). The excised lymph node is serially sliced transverse to its long axis at 2 mm intervals and all processed for histology, supplemented by levels of the paraffin block and cytokeratin immunohistochemistry where indicated. Diagnostic needle core biopsies are usually 14 G and measure up to 15–20 mm long. Delicate painting with alcian blue allows visualization at the paraffin block cutting stage. One representative section is usually sufficient for lesions other than radiological calcifications. The latter often require examination of multiple histological levels until any represented mammographic abnormality is detected. Specimen x-ray is used to check for the presence of calcifications to ensure that there has been sampling of the mammographically abnormal area. Calcifications will be identified histologically in most cases. If they are not present, a decision should be made by the MDT whether further sampling is necessary or not.

Gross Description

Specimen Type and Laterality

- FNA/needle core biopsy / VAB or VAE/localisation biopsy/excision biopsy/wide local excision/segmental excision/mastectomy. Optimal fixation is important in assessing tumour type, grade, lymphovascular invasion and hormone receptor/HER2 expression. This can be facilitated in larger specimens by making an initial cut for fixation purposes, immediately after specimen receipt and inking of relevant margins.
- Axillary lymph nodes: sentinel biopsy/sampling/clearance.
- Size in three dimensions (mm) and weight (g).

Tumour

- Quadrant: 50% upper outer (UOQ), 15% upper inner (UIQ), 10% lower outer (LOQ), 17% central, 3% diffuse (massive or multifocal). Breast cancer occurs either as a localised lesion, or multiple invasive foci not connected by DCIS and clearly separated by normal breast tissue. The latter may arise from multifocal field change within a number of abnormal ductulolobular units or as a result of seeding from involved lymphovascular channels.
- Distances (mm) from the nipple and resection limits.

Size

- Measure the size in three dimensions of all macroscopically visible tumour deposits. In the case of multiple deposits, describe their relationship to one another, bearing in mind that the most relevant factors are the size of the largest deposit (this is used to determine stage) and the distance from each margin to the nearest tumour deposit (Fig. 22.1).
- In post-neoadjuvant chemotherapy specimens an estimate of the size of the tumour bed should be provided. This may be appreciable as a poorly-defined area of oedema and fibrosis. Good clinical information is particularly important in these cases since there may have been significant tumour response to treatment.

Appearance

- Scirrhous/fleshy/mucoid/cystic/diffuse thickening.
- Ductal carcinoma tends to form a discrete mass lesion whereas lobular carcinoma can be difficult to define clinically, radiologically, cytologically and at the laboratory dissection bench. This has implications for completeness of excision in patients treated with breast conserving surgery and for the pathological assessment of the surgical margins. If the core biopsy types the breast cancer as infiltrating lobular, breast MRI scan may be performed to determine more accurately the extent of the lesion and to detect any satellite lesions that could preclude conservative surgery.

Edge

- Circumscribed/irregular. Mucinous and medullary carcinomas often have circumscribed pushing margins.

Histological Type

In Situ Carcinoma

Ductal Carcinoma In Situ (DCIS)

- Bound by basement membrane involving more than 2 duct spaces or more than 2–3 mm in maximum dimension. Epithelial proliferation of lesser extent is designated *atypical*

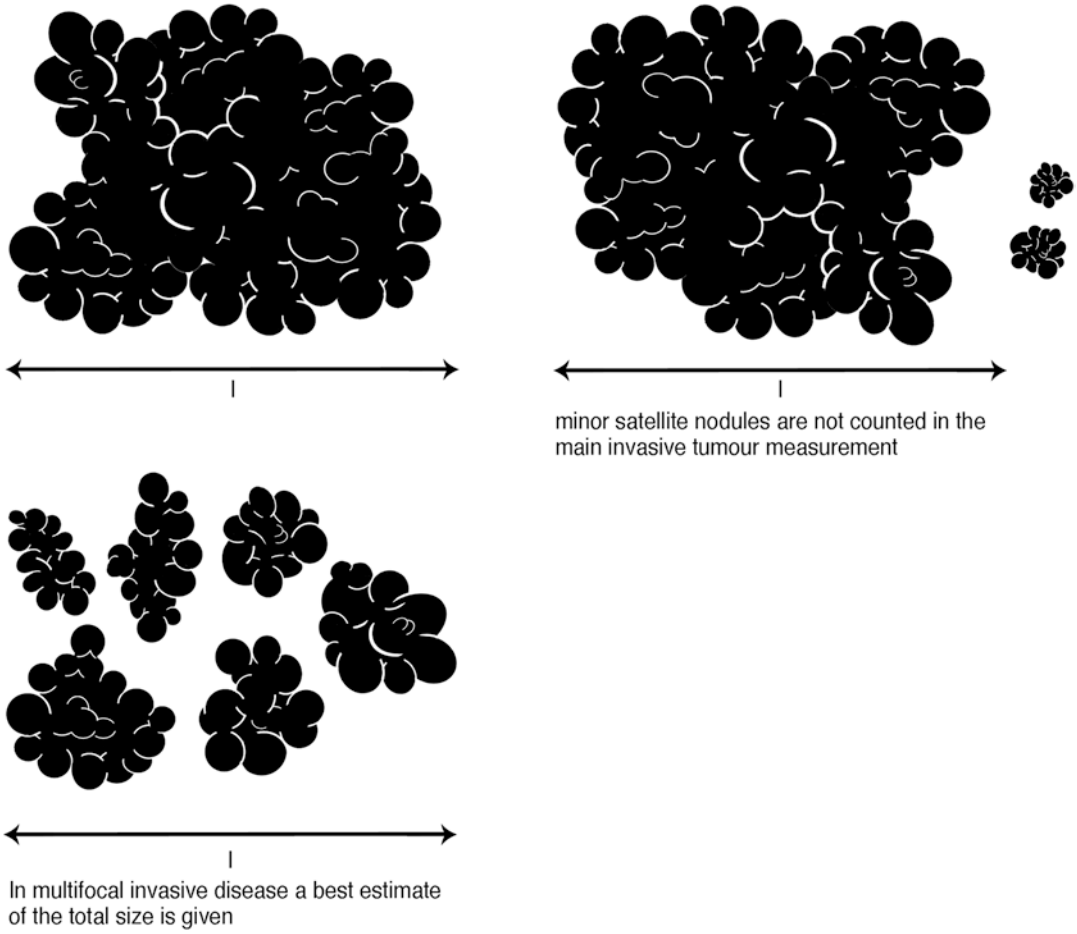


Fig. 22.1 Breast carcinoma: maximum invasive tumour measurement (I) in cases with more than one invasive focus

ductal hyperplasia (ADH) unless of intermediate or high cytological grade or with comedonecrosis (Fig. 22.2).

- *Nuclear grade:* low, intermediate, high.
  - Low grade has monomorphic evenly spaced cells with small central nuclei and few mitoses. Necrosis is rare.
  - High grade has pleomorphic irregularly spaced and usually crowded cells with large irregular nuclei, coarse chromatin, ≥1 nucleolus, frequent mitoses and often necrosis. There is loss of cell polarity.
- Necrosis: comedo or punctate. Comedo = central eosinophilic necrosis containing five or more pyknotic nuclei.
- Cell polarisation: present or absent.
- *Architectural patterns:* solid
  - Cribriform
  - Papillary
  - Micropapillary.

Rarer forms include signet ring cell, apocrine, clear cell, cystic hypersecretory and neuroendocrine variants.

Pure DCIS of limited size (<40 mm) tends to be unicentric, albeit ramifying through the involved duct system. It is usually treated by breast conserving surgery, either *microdochectomy* or *wide local excision* depending on its site and extent, and *adjuvant radiotherapy*. If a larger area is found to be involved preoperatively, axillary sentinel lymph node biopsy may be carried out since there is a small risk that occult invasive disease will be present. If DCIS

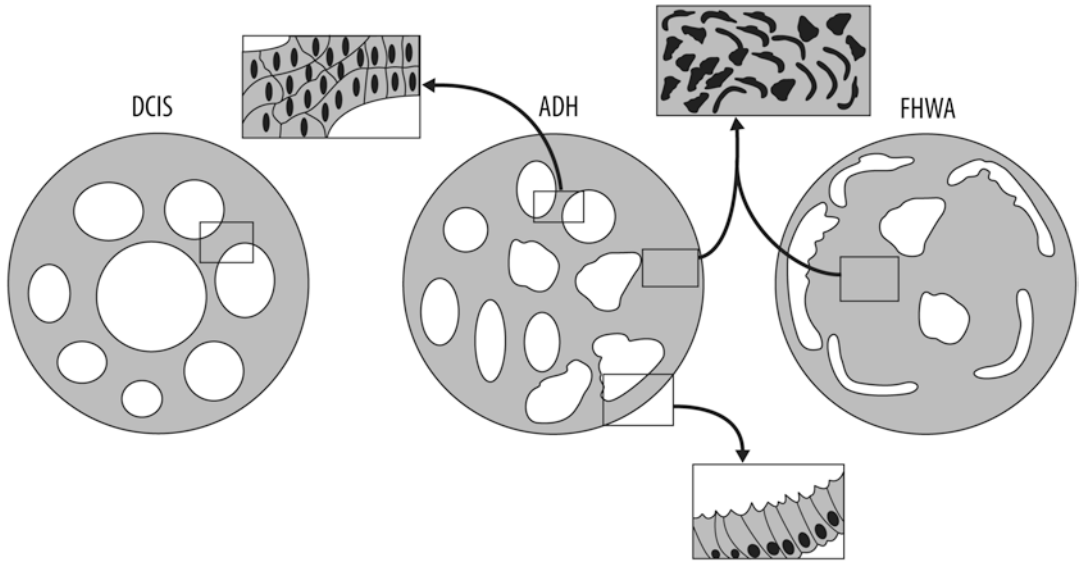


Fig. 22.2 Ductal carcinoma in-situ (DCIS) versus atypical ductal hyperplasia (ADH) versus florid hyperplasia without atypia (FHWA): cytology and histology. DCIS features smooth, punched-out luminal borders within involved basement membrane-bound space. The cytological features are regular and present throughout the entire population of at least two basement-membrane-bound spaces. FHWA is the most densely cellular and extensive of the proliferative disease without atypia lesions, also called "papillomatosis". There are ragged, often slit-like luminal borders. The nuclei throughout the involved area show variablity and tendency to a swirling pattern, as illustrated. ADH has features predominantly of non-comedo, cribriform DCIS, but also some features of proliferative disease without atypia or normally polarized cells within the same basement-membrane-bound space. (Page DL, Rogers LW. Combined histologic and cytologic criteria for the diagnosis of mammary atypical ductal hyperplasia. *Hum Pathol* 1992;23:1095–1097. ©1992. Reproduced, with permission, from Elsevier)

is mammographically extensive *mastectomy* is the preferred treatment. DCIS is also assessed for ER status (>80% are positive) as a guide to potential *hormonal treatment* response, e.g. with tamoxifen or aromatase inhibitors. Note that ER negative high grade DCIS is not responsive to hormone therapy and has a higher recurrence rate.

Lobular Carcinoma In Situ (LCIS)

- Uniform cells populating the lobule.
- No lumen in the acini.
- ≥50% of the acini in the lobule are expanded and filled.
- ±pagetoid spread into ducts.
- Potentially multifocal (70%) and bilateral (30–40%).
- Epithelial proliferation of lesser extent (e.g. with preservation of lumina) is designated *atypical lobular hyperplasia (ALH).*

The morphological spectrum of *LN (Lobular Neoplasia)* comprising ALH and LCIS can be encountered in a needle core biopsy, but is often not diagnosed preoperatively and is seen as an incidental finding in excision specimens. It is a *marker for an increased risk of malignancy* (greater for LCIS) and has a peak incidence between the ages of 40–50 years, some 10 years younger than that of DCIS. After diagnosis on an excision specimen, and if a concurrent carcinoma is not present, current recommendations are for continued follow up although the most appropriate duration of follow up is not clear.

Distinction between DCIS and LCIS is not always easy, e.g. when there is lobular cancerisation by low grade DCIS. Rarely, mixed lesions occur. Atypical lobular proliferations comprise a uniform population of dyscohesive cells with intracytoplasmic lumina. Loss of E-cadherin expression favours a lobular proliferation.

Microinvasion

- ≤1 mm from the adjacent basement membrane with infiltration of non-specialised interlobular stroma.

Microinvasion must be distinguished from the more frequent *cancerisation of lobules* (lobular architecture/intact basement membrane), and clinically is managed similarly to DCIS as the incidence of axillary lymph node metastases is very low. The likelihood of invasion increases with the grade of DCIS and presence of comedonecrosis. Extra blocks and levels should be assessed. Usually the type of invasive carcinoma correlates with the type of DCIS but LCIS can be associated with invasive carcinoma of ductal or lobular type.

Invasive Carcinoma

As well as indicating whether invasive disease is present, it should be subtyped (as follows below) since this provides important prognostic information.

Ductal

- No special type (NST): *70–75% of breast cancer.*

Lobular

- *15% of breast cancer.*
- *Classical:* 40%; single files of small cells in a targetoid periductal pattern, and with AB-PAS positive intracytoplasmic lumina.
- *Alveolar:* a nested pattern of 20 or more cells.
- *Solid:* sheets of tumour cells.
- *Trabecular:* bands of cells two to four across.
- *Signet ring cell.*
- *Pleomorphic:* classical pattern but with high grade cytological atypia and *aggressive* clinical behaviour. Regarded by some as having mixed lobular and ductal characteristics.
- *Tubulolobular:* classical pattern with focal microtubules which are less distinct than in tubular carcinoma.

Special Types

Pure special type carcinomas should show the defining histological features in at least 90% of the tumour cells.

- *Tubular:* round, ovoid, angular tubules with a single cell layer, cytoplasmic apical snouts and a fibrous/desmoplastic stroma.
- *Cribriform:* invasive cords and islands with the morphology of cribriform DCIS comprising punched out lumina and cytoplasmic apical snouts.
- *Colloid (mucinous):* pushing margins, extracellular mucin with small clusters (10–100 cells) of uniform epithelial cells.
- *Papillary:* solid or encysted/encapsulated (intracystic). There is absence of a peripheral myoepithelial layer and increased expression of invasion associated biomarkers (e.g. matrix metalloproteinases). Because of this, papillary carcinoma is generally regarded as a *low-grade invasive encapsulated carcinoma* with an expansile growth pattern and indolent behaviour, rather than an in situ carcinoma. Invasion is either (a) a solid, dominantly invasive carcinoma with a pushing margin and papillary pattern, or (b) an encysted papillary carcinoma with focal invasion (the invasive component can be papillary or ductal, NST). Note also and distinguish from *invasive micropapillary carcinoma* (micropapillae without cores set in clear spaces—the "inside-out" tumour with an external rim of apical cytoplasm), which correlates with *lymphovascular* and *axillary node metastases*. This pattern can also be seen as a component of 5% of ductal, NST tumours.
- *Invasive carcinoma with medullary features:* includes tumours that would previously have been described as classical medullary carcinoma, atypical medullary carcinoma, and ductal carcinoma, NST with medullary features. They have a pushing margin, high nuclear grade, syncytial growth pattern and a prominent lymphocytic infiltrate.

Mixed Types

- *10% of breast cancers.*
- *Mixed differentiation ductal and lobular.*
- *Tubular mixed*: stellate mass, central tubules with peripheral less differentiated adenocarcinoma.

Others

- *Metaplastic:* biphasic epithelial (ductal in situ/invasive NST grade 2/3, or, squamous cell) and sarcomatous elements (carcinosarcoma), or pure monophasic spindle cell carcinoma (cytokeratin/EMA/p63 positive). The sarcomatous element is either fibrosarcomatous/malignant fibrous histiocytoma like or chondro-, osteo-, leiomyo-, rhabdomyo- or liposarcomatous. Represents carcinoma with a spectrum of malignant spindle cell stroma which can be homologous or heterologous in character. *It behaves as a high-grade carcinoma*. The epithelial component may be minor and require multiple blocks to demonstrate. Metaplastic carcinoma often presents at a *more advanced stage* than high risk infiltrating duct cancer, with a tendency for *recurrence* and *decreased survival*. This relates particularly to lymph node status and the presence of a squamous cell component. Metastases can comprise either the epithelial or spindle cell elements.
- *Pleomorphic carcinoma:* high-grade ductal cancer with background spindle cells and >50% bizarre giant cells (cytokeratin positive). Presents with advanced disease.
- *Carcinoma with osteoclast giant cells (CD68 positive).*
- *Signet ring cell carcinoma:* gastric carcinoma analogue.
- *Small cell:* rare, a poorly differentiated/high-grade neuroendocrine carcinoma, aggressive, chromogranin±/synaptophysin/CD56/positive with a high Ki67 proliferation index.
- *Other neuroendocrine:* invasive ductal carcinoma with neuroendocrine differentiation (variable nests, spindle cells or large cells) or low-grade/well differentiated neuroendocrine (carcinoid) tumour. Note that metastatic neuroendocrine carcinoma from lung (TTF-1 positive) or gastrointestinal tract (CDX-2 positive) can mimic both in situ and invasive primary mammary carcinoma.
- *Secretory:* one third are in children, indolent, and of good prognosis. Two thirds are in adults and more aggressive. Shows tubular/solid/honeycomb patterns and PAS/AB-diastase positive luminal secretions.
- *Squamous cell:* primary, or secondary from breast skin, or metastatic, e.g. lung. Also distinguish from metaplastic breast carcinoma which often has an identifiable component of usual in situ or invasive breast cancer.
- *Clear cell:* glycogen rich with worse prognosis.
- *Mucoepidermoid:* grade determines the prognosis with cystic/mucin secreting better than solid/epidermoid variants.
- *Adenoid cystic:* indolent with late recurrence. As for mucoepidermoid carcinoma it is a salivary gland tumour analogue.
- *Apocrine:* rare, cytoplasmic apical snouts, GCDFP-15 and androgen receptor positive.
- *Adenomyoepithelioma:* in the elderly, of low malignant potential and characterised by sheaths of proliferating clear myoepithelial cells around epithelial lined spaces. Occasionally malignant myoepithelioma (spindle cells with mitoses).

Pure Carcinoma

- ≥90% of the tumour volume.

Mixed Carcinoma

- 50–90% of the tumour comprises a special type component.

Metastatic Carcinoma

- Often solitary and in the UOQ at a late stage in known carcinomatosis. The majority of childhood breast malignancy is metastatic, e.g. alveolar rhabdomyosarcoma. In adults, it is usually spread from lung (small cell carcinoma), malignant melanoma, malignant lymphoma/leukaemia, but may also be a secondary from ovary, contralateral breast (usually this represents a metachronous primary), gastrointestinal tract, kidney, thyroid carcinomas or small intestinal neuroendocrine tumour. *A relevant clinical history, absence of in situ change and multiple intravascular deposits are pointers to metastases.* Specific combinations of antibodies, e.g. CK7/TTF-1/Napsin-A (pulmonary adenocarcinoma), paranuclear dot CAM 5.2/synaptophysin/CD56 (small cell carcinoma), S100/SOX10/HMB-45/Melan-A (malignant melanoma), CD45/CD20/CD3/CD68/myeloperoxidase

(malignant lymphoma/myeloid leukaemia), CA125/CK7/WT-1 (ovary), CK20/CDX-2 (colorectal), RCC ab/EMA/CD10/vimentin (kidney), thyroglobulin/TTF-1 (thyroid) and markers of breast profile (e.g. ER/PR, cytokeratin 7, GATA3, CEA, GCDFP-15) may be helpful in distinguishing between primary breast and non-mammary disease. *Metastatic tumour should be considered in any breast lesion with unusual clinical, radiological, gross or histological features.*

Invasive Tumour Grade

Grade 1/2/3.

The Elston-Ellis modification of the Scarff-Bloom-Richardson system is used. There can be tumour grade heterogeneity and tubule formation takes this into account as it is based on the whole tumour area. However nuclear features and mitoses are assessed on the least differentiated area. Three parameters are assessed and scored:

	Score
Tubule formation	
Majority of tumour (>75%)	1
Moderate (10–75%)	2
Little or none (<10%)	3
Nuclear pleomorphism	
Small regular, uniform	1
Larger with variation	2
Marked variation in size and shape (± multiple nucleoli)	3

Mitoses (tumour periphery and most active areas rather than the paucicellular centre). Poor fixation may reduce mitotic counts: should doubt regarding mitotic count relate to fixation in excision specimens then counts may be based on core biopsy material. The mitotic count (number of mitoses per 10 high-power fields) is related to the objective field diameter which should be known for the microscope in use.

Total score	Grade
3–5	1
6–7	2
8–9	3

Extent of Local Tumour Spread

Border: pushing/infiltrative.

- Lymphocytic reaction: prominent/sparse.
- Paget's disease.
- Skin involvement (direct extension or lymphatics).

The pT stage is based on the maximum dimension of invasive cancer and not whole size measurements that include associated DCIS.

The maximum dimension of the invasive tumour should be recorded in millimetres. Measurement of invasive size and whole size can be difficult, especially in situations where, for example, there are multiple areas of invasion in a field of DCIS (see Fig. 22.3).

The TNM8 classification applies only to carcinoma of the female and male breast.

pTis	Carcinoma in situ: DCIS, LCIS or Paget disease with no malignancy in the underlying breast parenchyma
pT1	Tumour ≤20 mm
T1 mi	≤1 mm
T1a	1 mm < tumour ≤5 mm
T1b	5 mm < tumour ≤10 mm
T1c	10 mm < tumour ≤20 mm
pT2	20 mm < tumour ≤50 mm
pT3	Tumour >50 mm
pT4	Tumour of any size with direct extension to chest wall (ribs, intercostal muscles, serratus anterior but not pectoral muscle) or skin[a] (ulceration or nodules)
(a)	Chest wall
(b)	Oedema including peau d'orange, skin ulceration or satellite nodules[b] in the same breast
(c)	4a and 4b
(d)	Inflammatory carcinoma: clinically sore and red due to tumour involvement of dermal lymphatics and often without an underlying palpable mass. It can be difficult to obtain tissue proof on FNAC or needle core biopsy. The malignant cells are usually ductal, NST, grade 3.

[a]Dermal invasion alone without ulceration, satellite nodules or inflammatory carcinoma does not constitute pT4
[b]Clinical or grossly apparent skin satellite nodules, not just histological foci

(See Figs. 22.4, 22.5, 22.6, 22.7, 22.8, 22.9, 22.10, and 22.11).

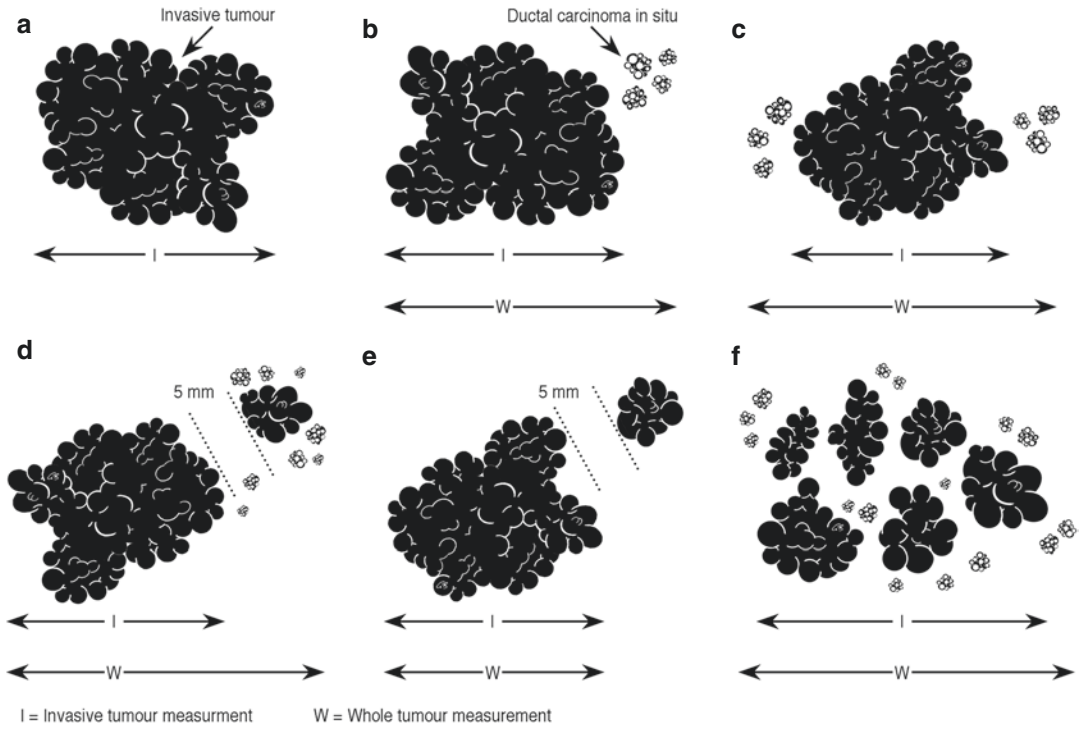


Fig. 22.3 Breast carcinoma measurement. (**a**–**f**) Measuring invasive (I) and whole (W) tumour sizes in various scenarios; (**d**–**e**). Occasionally, it can be extremely challenging to determine whether two adjacent foci represent satellite foci or one lesion mimicking this process due to plane of sectioning. The presence of intervening normal tissue and increasing distance between foci are features that indicate that these are more likely to be multiple foci than a localised process. A distance of 5 mm or greater is often used to define a separate focus; (**f**) The multiple apparent areas of invasion within extensive DCIS are together best regarded as the invasive tumour size. Adapted with permission from *Ellis IO, et al. Pathology reporting of breast disease in surgical excision specimens incorporating the dataset for histological reporting of breast cancer*. The Royal College of Pathologists, 2016

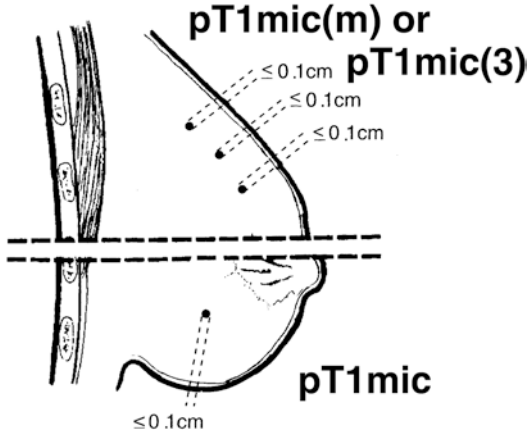


Fig. 22.4 Breast carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

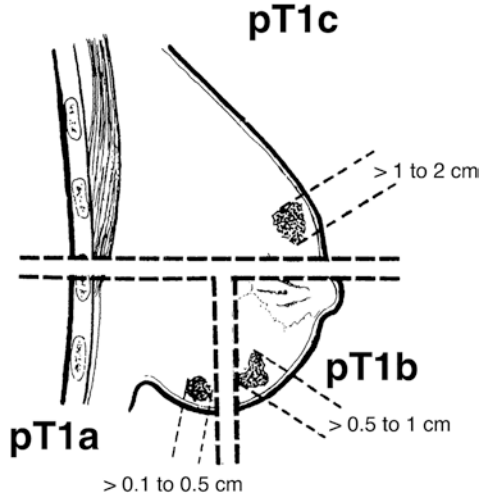


Fig. 22.5 Breast carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

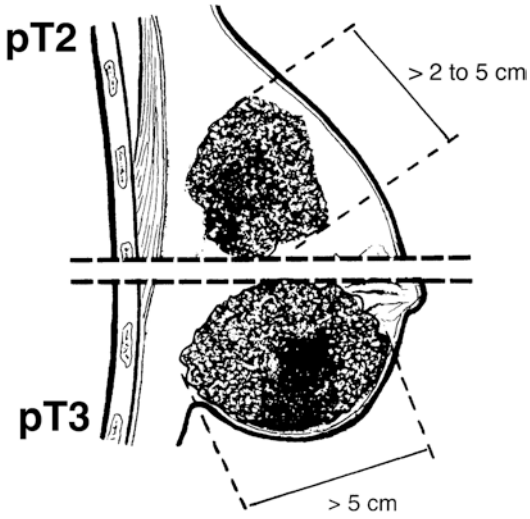


Fig. 22.6 Breast carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

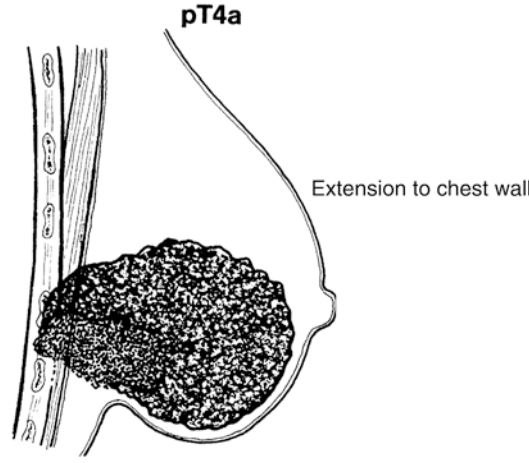


Fig. 22.7 Breast carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

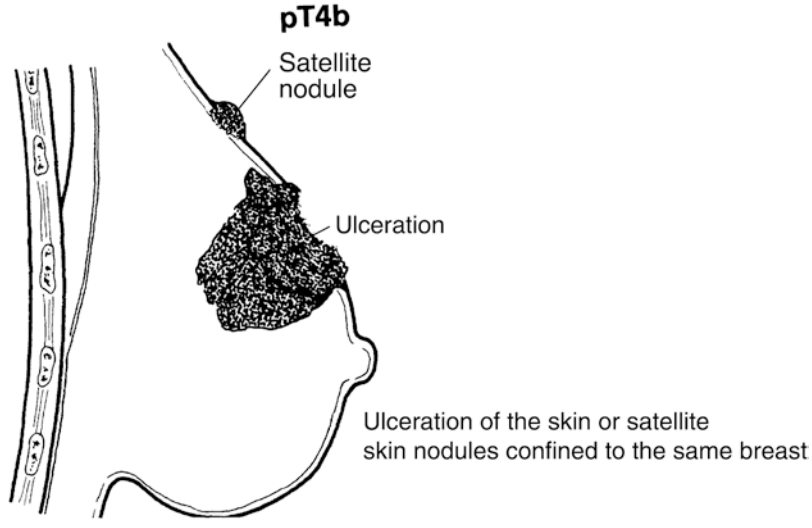


Fig. 22.8 Breast carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

In Situ change

- Present/absent.
- Intra−/extratumoural

Lymphovascular Invasion

- Present/absent.
- Intra−/extratumoural

The most common site for vascular invasion is at the tumour edge. It is present in about 25–35% of cases. Its presence can *double the risk of local recurrence*.

Lymph Nodes

- Site/number/size/number involved/extracapsular spread.

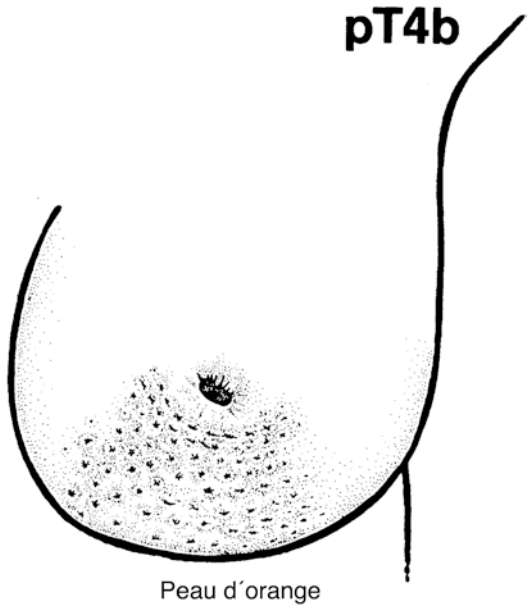


Fig. 22.9 Breast carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

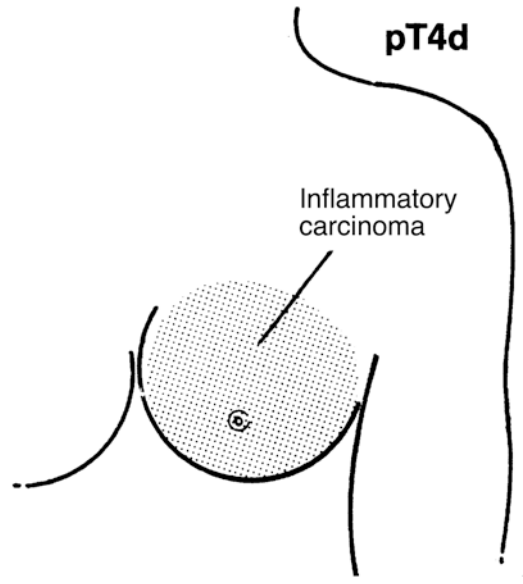
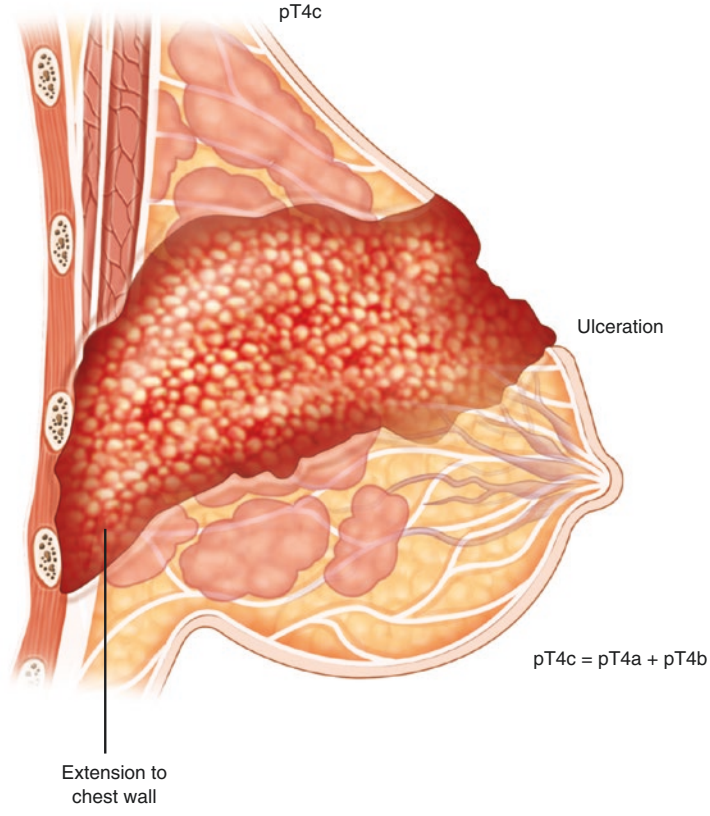


Fig. 22.11 Breast carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

Fig. 22.10 Breast carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag



Regional nodes: axillary (levels 1, 2, 3, and intramammary), ipsilateral infraclavicular, ipsilateral internal mammary and ipsilateral supraclavicular. Any other lymph node metastasis is regarded as a distant metastasis pM1, including cervical or contralateral internal mammary. A regional lymphadenectomy will ordinarily include a minimum of six lymph nodes (level 1) and in practice more often between 15 and 30 (levels 1, 2 and 3).

Axillary Lymph Nodes

They receive ≥75% of the lymphatic flow.

Level 1	Low axilla. Lymph nodes lateral to the border of pectoralis minor muscle
Level 2	Mid-axilla. Lymph nodes between the medial and lateral borders of the pectoralis minor muscle
Level 3	Apical axilla. Lymph nodes medial to the medial margin of the pectoralis minor muscle.

pN0	No regional lymph nodes metastasis
pN1	(a) metastasis in 1–3 ipsilateral axillary lymph node(s)
	(b) internal mammary lymph node(s) with microscopic metastasis by sentinel lymph node biopsy but not clinically apparent[a]
	(c) = a + b
pN2	(a) metastasis in 4–9 ipsilateral axillary lymph nodes
	(b) in clinically apparent internal mammary lymph node(s) but without axillary lymph nodes.
pN3	(a) metastasis in ≥10 axillary or infraclavicular lymph node(s)
	(b) metastasis in clinically apparent ipsilateral internal mammary lymph node(s) with axillary lymph nodes, or, metastasis in >3 axillary lymph nodes and in internal mammary lymph nodes with microscopic metastasis by sentinel lymph node biopsy but not clinically apparent.
	(c) metastasis in ipsilateral supraclavicular lymph node(s)

[a]clinically apparent = detected by clinical examination or by imaging studies (excluding lymphoscintigraphy) or grossly visible pathologically

Sentinel lymph node staging is designated as a suffix in brackets, e.g. pN1(sn) (Fig. 22.12).

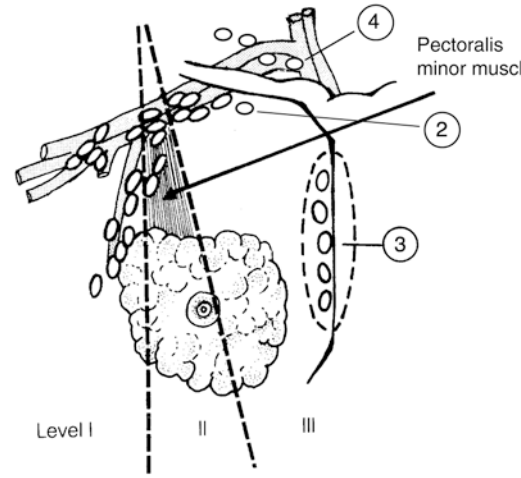


Fig. 22.12 Breast carcinoma: axillary lymph nodes. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

Cytokeratin markers are useful where the morphological appearances are suspicious, but not diagnostic of metastatic carcinoma, e.g. sinusoidal lobular carcinoma cells versus sinus histiocytosis. The significance of lymph node *micrometastases* (≤2 mm: pN1 (mi)) remains uncertain with some regarding it as an adverse prognostic indicator but others less convinced. From a practical viewpoint it does influence choice of systemic adjuvant chemotherapy and hormonal therapy and should be reported. The biological status of *isolated tumour cells* (≤0.2 mm: pN0 (i+)) is not established. Histological levels of serial slices and cytokeratin immunohistochemistry may be necessary particularly if *sentinel lymph node biopsy* (95% positive predictive value for axillary lymph node metastasis) alone is used for staging purposes in clinically node negative patients. Other approaches to axillary disease are *axillary lymph node sampling* (3 or 4 level 1 lymph nodes for staging only), particularly if operative identification of a sentinel lymph node is uncertain, or *axillary lymph node clearance* (for staging and treatment). Axillary lymph node involvement is seen in *30–40% of cases.* The Nottingham group has shown that a *threshold of three involved*

lymph nodes is associated with *decreased breast cancer specific survival* and *distant metastasis free survival.* Of patients with axillary lymph node disease there can also be involvement of the internal mammary chain (22%), and supraclavicular lymph nodes (20%). *Distant metastases* are most often to the skeleton, lung and pleura, liver, ovary, adrenal gland and central nervous system.

Presentation with metastatic tumour in axillary lymph nodes is usually due to either breast carcinoma or malignant melanoma. This can be resolved with immunohistochemistry. The source of the breast carcinoma is usually the ipsilateral breast or axillary tail of breast and the lesion can be clinically occult and difficult to locate as its size is often less than 20 mm in diameter. Invasive lobular carcinoma has a greater tendency than ductal carcinoma to metastasise to retro−/peritoneum, meninges, gastrointestinal and female genital tracts.

Excision Margins

Measure the distances (mm) to the nearest margins (superficial, deep, lateral, medial, inferior, superior). Differential block labelling and use of multi-coloured inks are important. Correlation of microscopy with macroscopic images taken during specimen dissection can prove extremely useful. Separate orientated cavity margin samples may also be submitted by the surgeon. In specimens submitted after breast conserving surgery the radial (superior, inferior, medial, lateral) margins are most important, since usually no deep or superficial breast tissue is left behind.

The definition of adequately cleared margins has not yet been agreed. However the UK Association of Breast Surgery has reached a consensus of ≥1 mm clearance for invasive carcinoma and ≥2 mm for DCIS. Since surgical practice continues to vary, it is recommended that margin status be reported as tumour at ink, <1 mm clear, or a whole number mm value.

Involved margins (defined as carcinoma cells *at* the ink) can *triple the risk of local recurrence* and can be an indication for either *radiotherapy* (deep, superficial margins), or *further surgery* as a local re-excision (site orientated cavity shavings) or conversion to a mastectomy. Note that margins can be particularly difficult to define and assess in lobular carcinoma when distinction from fibrous breast tissue can be problematic. *Margin involvement in DCIS* is a *strong predictor of local recurrence* about *50% of which will be as invasive disease*, particularly so for high cytonuclear grade DCIS and often correlating with high grade cancer. The presence of lobular neoplasia (LN) at a surgical margin does not need to be recorded but may be included as part of a descriptive report.

Other Pathology

Assess breast tissue away from the tumour for: a*typical hyperplasia, carcinoma in situ, satellite invasive foci, lymphovascular invasion (LVI).*

Carcinoma in situ and LVI: when present away from (>1 mm) the tumour they are *strong prognostic indicators of local and lymph node recurrence* and are important in selecting appropriate postoperative adjuvant therapy or further surgical excision. Relationship of *satellite invasive foci* to resection margins must also be assessed.

Atypical hyperplasia (ductal and lobular: ADH/ALH): regarded as having ×4–5 increased risk of subsequent carcinoma, and in situ change ×10–11 increased risk over control populations. The precancerous nature of atypical hyperplasia is illustrated by its shared molecular abnormalities with carcinoma in situ. There is also evidence for a *FEA (flat epithelial atypia/columnar cell hyperplasia with atypia)—ADH—DCIS—low-grade cancer sequence.* These risk lesions and *lobular neoplasia (LN)* have a strong association with *low grade breast cancer.* Conversely *high grade DCIS* tends to progress to *high grade invasive disease*. LCIS is usually an incidental microscopic finding, e.g. adjacent to a simple cyst or (60%) in the vicinity of invasive lobular cancer, and it is potentially multifocal and bilateral. DCIS may present as Paget's disease ± nipple discharge, a tumour forming mass (especially

comedo type with a greater propensity for invasive carcinoma), or adjacent to a symptomatic invasive breast cancer. In many cases it is found on biopsy for an impalpable lesion detected on radiological screening (15–20% of screening cancers). This can be either as linear, branching calcifications, or within the context of a radiologically suspicious but histologically benign lesion, e.g. radial scar/complex sclerosing lesions (RS/CSLs). The immunohistochemical markers smooth muscle myosin, p63, CK5/6 and CK14 are useful in demonstrating a myoepithelial cell layer as an aid to distinction from invasive carcinoma. These *RS/CSLs are often associated with foci of ADH/DCIS* and are regarded as *an independent marker of increased risk* for subsequent development of carcinoma.

Clinicopathological correlation: it is essential to *correlate the clinical mammographic abnormality with the excised specimen.* This requires *dissection guided by the postoperative specimen radiograph* demonstrating the lesion in question, with or without an in situ guide wire localisation needle. The histological slides must contain the abnormality, e.g. carcinoma or microcalcification, and if not, all of the residual tissue processed or further blocks selected according to radiographic study of the specimen serial slices. Usual calcification (calcium phosphate) is easily recognisable as basophilic in routine sections. A minority (10%) is oxalate in character, can be partially removed by tissue processing and is recognised by being doubly refractile on polarisation. It is usually seen in benign disease, e.g. fibrocystic disease. Similarly *needle core biopsy for microcalcification requires x-ray of the cores* to ensure that the relevant area has been sampled.

Paget's disease affects 2% of breast cancer patients and is distinguished immunohistochemically from malignant melanoma and Bowen's disease by being mucin, CEA, EMA, ER, HER2 (50%) and cytokeratin 7 positive, and S100, HMB-45 and melan-A negative. *DCIS* is nearly always identified in the subareolar duct system and there is associated *invasive breast carcinoma in 35–50% of cases.* Depending on the mammographic extent of the underlying lesion (size, in situ versus invasive disease,) breast conserving surgery with removal of the nipple-areolar complex (NAC), or mastectomy is carried out.

Neoadjuvant chemo−/radiotherapy: effects include tumour cell necrosis, degeneration, apoptosis, vacuolation, inflammation and loose fibrosis. Neoadjuvant treatment has previously been reserved for large, high grade *clinically advanced T3/T4 tumours*. It now has an increasing role in treatment of early breast cancer since, among other advantages, it can permit breast conserving surgery instead of mastectomy, and can allow more conservative management of the axilla.

Tumour typing and grading can be affected by neoadjuvant chemotherapy, meaning these features are better assessed on the pre-treatment needle core biopsy. Tumour size may also need to be based on the radiographic or ultrasound measurements. *Postoperative radiotherapy* is used for the control of local recurrence of breast cancer in the presence of positive deep or superficial margins of excision. It can result in diagnostically confusing cytological atypia in native ductulolobular unit epithelium. The presence of widespread metastatic disease should be clinically determined prior to surgical resection so that *palliative systemic therapy* can be considered.

Treatment of localised disease is by *wide local excision* ensuring 10–20 mm palpable margins of clearance around the tumour. Conversion to *mastectomy* may be necessary if adequate surgical excision cannot be achieved. Otherwise the residual breast receives *radiotherapy*. *Mastectomy (about 30% of cases)* is indicated as initial treatment if the tumour is centrally situated (behind the nipple), >3 cm in diameter and/or associated with radiological or biopsy evidence of extensive DCIS, or if it is the patient's preference. *Adjuvant hormonal therapy* is determined by the tumour ER/PR status. *Radiotherapy* after mastectomy is indicated for a large tumour, when there are more than 4 lymph nodes positive or there is vascular invasion. *Chemotherapy* is usually indicated in high-grade and lymph node positive patients particularly in the younger age group. More recently, gene expression profiling of tumour tissue has become useful to predict the likely benefit of chemotherapy in patients with early stage ER

positive HER2 negative cancer. HER2 status also influences chemotherapy decisions and any indication for *trastuzumab therapy* in locally recurrent disease or distant metastases.

Triple assessment: concordance of its three modalities *(clinical examination, radiology, needle core biopsy with or without cytology)* has replaced frozen section and the majority of open biopsies in the diagnosis of breast carcinoma. This allows a one stop assessment and progression to definitive breast conserving or more radical surgery. *FNAC* cannot accurately distinguish between in situ and invasive malignancy and should not be used alone in the diagnosis of malignant disease. Note that diagnostic core biopsy can underestimate cancer grade and may not accurately reflect tumour subtype compared with the resection specimen, e.g. for reasons of tumour heterogeneity in both cellularity and differentiation. Core biopsy, unlike cytology, can also provide a positive benign diagnosis and can confirm the presence of any calcification detected radiologically. Core biopsy is also necessary so that decisions can be made about suitability for neoadjuvant treatment. FNAC advantages are convenience to the patient and clinician, speed of results and low cost. It is also important in preoperative assessment of the axilla.

Potential *diagnostic pitfalls in core biopsies* are overcall of: RS/CSL as tubular carcinoma, apocrine atypia as DCIS, chronic inflammation (cytokeratin negative) as lobular carcinoma and radiotherapy changes as carcinoma. *False negative diagnoses* include the reverse of the above, metaplastic carcinoma mistaken for a fibrous scar, undercalling of DCIS as ADH, and, invasive cancer being missed due to sampling limitations.

Reporting Categories for Breast Fine Needle Aspirates and Wide Bore Needle Core Biopsies

FNAC is highly efficient and accurate at diagnosing a wide range of breast disease when interpreted in conjunction with the patient's age, clinical history, clinical features of the lesion and its radiological appearances. Two basic patterns are encountered:

1. Benign: a biphasic pattern of cohesive breast epithelium and background bare nuclei with low to moderate cellularity (except fibroadenoma which may be of high cellularity).
2. Malignant: dyscohesive clusters and singly dispersed variably atypical epithelial cells. Cytoplasmic preservation in dispersed cells. Absence of bare nuclei. Moderate to high cellularity for the patient's age.

FNAC reporting categories are:

C1	An inadequate specimen: insufficient epithelial cells, epithelial cell content obscured by inflammation or a technically poorly prepared smear
C2	An adequate benign specimen: of sufficient cellularity and showing a benign biphasic pattern
C3	Atypia, likely benign: showing some mild nuclear change or cellular dissociation but within an essentially benign pattern
C4	Atypia, suspicious for malignancy: a pattern and cell constitution suspicious for but not diagnostic of malignancy for quantitative (inadequate cellularity) or qualitative (insufficient atypia) reasons
C5	Malignant: a cellular specimen showing an unequivocally malignant pattern and individual malignant cells.

Wide bore needle core biopsy reporting categories are:

B1	Normal tissue only, or unsuitable for diagnosis.
B2	Benign abnormality: e.g. fibroadenoma, sclerosing adenorsis, columnar cell change, apocrine metaplasia, epithelial hyperplasia of usual type.
B3	Benign but of uncertain malignant potential: benign lesions associated with the presence of cancer and/or the risk of developing it, e.g. flat epithelial atypia (FEA), radial scar/complex sclerosing lesion (RS/CSL), ADH, ALH/LCIS, phyllodes tumour, papillary lesions. When reporting B3 lesions it should be explicitly stated whether an atypical epithelial proliferation is present.
B4	Suspicion of malignancy: epithelial proliferation suspicious but not diagnostic of malignancy for quantitative or qualitative reasons.
B5	Malignant: (a) in situ
	(b) invasive
	(c) not possible to classify as in situ or invasive

Reporting categories are a useful tool in the day-to-day management of individual cases and crucial for clinicopathological audit purposes. It is imperative that FNAC and wide bore needle core biopsy material are closely correlated with their respective surgical specimens. There is an increasing trend towards the use of needle core biopsy alone. This is partly because of a lack of widespread cytopathological expertise in interpretation of FNACs, but mostly because of its higher sensitivity and specificity for designating benign, indeterminate, non-palpable and calcified lesions, as well as its suitability for assessment of hormone and HER2 receptor status.

Prognosis

Prognosis relates to patient age, tumour type, size, grade, lymphovascular invasion, lymph node involvement and hormonal status. ER negative, lymph node positive and HER2 positive cancers confer higher risk.

Nottingham Prognostic Index (NPI) = 0.2 × invasive tumour size (cm) + grade + lymph node score.

	Score
No nodes involved	1
1–3 axillary nodes involved **or** positive internal mammary node	2
4 or more nodes involved and/or apical node involved, or any axillary node and any intramammary node	3

NPI score	Prognosis	5 year survival
<3.4	Good	88%
3.4–5.4	Intermediate	68%
>5.4	Poor	21%.

Prognosis according to *histological type* (10 year survivals):

Excellent (>80%)	Tubular, cribriform, mucinous,
	Tubulolobular, encysted papillary
Good (60–80%)	Tubular mixed, alveolar lobular
	Mixed ductal NST/special type
Intermediate (50–60%)	Classical lobular, medullary
	Invasive papillary
Poor (<50%)	Ductal (NST), mixed ductal and lobular
	Solid and pleomorphic lobular, metaplastic

Higher stage of disease at presentation, decreased response to conventional chemotherapy and lower patient survival are associated with tumours that are ER/PR negative, are DNA aneuploid, over express p53 and HER2, and have a high Ki-67 proliferation index. These are usually high-grade ductal cancers of no special type.

Hormonal and HER2 Receptor Status

Oestrogen/Progesterone Receptor (ER/PR) Expression

Positive in 70–80% of ductal (usually grade 1 /2) cancers and 80–90% of infiltrating lobular carcinomas. ER status is used to determine whether a patient will benefit from endocrine therapy. PR expression is a prognostic marker and may also indicate responsiveness to hormone therapy.

There are several scoring methods, none of which is universally accepted. Cases which have 1% or more tumour cells positive are regarded as ER or PR positive for management purposes.

Scoring System: "H-score"

In the H-score, each cell is assessed as:

0	No nuclear staining
1	Weak nuclear staining
2	Moderate nuclear staining
3	Strong nuclear staining.

The percentage of cells showing each intensity of staining is estimated over as much of the section as possible. The H-score is calculated by multiplying the intensity score by the percentage of tumour cells showing that intensity: e.g. a tumour with 50% of cells strongly stained, 25% moderately stained and 25% weakly stained would score (50 × 3) + (25 × 2) + (25 × 1) = 225.

Scoring System: Allred or "Quick Score"

In the Allred score, the proportion of cells staining and their intensity are assessed as:

Proportion	
0	No nuclear staining
1	<1% nuclei staining
2	1–10% nuclei staining
3	11–33% nuclei staining
4	34–66% nuclei staining
5	67–100% nuclei staining

Intensity	
0	No staining
1	Weak staining
2	Moderate staining
3	Strong staining

Adding the scores together gives a maximum score of 8.

Individual cancers can show heterogeneity of ER expression and in some respects the H-score and Allred score can take this into account. Carcinoma in situ (low to intermediate grade), infiltrating lobular carcinoma, low grade invasive ductal carcinoma and postmenopausal cancers tend to be ER positive, while high grade in situ and invasive ductal lesions and a significant number of premenopausal carcinomas are ER negative. In practice with improved immunohistochemistry the vast majority of breast cancers are either strongly positive or completely negative for ER and assessable visually at a glance. PR expression can be less clear cut. Expression is also amenable to quantitation by automated image analysis. Hormonal therapy is also considered in patients with low ER but high PR scores.

HER2 neu (c-erbB2) Expression

HER2 overexpression *(13–20% of breast cancers)* is associated with high grade in situ and grade 3 invasive ductal cancers, pleomorphic infiltrating lobular cancers, and ER negative, recurrent or metastatic tumours. It can indicate *potential resistance to tamoxifen and CMF (Cyclophosphamide, Methotrexate, Fluorouracil) chemotherapy* but *benefit from high dose adriamycin or Herceptin/trastuzumab monoclonal antibody therapy.* When combined with neoadjuvant chemotherapy trastuzumab treatment can potentially double the rate of complete pathological response, and in the adjuvant setting reduces recurrence and improves survival. HER2 status is assessed immunohistochemically (IHC) as negative (0–1+), equivocal (2+) or positive (3+). Equivocal cases (2+) are then subjected to fluorescent, chromogenic or dual colour dual hapten in situ hybridisation (FISH/CISH/DDISH) analysis for HER2 gene amplification, therapy being indicated in IHC 2+/ISH positive cases.

HER2 immunohistochemical assessment.

0	No membrane staining or incomplete membrane staining in <10% of the carcinoma cells
1+	Faint, partial membrane staining in >10% of the carcinoma cells
2+	Weak to moderate complete membrane staining in >10% of the carcinoma cells
3+	Strong complete membrane staining in >10% of the carcinoma cells.

ER, PR and HER2 status tends to be performed on the *diagnostic core biopsy* due to more standardised fixation and antigen preservation compared to that achieved in resection specimens, and to allow the results to be discussed before any operative treatment is undertaken. ER, PR and HER2 status are also determined on recurrent carcinoma deposits, e.g. skin, chest wall muscle, liver.

Immunophenotype: Miscellaneous Markers for Breast Cancer

- Cytokeratin (CAM 5.2, AE1/AE3, CK7), GCDFP-15, EMA, CEA positive.
- CK7 positive/CK20 negative: the reverse of this is seen in intestinal tract tumours and this can be useful in metastatic carcinoma of uncertain origin, e.g. signet ring cell carcinoma.
- Myoepithelial markers to distinguish RS/CSLs from tubular carcinoma and in situ from invasive ductal cancer: smooth muscle myosin, CK5/6, CK14, p63.
- E-cadherin: loss of expression in ALH/LCIS and infiltrating lobular carcinoma but retention in ductal lesions. Conversely 34βE12 is expressed in LCIS but lost in DCIS.
- Usual ductal epithelial hyperplasia shows mosaic positivity with CK5/6 and CK 14. ADH/DCIS is negative for these basal epithelial markers but positive for luminal epithelial antigens CK 8 and CK 18, indicating an absence of the mixed luminal/basal epithelial differentiation that is present in usual hyperplasia. ADH and DCIS are also usually uniformly ER positive whereas usual epithelial hyperplasia shows heterogeneous expression.

Other Malignancy

Phyllodes Tumour

- Benign, borderline or malignant comprising a *biphasic proliferation* of double layered hyperplastic *epithelium* and abundant, cellular *stromal* mesenchymal elements with a *leaf like architecture* (from Greek*: phullon* leaf).

  Designation is based on:

1. Circumscribed or infiltrative margins
2. Stromal cellularity

- Overgrowth (absence of epithelium in one low power field)
- Atypia
- Mitotic activity (probability of malignancy increases with mitotic activity)

Most are benign, but *local recurrence is not uncommon (21% of cases)* and a small number develop haematogenous metastases to lung and bone. Axillary lymph node metastases are rare. At the benign end of the spectrum the differential diagnosis includes cellular fibroadenoma. At the malignant end it includes metaplastic breast carcinoma (cytokeratin positive) and sarcoma. Mammography and FNAC are not particularly accurate at diagnosing phyllodes tumour. Patients are usually at least 40–50 years of age and diagnostic core biopsies show characteristic high percentage (>85%) stromal content with variable cellularity, pleomorphism and mitotic activity. *Wide local excision (1 cm margins)* is needed for histological designation and to reduce the risk of local recurrence, although this can occur even with benign and borderline lesions. Those classified as malignant have metastatic potential.

Sarcoma

Sarcoma forms <1% of breast malignancies, e.g. angiosarcoma (usually post radiotherapy), and other primary soft tissue sarcomas (in decreasing order of frequency): malignant fibrous histiocytoma, fibrosarcoma, rhabdomyosarcoma, liposarcoma, leiomyosarcoma. Prognosis relates to high histological grade, mitotic counts and infiltrating margins. Important and more common differential diagnoses are *metaplastic breast carcinoma* (identify an epithelial component, cytokeratin positive), and *malignant phyllodes tumour* (biphasic and typical architecture).

Angiosarcoma is the commonest primary breast sarcoma, occurs in middle aged to elderly women, and is usually found in the field of *prior irradiation* after a period of 3 or more years. It is on average 5 cm in diameter with poorly defined margins. It must be distinguished from haemangioma, pseudoangiomatous stromal hyperplasia (PASH), and atypical vascular proliferation occurring in the dermis after recent breast conserving surgery. Grade 1 lesions (40%) have an 81% 10 year survival. Grade 3 lesions (40%) mimic poorly differentiated carcinoma and have 10% 10 year survival with metastases to lungs, liver, skin, bone and brain. Diagnosis can be difficult on FNAC and biopsy is required. Angiosarcoma is variably factor VIII, CD 34 and CD 31 positive.

Other spindle cell lesions of the breast to be considered in a differential diagnosis are: metastatic malignant melanoma and sarcomatoid renal cell carcinoma, fibromatosis and myofibroblast-related lesions (inflammatory myofibroblastic tumour, myofibroblastoma).

Haematological Malignancy

Primary lymphoma of the breast is rare. It usually presents as a painless palpable mass. The breast may also be involved in systemic lymphoma. Most primary lesions are of B cell origin, with diffuse large B cell lymphoma by far the most common. Breast implant-associated anaplastic large cell lymphoma is extremely rare but is an important consideration when fluid and/or a mass forms around a synthetic implant.

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Part VI

Gynaecological Cancer

- Ovarian Tumours (With Comments on Fallopian Tube)
- Endometrial Carcinoma
- Cervical Carcinoma
- Vaginal Carcinoma
- Vulval Carcinoma
- Gestational Trophoblastic Tumours

Ovarian Tumours (with Comments on Fallopian Tube)

23

Rajeev Shah and Aaron Ervine

Introduction

Ovarian cancer accounts for 30% of female genital tract malignancies and some 50% of its cancer related deaths. High parity and use of oral contraceptives are protective, while long term oestrogen replacement therapy in postmenopausal women is a risk factor. About 10% of cases are on the basis of hereditary susceptibility with germline mutations in BRCA1 and BRCA2 genes conferring 20–50% and 10–25% lifetime risks of developing ovarian cancer, respectively. Serous carcinoma is the commonest ovarian epithelial malignancy overall (70–80% of cases) and responsible for 90% of deaths from ovarian cancer.

Ovarian cancer has a poor prognosis with an average 5 year survival of 35–40%, although this is improving due to screening, more effective chemotherapy and changes in surgical practice. This adverse outlook is due to late presentation in 70% of cases at an advanced stage of disease (FIGO II-IV), and with non-specific symptoms such as abdominal fullness or swelling. A high risk malignancy index equates to postmenopausal status, a solid or cystic lesion with septation, papillae or nodules on ultrasound scan, and elevated serum CA125 > 35 kU/L. Elevated levels of serum CA125 can also be seen in pregnancy, menstruation endometriosis and other cancers e.g. breast, uterus, lung and pancreas. Further investigations include MRI scan, CT scan chest/abdomen/pelvis, and peritoneal fluid aspiration for cytology.

Radiological imaging is an effective tool for detection of ovarian neoplasia but cannot distinguish between primary and metastatic disease. If a benign cyst is suspected FNAC (fine needle aspiration cytology) may be used, and cystectomy or unilateral salpingo-oophorectomy considered, particularly in a young woman of child bearing age. Percutaneous biopsy is only used where there is an adnexal mass associated with extensive peritoneal disease that is considered inoperable, and a definite tissue diagnosis would provide a basis for neoadjuvant or palliative chemotherapy. Otherwise suspected malignant ovarian lesions are treated by total abdominal hysterectomy and bilateral salpingo-oophorectomy with omentectomy and peritoneal cytology. If diagnostic ascitic fluid has not been previously submitted or is not present at operation, peritoneal staging washings are carried out. The pathologist should integrate the histological and cytological findings to determine an appropriate tumour stage. Distinction between hyperplastic mesothelial cells and borderline/malignant serous epithelial cells can be particularly problematic emphasising the need for close correlation. Pelvic and retroperitoneal lymph nodes are clinically and radiologically

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_23

assessed but not usually resected in stage I disease. Intraoperative frozen section may be necessary to determine lymph node status prior to undertaking radical surgery inclusive of lymphadenectomy, as it may be precluded by the presence of metastatic disease. More limited surgery and staging are options in young women who wish to preserve their fertility.

As commented on above, radiologically guided needle core biopsy of omental disease may be required for a definitive diagnosis, and also in patients not suitable for primary surgical debulking. Neoadjuvant chemotherapy can then be given, and if there is a good tumour response in a medically fit patient, subsequent cytoreductive salvage surgery or clearance of macroscopic abdominopelvic disease undertaken. Optimal debulking is defined as ≤10 mm of residual disease after completion of surgery. Due to changes of tumour regression after neoadjuvant chemotherapy it is difficult to subtype, grade and stage the ovarian cancer, although immunophenotypical expression similar to that of the chemonaïve tumour is often retained. Designation and staging is therefore based on the pre-treatment needle core sample and clinical disease distribution. Tumour type may also be an indicator as to potential chemoresponsiveness: e.g. mucinous and clear cell adenocarcinomas and low-grade serous carcinomas respond less. Postoperative chemotherapy is used for tumours that have spread beyond the ovary, or, if ovary confined but with any of: high-grade, capsule rupture, ascites or positive peritoneal washings.

In general ovarian cancer is split into two types based on morphological subtype and tumour grade. Type 1 or low-grade cancers show more indolent behaviour and early stage disease with good to intermediate prognosis (endometrioid/mucinous: 90% 5 year survival: clear cell: 70% 5 year survival), and can be treated by optimal surgical debulking. The much commoner Type 2 or high-grade cancers are more aggressive with advanced stage (III/IV) disease, poor prognosis (high-grade serous: 20–40% 5 year survival, <1% ovary confined), and require a combination of radical surgery and chemotherapy.

Gross Description

Specimen

- FNAC/wedge biopsy/oophorectomy and/or cystectomy/uni−/bilateral salpingo-oophorectomy ± hysterectomy/omentectomy/lymphadenectomy.
- Weight (g) and size (mm).

Tumour

Site

- Ovarian (cystic, cortical, medullary, hilar or serosal)/paraovarian/broad ligament.
- Serosal tumour is associated with a worse prognosis than an equivalent cortical or intracystic lesion. Medullary, hilar or paraovarian tumour nodules may indicate a metastatic deposit rather than a primary lesion.
- Unilateral/bilateral (30–40% of serous epithelial lesions).

Size

- Length × width × depth (mm) or maximum dimension (mm).

Appearance

Capsule: intact/deficient, smooth/rough.

Cut surface:

- Cystic: uni-/multilocular
  - Warty growths/nodules
  - Fluid contents: serous/mucoid
  - Sebaceous content: hair/teeth/colloid (struma ovarii)
- Solid: partially/totally (mm)
- Necrosis/haemorrhage.
- Ovarian cancer tends to have a mixed cystic and solid appearance. The former comprises uni-/multilocular thin walled cysts with warty, nodular, papillary or solid areas of tumour growth which can be internal (endophytic) or serosal (exophytic). In lesions with a smooth external surface, areas of capsular deficiency should be actively sought and the relationship to any tumour noted, either caused by it (due

to capsular infiltration), or, overlying or away from it. The latter may be due to surgical dissection through a plane of adhesions or intraoperative rupture because of the size or cystic nature of the lesion. It is therefore important to determine the mechanism and part of the tumour that is deficient or ruptured in assessing potential spillage of benign, borderline or malignant cells into the peritoneal cavity so that the multidisciplinary meeting can assign an appropriate FIGO stage. The solid component of ovarian cancer tends to be somewhat friable and pale in appearance. Other visual diagnostic clues include: granulosa cell tumour (pale/fleshy/cystic), steroid cell and carcinoid tumours (yellow), thecoma/fibroma (white, whorled cut surface with yellow areas, lobulated), metastatic malignant melanoma (pigmented), immature teratoma (dermoid cyst with solid areas other than calcification/teeth), malignant lymphoma (pale/fleshy) and metastases (multiple, necrosis, nodular corticomedullary/serosal/hilar/paraovarian/paratubal deposits).

Edge

- Circumscribed/irregular.

Fallopian tube: length (mm); infiltration of paratubal connective tissue. Tumour nodules in tubal mucosal fimbriae.

Omentum: weight (g) and size (mm); tumour nodules: number/maximum dimension (mm).

Histological Type

Epithelial and sex cord stromal lesions form 60–70% of ovarian tumours (75% of which are benign) and *90–95% of primary ovarian malignancy*. These have traditionally been considered to arise from the surface (coelomic) epithelium or cortical epithelial inclusion cysts. More recent evidence points towards an origin from the *fallopian tube fimbrial epithelium* for a high proportion of ovarian serous adenocarcinomas. Epithelial tumours are classified according to their *cell type, growth pattern* (solid, cystic, surface), *amount of fibrous stroma* and *neoplastic potential of the constituent epithelium* (benign, borderline or malignant/invasive). *Germ cell tumours comprise 25% of ovarian tumours* and the vast majority of these are benign. They form 60–70% of childhood ovarian tumours and while the majority of these are benign (cystic teratoma) there is a greater proportion of malignant germ cell tumours (immature teratoma, yolk sac tumour) than in adults.

Epithelial

– *Serous*	55%} benign, borderline or malignant lesions
– *Mucinous*	5–10%} benign, borderline or malignant lesions
– *Endometrioid*	15%} benign, borderline or malignant lesions
– *Clear cell*	10%} benign, borderline or malignant lesions
– *Brenner*	2%} benign, borderline or malignant lesions

- The *commonest primary ovarian carcinomas* are high-grade serous carcinoma (70%), clear cell carcinoma (10%), endometrioid carcinoma (10%), mucinous carcinoma (2–4%) and low-grade serous carcinoma (2%).
- *Mixed*: either of different epithelial subtypes or differentiation within one subtype
- Mucinous cystadenoma/Brenner tumour: a relatively common finding.
- Clear cell/endometrioid (both can arise ex-endometriosis), serous/endometrioid or serous/clear cell adenocarcinomas. Classification is subject to considerable observer variation and true mixed differentiation is relatively rare with the common epithelial subtypes. Each component must comprise at least 10% of the tumour area. The prognosis is often determined by the nature of the major component. However, the presence of a minor component of serous or undifferentiated carcinoma in an otherwise endometrioid adenocarcinoma adversely affects prognosis with the latter probably representing dedifferentiation from the low-grade endometrioid adenocarcinoma. High-grade serous adenocarcinomas

may also show endometrioid and clear cell like areas mimicking these two subtypes.
- Endometrioid adenocarcinoma with squamous cell differentiation (benign or malignant cytology).
- *Undifferentiated*:
- Small cell carcinoma of either: (a) hypercalcaemic type (young, bilateral, small cells with follicle like structures) or; (b) pulmonary type (lung small cell carcinoma analogue), both of which are of poor prognosis and show variable EMA/cytokeratin positivity ± chromogranin/synaptophysin/CD56. There is also a large cell neuroendocrine carcinoma variant.
- Non-small cell: immunohistochemistry may be necessary to distinguish it from malignant lymphoma, malignant melanoma, epithelioid variants of sarcoma, granulosa cell tumour and rare ovarian cancers, e.g. transitional cell carcinoma or squamous cell carcinoma.
- Osteoclast like.
- Trophoblastic differentiation.

Malignant Mixed Mesodermal Tumour (Carcinosarcoma)

- Old age, *poor prognosis*, *<1% of ovarian tumours*.
- Homologous: ovarian type adenocarcinoma (serous, endometrioid, clear cell, undifferentiated) with cytokeratin positive spindle cells indicating that this lesion is a carcinoma with variable malignant mesenchymal differentiation .
- Heterologous: ovarian type adenocarcinoma with foci of immature/malignant cartilage, striated muscle, osteoid.

Sex Cord/Stromal

- *8% of ovarian neoplasms*.
- *Fibroma/thecoma* (85%): fibroma (storiform spindle cells/collagenous stroma) is one of the commonest ovarian tumours. Thecomatous elements are fat stain positive. Fibroma/thecoma can be associated with Meig's syndrome (a benign ovarian tumour associated with ascites and pleural effusion) and endometrial hyperplasia. Sclerosing stromal tumour is a related variant. Fibrosarcoma shows cytological atypia and increased mitoses and is very rare.
- *Granulosa cell tumour* (12%):
- *Adult*: micro-(macro)follicular (Call-Exner bodies), trabecular, insular, watered-silk, solid, sarcomatoid patterns, and longitudinal nuclear grooves.
- *Juvenile*: solid or cystic, follicular patterns of small cells ± mitoses. It is less aggressive than the adult counterpart with tumour recurrence and metastases rare.
- *Sertoli-Leydig tumour*: well/moderate/poor differentiation with varying proportions of tubules lined by Sertoli cells, Leydig cells and spindle cells ± heterologous elements, e.g. mucin secreting glands. Sertoli cells are cytokeratin positive, and the stroma is inhibin positive.
- *Mixed and unclassified variants* (10% of cases).
- *Gynandroblastoma*: an equal mix of android and gynaecoid elements: i.e. granulosa-theca/Sertoli-Leydig.
- *Gonadoblastoma*: mixed germ cell/sex cord cell elements usually dysgerminoma/Sertoli/granulosa like cells.
- *Sex cord tumour with annular tubules (SCTAT)*: associated with Peutz-Jeghers syndrome and adenoma malignum of the cervix.
- *Immunohistochemistry*: the sex cord stromal tumours are variably inhibin/melan-A/calretinin/WT-1/SF-1/FOXL2 and CAM5.2/CD99(both focal) positive, but CA125/CK7/EMA/BerEP4/PAX-8/SALL4 negative.

Steroid Cell Tumours

- Rare (0.1%), hormonally active with virilisation (75%).
- Polygonal eosinophilic cells, inhibin/calretinin/melan-A/SF-1 positive.
- 30% are malignant based on size (>7 cm), mitoses, atypia, and necrosis.

Germ Cell Tumours

- *Teratoma*: mature/cystic.
  - Immature/solid.
  - Monodermal e.g. carcinoid tumour, struma ovarii (thyroid tissue).
  - Malignant transformation, e.g. squamous cell carcinoma (80% of malignant cases), carcinoid tumour, adenocarcinoma (no specific type or mucinous), thyroid papillary carcinoma.
- *Dysgerminoma* (seminoma analogue: PLAP/CD117/OCT3/4/SALL4 positive), *yolk sac tumour* (children/young adults, reticular/microcystic/endodermal sinus/tubulopapillary patterns, AFP/glypican 3/SALL 4 positive, chemosensitive), *embryonal carcinoma* (CD30/CAM5.2/OCT3/4/SALL 4/SOX 2 positive). SALL4 is a robust pluripotential pan-germ cell marker. It is positive in normal germ cells, immature teratoma, embryonal carcinoma, dysgerminoma and yolk sac tumour. OCT3/4 shows similar immunoexpression but is negative in yolk sac tumour.
- *Mixed germ cell tumour* (8% of cases) e.g. dysgerminoma and yolk sac tumour.
- *Choriocarcinoma*
  - Primary: rarely, primary prepubertal or as part of a mixed germ cell tumour.
  - Secondary: to gestational uterine, tubal or ovarian lesions (better prognosis).

Metastatic Carcinoma

- *10–15% of malignant ovarian tumours* often *mimicking a primary lesion* clinically and pathologically: especially from colorectum, appendix, stomach, pancreas, endocervix, endometrium and breast (infiltrating lobular). With any *ovarian mucinous tumour, metastases from elsewhere is a more common finding* and must be excluded by past medical history, clinical and radiological investigation.
- *Krükenberg tumours*: classically bilateral signet ring cell metastases from stomach and mucin positive with a reactive fibrous ovarian stroma ± luteinisation. Spread is transperitoneal and rare differential diagnoses include primary ovarian signet ring cell carcinoma, ovarian goblet cell carcinoid tumour (syn: crypt cell adenocarcinoma), and sclerosing stromal tumour. Other sources for Krükenberg tumours are large bowel, appendix, gall bladder, pancreas and breast.
- *Direct spread*: colorectal carcinoma, carcinoma of fallopian tube, endometrium and cervix.
- *Distant spread*: lung, malignant melanoma, breast, kidney, thyroid. Small cell ovarian tumours (juvenile granulosa, small cell carcinoma ± hypercalcaemia) must be distinguished from metastatic small cell carcinoma of lung.

Features favouring an ovarian primary are: unilaterality, large size with a smooth external surface and an expansile microscopic growth pattern. Other indicators of a primary lesion are associated ovarian pathology, e.g. benign cystic teratoma (dermoid cyst), Brenner tumour, Sertoli-Leydig tumour, or a mural nodule (see Sect. 9). *Approximately 70% of secondary carcinomas* are bilateral, most of which are <10 cm in diameter. Additional clues are solid, discrete, corticomedullary nodular deposits, surface or hilar deposits, prominent infiltrative stromal desmoplasia, lymphovascular invasion, extensive necrosis, colloid and signet ring carcinomas, pseudomyxoma peritonei, and lack of CK7 positivity. *Prognosis of metastatic carcinoma to the ovary is poor as this represents advanced disease.* Conversely primary ovarian mucinous carcinomas are usually low-grade and have limited extent early stage disease. A number of metastases mimic primary ovarian carcinoma histologically, e.g. gastrointestinal tract including appendix (endometrioid/mucinous), renal cell (mesonephroid/clear cell), thyroid (struma ovarii) and hepatocellular (yolk sac tumour) carcinomas. A relevant clinical history is crucial in designation and further clinical investigation, e.g. CT scan abdomen, serum AFP or CEA may be necessary to discover an occult primary lesion. See section "Other Pathology" for further discussion.

Differentiation

Adenocarcinoma

- Well/moderate/poor/undifferentiated, or, Grade 1/2/3/4.
- Grading for epithelial ovarian tumours is based on the degree of architectural differentiation (glandular/papillary/solid), cytological pleomorphism, and mitotic activity ± overt invasion of the stroma or capsule. The *presence and extent of stromal invasion is a strong prognostic indicator.*
- In serous adenocarcinoma there is usually correlation between cytoarchitectural differentiation features. Poorly differentiated tumours form the majority of cases, and are solid with tubulopapillary slit like spaces and high nuclear grade. Less common patterns are pseudoendometrioid, transitional cell like and microcystic. Well to moderately differentiated tumours are much less common (2% of cases), and are partially cystic with glands, papillae and lower nuclear grade. An exception to this with cytoarchitectural disparity is the grade 1 solid/nested psammomatous serous adenocarcinoma. In general, on morphological, molecular and behavioural grounds, ovarian serous adenocarcinomas are regarded as being either *low-grade* or *high-grade*, with the distinguishing features of *marked cytological atypia*, and, a threshold of *≤12 or > 12 mitoses per 10 high power fields*. This model has been extended to postulate *two pathogenetic types of ovarian carcinoma.*
  - *Type 1 (low-grade)*: including borderline epithelial lesions, low-grade serous adenocarcinoma, mucinous, low-grade endometrioid and malignant Brenner tumour
  - *Type 2 (high-grade)*: including high-grade serous adenocarcinoma, clear cell adenocarcinoma, undifferentiated carcinomas and carcinosarcomas.
  - *Type 1 cancers* have an indolent transition to malignancy from benign Mullerian neoplasia (e.g. cystadenoma/adenofibroma), metaplasia (e.g. endometriosis) or potentially fallopian tube fimbrial epithelial implants on the ovarian surface. They present with early stage disease, are treated by total surgical debulking, and are in general non-chemoresponsive. They show a variety of gene mutations such as BRAF, KRAS, PTEN and microsatellite instability.
  - *Type 2 cancers* undergo abrupt malignant change usually from a fallopian tube fimbrial epithelial precursor *(STIC – serous tubal intraepithelial carcinoma),* and occasionally ovarian cortical inclusion cysts or surface coelomic epithelium. They typically show p53 mutations and spread throughout the surfaces of the peritoneal cavity. They form >90% of advanced stage ovarian cancers requiring a combination of radical surgery and chemotherapy.
- A suggested scheme for endometrioid carcinoma is similar to its uterine counterpart based on the percentage of non-squamous/non-morular solid growth pattern: grade 1 (≤5%), 2 (6–50%), 3 (>50%).
- In endometrioid and mucinous tumours disproportionate nuclear atypia raises the grade by one level e.g. 1 → 2. In serous and clear cell carcinomas (both grade 3), *high nuclear grade* and *tumour subtype* take precedence over architecture.
- In mucinous tumours the *presence of invasion* outweighs cytoarchitectural grading features.

Borderline (Low Malignant Potential)

- *Serous borderline epithelial lesions* are of excellent prognosis regardless of stage and are bilateral in 20–25% occurring in a younger age group (40–50 years) than established carcinoma. They form 10–15% of epithelial tumours comprising epithelial complexity with budding, atypia, mitoses and nuclear layering but no destructive stromal invasion. There is peritoneal recurrence in 10–15% of cases. The outlook for *mucinous epithelial borderline tumours* (≤3 nuclei deep) depends on the subtype: i.e. the rare Mullerian endocervical (good outlook), or the much commoner intestinal variant (worse outlook). This

is due to the latter being associated with appendiceal mucinous lesions (see pseudomyxoma peritonei).

Micropapillary serous carcinoma: requires no demonstrable invasion but is designated on the degree of micropapillary/cribriform epithelial complexity. It is an exophytic lesion often associated with invasive peritoneal implants, bilaterality and advanced stage. It is of worse prognosis than usual serous borderline tumours, *behaving in effect as a low-grade adenocarcinoma*.

Sex Cord/Stromal

- Well/moderate/poor differentiation but weak correlation with prognosis, and grading is more dependent on the specific tumour type.

Functional: e.g. oestrogenic drive to endometrium in thecoma (25% of cases) and granulosa cell tumour. Virilisation in Sertoli-Leydig tumour.

Prognosis: of sex cord/stromal tumours relates to size (< or >5 cm), an intact or deficient capsule, bulk of extraovarian disease, atypia, mitoses (per 10 high power fields), necrosis and bilaterality.

Recurrence: in 30% of patients and tends to be local. It may be extra-pelvic and after a considerable *lag period of 10–20 years* although recurrent juvenile granulosa and Sertoli-Leydig tumours recur within 3 years. Raised serum inhibin levels may be useful in detecting recurrent granulosa cell tumour. Note that artifactual displacement of granulosa cells due to surgical trauma or cut up can mimic vascular invasion.

Germ Cell

- *Mature*: cystic (95% of cases). Common tissues represented are skin and appendage structures, muscle, fat, ganglia, neuroglia, respiratory, gastrointestinal and pancreatic glandular tissue, retinal elements, cartilage, teeth and bone.
- *Immature*: solid with histologically identifiable immature tissues especially cartilage, neuroepithelium, striated muscle and immature cellular mesenchyme.
- Grade 1: mostly mature tissue, loose mesenchyme, immature cartilage, focal (<1 low power field/slide) immature neuroepithelium.
- Grade 3: scant mature tissue, extensive (>3 low power fields/slide) immature neuroepithelium ± peritoneal implants which can be mature (e.g. gliomatosis peritonei) or immature, and are graded separately.
- ±Carcinoma (e.g. squamous cell, adenocarcinoma) or sarcoma (e.g. rhabdomyosarcoma, sarcoma of no specific type) in mature or immature lesions. *Development of a somatic type malignancy is an adverse indicator*.
- GFAP, synaptophysin and SALL 4 can help in identification of immature neuroepithelial elements.
- Combination chemotherapy for grade 2/3 tumours and those with peritoneal implants gives 90–100% survival.

Extent of Local Tumour Spread

- Border: pushing/infiltrative.
- Lymphocytic reaction: prominent/sparse.

Capsule/serosa/paratubal connective tissue/contiguous fallopian tube/uterus. Involvement of fallopian tube was traditionally considered to be secondary to primary ovarian disease wherein the bulk of tumour resided. Extension of tumour along the tubal mucosa was regarded as a mimic of carcinoma in situ and a tubal origin. Current evidence indicates *an origin for a significant majority of ovarian and primary peritoneal serous cancers from fallopian tube fimbrial serous tubal intraepithelial carcinoma (STIC).* Spread to uterus is usually as a serosal plaque of friable tumour with invasion of outer myometrium and/or its underlying vessels.

Extensive sampling of ovarian epithelial cystic lesions is necessary (1 block/cm maximum dimension of lesion) as there can be marked *heterogeneity* and *coexistence of benign, borderline and malignant features* e.g. mucinous

lesions. In this respect more blocks are required than in an obviously malignant homogeneous tumour, and nodular/solid areas should be preferentially sampled in an otherwise cystic lesion. *Microinvasion ≤10 mm² (or approximately 3 mm diameter)* can be difficult to distinguish from crypt epithelial complexity and invagination into stroma (desmoplasia is a useful feature in adenocarcinoma). It is occasionally seen in otherwise borderline serous tumours but does not alter the prognosis. *Invasion >5 mm* may help to discriminate between mucinous and endometrioid borderline and adenocarcinoma lesions with a worse clinical outcome.

Invasion of stroma and/or capsule remains the hallmark of adenocarcinoma but not infrequently its presence is difficult to assess or it is not evident. This is particularly problematic in mucinous lesions, where a designation of non-invasive carcinoma or intraepithelial carcinoma may be made on the basis of *epithelial complexity* and *cellular atypia* alone, e.g. nuclear stratification ≥4 deep, a confluent glandular or cribriform epithelial pattern, or stroma-free papillae of epithelial cells. Further sampling is necessary to exclude frankly invasive areas warranting the more usual designation of adenocarcinoma. A 'destructive' infiltrative pattern of invasion also has a poorer outlook than an 'expansive' confluent invasive edge.

Minimal staging requires removal of the ovarian primary lesion, biopsy of the contralateral ovary, biopsy of omentum and peritoneal surfaces, and peritoneal washings for cytology if ascitic fluid is not present.

FIGO

FIGO staging alone is recommended for all gynaecological cancers except cervical cancers (Figs. 23.1, 23.2 and 23.3). FIGO staging in ovarian carcinomas is broadly comparable to TMN8.The classification applies to malignant surface epithelial-stromal tumours including those of borderline malignancy. Non-epithelial ovarian cancers may also be classified using this scheme.

TNM Staging (FIGO Staging in Parenthesis)

TX: Primary tumour cannot be assessed
T0: No evidence of primary tumour
T1(I): Tumour limited to the ovaries
T1a (IA): Tumour limited to one ovary (capsule intact) or fallopian tube surface; no malignant cells in ascites or peritoneal washings
T1b (IB): Tumour limited to one or both ovaries (capsules intact) or fallopian tubes; no tumour on ovarian or fallopian tube surface, no malignant cells in ascites or peritoneal washings
T1c (IC): Tumour limited to one or both ovaries or fallopian tubes with any of following:
T1c1 (IC1): Surgical spill
T1c2 (IC2): Capsule ruptured before surgery or tumour on ovarian or fallopian tube surface
T1c3 (IC3): Malignant cells in ascites or peritoneal washings
T2 (II): Tumour involves one or both ovaries or fallopian tubes with pelvic extension below pelvic brim or primary peritoneal cancer
T2a (IIA): Extension and/or implants on uterus and/or fallopian tubes and/or ovaries; no malignant cells in ascites or peritoneal washings
T2b (IIB): Extension to other pelvic intraperitoneal tissues
T3 and/or N1 (III): Tumour involves one or both ovaries or fallopian tubes, or primary and/or peritoneal cancer, with cytologically or histologically confirmed spread to the peritoneum outside the pelvis and/or metastasis to the retroperitoneal lymph nodes
N1: Retroperitoneal lymph node metastasis only
N1a (IIIA1i): Lymph node metastasis up to 10 mm in greatest dimension
N1b (IIIA1ii): Lymph node metastasis more than 10 mm in greatest dimension
T3a any N (IIIA2): Microscopic extrapelvic (above the pelvic brim) peritoneal involvement with or without positive retroperitoneal lymph nodes including bowel involvement
T3b any N (IIIB): Macroscopic peritoneal metastasis beyond pelvic brim up to 2 cm in greatest dimension, including bowel involvement with or without retroperitoneal lymph node metastasis
T3c any N (IIIC): Peritoneal metastasis beyond pelvic brim more than 2 cm in greatest dimension with or without retroperitoneal lymph node metastasis (includes extension of tumour to capsule of liver and spleen without parenchymal involvement of either organ)
M1 (IV): Distant metastasis excluding peritoneal metastases
M1a (IVA): Pleural effusion with positive cytology
M1b (IVB): Parenchymal metastases and metastases to extra-abdominal organs (including inguinal lymph nodes and lymph nodes outside the abdominal cavity)

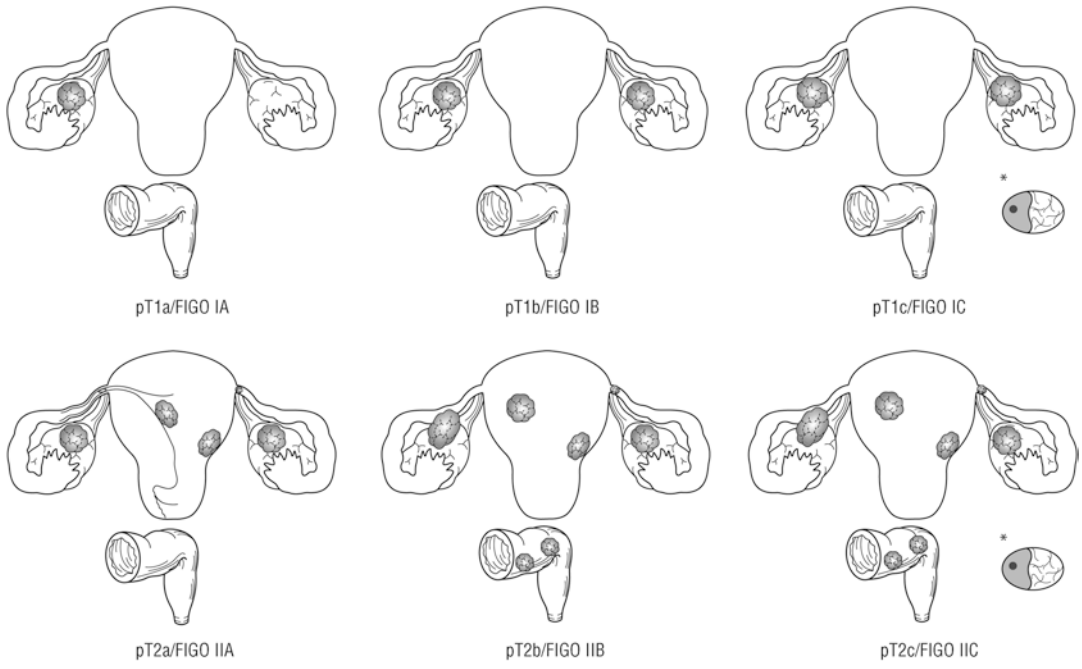

Fig. 23.1 Ovarian carcinoma. Reproduced, with permission, from *Histopathology Reporting: Guidelines for Surgical Reporting*, *2nd ed.,* © 2006, Springer

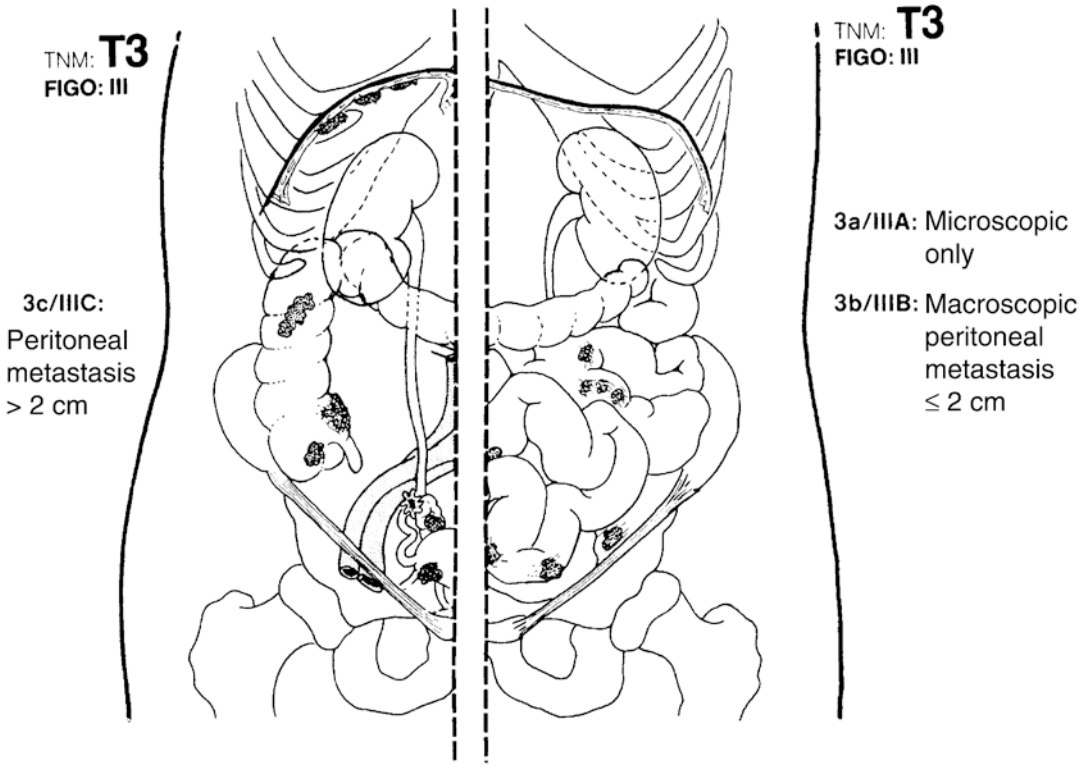

Fig. 23.2 Ovarian carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

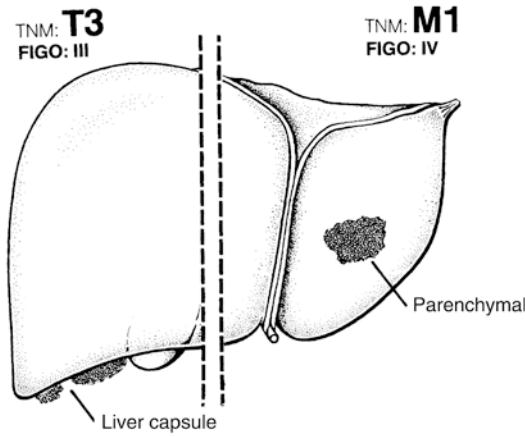

Fig. 23.3 Ovarian carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

The commonest pattern of spread is the *contralateral ovary, peritoneum, para-aortic and pelvic lymph nodes and liver. Lung is the preferred extra-abdominal* site and occasionally presentation can be at other abdominopelvic e.g. sigmoid colon, or unusual sites, e.g. breast, clinically mimicking primary intestinal and mammary disease, respectively.

Lymphovascular Invasion

- Present/absent.
- Intra-/extratumoural.
- Look for particularly in the paraovarian/paratubal connective tissues.

Lymph Nodes

Site/number/size/number involved/extracapsular spread.

Regional nodes: obturator (hypogastric), common iliac, external iliac, lateral sacral, para-aortic, inguinal. A regional lymphadenectomy will ordinarily include a minimum of 10 lymph nodes.

- Beware of overcalling intranodal endosalpingiosis or Müllerian inclusions which can be associated with borderline changes and difficult to distinguish from microscopic metastases. Comparison with the index ovarian lesion and disease elsewhere, e.g. peritoneum is important in designation. They are not thought to be associated with an adverse outcome.

Omentum/Peritoneum

Some 40% of serous borderline tumours, especially exophytic lesions, are associated with foci of peritoneal serous epithelial proliferation particularly in the omental and pelvic peritoneum.

Endosalpingiosis

- *No epithelial atypia*: ± a benign or borderline ovarian lesion.
- A metaplastic process in serosal epithelial inclusions.

Implants

- *Epithelial atypia*: usually associated with a *borderline ovarian serous lesion* (rarely, mucinous). The implants are assessed independently of the ovarian tumour as: *non-invasive* or *invasive* (destructive infiltration of underlying tissue disrupting the omental lobular architecture), and, *epithelial* (proliferative) or *desmoplastic/stromal* (>50–75% loose stroma/granulation tissue with small nests or papillae of epithelial cells).
- Probably represents *multifocal neoplasia* arising in peritoneal inclusions. Endosalpingiosis, desmoplastic and non-invasive proliferating implants should be noted and follow up recommended. *Invasive proliferating implants are regarded as low-grade carcinoma* as they progress in 80% of cases with a 10 year survival of about 35%. Non-invasive proliferating implants are distinguished from surface serous adenocarcinoma by greater epithelial architectural and nuclear atypia in >25% of the lesion area in adenocarcinoma.

Primary Peritoneal Carcinoma

- Young to middle aged females.
- *Serous adenocarcinoma in type*: CK7/WT1/p16/ER positive/p53 mutational.
- There is *extensive peritoneal disease ± an ovarian serosal component* with otherwise normal ovaries (any ovarian invasion <5 mm^2). It may arise from fallopian tube STIC or surface coelomic epithelium. It can also be associated with EIC (endometrial intraepithelial carcinoma), or low volume high-grade endometrial serous carcinoma.
- Treated by surgical cytoreduction and chemotherapy. The psammomatous-rich adenocarcinoma variant has a better prognosis and indolent course.

Pseudomyxoma Peritonei

Pseudomyxoma peritonei is strongly associated with *appendiceal mucinous neoplasia* and there can be *concomitant cystic ovarian mucinous borderline tumours of intestinal type*. The peritoneal cavity fills with abundant mucin with or without a component of either cytologically bland, proliferating or malignant epithelium. Appendiceal and ovarian lesions coexist in 90% of pseudomyxoma peritonei cases and the latter are now considered to represent direct implantation from the former due to spillage of neoplastic appendiceal epithelium into the peritoneal cavity. Appendicectomy may be indicated particularly with bilateral cystic ovarian tumours and any clinical suggestion of pseudomyxoma peritonei or an appendiceal mucocoele.

Immunohistochemistry may be of help in that ovarian lesions of appendiceal origin may be CK20 positive/CK7 negative but primary ovarian tumours CK7 positive/CK20 negative. A CK20 positive/CK7 positive phenotype can indicate either origin as 50% of LAMN (Low grade Appendiceal Mucinous Neoplasm) are of this immunophenotype and ovarian mucinous tumours can show patchy CK20 positivity. In this case consideration also has to be given to a cystic metastasis from the pancreaticobiliary tree which can also co-express CA125 and show 'maturation phenomenon' with coexistence of benign, borderline and malignant morphology.

Metastatic Adenocarcinoma

- From primary ovarian adenocarcinoma usually forms either macroscopically obvious confluent tumour nodules, or a diffusely thickened omental cake.

Other Pathology

Hereditary factors: responsible for 5–10% of ovarian carcinomas. Mutations in the *BRCA1* and *BRCA2* genes carry a *20–50% risk* of ovarian cancer up to the age of 70 years. The tumours occur 5 years earlier than sporadic ovarian carcinoma, are mainly of serous histological type, and have a strong association with breast cancer (BRCA1: 87% risk). Affected patients have a higher proportion of solid undifferentiated serous cancers with increased mitoses and prominent tumour infiltrating lymphocytes (TILs). There is also an increased 9% lifetime risk of ovarian cancer in *hereditary non-polyposis colorectal cancer (HNPCC)* due to abnormality in DNA mismatch repair genes (usually MSH2/MSH6), and typical ovarian cancer types are endometrioid and clear cell. Prognosis of hereditary ovarian cancer is grade and stage dependent similar to that of sporadic ovarian carcinoma. There is some evidence of a slightly better prognosis due to a better response to chemotherapy. Lesions in hereditary tubo-ovarian neoplasia may be early or microscopic and require serially slicing and processing *in toto* of both ovaries and fallopian tubes (STIC).

Meig's syndrome: ascites, ovarian fibroma and right sided pleural effusion.

Contralateral ovary and tube: synchronous/metastatic disease of parenchyma or serosa (e.g. 40% of serous papillary lesions). Clues to metastatic disease in the non-dominant ovary are multiple nodules, surface implants and lymphovascular invasion. Synchronous primary lesions will tend to show similar tumour distribution

and appearance, e.g. size and cystic with solid components.

Uterus: synchronous/metastatic disease of endometrium, endocervix or serosa i.e. multifocal Müllerian neoplasia. When tumour is present in the ovary and endometrium (usually endometrioid adenocarcinoma, sometimes serous adenocarcinoma) it can be difficult to tell which is the primary/metastatic site or if they are independent primary tumours: i.e. stage II/III versus synchronous stage I disease. The *tumour site* and *distribution* (dominant bulk/depth of invasion/multiple serosal nodules/hilar involvement/lymphovascular invasion), and evidence of any *precancerous lesions* (endometrial hyperplasia/fallopian tube fimbrial STIC/ovarian endometriosis) are useful clues. The good prognosis of endometrioid adenocarcinomas confined to the endometrium and one or both ovaries suggests that they usually represent independent synchronous tumours, but in other cases with extensive disease, distinction can be somewhat arbitrary and best attributed in light of full clinicopathological details. Ovarian carcinoma metastasizing to endometrium is rare but involvement of the outer myometrium is not uncommon.

Endometriosis: concurrent *ovarian endometriosis* (± atypical hyperplasia) and endometrial carcinoma are seen in up to 25% of ovarian endometrioid adenocarcinomas. The frequency of associated disease is lower in ovarian clear cell (mesonephroid) adenocarcinoma which may also be related to foci of ovarian endometriosis (10–20%), with the latter now considered in some cases to be a potentially premalignant lesion. Clear cell adenocarcinoma should be distinguished from yolk sac tumour, dysgerminoma and metastatic renal cell carcinoma (see below).

Ploidy: DNA aneuploidy in borderline ovarian epithelial lesions and ovarian adenocarcinoma is generally regarded as an adverse prognostic indicator, although it is not routinely assessed in daily practice.

Mucinous borderline tumours: these are either *intestinal* (85% of cases: associated with appendiceal mucinous neoplasia ± pseudomyxoma peritonei), or *Müllerian* (endocervical; 30% are associated with endometriosis) in type. They have differing pathological and clinical features representing *high-grade* and *low-grade proliferating mucinous tumours*, respectively. A wide spectrum of benign/borderline/malignant intestinal differentiation can be seen and *metastases* from appendix, colon, stomach, and pancreas need to be excluded clinically. *Primary ovarian lesions are now recognised to comprise only a minority (2–10%) of ovarian mucinous tumours* of borderline or malignant character. Primary mucinous tumours can also rarely show a spectrum of *mural nodules* of varying size and appearances ranging from anaplastic carcinoma (cytokeratin positive large/spindle cells: aggressive) and sarcoma (fibrosarcoma, rhabdomyosarcoma) to benign behaving sarcoma like (pseudosarcomatous) lesions with cytokeratin negative giant cells and spindle cells. Pseudomyxoma ovarii is commoner in borderline and malignant lesions, particularly those with pseudomyxoma peritonei of appendiceal origin. Mucinous (intestinal type) and endometrioid ovarian carcinomas and Sertoli-Leydig tumours can closely mimic or be imitated by colorectal and other gastrointestinal adenocarcinomas. These secondary tumours can occur after, concurrently, or even predate the detection of the primary tumour.

Immunophenotype in the Differential Diagnosis of Ovarian Tumours

Colorectal metastases to the ovary tend to be bilateral, solid with areas of necrosis, and on microscopy show crescentic garland type strips of tumour with segmental and dirty necrosis. There is diffuse cellular staining with CEA and CA19-9, and CA125 is usually negative. *Ovarian serous and endometrioid carcinomas* tend to be cystic with solid areas, uni- or bilateral, show PAX-8 positivity, CEA staining, ± CA125/WT1 and variable CA19-9. *Ovarian mucinous lesions* are also often ER, PR, WT1 and CA125 negative negating the use of these markers in the distinction from secondary carcinoma. Additionally, CDX-2 can be positive in intestinal phenotype ovarian lesions making it unsuitable in this context for distinction from colorectal cancer. Mucin

is scanty in endometrioid variants and negative in Sertoli-Leydig tumours.

The *clinical and radiological anatomical distribution of disease* is important and commonly a rectosigmoid primary cancer will also show locoregional mesenteric lymph node disease and peritoneal involvement. At *intraoperative frozen section* the site of origin of a mucinous adenocarcinoma cannot be given with confidence, whereas this distinction can be made on morphological grounds for ovarian serous or clear cell adenocarcinoma invading large bowel. Despite this the differential diagnosis between ovarian and colorectal cancer can still be difficult and *in any ovarian mucinous or endometrioid tumour the possibility of a gastrointestinal origin must be excluded clinically. Differential cytokeratin expression* may be of use with intestinal tumours showing a different profile (CK20 positive/7 negative) to ovarian tumours (CK7 positive/CK20±, patchy). Gastrointestinal cancers are usually p53 positive. Note that *gastric and pancreaticobiliary cancers* are also variably CK7/20 positive and may even express CA125 as do some endometrial cancers. There is loss of DPC4 (SMAD4) in 50% of pancreaticobiliary cancers but not in ovarian lesions. *Endocervical adenocarcinoma* is often p16 (HPV surrogate marker) positive. Metastatic *breast carcinoma* shows a similar cytokeratin profile (CK7+/CK20−, ER/PR positive) but is also GCDFP-15 and typically GATA-3 positive. Infiltrating lobular breast cancer is particularly prone to gynaecological tract metastasis and a relevant clinical history is important. Other metastatic tumours mimicking primary ovarian cancer are *renal cell carcinoma* (CT abdomen, sinusoidal vascular pattern, less hob-nail cells, RCC ab/CD10/EMA/vimentin positive) and *transitional cell carcinoma of the urinary tract* (uroplakin III/CK7/CK20/GATA-3 positive). In peritoneal fluid cytology specimens BerEP4 is helpful in distinguishing between mesothelial and epithelial cells.

Serum levels and tissue expression of various antigens are detectable in a range of ovarian neoplasms but characteristically strong associations are:

CK7/CA125/WT-1/p53/p16	Tubal/ovarian/peritoneal adenocarcinoma (serous type)
HNF-1β/Napsin-A	Ovarian clear cell carcinoma
AFP/glypican 3/SALL 4	Ovarian yolk sac tumour
βHCG/cytokeratins	Ovarian choriocarcinoma
PLAP/CD117/OCT3/4/SALL 4	Dysgerminoma
Inhibin/CD99/calretinin/SF-1	Granulosa cell tumour

SALL4 is a robust pluripotential pan-germ cell marker. It is positive in normal germ cells, immature teratoma, embryonal carcinoma, dysgerminoma and yolk sac tumour. OCT3/4 is similar but is negative in yolk sac tumour.

Prognosis

Prognosis of ovarian carcinoma relates to morphological features such as histological type and grade, volume percentage epithelium and mitotic activity index as well as large volume of disease after cytoreduction, high volume ascites, high postoperative CA125 levels, the age and BRCA gene status of the patient. Over expression of markers such as p53 and HER2 may also be adverse. However, *the predominant factor is stage of disease* and the *degree of extra-ovarian spread and omental involvement. Early stage disease* confined to the ovary or pelvis has an *80% 5 year survival rate* whereas the *majority (70%) of patients present late* with widespread metastatic disease (FIGO III/IV) and *20–30% 5 year survival.* Undoubtedly there are different types of ovarian adenocarcinoma according to their origins and behavior: i.e. *type 1/low-grade cystadenocarcinoma* arising from a cystic ovarian neoplasm/Mullerian metaplasia with indolent behaviour and early stage disease, or, *type 2/high-grade adenocarcinoma* arising from fallopian tube fimbrial epithelium or a thin rim of outer cortex and showing aggressive behaviour with disproportionately extensive local spread and involvement of adjacent structures. *Overall survival probability at 5 years is about 35–40%.* Serum CA125 levels are useful for monitoring disease

progression and response to treatment but will be elevated in only 50% of early, curable disease. A suggested screening strategy is a combination of clinical examination, serum CA125 levels and abdominopelvic ultrasound examination. FNAC has a role to play in the distinction between functional cysts (e.g. granulosa/luteal—inhibin positive) and benign or malignant epithelial lesions (cytokeratin/BerEp4 positive). Accurate surgical assessment is needed to avoid understaging of ovarian cancer, with *surgical excision* the mainstay of treatment. Cytoreductive surgery is also used for extensive disease with *adjuvant therapy*. The majority of patients with high volume and high-grade serous carcinoma initially respond to treatment but *70–80% eventually relapse with local recurrence*. In some cases a subsequent "second-look" operation is carried out to assess the response to therapy and as a prequel to further chemotherapy. Borderline serous and endocervical mucinous ovarian epithelial neoplasms are uniformly of excellent prognosis (95–100% 5 year survival), even if microinvasion (<1–3 mm diameter) is present, with uni- or bilateral adnexectomy as effective as radical surgery. Prognosis is worse with invasive peritoneal implants. Stage I mucinous adenocarcinoma has a good prognosis but may metastasise if extensive stromal invasion is present. The possibility of an ovarian mucinous tumour representing spread from appendix, colorectum, endocervix or pancreaticobiliary tree should always be considered. Clear cell adenocarcinoma is intermediate-grade with a worse outlook than mucinous or endometrioid ovarian cancer, e.g. 70% 5 year survival for stage I disease. Undifferentiated carcinoma and malignant mixed mesodermal tumour (carcinosarcoma) have a poor prognosis. The vast majority of *sex cord/stromal tumours are stage I with 85–95% 5 year survival.* Higher stage and tumour rupture are adverse indicators but only about *10–30% of cases subsequently recur*, often at a late stage with 10 and 20 year survivals of 60–90% and 40–60% respectively. Malignant ovarian germ cell tumours are unusual and occur mainly in children, adolescents and young adults. 60% present with stage I disease (5 year survival 90%) with a 70–80% 5 year survival rate for all stages of disease. Serum βHCG and AFP levels are useful in postoperative monitoring and postoperative chemotherapy is used for tumours other than stage I, grade I immature teratoma.

Other Malignancy

Malignant Lymphoma

- *Primary* or more commonly *secondary* to systemic disease, particularly if low-grade B cell malignant lymphoma.
  - *Burkitt's/Burkitt's-like:* childhood, young adults, and associated with bilateral breast lymphoma in young women.
  - *Non-Hodgkin's diffuse large B cell:* average survival 3 years, often shows sclerosis.
- *Differential diagnoses*: includes the full range of malignant small blue cell tumours as well as granulosa cell tumour and dysgerminoma. Confirm by immunohistochemistry for lymphoid markers and negativity for inhibin, calretinin, PLAP, OCT3/4, SALL4, desmin, CD99, and neuroendocrine markers (CD56/synaptophysin/chromogranin).

Leukaemia

- Granulocytic sarcoma (CD34/CD43/CD68/CD117 and chloroacetate esterase/myeloperoxidase positive).
- 10–20% of acute and chronic leukaemias; a site of relapse for ALL during bone marrow remission.

Sarcoma

- *Leiomyo-rhabdomyo-/angio-/chondro-/osteo-/neurofibrosarcoma*: these are all rare and more often part of a malignant mixed mesodermal tumour or (immature) teratoma.
- *Endometrioid stromal sarcoma*: granulosa like cells with distinctive vascular pattern of spiral arteriole type vessels. CD10 positive

and smooth muscle actin/desmin focally positive, and, inhibin/h-caldesmon negative. Exclude spread from a uterine lesion.
- *Undifferentiated (high-grade) sarcoma*: fibro-/rhabdomyosarcoma like with atypia and mitoses.

Malignant Melanoma

- *Secondary*: present in 20% of fatal cases.
- *Primary*: within the wall of a dermoid cyst.

Others

Secondary involvement or clinical mimicry of an ovarian tumour by peritoneal *malignant mesothelioma* (well-differentiated papillary/multicystic, or of no special type), *intra-abdominal desmoplastic small round blue cell tumour* (with divergent differentiation: cytokeratin, EMA, vimentin, synaptophysin, desmin positive; in the pelvis and abdomen of young people), *Ewing's sarcoma/PNET, neuroblastoma* (neurofilament/synaptophysin), *rhabdomyosarcoma* (desmin/myoD1/myogenin) and retroperitoneal liposarcoma.

Comments on Fallopian Tube Carcinoma

Primary carcinoma of the fallopian tube has traditionally been considered to be rare (<1% of primary genital tract malignancy: increased risk in BRCA1/BRCA2 gene mutations), and greatly outnumbered by direct tubal extension from ovarian carcinoma (where the bulk of tumour is in the ovary) and uterine carcinoma (where the bulk of tumour is in the uterus). However previous comments (above) on the *fallopian tube fimbrial epithelial origin (STIC) for a significant proportion of ovarian/peritoneal serous carcinomas* should be noted. The tumour should be located macroscopically within the tube or its fimbriated end, and the uterus and ovaries should be grossly normal with any malignancy conforming to features of metastases or, alternatively, in keeping with the size and distribution of an independent primary. In primary fallopian tube carcinoma the tumour may be microscopic or the tube is distended by a solid or papillary tumour. Histologically the cancer is *invasive papillary adenocarcinoma usually similar to ovarian serous adenocarcinoma.* However, the full spectrum of Müllerian subtypes has been reported e.g. endometrioid, mucinous, clear cell, transitional and unusual tumours e.g. squamous cell carcinoma. Prognosis depends mostly on the stage of disease, with 5 year survival rates of 77% for stage I and 20% for stage III. Tumour recurrence is intra-abdominal and spread is similar to that of ovarian carcinoma. Other rare malignant tumours recorded are malignant mixed mesodermal tumour, leiomyosarcoma and gestational choriocarcinoma. Benign adenomatoid tumour is the commonest neoplasm occurring within the wall usually near the uterine cornu.

Broad ligament lesions include female Wolffian adnexal tumour (solid, sieve like trabecular/tubular pattern and CK7/inhibin/calretinin/androgen receptor positive, benign), and cystic lesions lined by Müllerian epithelium of variable type (e.g. serous, mucinous etc.) and character (usually benign/occasionally borderline or malignant).

FIGO

For details see ovarian carcinoma.

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Endometrial Carcinoma

24

Rajeev Shah and Aaron Ervine

Introduction

The uterus and the ovary are the commonest sites for female genital tract cancer with risk factors for endometrial cancer including obesity, diabetes, nulliparous status, hypertension and late menopause. The majority (>80% of cases) are type I endometrial adenocarcinoma arising as a result of unopposed exogenous or endogenous oestrogens from a background of endometrial hyperplasia, and presenting confined to the endometrium and with a good prognosis. Type II cancers occur in older women, are oestrogen independent and >50% present with extrauterine spread due to early lymphatic dissemination. Prognosis is poor, worsening in the following order: clear cell adenocarcinoma, serous adenocarcinoma and carcinosarcoma.

Most endometrial cancers present with abnormal vaginal bleeding and this is particularly significant in a postmenopausal patient with 88% of patients over 50 years and a peak incidence of 60–64 years of age. Other symptoms include an abdominopelvic mass, feeling of fullness in the abdomen and uterine prolapse. There may also be constipation or urinary frequency. Investigation is by dilatation and curettage under general anaesthetic, but now more commonly outpatient pipelle endometrial sampling. The retrieved fragments are usually very scanty and may require filtering from the formalin fixative. The role of the pathologist is not to phase the endometrium but to comment on whether any functional endometrium is actually represented, and if so, if it is benign, atypical or malignant. Atypical endometrium may represent a false negative sample of a concurrent adenocarcinoma. Investigation also includes transvaginal ultrasound scan which can relate the endometrial stripe thickness to the menopausal status (postmenopausal is usually <5 mm), and detect any focal lesions, e.g. polyps. Hysteroscopy allows direct visualisation of the uterine cavity and more extensive sampling. Transcervical resection of the endometrium (TCRE) is reserved for benign dysfunctional endometria in premenopausal patients. If there are histological features suspicious of or diagnostic of malignancy in biopsy material, MRI scan is used to assess tumour stage, in particular the depth of myometrial invasion, and the presence of cervical or extrauterine involvement. CT scan assesses more distant spread.

A significant proportion of patients with atypical hyperplasia and early stage well differentiated endometrioid adenocarcinoma may respond to progesterone therapy, although this is excluded by radiological evidence of myoinvasive disease. In general treatment of uterine cancers (adenocarcinoma, carcinosarcoma, sarcoma) is by hysterectomy and bilateral

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*, https://doi.org/10.1007/978-3-030-27828-1_24

salpingo-oophorectomy, with peritoneal washings as part of the staging procedure. The surgical approach is often laparoscopic and vaginal. Modified radical hysterectomy (inclusive of vaginal cuff, parametria, omentectomy and limited regional lymphadenectomy) is considered for deeply myoinvasive cancers, those with cervical involvement, or high-grade cancers (serous, clear cell, undifferentiated, squamous cell). They, and cancers with lymphovascular invasion, may also require preoperative chemotherapy and/or post-operative chemoradiotherapy. Occasional locally advanced tumours are not amenable to resection and chemo−/radiotherapy is used as the first line of management. This can result in marked cytological changes, e.g. radiation atypia mimicking serous intraepithelial carcinoma, or taxane induced ring mitoses. Furthermore, significant tumour regression can lead to only microscopic residual foci of tumour masked by a necroinflammatory response, making assessment of pathological stage difficult. Vaginal vault recurrence of uterine cancer is relatively common and treated with further local surgery and/or radiotherapy. A rare, late complication is post irradiation sarcoma or carcinoma, within the field of treatment after a latent period of several years.

Gross Description

Specimen

- Curettage/pipelle sample (on an outpatient basis: some cases are also detected by routine cervical smear)/TCRE chippings.
- Subtotal/total/radical hysterectomy/bilateral salpingo-oophorectomy/limited pelvic lymph node dissection, omentectomy.
- Size (mm) and weight (g).
- Suboptimal fixation of the endometrium in a hysterectomy specimen can make accurate histological assessment problematic. This can be countered by post-surgical injection of formalin with a narrow caliber plastic stylet through the cervical os.

Tumour

Site

- Fundus, body, isthmus. Involvement of the lower uterine segment is unfavourable.

Size

- Length × width × depth (mm) or maximum dimension (mm).

Appearance

- Polypoid/papillary/solid/ulcerated/infiltrative/necrotic/haemorrhagic.
- Malignant mixed mesodermal tumours are typically fundal and polypoid in an elderly patient and may protrude inferiorly through the internal cervical os.

Edge

- Circumscribed/irregular.

Extent

- Infiltration endometrium, myometrium, serosa, cervix, parametrium.

Adjacent Endometrium

- Atrophic, hyperplastic, polypoid.

Histological Type

The vast majority of uterine cancers are *adenocarcinoma of two main types,* although there is overlap between the categories

- *Type I (prototype: endometrioid adenocarcinoma):* peri-/postmenopausal, low parity, high socio-economic status, obesity, diabetes, hypertension, hyperoestrogenism (hormonal therapy, endogenous or secreting tumour, e.g. ovarian sex cord-stromal), ER positive, background endometrial hyperplasia. Microsatellite instability/PTEN (phosphatase and tensin homologue) mutations.
- *Type II (prototypes: serous and clear cell adenocarcinomas, carcinosarcoma):* older patients, more aggressive, atrophic endometrium with precursor serous endometrial intraepithelial carcinoma (EIC), ER±. p53 mutations.

A minority of endometrial cancers are *familial*, or associated with *hereditary non-polyposis colorectal cancer (HNPCC)* where incidence (2%) approximates that of bowel cancer. Adenocarcinoma is usually of endometrioid type, but distinctive clues to these microsatellite unstable cancers are tumour in the lower uterine segment and of undifferentiated morphology with a prominent infiltrate of peri-/intratumoural lymphocytes (TILs). This can be further explored with mismatch repair immunohistochemistry. Significance as to prognosis is uncertain. Cumulative dose of *tamoxifen in patients with breast cancer* is also a risk factor for endometrial carcinoma.

Endometrioid Adenocarcinoma

- *70–80% of cases.*
- Typical: low-grade well differentiated endometrial type glandular pattern, in perimenopausal patients, and due to unopposed oestrogenic drive ± adjacent endometrial hyperplasia.
- Variants:
  - *With squamous differentiation*—up to 30% of cases. The tumour is graded on the glandular component as it can be difficult to tell if the squamous cell element is benign or malignant. Where both elements are well differentiated the previous designation of adenocanthoma is now rarely used.
  - *Secretory carcinoma*—the cells resemble secretory endometrium.
  - *Ciliated carcinoma*—rare, the cells resemble tubal epithelium.
  - *Villoglandular carcinoma*—low-grade, well differentiated and papillary. Exclude high-grade serous carcinoma (high-grade nuclear characteristics with a tufted papillary pattern).
  - *Sertoliform carcinoma.*

Serous (Papillary) Adenocarcinoma

- *5–10% of cases.*
- *High-grade* in the elderly and de-novo with no adjacent hyperplasia but associated with *serous EIC.*
- Typically shows *disproportionate lymphovascular/myometrial invasion* and *omental spread* relative to the amount of endometrial disease. This means that EIC or stage I serous carcinoma can be associated with *extensive extrauterine spread* and present as a *primary peritoneal serous adenocarcinoma* but no clinically or radiologically obvious uterine or ovarian mass lesion.
- Potentially *multifocal* with extrauterine lesions e.g. ovary, fallopian tube.
- High-grade nuclear characteristics, usually a papillary pattern but occasionally tubuloglandular.
- Necrosis and psammoma bodies are often seen. p53 typically mutated (immunochemistry either overexpressed or "null" type), HER2, p16, PTEN, HMGA2 and Ki-67. ER ±/PR-. It is also WT-1 negative in distinction from ovarian papillary serous adenocarcinoma which is positive and this is a useful feature in differential diagnosis and staging.
- *Poor prognosis* with *30% 5 year survival.*

Clear Cell Adenocarcinoma

- *1–5% of cases;* postmenopausal. Not related to diethylstilboestrol and *aggressive* with myometrial invasion.
- >50% of the cells are clear cell, mixed solid/glandular/tubulocystic/papillary architecture.

Mucinous Adenocarcinoma

- >50% cells have stainable mucin.
- *Usually low-grade, minimally invasive and good prognosis.*
- Distinguish from cervical adenocarcinoma by differential biopsy/curettage, and exclude a gastrointestinal primary (clinical history, CK20/CDX-2 positive/CK7 negative).

Squamous Cell Carcinoma

- In old age often with cervical stenosis and pyometra; exclude spread from a cervical carcinoma.

Mixed

- Second component is >10% of the tumour area, e.g. composite endometrioid/serous/clear cell adenocarcinoma. Any carcinoma with *5–10% serous characteristics* tends to behave more aggressively and should be designated as such. Adenocarcinoma with squamous cell differentiation is excluded. Adenosquamous carcinoma (where both components are obviously malignant) is a mixed lesion with a poor prognosis.

Undifferentiated Carcinoma

- Small cell/not otherwise specified: *aggressive.*
- Spread from a cervical primary or lung primary must be excluded in small cell carcinoma.
- It can be difficult to distinguish between *high-grade endometrioid, serous and undifferentiated uterine carcinomas* and more recognisable foci should be sought with additional tissue blocks. *Undifferentiated carcinoma* shows a sheeted dyscohesive pattern of large, monomorphic, mitotically active cells sometimes with rhabdoid cells, giant cells, necrosis and myxoid stroma. It lacks a minor component of more usual serous or endometrioid cancer and is only focally cytokeratin positive. More than *50% present with advanced stage* and *ultimately fatal disease.* Occasional cases are in younger patients, in the lower uterine segment and associated with DNA mismatch repair abnormalities.

Malignant Mixed Müllerian Tumours (MMMT)

- *Low-grade malignancy:* adenosarcoma; carcinofibroma. Both are rare.
- *High-grade malignancy:* carcinosarcoma/sarcomatoid carcinoma is a carcinoma comprising either serous or endometrioid endometrial adenocarcinoma with a *biphasic pattern* and component of vimentin/cytokeratin positive malignant spindle cells. Lesions can be either *homologous* or *heterologous* (containing tissues alien to the uterus, commonly immature/malignant cartilage, striated muscle, bone). Those tumours with heterologous elements have a worse prognosis than those where the mesenchymal component is homologous. Carcinosarcoma is the commonest malignant mixed tumour and 50% contain heterologous elements. It is considered a malignancy of epithelial origin and staged as other endometrial carcinomas (see section "Other Pathology" for further discussion).

Metastatic Carcinoma

- *Direct spread:* cervix, bladder, rectum.
- *Distant spread:* infiltrating lobular breast carcinoma, kidney, malignant melanoma, stomach, pancreas. The commonest are breast, stomach, colon and pancreatic carcinomas. Often myometrial with an endometrial component, metastases can mimic primary endometrial disease e.g. infiltrating lobular breast carcinoma (endometrial stromal sarcoma), renal carcinoma (clear cell adenocarcinoma), and colorectal carcinoma (mucinous adenocarcinoma). A relevant *clinical history* and *comparison with previous histology* are crucial in making the distinction. Metastases should be considered in any endometrial cancer of unusual appearance, multinodular growth pattern, with prominent lymphovascular involvement or lack of precancerous endometrial changes.

Differentiation

- FIGO grade 1/2/3 for endometrioid adenocarcinoma. Based on architectural and nuclear features.

The glandular component of endometrioid adenocarcinomas is graded 1 (<5%), 2 (6–50%), and 3 (>50%) based on the proportion of non-squamous, non-morular solid growth pattern

(figures in parenthesis indicate percentage solid component for that grade). The presence of high grade nuclear features in >50% of the tumour raises the overall grade by 1 of architecturally grade 1 and 2 tumours: nuclear grade 1 (oval nuclei, even chromatin, inconspicuous nucleolus, few mitoses) and nuclear grade 3 (irregular, rounded nuclei, prominent nucleoli, frequent mitoses). Nuclear grade 2 is intermediate between grades 1 and 3. Some cancers show grade heterogeneity indicative of tumour progression. In tumours with squamous cell differentiation, grading is based on the glandular component. Serous, clear cell, carcinosarcoma and undifferentiated carcinomas are automatically considered overall grade 3 with nuclear grade taking precedence over architecture. Mucinous carcinomas are generally grade 1. There may be a discrepancy in grading between biopsy and resection material and a provisional grade is often provided on initial material.

Extent of Local Tumour Spread

- Border: pushing/infiltrative.
- Lymphocytic reaction: prominent/sparse.

Endometrium

- *EIN (endometrial intraepithelial neoplasia)* is an umbrella term encompassing and highlighting the diagnostic difficulties there are in distinguishing between entities on the overlap spectrum of complex endometrial hyperplasia with atypia, and, intra-endometrial adenocarcinoma. Progression along this spectrum is characterised by increased *glandular crowding and complexity*, *intraglandular necrosis* and *cytological atypia* with *reduction in intervening stroma*. An important differential diagnosis is a benign mimic with focal glandular crowding e.g. endometrial polyp (look for covering epithelium and prominent vascular stroma with thick walled vessels). Another possible consideration is atypical polypoid adenomyoma arising from the isthmus in younger patients (look for a glandular proliferation in abundant smooth muscle).
- *EIC (endometrial intraepithelial carcinoma)* is effectively serous adenocarcinoma in situ of the endometrial surface epithelium and is present *adjacent to or overlying 90% of serous adenocarcinomas*. p53 is typically mutated (either overexpressed or "null" type) and the Ki-67 proliferation index is high. It is also occasionally seen with clear cell carcinoma and even extrauterine peritoneal disease in the absence of invasive endometrial cancer.
- *EIN* and *EIC* are to be distinguished from the range of relatively commonly occurring *endometrial metaplasias* and *reactive epithelial changes.* These include ciliated tubal, mucinous, squamous morular and intestinal metaplasias, and surface syncytial, papillary, eosinophilic and clear cell changes.

Myometrium

- Proportion of wall involved <50%, ≥50%.
- If ≥50% on MRI scan a radical hysterectomy is considered. Outer myometrial involvement is seen in about 25% of cases.

Extent of myometrial invasion relates to the histological type and grade of carcinoma. True myometrial invasion must be distinguished from both a normal irregular outline or expansion of the endo-/myometrial junction (look for a continuous line of myometrial vascular structures in a compressed atrophic myometrium), and, abnormal epithelium in pre-existing adenomyosis (look for periglandular endometrial stroma—CD10 positive). Invasive stromal desmoplasia and inflammation are useful diagnostic clues although often not present in carcinoma. Alternative patterns of invasion should also be considered e.g. diffuse along a broad pushing front, or, MELF (microcystic, elongated, fragmented). Endometrial/myometrial junction and endometriosis containing carcinoma usually have a broad or lobulated front and smooth outline associated with native endometrial stroma. To some extent this dilemma is superseded by FIGO staging with stage I disease

encompassing any carcinoma confined to the endometrium or up to <50% of the myometrium. An approximate landmark for the latter is invasion less than or beyond the myometrial vascular arcuate plexus. Occasionally a patient is a non-operable surgical candidate due to co-morbidity and a TCRE specimen is provided—assessment of myoinvasion can be problematic due to the random orientation of the chippings.

Serosa

- Distance (mm) of the deepest point of invasion from the nearest serosal surface. Direct serosal spread is relatively unusual but is associated with a 40% 5 year survival rate and a tendency for recurrences in extra-abdominal sites.

Vagina

- Vaginal recurrences are not uncommon and treated by further local surgery or radiation therapy. The rare situation of clinical presentation of endometrial carcinoma with a vaginal metastasis is adverse with a median survival of 1–2 years.

Parametrium

- Parametrial involvement either by direct extension or lymphatic invasion is an *indicator of poor prognosis*. It can only be assessed when there has been a modified radical hysterectomy, i.e. for a preoperative diagnosis of a high-grade cancer or where imaging has suggested cervical or deep myometrial involvement.

Endocervix/Exocervix

- *10% of cases* usually by direct invasion, lymphatic spread or occasionally by implantation following curettage. *Stromal invasion* increases the *risk of pelvic lymph node metastases.* It must not be confused with post biopsy or cut-up artifactual 'carry-in', florid reactive changes following biopsy sampling or curettage, or other benign mimics of neoplasia, e.g. mesonephric remnants or tuboendometrial metaplasia. There is considerable interobserver variation in assessing whether there is tumour involvement of endocervical glands versus cervical stroma. Distinction between an *endometrial* and *cervical origin for a tumour* can be difficult clinically, radiologically and histologically in curettage samples. Some reliance is placed on the *nature of the tissue from which the carcinoma appears to be arising,* e.g. dysplastic cervix or hyperplastic endometrium. *Immunohistochemistry* may also be of help in *low-grade adenocarcinomas* in that uterine endometrioid adenocarcinoma is usually vimentin/ER positive and CEA negative while cervical adenocarcinoma shows the reverse. In addition, p16 antibody also stains more strongly in cervical adenocarcinoma. Alternatively with high-grade or undifferentiated carcinoma endometrial lesions may be p53+/p16− and cervical cancers the reverse. Cervical squamous carcinoma may also be strongly p63 positive.

Fallopian Tubes/Ovaries

- Either by direct extension or metastatic spread. Lymphovascular invasion favours the latter. If extrauterine disease is confined to the ovaries or fallopian tubes 5 year survival is still relatively favourable at 75%.

Omentum

Involved/Not involved.

FIGO

FIGO staging is recommended and applicable to uterine carcinoma and carcinosarcoma.

I Tumour confined to the corpus:
A. Invades endometrium or less than half of the myometrium
B. Invades half or more of the myometrium

II	Tumour invades cervical stroma but does not extend beyond the uterus
III	Local and/or regional lymph node tumour spread:
	A. Invades uterine serosa and/or adnexa(e)
	B. Vaginal and/or parametrial involvement
	C. Metastasis to pelvic (C1) and/or para-aortic (C2) lymph nodes
IV	Tumour invades:
	A. Bladder and/or bowel mucosa[a] and/or
	B. Distant metastases including intraabdominal and/or inguinal lymph nodes

[a]Requires histological confirmation by biopsy. Invasion of the rectal wall or bladder wall is pT3. Positive peritoneal cytology is reported separately without changing the stage. "Frozen pelvis" is a clinical term meaning tumour extension through the parametrium to the pelvic wall(s) i.e. IIIB (Figs. 24.1, 24.2, and 24.3)

Lymphovascular Invasion

- Present/absent.
- Intra−/extratumoural. Usually at the invasive front of the carcinoma.
  - Seen particularly in *serous* and deeply *myoinvasive endometrioid adenocarcinomas.* Lymphovascular invasion in the outer myometrium is an adverse prognostic indicator but it does not upstage an otherwise superficially invasive (FIGO IA) carcinoma.
  - Beware vascular *pseudoinvasion* in laparoscopic hysterectomy (LAH) specimens and autolysis related cut-up *smear artifact.* It can also be difficult to separate true lymphovascular invasion from intramyometrial peritumoural *retraction artifact*. These distinctions are important as *lymphovascular invasion* is an *indicator for postoperative adjuvant therapy*. The presence of red blood cells, a perivascular lymphocytic infiltrate and a demonstrable endothelial lining (CD34, D2–40) are useful pointers. True vascular invasion is also unusual in early stage low-grade cancers. Note that it can also be mimicked by the cystic spaces (lined by epithelium rather than endothelium) of the MELF (Microcystic Elongated and Fragmented) pattern of myometrial invasion.

Lymph Nodes

Site/number/size/number involved/extracapsular spread.

Regional nodes: pelvic (obturator and internal iliac), common and external iliac, parametrial, sacral and para-aortic. A regional lymphadenectomy will ordinarily include a minimum of 10 lymph nodes.

The commonest sites of *extrauterine spread* are the *regional lymph nodes* and *ovaries.* Lymph node disease relates to the tumour grade, type, depth of invasion and lymphovascular involvement. *Invasion of the outer third of the myometrium* is associated with *lymph node metastasis in up to 33% of cases*. Pelvic and para-aortic lymph node metastases are associated with 65–85% and 35–45% 5 year survivals respectively. Initial spread is to pelvic lymph nodes but there can be skip metastasis to involve para-aortic lymph nodes alone. *Recurrences* are in the pelvis and vaginal vault. High-grade serous carcinoma tends to show extensive omental and pelvic disease. *Distant metastases* of uterine carcinoma are to lung, liver, bone, central nervous system and skin of the scalp.

Excision Margins

Distances (mm) to the serosa, tubal and inferior vaginal limits.

Other Pathology

Uterus

- *Polyp(s), hyperplasia (simple, or, complex with architectural ± cytological atypia), adenomyosis*.

Carcinoma occasionally develops within a preexisting *endometrial polyp* although this is increased in *tamoxifen therapy* (see below). It can be of either usual low-grade endometrioid or high-grade serous type.

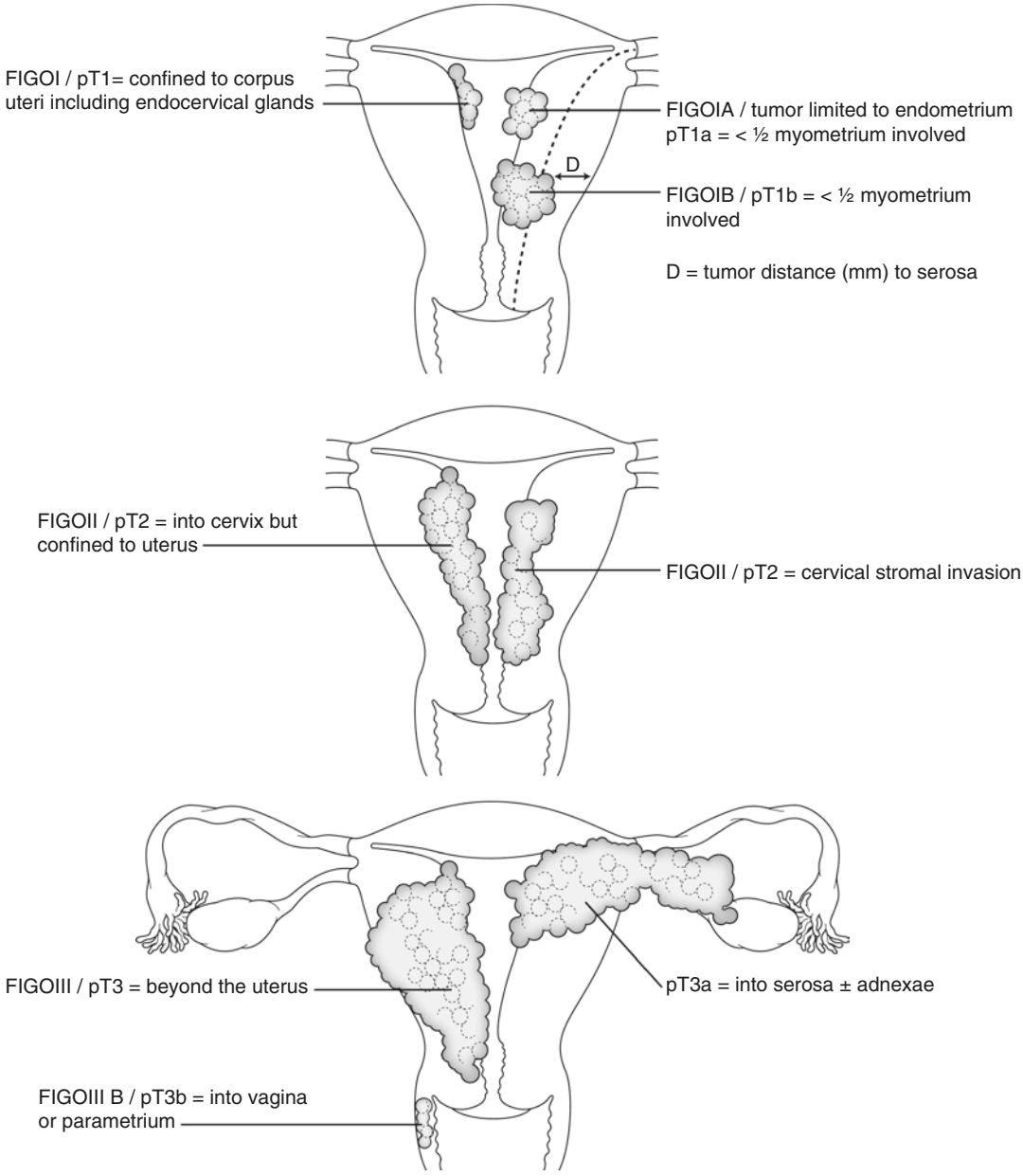

Fig. 24.1 Uterine carcinoma. Reproduced, with permission, from *Histopathology Reporting: Guidelines for Surgical Reporting*, *2nd ed.,* © 2006, Springer

About *25% of untreated atypical hyperplasias progress to adenocarcinoma* and up to *40% are associated with concurrent disease*. Features favouring *adenocarcinoma* over complex hyperplasia with cytological atypia are: *intraglandular epithelial bridges, stroma free papillary and cribriform epithelial patterns, intraglandular polymorphs and necrosis, cytological atypia, mitoses and stromal elimination and invasion.* Criteria for stromal invasion include: a. irregularly infiltrating glands associated with a fibroblastic or desmoplastic response, and/or, b. extensive papillary or confluent glandular (cribriform) growth patterns. Stromal and superficial

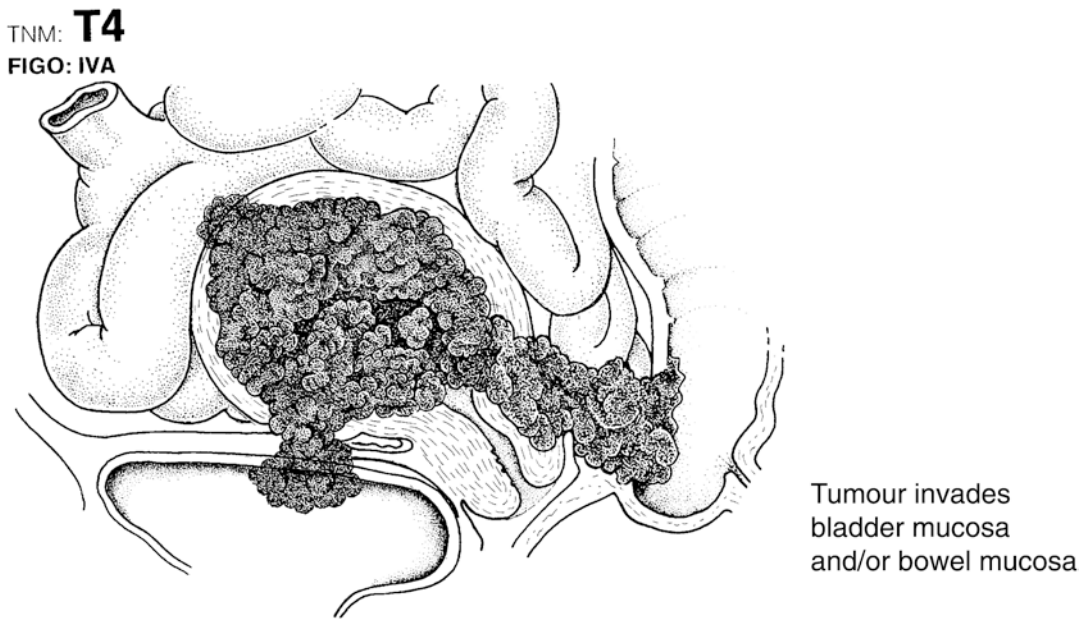

Fig. 24.2 Uterine carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

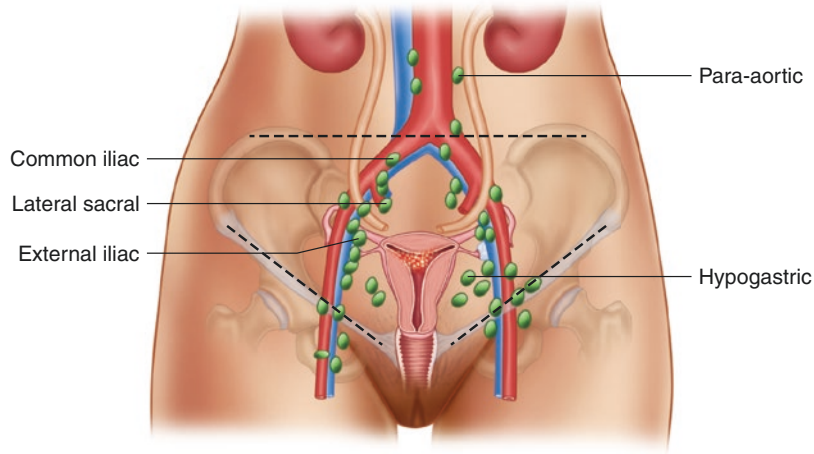

Fig. 24.3 Uterine carcinoma: regional lymph nodes. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

myometrial invasion are useful in distinguishing between intra-endometrial and invasive adenocarcinoma in curettage specimens. Inhabitation of *adenomyosis* by complex hyperplasia or adenocarcinoma usually shows circumscribed foci with surrounding native endometrial stroma.

Ovary

- *Thecoma* and *granulosa cell tumour:* can result in oestrogenic drive to the endometrium resulting in simple or complex hyperplasia, or adenocarcinoma.

- *Accompanying ovarian carcinoma*: this is seen in 5–10% of endometrial carcinomas. Distinction between *synchronous primary lesions* and *ovarian secondaries* rests on the latter being bilateral, multinodular, often serosal and with ovarian stromal lymphovascular invasion. The uterine primary will usually be high-grade with deep myometrial and lymphovascular invasion. There is a higher frequency of *concurrent early stage primaries in endometrioid adenocarcinoma of the uterus and ovary (25%)* than in the other cancer subtypes.

Other Comments

Tamoxifen effects: tamoxifen related polyps, hyperplasia, adenocarcinoma, adenosarcoma, carcinosarcoma. Tamoxifen is an anti-oestrogen but has paradoxical oestrogenic effects on the endometrium leading to an increased frequency of a range of endometrial neoplasms some of which are prognostically adverse. Polyps can be large, multiple, necrotic, have areas of glandular, papillary or clear cell metaplasia, and may even harbour adenocarcinoma, often serous in type.

Carcinosarcoma: is often polypoid at the fundus of an elderly patient with deep myometrial and lymphovascular invasion. About *30% present with stage III/IV disease.* The carcinomatous component is usually *high-grade glandular* (endometrioid, clear cell, serous or undifferentiated), and the *sarcomatous element homologous* (cf. endometrial stroma, leiomyosarcoma, fibrosarcoma) or *heterologous* (striated muscle, cartilage, bone, fat). Heterologous elements portend a worse outcome. Immunohistochemistry for epithelial and mesenchymal markers, e.g. desmin may be necessary to confirm the diagnosis. This *aggressive neoplasm has a 20–40% 5 year survival.*

Adenosarcoma: the majority (80%) of adenosarcomas arise in the postmenopausal endometrium. They are polypoid with proliferative type glands and usually homologous stromal type sarcoma distributed in a condensed periglandular cambium layer. *Recurrence (30%)* relates to mitoses, stromal overgrowth and myoinvasion in this *low- to intermediate-grade malignancy*.

Immunophenotype

Endometrioid adenocarcinoma usually co-expresses cytokeratins (7, 8, 18, 19) and vimentin. Oestrogen and progesterone marking is common relating to histological type and grade, and is of some use in assessing potential response to hormonal therapy in disseminated disease. CEA stains weaker than in cervical carcinoma. The squamous morules seen in endometrioid carcinoma stain positively with CDX-2. In contrast to grade 3 endometrioid adenocarcinomas, high-grade *serous adenocarcinomas* over express p53, p16, PTEN, HMGA2 and may be negative for ER/PR. These immunoprofiles may be of use in distinguishing high-grade endometrioid adenocarcinoma from serous adenocarcinoma with a tubuloglandular pattern. Uterine serous adenocarcinoma is also WT1 negative in contrast to ovarian serous adenocarcinoma. DNA aneuploidy is an index of high-grade, advanced stage tumours and over expression of Ki-67, p53 and HER2 (20–40% of cases) is adverse.

Prognosis

Radical hysterectomy is considered for endometrial carcinoma if there is ≥50% myometrial invasion with grade 2 or 3 histology, invasion of the cervix, undifferentiated, clear cell or serous adenocarcinoma, lymphovascular invasion or suspicious lymph nodes on CT scan. *Preoperative adjuvant chemo−/radiotherapy* may also be used in these circumstances. *Intraoperative frozen section* of suspicious lymph nodes is an important prequel to radical resection, and if positive a more conservative approach adopted.

Overall 5 year survival for endometrial carcinoma is *80–85%* with *type I* oestrogen-related cases arising from a background of hyperplasia typically presenting with stage I disease, and doing better than *type II* non-oestrogen-dependent lesions *(30–70% 5 year survival).* Serous,

undifferentiated, squamous cell and clear cell carcinomas are more aggressive than equivalent stage endometrioid tumours. *Lymphovascular invasion,* which correlates with progressing tumour grade, myometrial invasion and stage (cervical and extra-uterine spread), is an *adverse prognostic factor* (70–75% 5 year survival). Prognosis also relates strongly to *tumour stage:* I, 82–95% 5 year survival; II, 60–80%; III, 30–50%. *Tumour grade* has an influence in that grade 1 tumours (87% 5 year survival) fare better than grade 3 (58% 5 year survival).

Other Malignancy

Endometrial stromal lesions can be benign (stromal nodule) or malignant, the latter having infiltrating margins. Biopsy or curettage samples impose limitations in making this assessment, which is more appropriately done on a hysterectomy specimen.

Endometrial Stromal Sarcoma

- A *low-grade malignancy resembling endometrial stroma* (stromal cells/spiral arteriole vascular pattern) with infiltrative margins and variable mitoses (usually <5–10/10 high-power fields). Shows characteristic *lymphovascular invasion* (previously called endolymphatic stromal myosis). It comprises 20% of uterine sarcomas and 2% of uterine malignancies. An *80–90% 5 year survival rate* if confined to the uterus, but prone to *local pelvic or abdominal recurrence (30%) after a lag period of many years,* and may cause pressure effects, e.g. hydronephrosis. *High stage tumours* have a *40% 10 year survival rate*. Sometimes hormone responsive to adjuvant progesterone therapy.

Undifferentiated Uterine Sarcoma

- Previous and current alternate designation as high-grade endometrial stromal sarcoma. Characterised by cellular pleomorphism, mitoses (>10/10 high-power fields) and destructive myometrial invasion with infiltrating margins and aggressive behaviour. *Size of tumour (>4 cm)* and *extrauterine extension* are adverse indicators in low- and high-grade lesions. Treatment is surgical although there is some evidence for partial response to chemotherapy and hormonal manipulation in metastatic disease.

FIGO staging for uterine sarcomas

I	Tumour confined to the uterine corpus
	A. ≤5 cm
	B. >5 cm
II	Tumour extends beyond the uterus, within the pelvis
	A. To adnexa(e)
	B. To extrauterine pelvic tissues
III	Tumour invades abdominal tissues
	A. One site
	B. Two sites
	C. Regional lymph nodes
IV	Tumour invades:
	A. Bladder and/or rectum
	B. Distant metastases.

Immunophenotype of mesenchymal tumours: endometrial stromal sarcomas are CD10, WT1, ER, PR and vimentin positive. Up to 50% are desmin positive but they are h-caldesmon negative. Low-grade lesions also preserve a pericellular reticulin pattern. Differential diagnosis of endometrial stromal sarcoma is leiomyomatous tumour (strongly desmin/h-caldesmon positive ± CD10), and that of undifferentiated uterine sarcoma is undifferentiated carcinoma (cytokeratin, EMA positive).

Leiomyomatous Tumours

- *Malignancy relates to a combination of:*
  - Infiltrative margins.
  - Cellular atypia.
  - Coagulative tumour cell necrosis.
  - Mitoses >10/10 high-power fields.
  - Leiomyosarcoma (1–3% of uterine malignancies/40–50% of uterine sarcomas) is usually a high-grade lesion with a bulky

mass, satellite nodules, areas of necrosis and variably poor outlook (20–70% 5 year survival). Surgical excision with or without adjuvant chemotherapy is the mainstay of treatment. Variants are typical (spindle cells), epithelioid and myxoid. Tumour cells are smooth muscle actin, desmin, h-caldesmon, calponin and vimentin positive with cross reactivity for cytokeratin (CAM 5.2) and variable ER expression.

- *Uncertain malignant potential:*
  - Cellular atypia and mitoses 5–10/10 high-power fields indicate probable malignancy if the atypia is moderate or severe.
- *Cellular leiomyoma:*
  - Benign; identify thick walled vessels and strong desmin positivity to distinguish from an endometrial stromal tumour.
- *Mitotically active leiomyoma:*
  - Benign if no significant cytological atypia, abnormal mitoses or coagulative tumour cell necrosis.
- *Cell type:*
  - Myxoid and epithelioid leiomyosarcomas have less tumour cell necrosis, cytological atypia and mitotic activity. Relatively bland myxoid lesions can locally recur and metastasise. An infiltrative growth pattern is a useful diagnostic feature.
- *Beware pseudomalignancy:*
  - Bizarre *symplastic leiomyoma*. Benign if the symplastic nuclear change is focal, the mitotic count is low (<3/10 high-power fields) and coagulative tumour cell necrosis is absent.
  - Changes after *gonadotrophin analogue treatment*, *viz*. haemorrhage and necrosis, symplastic type nuclear atypia and apparent hypercellularity. Extensive coagulative necrosis after uterine artery embolisation can also confuse, but other features of malignancy such as atypia and mitoses are usually absent.
  - *Intravenous leiomyomatosis* with vascular invasion and "metastases" but not malignant.

Choriocarcinoma/Placental Site Trophoblastic Tumour (PSTT)

- After abortion, normal or molar pregnancy.
- See Chap. 28.

Malignant Lymphoma/Leukaemia

- See Chap. 25.

Others

- Haemangiopericytoma, angiosarcoma, soft tissue sarcomas and germ cell tumours are all rare.

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Cervical Carcinoma

25

Rajeev Shah and Aaron Ervine

Introduction

Cervical cancer is the second commonest cancer in females worldwide. Its incidence in Western Society is decreasing due to screening programmes and this will be improved upon by HPV (Human Papilloma Virus) vaccination Schemes. A majority of deaths related to cervical cancer are in developing countries. Risk factors for cervical intraepithelial neoplasia and cancer are HPV infection, early age at onset of sexual intercourse, multiple sexual partners, and smoking.

Cervical epithelial lesions are often detected because of an abnormal smear as part of a cervical screening programme. A persistent or high-grade abnormality is referred for colposcopic visualisation of the squamocolumnar epithelial transformation zone to delineate abnormal areas of mucosa characterised by punctation, mosaicism and loss of uptake of iodine (acetowhite epithelium). Cervical punch biopsy determines the nature of the abnormality, which if localised, is thermally ablated or resected by large loop or cone biopsy depending on its extent. The aim is to completely excise any precancerous lesion. Specimens are orientated, serially sliced and all processed with standard step sections to establish the nature (squamous cell or glandular) and grade of the lesion, the presence of any invasive component, and relationship to the ectocervical, endocervical and deep margins. Close histocytological correlation is required for accurate reporting and smear follow up of completely excised lesions is for 5–10 years with subsequent return to usual screening programme intervals. A proportion of established cervical cancers are asymptomatic, in the older age group and undiscovered due to non-attendance at cervical smear appointments. Some result from misinterpretation and undercalling, or non-representative sampling of previous smears in what is a screening programme with inevitable false negative cases. Symptomatic disease (e.g. postcoital bleeding) requires clinical examination, and if a cancer is suspected, a targeted wedge biopsy rather than a punch biopsy taken as this has a greater chance of establishing invasive disease. Tumour staging is by MRI scan (for local spread) and CT scan (for distant spread). Occasionally PET scan is done for distant metastases, and if imaging cannot exclude bladder or bowel involvement, cystoscopy and sigmoidoscopy may be required.

Cold cone knife biopsy may be considered for small cancers or if a cervical glandular lesion is suspected. However, in general, with tumours greater than FIGO stage IA, radical hysterectomy inclusive of pelvic lymphadenectomy is carried out. Laparoscopic vaginal hysterectomy is used for tumours ≤2 cm. Intraoperative frozen section

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_25

of clinically suspicious lymph nodes or sentinel lymph node mapping is sometimes indicated before proceeding to radical surgery. In occasional cases in young women with a FIGO IB tumour ≤2 cm a fertility sparing radical trachelectomy (upper vagina, cervix, parametria) with laparoscopic lymphadenectomy is performed. It can be associated with up to a 50% chance of successful subsequent pregnancy. Postoperative chemoradiation is indicated for patients with positive pelvic lymph nodes, and, radiotherapy for lymph node negative patients but with more than one third depth stromal invasion, lymphovascular invasion or tumour diameter >4 cm. Indications for pelvic exenteration are invasion of adjacent organs, recurrent disease and severe pelvic irradiation necrosis, in the absence of distant extrapelvic metastases on CT/PET scan. Advanced disease may present with ureteric obstruction and chronic renal failure, haematuria or rectal symptoms. It can be treated by primary chemoradiation with subsequent salvage hysterectomy, or exenteration if deemed appropriate by the clinical multidisciplinary team. Less than 5% of patients with recurrent disease are alive at 5 years.

Gross Description

Specimen

- Cervical smear/punch or wedge biopsy/diathermy (hot) or knife (cold) cone biopsy/LLETZ (large loop excision of transformation zone)/ hysterectomy/trachelectomy with laparoscopic lymphadenectomy/radical (Wertheim's) hysterectomy with vaginal cuff, parametria and lymphadenectomy/ pelvic (anterior/posterior/ total) exenteration (components: bladder, ureters, uterus, vagina, tubes and ovaries, rectum)
- Size (mm) and weight (g).

Tumour

Site

- Endocervix/ectocervix.
- Anterior/posterior.
- Lateral (right/left).

Size

- Length × width × depth (mm) or maximum dimension (mm).
- Early stromal invasion is breech of the basement membrane with scant stromal penetration <1 mm in depth.
- Microinvasion is a term that was inconsistently used and is no longer favoured. *Early cancer* is more appropriately designated by FIGO stage as defined by the depth and horizontal extent of invasive disease:
  - *Depth*: ≤3 mm (FIGO IA1) or if >3 mm and ≤5 mm (FIGO IA2) depth of invasion from the nearest (surface or glandular) basement membrane, usually involved by CIN/CGIN (cervical intraepithelial neoplasia/cervical glandular intraepithelial neoplasia).
  - *Horizontal extent*: ≤7 mm.
  - *Vessels*: venous or lymphatic permeation *does not alter the staging* but is taken into account for management decisions by the gynaecological oncologist.

Appearance

- Polypoid/papillary/nodular/solid/ulcerated/ burrowing. Ulcerated cancers generally infiltrate more deeply than polypoid ones.

Edge

- Circumscribed/irregular.

Extent

- Infiltration of cervical wall, parametria/paracervix, corpus uteri, vagina.

Histological Type

Squamous Cell Carcinoma

- *80% of cases.*
- Classical:
  - Keratinising
  - Non-keratinising: large cell/small cell. Non-keratinising large cell is recognisably squamous in character with cell stratification and intercellular bridges but no keratin pearls are present.

- Variants:
- *Verrucous:* exophytic and locally invasive, it may recur after excision and radiotherapy. It shows bland cytology with bulbous processes and a pushing deep margin.
- *Warty:* surface koilocytosis and an invasive deep margin.
- *Spindle cell:* upper aerodigestive tract analogue with tumour cell fibroplasia (sarcomatoid carcinoma).
- *Papillary:* two types of papillary neoplasm with either CIN like changes/in situ squamous cell epithelium, or, squamotransitional cell type epithelium. The latter occurs in post menopausal women and is associated with late recurrence and metastases (25%). Invasion at the base may be superficial or associated with more usual squamous cell carcinoma.
- *Basaloid:* an aggressive neoplasm comprising nests of basaloid cells with peripheral palisading and central keratinisation or necrosis.
- *Lymphoepithelioma like:* circumscribed margin, lymphocytic infiltrate, large uniform cells with a prominent nucleolus. It may have a better prognosis and is radiosensitive. ±EBV (Epstein Barr Virus) positive.

Adenocarcinoma

- *10–15% of cases*.
- *Endocervical:* 70% of cervical adenocarcinomas and variably glandular/mucinous related to the degree of differentiation which is usually well to moderate.
- *Endometrioid:* 25% of cervical adenocarcinomas, and exclude a uterine adenocarcinoma extending to cervix. Typically at the junctional zone arising from endometriosis/tuboendometrial metaplasia, and may coexist with usual endocervical type adenocarcinoma. A minimal deviation variant exists.
- *Minimal deviation (adenoma malignum):* late presentation and poor prognosis with bland epithelium showing mitoses and irregular gland extension deep (>50%) into the cervical stroma. CEA and p53 over expression may be of diagnostic help. Associated with Peutz-Jeghers syndrome.
- *Villoglandular:* good prognosis in young females. Papillary with CGIN type covering epithelium, connective tissue cores and indolent invasion at the base. More aggressive moderately differentiated variants occur and it can be associated with more usual cervical cancer subtypes. Also consider implantation from an endometrial primary.
- *Clear cell:* clear, hobnail cells, glycogen PAS positive, solid, tubules, papillae. Some are associated with in utero exposure to diethylstilboestrol.
- *Serous papillary:* poor prognosis and potentially multifocal in endometrium and ovary. High-grade cytological appearances ± psammoma bodies—exclude low-grade villoglandular adenocarcinoma.
- *Mesonephric:* from mesonephric duct remnants deep in the posterior or lateral cervical wall. Small glands with eosinophilic secretions.
- *Non-Müllerian mucinous:* intestinal including colloid and signet ring cell carcinomas. There is also a gastric type. Poor prognosis compared to usual endocervical-type adenocarcinoma (30% vs. 70% 5 year survival), and exclude a gastrointestinal secondary adenocarcinoma.

Poorly Differentiated Carcinoma

- *Scirrhous, undifferentiated.*
- In undifferentiated carcinomas also consider differential diagnoses such as sarcoma (epithelioid leiomyosarcoma), malignant melanoma and malignant lymphoma.

Mixed Tumours

- *Mixed type* (e.g. squamous cell/adenocarcinoma) and *differentiation* (e.g. endocervical/endometrioid adenocarcinoma).
- *Adenosquamous, solid with mucus production*: varies from well differentiated glandular and squamous cell components, to solid poorly differentiated squamous cell carcinoma with stainable PAS positive mucin production (up to 30% of cases) and which is *more aggressive* than usual squamous cell carcinoma.

Miscellaneous Carcinoma

- *Glassy cell:* a poorly differentiated adenosquamous carcinoma in young women.
- *Adenoid basal:* indolent and often an incidental finding at hysterectomy or cone biopsy. Organoid lobules and nests of cells with punched out lumina ± eosinophilic secretions. Strong association (90%) with overlying high-grade CIN or microinvasive squamous cell carcinoma.
- *Mucoepidermoid* and *adenoid cystic*: low-grade and indolent behaviour although recurrence/metastasis if incompletely removed.

Small Cell Carcinoma

- *Primary* or *secondary*. A poorly differentiated/high-grade neuroendocrine carcinoma. Chromogranin(focal)/synaptophysin/CD56 positive with poor prognosis. High Ki-67 index, TTF-1/CD99/p16/p63±. Treated by chemotherapy and not surgery.

Other Neuroendocrine Tumours

- *Atypical carcinoid* like tumour of intermediate-grade malignancy as well as classical well differentiated/low-grade *carcinoid tumour* (rare), and high-grade *large cell neuroendocrine carcinoma*.

Metastatic Carcinoma

- *Direct spread*: endometrium (commonest), colorectum, bladder.
- *Distant spread*: breast (especially infiltrating lobular), stomach, ovary.

Differentiation

Varies according to lesion type: e.g. keratinising squamous cell carcinoma is well to moderately differentiated, non-keratinising large cell moderate, and non-keratinising small cell poorly differentiated. About 60% are moderately differentiated. Tumour grade does not reliably predict prognosis and is only broadly indicative. However, grade 1 (small amount (≤10%) of solid growth with mild nuclear atypia) has a better prognosis than grade 3 (solid pattern (>50%) with severe nuclear atypia) adenocarcinoma. Undifferentiated carcinomas show no squamous cell or glandular differentiation (grade 4). Small and large cell neuroendocrine carcinomas are high-grade or poorly differentiated.

Extent of Local Tumour Spread

- Border: pushing/infiltrative.
- Lymphocytic reaction: prominent/sparse.

Infiltration

- Cervical wall, parametria, endometrium, myometrium, vagina.
- Depth through the cervical stroma and parametrium and distance to the nearest parametrial resection margin (mm).
- Infiltration of the superficial and deep thirds of the cervix have average disease free intervals of 94% and 73% respectively.

FIGO/TNM

FIGO staging is recommended with additional TNM8 comments on lymph node status. The classifications are applicable to cervical carcinomas

I	Carcinoma confined to the uterus (extension to the corpus is disregarded)
IA	Lesions detected only microscopically; maximum size 5 mm deep and 7 mm across; venous or lymphatic permeation does not alter the staging
IA1	Stromal invasion ≤3 mm deep and ≤7 mm horizontal axis
IA2	Stromal invasion >3 mm to ≤5 mm deep and ≤7 mm horizontal axis
IB	Clinically apparent lesion confined to the cervix or microscopic lesion larger than stage IA2 (occult carcinoma)

IB1	clinical lesions ≤4 cm in greatest dimension
IB2	Clinical lesions >4 cm in greatest dimension
II	Invasive carcinoma extending beyond the uterus but not to pelvic wall or lower third of vagina.
IIA	Without parametrial invasion
IIA1	Clinical lesion ≤4 cm in greatest dimension
IIA2	Clinical lesion >4 cm in greatest dimension
IIB	With parametrial invasion
III	
IIIA	Tumour involves lower third of vagina
IIIB	Tumour extends to pelvic wall wall, or causes hydronephrosis or non-functioning kidney
IV	Tumour involving mucosa[a] of urinary bladder mucosa and/or rectum or extends beyond the true pelvis. Adjacent organ involvement (IVA) or distant metastases (IVB).

[a]Bladder/rectal mucosal involvement requires confirmation by biopsy and involvement of bladder/rectal wall only is FIGO stage III

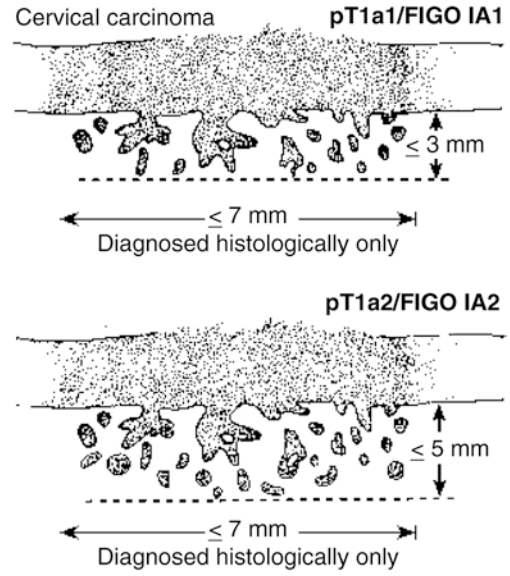

Fig. 25.1 Cervical carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

FIGO III refers to grossly or histologically evident continuous invasion beyond the myometrium into the parametrium and to "frozen pelvis"—a clinical term meaning extension to pelvic wall(s). Parametrial involvement is an indicator of poor prognosis and likely lymph node metastases. Positive peritoneal fluid is not considered in the FIGO classification.

Tumour spread: is typically to vagina, uterine corpus, parametria, lower urinary tract (ureters) and uterosacral ligaments. Involvement of regional lymph nodes relates to the stage of disease with lungs, brain and bone the commonest (5–10%) sites of distant metastases (Figs. 25.1, 25.2, 25.3, 25.4, 25.5, 25.6, and 25.7).

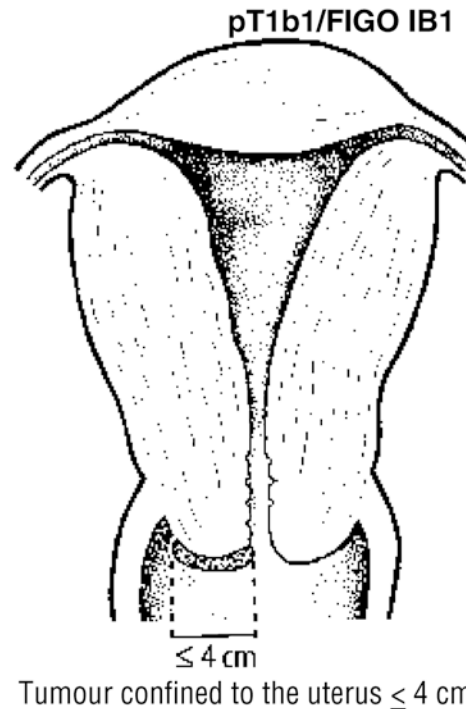

Fig. 25.2 Cervical carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

Lymphovascular Invasion

- Present/absent.
- Intra−/extratumoural.
- Lymphovascular invasion should be noted on biopsy material as it may influence the choice of more extensive surgical resection. Its presence and extent are a *strong indicator of poor*

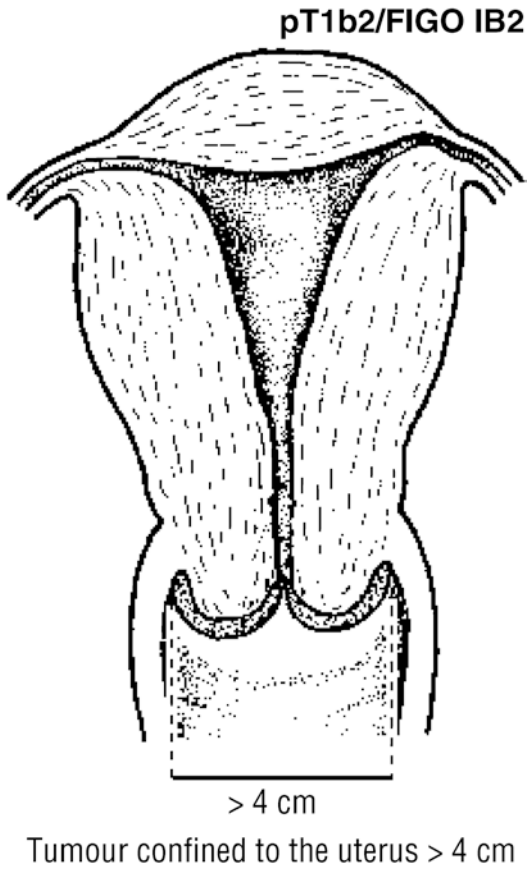

Fig. 25.3 Cervical carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

prognosis and the likelihood of lymph node metastasis and recurrence of tumour.

Lymph Nodes

- Site/number/size/number involved/extracapsular spread.
- Parametrial involvement increases regional lymph node metastases to about 35% of cases.
- Regional nodes (Fig. 25.8): paracervical[1], parametrial[2], hypogastric[3] (internal iliac, obturator), common[5] and external iliac[4], presacral[6], lateral sacral[7]. Para-aortic lymph nodes are not regional. A regional lymphadenectomy will ordinarily include a minimum of six lymph nodes. Intraoperative frozen section examination of suspicious lymph nodes may be done as a prequel to radical surgery, and if positive, a more conservative approach adopted.

pN0	no regional lymph node metastasis.
pN1	metastasis in regional lymph node(s).

Excision Margins

Distances (mm) to the nearest deep cervical (anterior and posterior), lateral parametrial and inferior vaginal resection margins.

In a trachelectomy specimen distances (mm) to the nearest deep cervical, lateral parametrial, proximal endocervical and distal ectocervical resection margins.

Other Pathology

HPV	Human papilloma virus infection causing an anogenital field change of viral lesions (flat koilocytosis/condyloma accuminatum), intraepithelial neoplasia and carcinoma.
CIN	Cervical intraepithelial neoplasia.
SIL	Squamous intraepithelial lesion (Bethesda system).
AIS/CGIN	Adenocarcinoma in situ (AIS) / high-grade cervical glandular intraepithelial neoplasia (CGIN).
SMILE	Stratified mucin producing intraepithelial lesion
VAIN	Vaginal intraepithelial neoplasia
VIN	Vulval intraepithelial neoplasia.
AIN	Anal intraepithelial neoplasia.
Bowenoid papulosis	A now less often used clinical term describing brown perineal patches in young women. HPV induced with histology of VIN 3 and a negligible risk of progression to carcinoma.

Evidence indicates that *"high-risk" HPV infection* results in *high-grade CIN (SIL)* with a higher rate of progression to carcinoma (high risk HPV types 16 and 18 are responsible for approximately 70% of cervical cancers). *"Low-risk" HPV* and *low-grade CIN* may potentially regress. HPV infection is also an aetiological factor in cervical glandular dysplasia (CGIN), which often

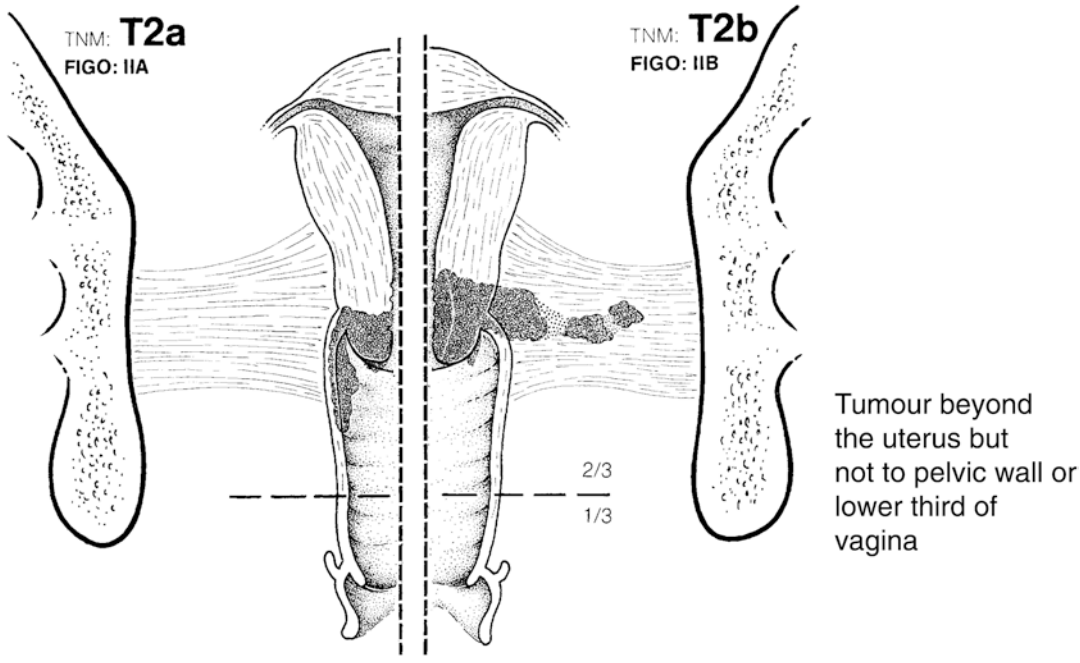

Fig. 25.4 Cervical carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

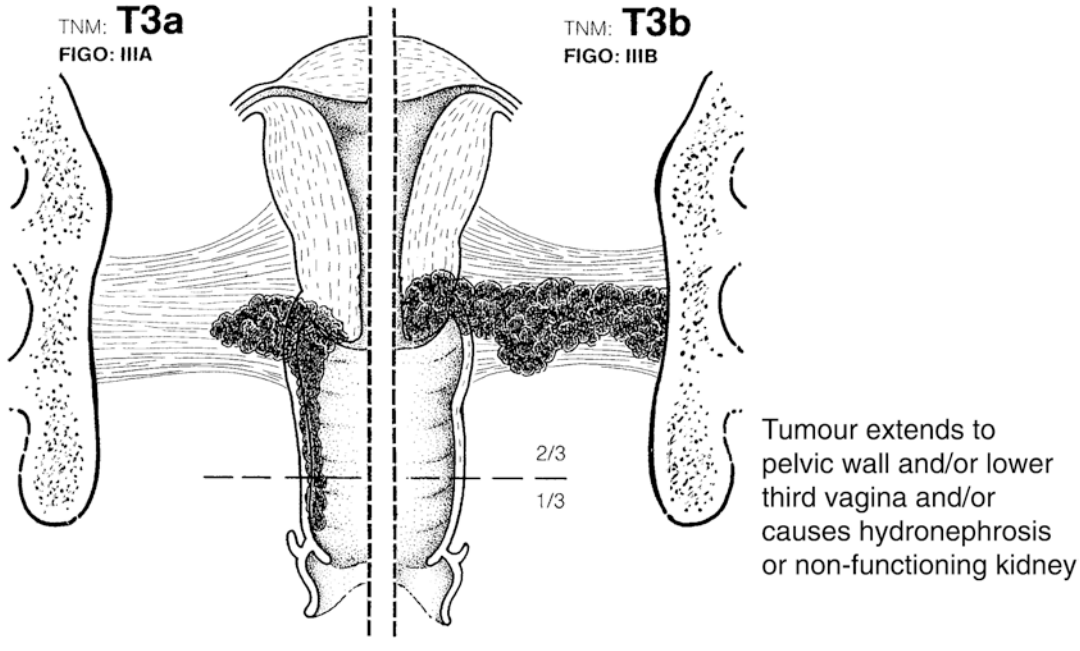

Fig. 25.5 Cervical carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

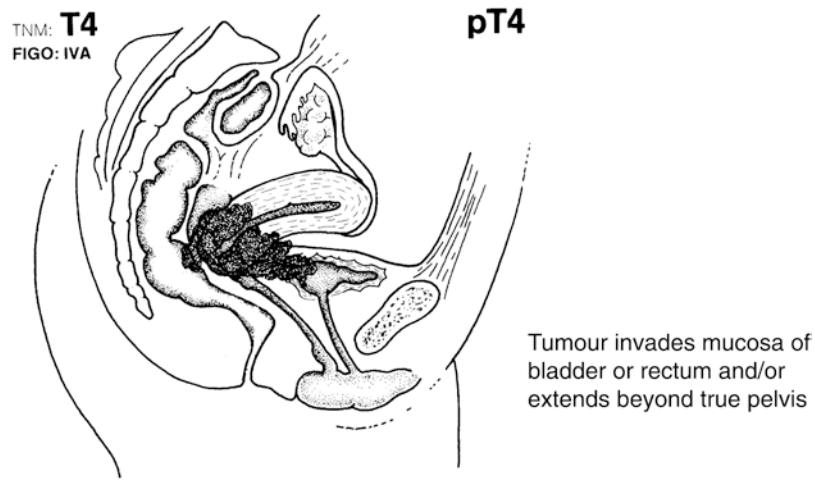

Fig. 25.6 Cervical carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

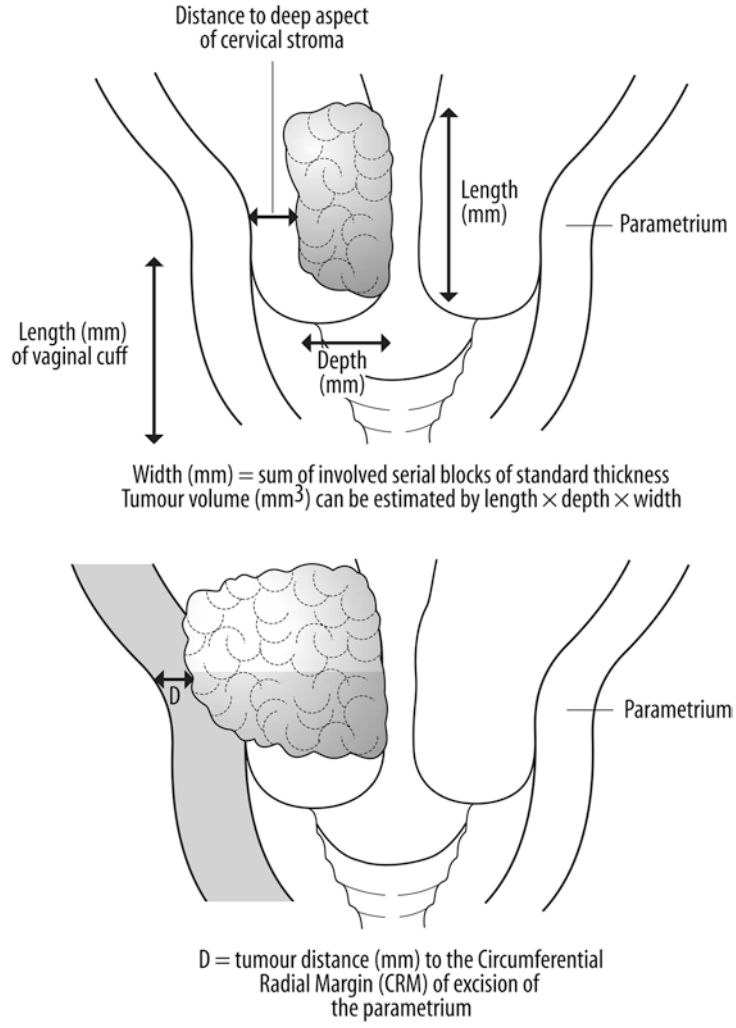

Fig. 25.7 Cervical carcinoma. Reproduced, with permission, from *Histopathology Reporting: Guidelines for Surgical Reporting*, *2nd ed.,* © 2006, Springer

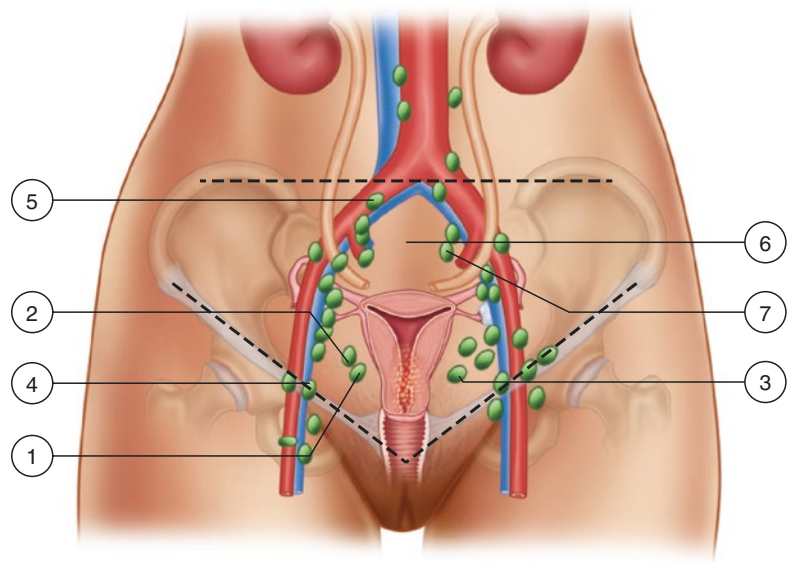

Fig. 25.8 Cervical carcinoma: regional lymph nodes. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

coexists with squamous epithelial dysplasia (CIN, SIL), and occasionally SMILE of intermediate squamocolumnar character.

High-grade CGIN also potentially progresses to invasive disease with a strong association between high-grade CGIN and invasive lesions. CGIN should also be distinguished from *benign mimics*: e.g. tuboendometrial metaplasia (TEM), endometriosis, endocervical microglandular hyperplasia, mesonephric remnants and endocervical gland tunnel clusters. CGIN shows an abrupt junction with normal epithelium, nuclear atypia, mitoses and apoptosis. Ciliation is less frequent than in metaplasia. Immunohistochemistry may also be of help in that CGIN has a high Ki-67 proliferation index (>10%), stains strongly with p53, shows strong diffuse "block" positivity with p16, and is focal or negative for bcl-2. Benign mimics such as TEM have a converse immunophenotype with this panel. Endocervical (commonest), intestinal and endometrioid variants are described. Ki-67 positive cells in the upper two thirds of the epithelium also reliably identifies CIN lesions in biopsy material. Retention of distinct immunophenotype may also help to distinguish neoplastic squamous or glandular epithelia from thermal cautery artifact at diathermy loop or conisation specimen tissue margins.

Low-risk	HPV types 6, 11	Koilocytosis, CIN 1
High-risk	HPV types 16, 18, 31, 33, 35, 39, 51	CIN 2/3
		Squamous carcinoma
		Typing by in situ hybridisation
CIN (SIL)	Low-grade	CIN 1, SIL 1
	High-grade	CIN 2, CIN 3, SIL 2

"High-risk" HPV DNA subtyping has a role in primary screening, quality assurance checking, and the closer follow up of women whose cervical smears shows BNA (borderline nuclear abnormalities)/ASC-US (atypical squamous cells of uncertain significance) changes.

Immunophenotype: cervical squamous cell carcinoma is AE1/AE3, CK5/6, p63 positive, and adenocarcinoma p16 and CEA positive.

FIGO IA Carcinoma

The higher the grade and larger the CIN lesion the more likely it is to show early invasion with a *35% risk of CIN 3 progressing to carcinoma over a ten year period.* FIGO IA carcinoma is not designated on small, limited biopsy samples but rather on a large biopsy specimen, e.g. cone

biopsy which allows removal and assessment of the whole lesion. Five year survival rates are about 95%. Risk factors for progression to clinically invasive carcinoma are increasing depth of invasion, increasing lateral extent (horizontal axis) of the lesion, lymphovascular invasion and incomplete removal by LLETZ/cone biopsy. Adverse factors in occult carcinoma (i.e. bigger than FIGO IA but not clinically detectable) are a depth of invasion >5 mm and lymphovascular invasion. Very occasional cases, where CIN has focal areas suspicious of penetrating the stroma but lacking definite evidence of invasion, may warrant a designation of "equivocal stromal invasion". Commonly occurring tangential cutting and extension of CIN into endocervical crypts must be excluded and is supported by an intact circumscribed basement membrane. An uncommon differential is squamous cell carcinoma with a CIN 3 like growth pattern, for which widespread, deep expansion with luminal necrosis are useful diagnostic clues. A rare mimic of cervical cancer is buried or entrapped atypical epithelium in the superficial cervical stroma following a previous biopsy.

The first stage of *early invasive squamous cell carcinoma* is recognised by budding of invasive cells with morphology similar to that of the overlying CIN lesion through the basement membrane. With lesion progression the tongues of tumour become *more differentiated or "hypermature" (so called paradoxical maturation/differentiation)* with cytoplasmic eosinophilia and nuclear clearing. A *stromal fibro-inflammatory reaction* is also seen. The distinction between adenocarcinoma in situ and early invasive adenocarcinoma is more difficult to define and measure with features such as *depth* beyond the normal endocervical gland field, *cribriform complexity and budding* of glandular architecture, *stromal fibroinflammatory reaction* and *proximity of abnormal glands to thick walled stromal vessels* of use.

Measurement of tumour extent in cervical carcinoma is readily obtained in the longitudinal and deep axes, whereas the transverse dimension depends on summation of the number of involved adjacent blocks of known thickness. About 10% of cervical carcinomas show multifocal stromal invasion characterised by clear separation of tumour by uninvolved tissue or an origin from different lips of the cervix. In this case the pathological stage is based on the width and depth of the largest tumour and not the combined dimensions of the various cancers.

Treatment

Treatment of cervical carcinoma is based on the tumour stage, patient age and consideration of fertility. Early invasive carcinoma may be treated with *loop or cold knife cone biopsy* ensuring a minimum 3 mm clearance of margins (note that there can be a small risk of skip or higher endocervical canal lesions), or *simple hysterectomy*. A fertility sparing procedure such as *radical trachelectomy* (removal of the upper vagina, cervix and surrounding parametria with laparoscopic lymphadenectomy) may be considered in young women with up to a FIGO IB1 tumour <2 cm in maximum dimension. *Radical hysterectomy* is indicated for larger tumours or where there is lymphovascular invasion. *Radiotherapy* produces tumour cell necrosis, degeneration, pleomorphism, maturation, inflammation and fibrosis. *Combination radio−/chemotherapy* (±brachytherapy) is used to augment radical surgery for tumours ≥ FIGO IB, or on its own for palliative control in high stage disease. Small cell neuroendocrine carcinoma is treated by chemotherapy rather than surgery.

Prognosis

Prognosis relates to tumour type and volume, invasion of endometrium, parametrium and vessels, and most importantly *stage of disease*. Overall 5 year survival rate is 55%, with stage I carcinoma 85–90% and 35%/10% for stages III/IV. Tumours with a glandular component, lymphovascular invasion and young age at diagnosis (<30 years) are more aggressive and more often positive for pelvic lymph node metastases. The incidence of cervical adenocarcinoma is increas-

ing and presents on average 5 years younger than squamous cell carcinoma. Anecdotally it has a worse prognosis than squamous cell carcinoma and radical surgery is undertaken. This adverse outlook may relate to presentation with more bulky disease and greater resistance to radiotherapy. However the risk of lymph node metastasis is broadly similar for equivalent early stage cervical adenocarcinoma and squamous cell carcinoma. Therefore, early FIGO 1A1 cervical adenocarcinoma can also be treated by local excision, or small 1B1 tumours by trachelectomy if preservation of fertility is a concern. Mixed differentiation tumours, and the coexistence of CIN and CGIN particularly on the surface and in crypts respectively, are sometimes seen. High-grade CGIN or adenocarcinoma in situ is usually treated by hysterectomy although conservative conisation may be used if the patient is young (<36 years) and wishes to remain fertile.

Other Malignancy

Malignant Melanoma

- Usually metastatic.
- Primary lesion is rare: 40% 5 year survival.

Embryonal Rhabdomyosarcoma

- Infancy/childhood.
- Syn. Sarcoma botryoides.
- Cellular subepithelial cambium zone/myxoid zone/deep cellular zone.
- Small cells/rhabdomyoblasts/desmin, myogenin and myo D1 positive.
- ±Heterologous elements.

Leiomyosarcoma

- 40–60 years.
- Cellular atypia, necrosis, and >5 mitoses/10 high-power fields.
- >10 mitoses/10 high power fields required if no atypia.

Adenosarcoma

- Polypoid.
- 25% have heterologous elements (striated muscle, cartilage, fat).
- A low-grade malignancy.

Stromal sarcoma and malignant mixed mesodermal tumour are more likely to represent spread to the cervix from an endometrial lesion rather than a primary cervical tumour.

Malignant Lymphoma

- More often secondary spread from systemic/nodal disease.
- Primary: 70% are intermediate to high-grade of large B cell type.
- 5 year survival is about 75%.

Leukaemia

- Granulocytic sarcoma as a presentation of chronic myeloid leukaemia.
- CD34/CD43/CD68/CD117/chloroacetate esterase /myeloperoxidase and neutrophil elastase positive.
- Relapse of AML, blast transformation of CML.

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Vaginal Carcinoma

26

Rajeev Shah and Aaron Ervine

Introduction

Vaginal pathology may be asymptomatic or present with bleeding, discharge, dyspareunia (pain at sexual intercourse), a feeling of discomfort or a mass. Clinical examination and direct visualisation by coloposcopy can show viral warts, dysplastic mucosal lesions vaginal intraepithelial neoplasia (VAIN), tumour and even changes related to diethylstilboestrol exposure (DES: see below). Vaginal smear, punch or wedge biopsy allow a tissue diagnosis, and the strong associations with any previous vulval, cervical and endometrial disease must be taken into account. Pelvic MRI scan is used to stage suspected tumour including the presence of pelvic or inguinal lymphadenopathy with the latter sometimes also amenable to investigation by fine needle aspiration cytology (FNAC). Surgery in the form of either wide local excision or radical vaginectomy is used for localised, or non-responsive or recurrent tumours, respectively, otherwise chemoradiation subject to assessment and discussion at a multidisciplinary meeting. Local excision, laser ablation and topical 5-fluorouracil are additional options for superficial mucosal wart or VAIN lesions. Pelvic exenteration is sometimes used for extensive local disease or post radiotherapy necrosis if there is no evidence of extrapelvic distant metastases on CT/PET scan.

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_26

Gross Description

Specimen

- Vaginal smear/biopsy/wide local excision/partial or subtotal vaginectomy/radical vaginectomy (with hysterectomy, salpingo-oophorectomy and lymphadenectomy)/pelvic exenteration.
- Weight (g) and size (mm), number of fragments.

Tumour

Site

- Anterior/posterior/lateral (right or left)/fornices. Usually anterior or lateral and upper third (50–60%).

Size

- Length × width × depth (mm) or maximum dimension (mm).

Appearance

- Polypoid/verrucous/papillary/sessile/ulcerated/pigmented.

- Exophytic lesions are commoner than endophytic and most are either nodular or ulcerative.

Edge

- Circumscribed/irregular.

Histological Type

Secondary carcinoma is more frequent than primary carcinoma in the vagina.

Squamous Cell Carcinoma

- *90–95% of primary vaginal carcinomas.*
- Keratinising/non-keratinising.
- Large cell/small cell.
- Mainly moderately differentiated keratinising.
- Variants
- *Verrucous:* exophytic, bland cytology with deep bulbous processes and a locally invasive pushing margin.
- *Warty (condylomatous):* with focal invasion at the base.
- *Spindle cell:* a cytokeratin positive sarcomatoid carcinoma.

Adenocarcinoma

- *Clear cell:* PAS positive for glycogen, solid/tubules/papillae. From 1970 to 2000 most patients were 14–25 years with in utero exposure to diethylstilboestrol (DES). Following withdrawal of DES and as this cohort ages this diagnosis is decreasing. Non-DES cases in the older age group are rare comprising clear/hobnail cells ± vaginal adenosis defined as the presence of any Müllerian type glandular epithelium, often endocervical, or, tuboendometrial in character. Differential diagnosis is vaginal adenosis with microglandular hyperplasia and Arias-Stella reaction in pregnancy or hormone therapy. Prognosis is relatively good (80% 5 year survival) if the tumour is small and superficial. Otherwise local recurrence and lymph node metastases usually occur within 3 years but sometimes late after many years.
- *Endometrioid:* possibly arising from previous endometriosis.
- *Mucinous:* endocervical or intestinal in type and the former may be associated with adenosis (endocervicosis). Note that rarely primary vaginal intestinal type adenoma of tubular/villous morphology can occur.
- *Mesonephric*: deep in the lateral vaginal walls arising from mesonephric remnants.

Adenosquamous Carcinoma

- Mixed differentiation and of worse prognosis.

Adenoid Cystic Carcinoma

- Indolent with late local recurrence and potential for metastases.

Adenoid Basal Carcinoma

- Indolent behaviour.

Small Cell Carcinoma

- Primary or secondary from cervix or lung. A poorly differentiated/high-grade neuroendocrine carcinoma. Paranuclear dot CAM5.2/synaptophysin/CD56/Chromogranin typically focally positive or negative, Ki-67 high proliferation index.

Undifferentiated Carcinoma

- No evident squamous cell or glandular differentiation.

Transitional Cell Carcinoma

- Primary (rare), or in association with concurrent bladder/urethral carcinoma.

Carcinoid Tumour

- Chromogranin/synaptophysin/CD56 positive. Low Ki-67 index (≤2%) and a well differentiated neuroendocrine tumour of low-grade malignancy.

Endodermal Sinus Tumour

- Yolk sac spectrum of appearances and AFP/glypican 3 positive in the posterior wall and fornices of infants. It is responsive to surgery and chemotherapy.

Malignant Melanoma

- *3% of cases* and mucosal junctional activity indicates a primary lesion. It is of *poor prognosis*.
- More often represents a *metastasis* e.g. from urethra or vulva.

Metastatic Carcinoma

- *Comprises 80% of malignant vaginal tumours, far outnumbering primary lesions*.
- *Direct spread*: cervix, endometrium, rectum, vulva, bladder, urethra.
- *Distant spread*: kidney, breast, gastrointestinal tract, ovary.

The commonest metastases *(cervix, endometrium, rectum)* are usually in the upper third of the vagina and may be submucosal. Other metastatic tumour types should also be considered e.g. vaginal recurrence of vulval or urethral malignant melanoma, or, uterine leiomyosarcoma.

Differentiation/Grade

Well/moderate/poor/undifferentiated, or, Grade 1/2/3/4.

There is no specific recommended grading system for vaginal tumours with squamous cell or glandular differentiation other than the above. Grade 4 (undifferentiated) tumours show no differentiation. Transitional cell carcinomas are designated WHO I/II/III based on cytonuclear grade.

Extent of Local Tumour Spread

- Border: pushing/infiltrative.
- Lymphocytic reaction: prominent/sparse.

FIGO

FIGO staging is recommended. It applies to primary carcinoma of the vagina only excluding secondary growths either by metastasis or direct extension, e.g. from cervix, vulva or rectum. A vaginal carcinoma occurring 5 years after successful treatment of a carcinoma of the cervix is considered a primary vaginal carcinoma.

Tumours invading ≤3 mm with no lymphovascular invasion have a low incidence of lymph node metastases.

I	Tumor confined to the vagina
II	Tumor invades paravaginal tissues but does not extend to pelvic wall
III	Tumor extends to pelvic wall[a]
IVA	Tumor invades mucosa of the bladder or rectum or shows direct extension beyond the true pelvis
IVB	Distant metastasis

[a]The pelvic wall is defined as muscle, fascia, neurovascular structures or skeletal elements of the bony pelvis

"Frozen pelvis" is a clinical term meaning tumour extension to the pelvic wall(s) and is classified as FIGO III.

Spread is mainly by early direct invasion and lymph node metastases, with 50% beyond the vagina (FIGO II) at presentation and 25% in the rectum or bladder (FIGO IVA).

IVB (M1) disease is either an upper two thirds vaginal tumour with inguinal lymph node metastases, or, a lower third vaginal tumour with pelvic lymph node metastases. Other distant sites include lung, liver and brain (Figs. 26.1, 26.2, 26.3, and 26.4).

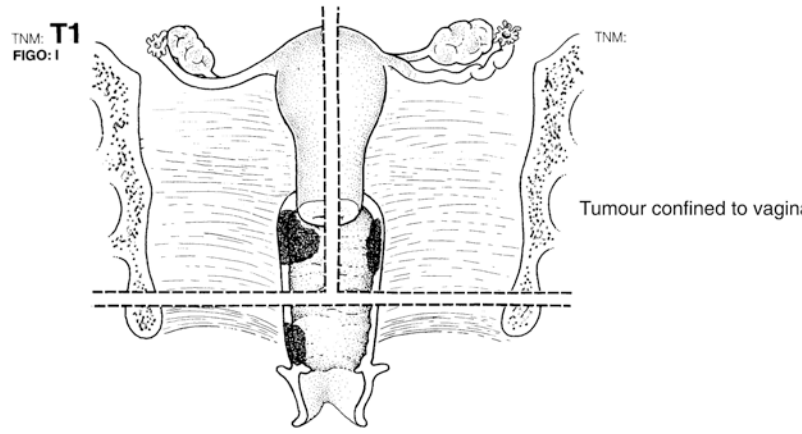

Fig. 26.1 Vaginal carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

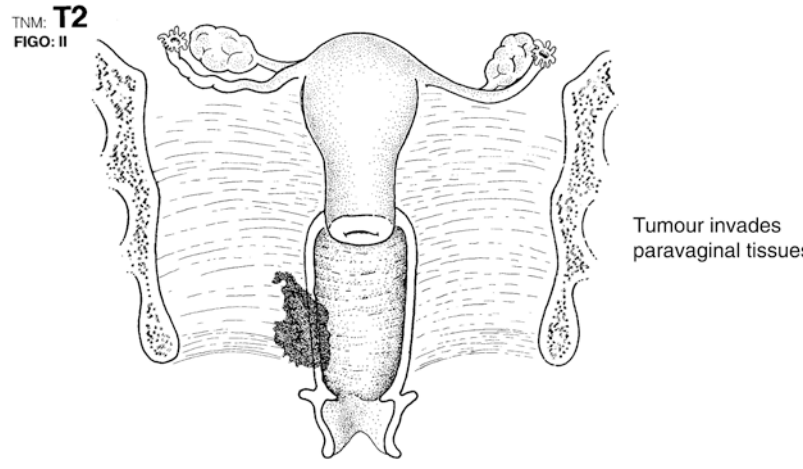

Fig. 26.2 Vaginal carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

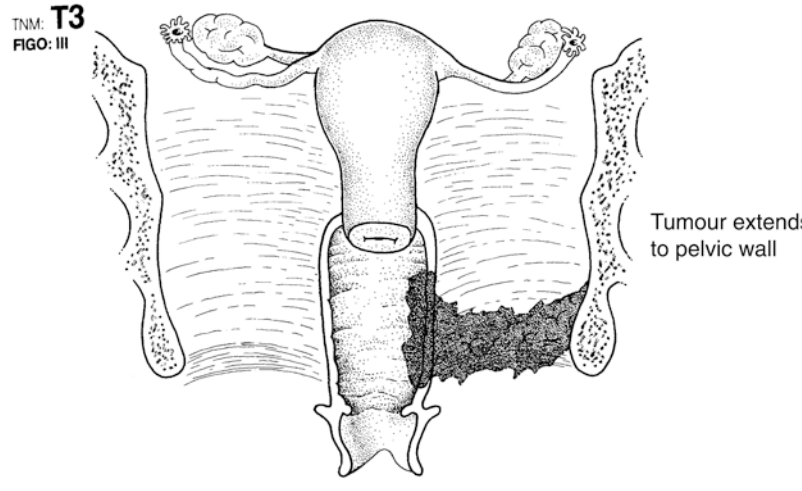

Fig. 26.3 Vaginal carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

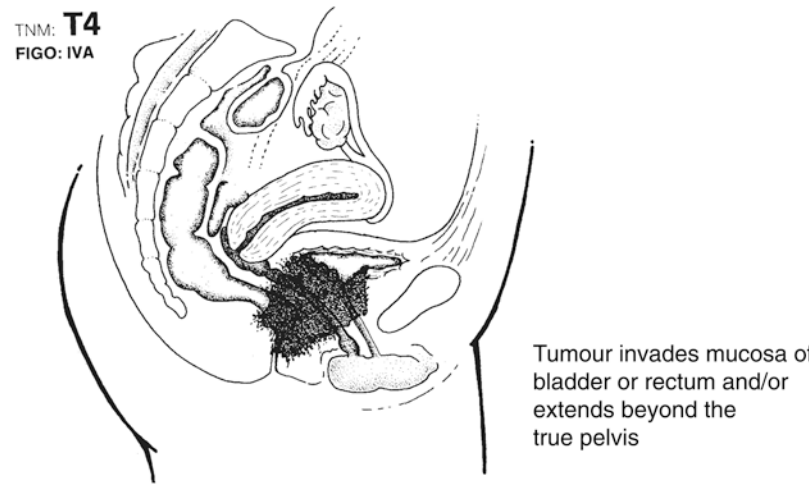

Fig. 26.4 Vaginal carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

Lymphovascular Invasion

- Present/absent.
- Intra−/extratumoural.

Lymph Nodes

- Site/number/size/number involved/extracapsular spread.
- Regional nodes: upper two thirds—obturator, internal iliac, external iliac, pelvic nodes; lower third—inguinal and femoral nodes.
- A regional lymphadenectomy will ordinarily include a minimum of six lymph nodes.

Excision Margins

Distances (mm) to the nearest longitudinal resection limit and deep circumferential radial margin. The presence of epithelial (squamous or glandular) dysplasia or atypical adenosis at a mucosal resection margin may increase the frequency of recurrent tumour.

Other Pathology

Vaginal Intraepithelial Neoplasia (VAIN): Grades I/II/III

- Rarer and less well established than the CIN or VIN - cancer sequences.

Cervical Intraepithelial Neoplasia (CIN)/Anal Intraepithelial Neoplasia (AIN)

HPV 16/18 is a common aetiology in CIN, VAIN, AIN and VIN (vulval intraepithelial neoplasia). It is instrumental in the *field change effect* of carcinogenesis in the female genital tract which results in synchronous or metachronous cancers in the vulva, cervix and vagina. The vagina is also a common site of direct spread from vulval and cervical carcinomas and it should be noted that *the commonest vaginal malignancies are secondary cervical, endometrial and rectal carcinomas.* Knowledge of a relevant past clinical history and availability of slides for comparison are, therefore, crucially important in designation of any vaginal lesion. A not uncommon differential diagnosis for adenocarcinoma on vaginal smear or vault biopsy is post-hysterectomy prolapsed fallopian tube or endometriosis.

Prognosis

Initial treatment of vaginal carcinoma is by *irradiation*, with better response for squamous cell carcinoma than adenocarcinoma, malignant melanoma and sarcoma. *Surgery* is used for localized early stage disease, non-responsive cases or local recurrence. Upper vaginal lesions tend to *recur locally* while lower vaginal tumours are prone to developing *distal or pelvic side wall disease*.

Prognosis relates strongly to *disease stage,* e.g. 43% 5 year survival with 70% for stage I and 30% for stage III. Vaginal malignant melanoma spreads early to pelvic soft tissues, lymph nodes, peritoneum, lung and bone with 5 year survival rates of 21%.

Other Malignancy

Malignant Lymphoma/Leukaemia

- Malignant lymphoma is usually secondary to systemic disease. Rare primary lesions are intermediate to high-grade large B cell in type.

Embryonal Rhabdomyosarcoma (Sarcoma Botryoides)

- Infants/children <5 years of age, anterior vaginal wall.
- Superficial subepithelial cellular cambium layer, intermediate myxoid zone, deep cellular zone, desmin/myo D1/myogenin positive.
- *Locally aggressive* necessitating primary chemotherapy ± surgery and irradiation.

Leiomyosarcoma

- Usually >3 cm, with cell atypia and ≥5 mitoses/10 high-power fields.
- A primary lesion is rare: consider metastases e.g. uterine leiomyosarcoma.

Müllerian Stromal Sarcomas and Other Sarcomas

- E.g. alveolar soft part, Undifferentiated pleomorphic sarcoma NOS, synovial sarcoma, extragastrointestinal stromal tumours (eGISTs) of the rectosigmoid septum.

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Vulval Carcinoma

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Rajeev Shah and Aaron Ervine

Introduction

Vulval cancer is relatively infrequently encountered, forms 5% of gynaecological malignancies and occurs mainly in women aged 60–80 years. However, its incidence is increasing in a younger patient demographic resulting in a bimodal distribution. Precursor lesions (vulval intraepithelial neoplasia (VIN), lichen sclerosus) appear to be increasing partly due to better clinical detection. Smoking and human papilloma virus (HPV) infection are known risk factors. High risk HPV appears to be the main driver in the increase in vulval neoplasia in younger women. In older women, neoplasia arises on a background of chronic inflammatory dermatoses, especially lichen sclerosus. Correspondingly, there are two main types of vulval squamous cell carcinoma—HPV related and HPV independent.

Vulval cancer can present as itch in an area of pallor or redness (leukoplakia/erythroplakia) on a background of atrophic or hypertrophic lichen sclerosus. A nodular, verruciform or ulcerating mass may be present and a diagnostic wedge or punch biopsy taken. The former is more likely to establish the presence of any invasive disease, and the latter is sufficient for abnormalities in a flat epithelium such as VIN. CT and MRI scans are used to detect and stage local soft tissue spread and inguinal lymphadenopathy which may also be amenable to fine needle aspiration cytology (FNAC). Because of the strong association with HPV, concurrent cervicovaginal and anal disease should be excluded.

Surgical treatment is geared to the patient's age, fitness, tumour site and stage with wide local excision for "early" FIGO stage IA disease. Lateral and central FIGO stage IB lesions may be treated by partial vulvectomy with ipsilateral or bilateral groin lymph node dissection, respectively. FIGO stage II cancers and above need radical vulvectomy which includes removal of the perianal skin and bilateral inguinal lymphadenectomy. Pre-operative radiotherapy may be given for tumours with extensive local invasion or involved lymph nodes in an attempt to downstage disease, facilitate surgery and avoid pelvic exenteration.

Gross Description

Specimen

- Biopsy/wide local excision/partial (hemivulvectomy)/simple/radical vulvectomy /uni-/bilateral inguinal lymphadenectomy/pelvic exenteration.
- Size (mm) and weight (g).

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_27

Tumour

Site
- Anterior/posterior.
- Lateral (right/left).
- Labia majora/labia minora/clitoris.
- Labia majora is the commonest site then labia minora and clitoris.
- Bilateral (25%)

Size
- Length × width × depth (mm) or maximum dimension (mm).

Appearance
- Polypoid/verrucous/ulcerated/necrotic/satellite lesions/pigmented.
- 50% are ulcerated, 30% exophytic.

Edge
- Circumscribed/irregular.

Histological Type

Vulval carcinomas show the full range of cutaneous cancers.

Squamous Cell Carcinoma

- *90% of malignant vulval neoplasms*.
- Keratinising or non-keratinising and of two main types
  - 60% of cases are in older women, independent of HPV, and a keratinising squamous cell carcinoma with a spectrum of adjacent epithelial changes, viz. lichen sclerosus, squamous cell hyperplasia, hyperkeratosis, or differentiated VIN
  - 30% of cases are in younger women, HPV 16/18 positive, of basaloid or warty histology and with adjacent VIN of classic/undifferentiated type (See Sect. 9. Other pathology).

Variants
- *Basaloid:* 28% of cases occurring at a younger age (<60 years) and associated with HPV, cervical and vaginal lesions. Comprises nests of basaloid cells with peripheral palisading, central necrosis, focal keratinisation and mitoses.
- *Warty:* associated with HPV and koilocytosis. Prognosis is intermediate between that of usual squamous cell carcinoma and verrucous carcinoma. Care must be taken to distinguish from pseudoepitheliomatous hyperplasia overlying lichen sclerosus, Crohn's disease or a granular cell tumour.
- *Adenoid:* a pseudoglandular/acantholytic pattern.
- *Verrucous:* exophytic with a pushing deep margin of cytologically bland bulbous processes. Prone to local recurrence after incomplete excision or radiotherapy.
- *Spindle cell:* a cytokeratin positive sarcomatoid carcinoma.

Basal Cell Carcinoma

- *20% local recurrence rate* and metastases are rare.

Distinguish from basaloid squamous cell carcinoma, Merkel cell carcinoma and secondary small cell carcinoma by morphology and immunohistochemistry.

Adenocarcinoma

- Rare.
- Appendage origin/Bartholin's gland/mesonephric duct remnants, or metastatic.

Paget's Disease

- *2% of vulval malignancy*.
- Characterized by a proliferation of *intraepithelial adenocarcinoma cells* probably arising from basal layer multipotential cells and differentiating along sweat gland lines. In *10–20% of cases there is a locoregional or extragenital malignancy* e.g. vulval appendage tumour or bladder carcinoma, cervical carcinoma, anorectal carcinoma, breast carcinoma. Immunohistochemistry may help to indicate a possible origin from bladder

(uroplakin III/CK7/20/GATA-3 positive) or anorectum (CK20/CDX-2, MUC2 positive). GCDFP-15 can be positive in vulval Paget's disease and metastatic breast cancer.
- *Multifocal:* check margins histologically as there is a *40% recurrence rate*.
- *Differential diagnosis:* mucin stains and immunohistochemistry (EMA, CEA, CAM 5.2, CK7, HER2 positive) may be necessary to distinguish from Bowenoid VIN (AE1/AE3, p63 positive, and CAM 5.2/CEA/CK7 negative) and superficial spreading malignant melanoma (S100, HMB-45, SOX-10, melan-A positive).
- *Prognosis:* Paget's disease without an associated neoplasm has a *very good prognosis if completely excised.* However, it may also progress to invasive carcinoma and lymph node metastases if beyond the microinvasive stage.

Merkel Cell Carcinoma

- Exclude secondary small cell carcinoma from lung.
- A *locally aggressive* and *potentially metastasising* poorly differentiated/high-grade neuroendocrine carcinoma.
- CAM 5.2 (paranuclear), CK20, chromogranin/synapophysin/CD56 positive. High Ki-67 index. Lung small cell carcinoma is CK20 negative/TTF-1 positive.

Malignant Melanoma

- *3–10% of malignant vulval neoplasms*.
- Usually mucosal and cutaneous involvement with Breslow depth and clinical stage the main prognostic indicators.

Metastatic Carcinoma

- *5% of malignant vulval neoplasms.*
- *Direct spread*: cervix (50% of cases), endometrium, vagina, urethra, bladder, anorectum.
- *Distant spread*: ovary, kidney, breast, lung, malignant melanoma, choriocarcinoma.

Secondary urethral tumours are squamous cell carcinoma, transitional cell carcinoma or malignant melanoma.

Differentiation

Squamous carcinomas are graded as well differentiated (grade 1), moderately differentiated (grade 2) and poorly differentiated (grade 3) according to the degree of keratinisation, intercellular bridges and pleomorphism, following a modified version of Broders' grading system. There is no agreed grading system for adenocarcinomas of the vulva.

- Well >50%; moderate 20–40% of cases.
- Grading for other vulval tumours should broadly reflect that of non-melanocytic cutaneous carcinomas. Undifferentiated tumours show no squamous cell or glandular differentiation.

Extent of Local Tumour Spread

- Border: pushing/infiltrative. An irregular infiltrative margin has a higher incidence of lymph node metastases.
- Lymphocytic reaction: prominent/sparse.
- Use of the term "microinvasion" should be avoided as some of these carcinomas will have lymph node metastases and invasive lesions >1 mm in depth may require radical surgery. *Superficially invasive squamous cell carcinoma* is defined as a single lesion measuring ≤2 cm diameter and with a depth of invasion ≤1 mm, i.e. FIGO IA. Over diagnosis of early invasion in VIN is avoided by a requirement in invasive disease for an irregular outline or contour of neoplastic epithelium, buds of abrupt epithelial differentiation (so called paradoxical maturation) with more plentiful eosinophilic cytoplasm compared to the overlying basaloid VIN 3, and an accompanying desmoplastic stromal response. Various mimics of invasion should be discounted such as tangential sectioning of adnexal epithelium involved by VIN 3, pseudoepitheliomatous

hyperplasia or displaced epithelium due to previous biopsy.
- Distance (mm) to the nearest painted surgical margin (lateral cutaneous, medial mucosal, deep subcutaneous).
- Involvement of vagina, urethra, perineum, anus.
- FIGO staging is recommended and applies to primary carcinomas of the vulva.

Stage I: Confined to vulva, negative nodes.
IA: Tumour confined to the vulva or perineum, ≤2 cm in size with stromal invasion[a] ≤1.0 mm, negative nodes.
IB: Tumour confined to the vulva or perineum, greater than 2 cm in size OR with stromal invasion[a] greater than 1.0 mm, negative nodes.
Stage II: tumour of any size extending to adjacent structures.
II: Tumour of any size with adjacent spread (lower third of urethra, lower third of vagina, anus) with negative nodes.
Stage III: tumour of any size with or without extension to adjacent perineal structures (lower third of urethra, lower third of vagina, anus) but with positive inguinofemoral lymph nodes.
IIIA: (1) With one lymph node metastasis ≥5.0 mm. (2) With one or two lymph node metastasis(es) of less than 5.0 mm.
IIIB: (1) With two or more lymph nodes metastases ≥5.0 mm. (2) With three or more lymph nodes metastases, less than 5.0 mm.
IIIC: Positive node(s) with extracapsular spread.
Stage IV: invasion of other regional structures (upper two-thirds of urethra, upper twothirds of vagina or distant metastases).
IVA: (1) Upper two-thirds of urethra, upper two-thirds of vagina, bladder mucosa, rectal mucosa OR fixed to pelvic bone. (2) Fixed or ulcerated inguinofemoral lymph nodes.
IVB: Any distant metastasis including pelvic lymph nodes.

[a]The depth of invasion is defined as the measurement of the tumour from the epithelial–stromal junction of the adjacent most superficial dermal papilla to the deepest point of invasion

In ulcerated tumours it may not be possible to provide a depth of invasion measurement if the epithelial-stromal interface cannot be determined. In such cases tumour thickness is measured from the base of the ulcer (in the case of ulcerated tumours) to the deepest point of invasion. However, depth of invasion should be provided whenever possible as this distinguishes FIGO stage IA from IB tumours.

Upper urethra (FIGO IV) is proximal, lower urethra (FIGO II/III) is distal. Spread is direct to the vagina, urethra, anus, inferior pubic and ischial rami and ischiorectal fossae (Figs. 27.1 and 27.2).

Lymphovascular Invasion

- Present/absent.
- Intra-/extratumoural.
- Carcinomas invading >1 mm have a higher incidence of lymphovascular invasion and lymph node metastases. This is facilitated by the vulval subepithelial connective tissues having a particularly rich vascular network.

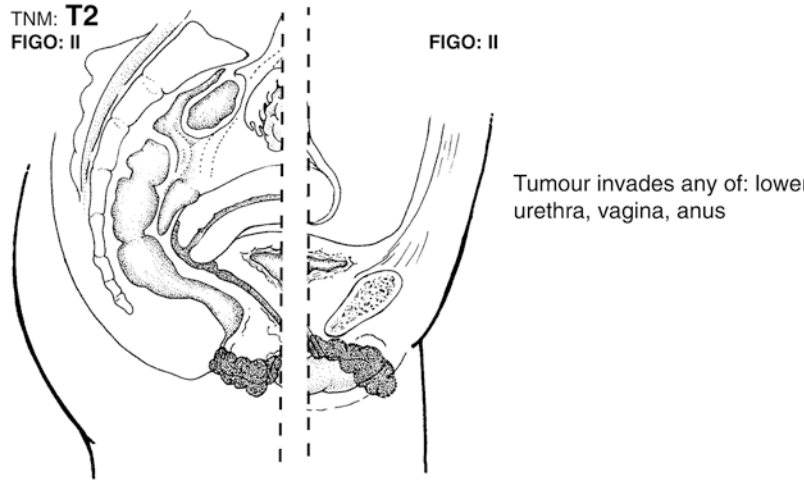

Fig. 27.1 Vulval carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

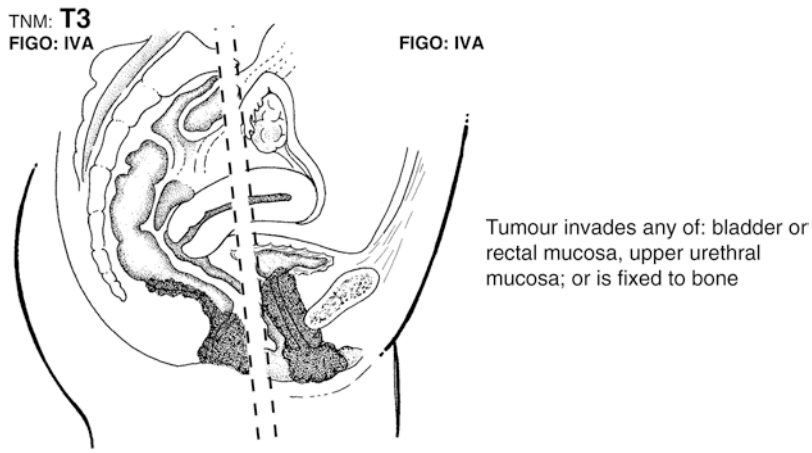

Fig. 27.2 Vulval carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

Lymph Nodes

- Site/number/size/number involved/extracapsular spread.
- Regional nodes: femoral and inguinal. A regional lymphadenectomy will ordinarily include a minimum of six lymph nodes.
- Labial tumours go initially to inguinal lymph nodes whereas clitoral lesions may go directly to deep pelvic lymph nodes. Ulcerated tumours can produce *reactive regional lymphadenopathy mimicking metastatic disease* and this can be further investigated by FNAC.

Excision Margins

Distances (mm) of tumour and VIN or Paget's disease to the nearest painted lateral cutaneous, medial mucosal and deep subcutaneous excision margins, and anal, vaginal, urethral limits.

Other Pathology

Lichen Sclerosus

- Atrophic, hyperplastic with hyperkeratosis, or both (mixed dystrophy).
- *Associated with (5–25% of cases)* but *low risk of progression to (5%)* carcinoma. Recurrent disease is linked to persistent VIN and lichen sclerosus.
- Can over expresses p53 and sometimes shows basal layer cytological atypia.

Condyloma

- Warty (condyloma accuminatum) or flat, and caused by HPV 6/11 with koilocytosis.

Bowenoid Papulosis

- Brown perineal patches in young women.
- HPV induced.
- Histology of VIN 3.
- There is a *negligible risk of progression* to carcinoma.

Vulval Intraepithelial Neoplasia (VIN) Grades 1/2/3

- The terms low grade squamous intraepithelial lesion (LSIL) high-grade squamous intraepithelial lesion (HSIL) are recommended by the World Health Organization but not widely used in the UK. LSIL refers to HPV related change and VIN 1; HSIL refers to VIN 2, VIN 3, Bowen's disease and Bowenoid dysplasia. These are prognostically relevant groups:

HPV independent precursor lesions or differentiated VIN (dVIN) comprise a third category. In the UK, "high grade" VIN includes both VIN 2 and VIN3.
- Typically multifocal and present in the adjacent epithelium of 60–70% of cases of squamous cell carcinoma.
- *Progression* to carcinoma is in the order of *10–20%.*
- Classic, or, variant types

Classic/undifferentiated (usual) type: includes *Bowenoid/basaloid* (resembles CIN 3) and *warty subtypes* (surface parakeratosis/koilocytosis), but with considerable morphological overlap and variable age presentation. Associated with HPV and multifocal genital tract cervicovaginal, perineal and anal disease. Shows diffuse positivity for p16—a surrogate marker for HPV infection.

Variant type: simplex or differentiated VIN with maturation, hyperplasia, hyperkeratosis, variable parabasal cytological atypia and over expression of p53.

Prognosis

Nearly *30% of vulval squamous cell carcinomas have metastasised* to inguinal or pelvic lymph nodes at presentation. *Prognosis relates to tumour size, an infiltrative tumour margin, depth of invasion, vascular involvement and in particular, lymph node disease.* HPV independent tumours appear to have a worse outcome than HPV positive lesions.

Stage I lesions have a 5 year survival of 90%, stage II 80%, stage III 60%, stage IV 20% and *overall 50–75%.* Unilateral lymph node involvement (60–70% 5 year survival) is better than bilateral lymph node disease (25% 5 year survival). Extranodal spread may necessitate postoperative radiotherapy. *Treatment* is by *partial or total vulvectomy* with *uni-/bilateral inguinal lymph node dissection*. A *limited local excision* with wide (1 cm) surgical margins may be used in early stage (well differentiated superficially invasive) disease or medically unfit patients. VIN 3 may be treated by topical therapy, laser, electrocoagulation, wide local excision, partial/total or skinning vulvectomy. *Pelvic exenteration* is reserved for locally extensive disease with no clinical or radiological evidence of extrapelvic metastases. Prognosis of *malignant melanoma* relates to tumour thickness and depth of invasion at the time of presentation, with *average 5 year survival of 30–35%.*

Other Malignancy

Malignant Lymphoma/Leukaemia

- Secondary to systemic disease usually diffuse large B cell in type.

Adnexal/Bartholin's Gland Carcinomas

- Rare and arising from eccrine or apocrine glands when distinction from metastatic ductal carcinoma of the breast can be problematic.

Bartholin's gland carcinoma forms *1–2% of vulval neoplasms* and shows a range of differentiation: squamous cell, adenocarcinoma, mixed adenoid cystic/mucoepidermoid carcinoma. Ideally an origin from adjacent Bartholin's gland structures should be demonstrable. Five year survival rates vary from 40% to 80% depending on the stage at presentation.

Aggressive Angiomyxoma

- Myxoid stroma, prominent vessels and spindle cells. A *locally infiltrative vulvovaginal tumour in young women* the edges of which are difficult to define surgically and therefore problematic to resect with *ischiorectal and retroperitoneal recurrence a likelihood*. Vimentin, actin positive ± desmin. Nuclear transcription factor HMGA2 positive in 50% of cases. They are also ER and progesterone receptor positive raising a possible role for hormonal therapy.

Sarcomas

- Leiomyo-/rhabdo-/liposarcoma.
- *Leiomyosarcoma:* >5 cm diameter, infiltrating margins, >5–10 mitoses/10 high-power fields, cellular atypia.
- *Rhabdomyosarcoma*: occurs in childhood and young adults, with vaginal disease being embryonal in type, and vulval alveolar. Desmin/myo D1/myogenin positive.
- *Liposarcoma:* well differentiated adipocytic/atypical lipoma in type.

Others

- Dermatofibrosarcoma protuberans, epithelioid sarcoma, malignant rhabdoid tumour.

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Gestational Trophoblastic Tumours

28

Rajeev Shah and Aaron Ervine

Introduction

Molar pregnancy affects 1–3 in every 1000 pregnancies with women less than 16 years and more than 50 years of age at higher risk. It is characterised by an abnormal conception with excessive placental and no or minimal fetal development. It usually presents with first trimester bleeding, a uterus larger than expected for gestational dates, absence of fetal parts or a snowstorm appearance on ultrasound examination, and markedly elevated serum βHCG (β subunit human chorionic gonadotrophin). A clinical diagnosis is made in 80% of complete moles and in 30% of partial moles—the remainder after routine histology of evacuated products of conception. Partial moles present with spontaneous abortion. Trophoblastic disease should be considered when there is continued vaginal bleeding following delivery or an abortion.

About 10% of hydatidiform moles result in one of the malignant forms of persistent gestational trophoblastic disease, *viz* invasive hydatidiform mole (commonest), choriocarcinoma, placental site trophoblastic tumour or epithelioid trophoblastic tumour. In the UK persistent trophoblastic disease has a centralised system for registration, clinical monitoring and treatment. Lung is the commonest site of metastatic disease resulting in cough, haemoptysis, chest pain or dyspnoea. Curative intent chemotherapy is highly effective and delivered with minimal morbidity. It is indicated in a number of circumstances: biopsy proven choriocarcinoma, evidence of metastases (brain, lung, liver, vulva, gastrointestinal tract), heavy vaginal or gastrointestinal bleeding, serum HCG > 20,000 IU/mL more than 4 weeks after evacuation, and rising or persistent serum HCG levels 6 months after evacuation. Response to treatment is assessed by falling serum HCG levels.

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Gross Description

Specimen

- Curetting/hysterectomy.
- Weight (g) and size (mm), number of fragments, villous diameter.

Tumour

Site

- Endometrial/myometrial/extrauterine: serosa
  - Parametria
  - Adnexae.
- Cavity: fundus, corpus, isthmus.

D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_28

Size

- Length × width × depth (mm) or maximum dimension (mm). Size >50 mm is prognostically adverse.

Appearance

- Haemorrhagic/necrotic/vesicular/nodular/polypoid masses.

Edge

- Circumscribed/irregular.

Histological Type

Choriocarcinoma

- Suspect on curettings if there is abundant necrotic/haemorrhagic decidua, bilaminar aggregates of exuberant syncytiotrophoblast and cytotrophoblast, and ***no*** chorionic villi.
- *50% are preceded by a molar gestation,* but also seen after normal pregnancy (20%) or spontaneous abortion (30%).
- *2–3% of complete moles progress* to choriocarcinoma.
- *Destructive myometrial and vascular invasion* are common, leading to *haematogenous metastatic spread* to lung (60–80%), vagina (30%), pelvis (20%), brain (17%) and liver (11%).
- HCG/cytokeratin/inhibin positive/HPL (human placental lactogen) focal.
- *5 year survival is >90%* with *chemotherapy* (uterine disease >95%, metastatic disease 83%).

Invasive Hydatidiform Mole (Chorioadenoma Destruens)

- *16% of complete moles*.
- Penetration into the myometrium or uterine vasculature ± adjacent structures of molar villi associated with variable degrees of trophoblast hyperplasia. Haemorrhage and perforation can occur.
- Haematogenous transport of "metastatic" nodules to vagina, lung and central nervous system. They do not affect the prognosis but may present with per vaginum bleeding or haemoptysis and respond well to chemotherapy.

Placental Site Trophoblastic Tumour (PSTT)

- Mostly following a normal term pregnancy (75%).
- A polypoid mass composed of monomorphic intermediate trophoblast—mononuclear cytotrophoblast ± multinucleated cells, dissecting myofibres without necrosis or haemorrhage. Peri−/intravascular growth patterns.
- HCG negative, HPL/alpha-inhibin/cytokeratin positive.
- *10–15% are malignant* (mitoses >2/10 high-power fields, deep invasion, clear cells). Not chemoresponsive and *requires surgical removal*.

Epithelioid Trophoblastic Tumour (ETT)

- Along with choriocarcinoma and PSTT a non-villous forming potentially malignant gestational trophoblastic tumour.
- Very rare, following normal pregnancy.
- Geographical areas of necrosis with islands of uninucleate polygonal eosinophilic cells.
- Cytokeratin/alpha-inhibin positive: mostly HCG/HPL negative.
- Behaviour is similar to PSTT rather than choriocarcinoma.

Differential diagnosis for choriocarcinoma, PSTT and ETT include persistent molar tissue, undifferentiated carcinoma/sarcoma and epithelioid leiomyosarcoma.

Differentiation

See above.

Extent of Local Tumour Spread

- Border: pushing/infiltrative
- Lymphocytic reaction: prominent/sparse. There is an improved prognosis with an intense tumour stroma interface inflammatory infiltrate in choriocarcinoma.

The TNM/FIGO classification applies to choriocarcinoma, invasive hydatidiform mole and placental site trophoblastic tumour. Histological confirmation is not required if the HCG level is abnormally elevated.

TNM (FIGO)

pT1 (I)	Tumour confined to the uterus
pT2 (II)	Tumour extends to other genital structures: vagina, ovary, broad ligament, fallopian tube by metastasis or direct extension
pM1a (III)	Metastasis to the lung(s)
pM1b (IV)	Other distant metastasis with or without lung involvement (brain, liver, kidney, gastrointestinal tract).

FIGO stages I–IV are subdivided into *A (low risk)* and *B (high risk)* categories according to a multiparameter prognostic score (Table 28.1). Prognostic grouping is assigned by recording stage with prognostic score, e.g. II:4 or IV:9 (Table 28.1).

Lymphovascular Invasion

- Present/absent.
- Intra−/extratumoural.
- Physiological trophoblast in placental site reaction is frequently endovascular with potential for myometrial invasion and this must not be over interpreted as malignancy.
- Molar tissue can potentially spread to cervix, vaginal wall and vulva through a much dilated pelvic vasculature.
- Choriocarcinoma typically shows *destructive myometrial and vascular invasion*. *Diagnostic biopsy* of metastatic deposits may be *contraindicated* due to the *risk of life threatening haemorrhage.*

Lymph Nodes

Usually tertiary metastases from a large extrauterine lesion and of poor prognosis. Classified as metastatic M1b (FIGO IV) disease.

Table 28.1 Prognostic score for gestational trophoblastic tumours

Prognostic score	0	1	2	3
Prognostic factor				
Age	<40	≥40		
Antecedent pregnancy	H. mole	Abortion	Term pregnancy	
Months from index pregnancy	<4	4–<7	7–12	>12
Pretreatment serum HCG (IU/ml)	$<10^3$	10^3–$<10^4$	10^4–$<10^5$	$\geq 10^5$
Largest tumour size including uterus	<3 cm	3–<5 cm	≥5 cm	
Sites of metastasis	Lung	Spleen, kidney	Gastrointestinal tract	Liver, brain
Number of metastasis		1–4	5–8	>8
Previous failed chemotherapy			Single drug	2 or more drugs

Risk categories
Total prognostic score 7 or less is low risk (add "A" to FIGO Stage)
Total prognostic score 8 or more is high risk (add "B" to FIGO Stage)

Excision Margins

Distances (mm) to the serosa and parametrial resection limits.

Other Pathology

Complete Hydatidiform Mole

- Androgenetic diploid 46XX.
- *Diffuse villous vesicular swelling* although this is only well developed beyond 12 weeks gestation by dates.
- *Central cistern formation*.
- *Circumferential/multifocal trophoblast.* Grading the degree of trophoblast proliferation is not of prognostic value.
- *Absence of fetal red blood cells and tissues* unless with a twin gestation.
- *Volume of placental tissue* is often abundant >100 g.
- *Early moles*: increasingly frequent with routine use of ultrasound examination. There can be a mixture of hydropic and non-hydropic villi or only lobulated villi making diagnosis problematic. Look for *branching and small sprouts* of secondary villi, some *invaginations in outline* and *trophoblast inclusions,* and a *myxoid stroma with nuclear debris*. Moderate trophoblast hyperplasia is also usually seen.
- p57 immunostain negative

The vast majority regress but *10–15% develop persistent trophoblastic disease* representing either incomplete removal of molar tissue, residual invasive mole within the myometrium or its vasculature, or choriocarcinoma.

Partial Hydatidiform Mole

- Biparental; triploid 69XXY, a minority are trisomy.
- ± A fetus (usually abnormal). Fetal death usually occurs around the eighth week leaving only necrotic debris or a persistent fetal circulation with open vessels and nucleated red blood cells.
- A *mixture* of focal villous vesicular swelling with central cisterns and normal sized villi.
- "Norwegian fjord" scalloped or dentate outline with *trophoblast inclusions*.
- Circumferential/multifocal *trophoblast hyperplasia*, usually syncytiotrophoblast.
- Volume of placental tissue normal.
- There is *persistent disease in up to 0.5–1% of cases.*
- p57 immunostain positive (if differential diagnosis is complete mole)

In molar change vesicles of 2–3 mm diameter are usually seen grossly.

Hydropic Degeneration

- Often trisomy or triploid.
- *Villi < 2–3 mm and rounded*.
- *No cisterns.*
- *Trophoblast is polar* in distribution and/or attenuated.

There is a risk of *overdiagnosis* of mole in ectopic tubal pregnancy due to the exuberant trophoblast that is associated with early non-molar gestational sacs.

Other Comments

Placental site reaction: a common localised phenomenon in curettings from abortions and must not be confused with gestational trophoblastic tumours. It comprises an exaggerated response of decidua, altered myometrial smooth muscle cells and intermediate trophoblast without myometrial destruction or invasion. It is usually associated with some immature villi. Placental site nodules or plaques are characterised by small size, circumscribed margins and hyalinisation. Both are benign and do not require staging or follow up.

Trophoblast: unlikely to be neoplastic if the last known pregnancy was recent, of short dura-

tion, aborted, and characterised by a mixture of villous and placental site trophoblast. The differential diagnosis between hydropic degeneration, partial and complete moles is often difficult and *monitoring serum β subunit HCG levels* is useful to ensure that they revert to normal in time. Trophoblastic disease is associated with *persistently abnormal levels* and if this is present after 60 days with a previous diagnosis of hydatidiform mole consideration is given to use of chemotherapy. Another differential diagnosis for choriocarcinoma is non-gestational carcinoma with trophoblast metaplasia e.g. ovary, endometrium.

In curettage specimens trophoblast can be categorised as

- Villous trophoblast: the usual post abortion finding.
- Simple non-villous trophoblast: distinction between syncytio- and cytotrophoblast cannot be made and usually occurs after abortion.
- Suspicious non-villous trophoblast: no villi, a bilaminar arrangement of syncytio- and cytotrophoblast but no tissue invasion.
- Non-villous trophoblast diagnostic of choriocarcinoma: myometrial fragments with demonstrable invasion by bilaminar trophoblast.

Interpretation should be in the context of clinical, radiological and biochemical findings.

Flow cytometry: can help to distinguish between a diploid complete mole and triploid partial mole, and, between a triploid partial mole and non-molar diploid hydropic abortion. Cell proliferation markers (e.g. Ki-67) are strongly over expressed in complete moles but are of limited practical value. The product of the maternally expressed gene CDKN1C, *p57 KIP2* can be stained immunohistochemically. It shows high levels of nuclear expression in the cytotrophoblast and villous mesenchyme of partial moles and hydropic abortions but is absent in complete mole and may prove a useful adjunct to the main morphological criteria in the designation of molar pregnancies. It has not been found to be of use in identifying the type of causative pregnancy in established gestational trophoblastic tumours.

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Part VII

Urological Cancer

- Renal Cell and Renal Pelvis/Ureter Carcinomas
- Bladder Carcinoma
- Prostate Carcinoma
- Urethral Carcinoma
- Testicular Cancer
- Penile Carcinoma

29 Renal Cell and Renal Pelvis/Ureter Carcinoma

Declan O'Rourke

Introduction

Carcinomas of the renal parenchyma and pelvis account for 2–3% of all cancers and 7000 cases in the UK per annum. Incidence is increasing partly due to improved diagnostic techniques and detection on radiological examination. Risk factors include smoking, obesity, exposure to carcinogenic industrial compounds through drinking water, and genetic factors, e.g. von Hippel-Lindau disease, hereditary papillary renal carcinoma, and hereditary non-polyposis colorectal cancer (HNPCC). Renal pelvis/ureter carcinomas are much less common than tumours of the renal parenchyma, comprising 4–5% of all urothelial cancers. Some 20–30% of upper urinary tract urothelial cancers show microsatellite instability and loss of mismatch repair protein antibodies and there is an association with HNPCC.

Up to one third of renal carcinomas are asymptomatic and an incidental finding on radiological examination. Conversely about 35% present late in their disease course with metastases. The classic triad of flank pain, mass and haematuria is infrequent (10%) and usually indicates advanced disease. Weight loss and painless haematuria are perhaps the most frequent presenting complaints. Other symptoms and signs are unexplained fever, anaemia, hypertension, hypercalcaemia and elevated ESR (erythrocyte sedimentation rate).

Investigation for renal cell carcinoma is by abdominal ultrasound and contrast enhanced CT scan which can distinguish between cystic and semi-cystic or solid lesions. They also provide staging information on lymph node, renal vein and inferior vena cava (IVC) involvement. MRI scan gives complementary information regarding the latter. Renal pelvic cancers are defined by retrograde pyelography and ureteropyeloscopy with cytological brushings and/or forceps biopsy. With increasing forceps size and improved technique and equipment, endoscopic biopsy can be diagnostic in 25–30% of cases. However care must be taken due to the limited material produced, benign mimics of neoplasia, e.g. polypoid urethritis/pyelitis, and artefact and distortion resulting in potential over diagnosis. This is particularly relevant in the absence of a clinically suspected tumour. Needle biopsy is done in a minority of renal cell cancers. This is usually when there is extensive spread for the purposes of obtaining a tissue diagnosis as a prequel to palliative adjuvant or immunotherapy, and, also to rule out a more treatable cause of the renal mass, e.g. malignant lymphoma. It is also used in the investigation of an indeterminate complex, cystic renal mass, or, in tandem with local tumour destruction by cryotherapy or RFA (high radiofrequency

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_29

ablation) in patients who are not medically fit for radical surgery, who have widespread disease or a solitary kidney. It is sometimes indicated prior to nephron sparing partial nephrectomy, or to justify observation only management.

Otherwise most imaging proven and kidney confined mass lesions require surgical resection. Omitting an invasive needle biopsy avoids disrupting local anatomical structures and any risk of upstaging the tumour. The mainstay of surgical treatment for renal cell carcinoma is radical nephrectomy comprising removal of the kidney outside Gerota's fascia, and ipsilateral adrenalectomy with or without regional lymphadenectomy. A laparoscopic approach is preferred with decreased patient morbidity and faster post operative recovery. Advances in preoperative imaging and staging have made partial nephrectomy an option for select patients, e.g. tumour <4 cm dimension, bilateral synchronous tumours, tumour in a solitary kidney, or, with a poorly functioning contralateral kidney. Although local surgical resection for renal carcinoma is often complete (R0), haematogenous metastases are not infrequent and may occur at an early or late stage of disease. Renal pelvis/ureter carcinoma requires nephrectomy with ureterectomy. Endoscopic resection for early low-grade lesions is also now available.

Gross Description

Specimen

- Fine needle aspiration cytology (FNAC)/needle core biopsy/partial nephrectomy/ nephrectomy ± ureterectomy/radical nephrectomy (kidney, pelvis, perirenal fat out to Gerota's fascia, adrenal gland, a length of ureter, ± regional lymph nodes)/segmental ureterectomy.
- Right/left: adrenal gland is superior, and the ureter lies behind the renal artery and vein and descends inferiorly.
- Weight (g) and size (mm).
- Length (mm) of attached ureter.
- adrenal gland: present/absent.

Tumour

Site

- Upper/lower pole, midzone, hilum, medullary, cortical, subcapsular, extracapsular, pelvic/peripelvic.
- Single/multiple (satellite nodules are present in 5% of renal cell cancers) or bilateral (1%).
- Most renal cell carcinomas are *solitary*, randomly distributed in the renal cortex and unilateral. *Multifocality* and *bilaterality* suggests a *hereditary cancer type.*
- Most renal cell carcinomas are centred on the cortex, urothelial carcinomas on the pelvis, or *multifocal* in the pelvicalcyceal and ureteric collecting system.

Size

- Length × width × depth (mm) or maximum dimension (mm).
- Average size for renal cell carcinoma is 7 cm but there is an increasing proportion of smaller tumours with better radiological detection.

Appearance

- Cystic/solid/lobulated: renal cell carcinoma.
- Necrotic/haemorrhagic/yellow/calcification: renal cell carcinoma.
- Circumscribed/tan/central scar: oncocytoma, chromophobe and papillary carcinomas.
- White/granular/scirrhous: sarcomatoid and collecting duct carcinomas.
- Papillary/sessile/scirrhous: renal pelvis carcinoma.

Edge

- Circumscribed/irregular.

Compression/Infiltration of Structures

- Perinephric fat, capsule, cortex, medulla, pelvis, peripelvicalyceal fat (renal sinus), adrenal gland, renal vein.
- Most renal cell carcinomas have a pushing margin. Diffuse infiltration of the adjacent kidney, perirenal and renal sinus fat is uncommon in contrast to infiltrating pelvic urothelial/transitional cell carcinoma.

Histological Type

Renal malignancy of childhood (nephroblastoma, clear cell sarcoma, rhabdoid tumour) is not discussed.

Renal Cell Carcinoma (WHO 2016 Classification of Renal Neoplasms)

- Clear cell renal cell carcinoma
- Multilocular cystic renal neoplasm of low malignant potential
- Papillary renal cell carcinoma
- Chromophobe renal cell carcinoma
- Collecting duct carcinoma
- Renal medullary carcinoma
- Hereditary leiomyomatosis and renal cell carcinoma-associated renal carcinoma
- MiT Family translocation carcinomas
- Succinate dehydrogenase (SDH) deficient renal carcinoma
- Mucinous tubular and spindle cell carcinoma
- Tubulocystic carcinoma
- Acquired cystic disease associated renal cell carcinoma
- Clear cell papillary renal cell carcinoma
- Renal cell carcinoma, unclassified

Adenocarcinoma

- *90% of cases.*
- *Clear cell: 70% of cases*. Of proximal convoluted tubule origin. Solid/trabecular/alveolar/tubuloacinar/cystic patterns with a prominent sinusoidal vascular stroma and areas of haemorrhage. Glycogen and fat rich clear to eosinophilic granular cytoplasm and variable nuclear morphology. *Multilocular cystic clear cell renal cell neoplasm of low malignant potential* is a rare distinct subtype of clear cell renal cell carcinoma with an excellent prognosis
- *Papillary: 10–15% of cases*. It is of better prognosis (80% 5 year survival) than clear cell carcinoma and up to 70% are intrarenal at diagnosis. Potentially multifocal, bilateral and familial (hereditary papillary renal carcinoma) arising on a background of precursor papillary adenoma(s). Formerly termed chromophil carcinoma. It is encapsulated, with solid and tubular patterns but at least 50–70% of the tumour area is papillary with stromal aggregates of foam cells, focal psammomatous microcalcifications and haemorrhage. It is the commonest renal carcinoma in dialysis patients.
  - *Type 1*: basophilic cuboidal cell, uniform bland appearance and more often multifocal. Trisomies 7, 16, 17 are often detected.
  - *Type 2*: eosinophilic columnar cell with nuclear stratification. It is of worse prognosis than type 1 (oncocytic pattern is increasingly recognized but not part of the 2016 classification). *Clear cell papillary renal cell carcinoma* was initially reported in end-stage kidneys, but is now most commonly sporadically in otherwise normal kidneys. Characteristically CK7 positive and AMACR negative.
- *Chromophobe: 3–5% of cases* arising from intercalated cells of cortical collecting ducts. It is of slightly better prognosis than clear cell carcinoma. Solid and nested patterns with a perinuclear halo, clear to flocculent cytoplasm (positive with Hale's colloidal iron and alcian blue), prominent ("koilocyte like") cytoplasmic membranes, and "wrinkled" raisinoid hyperchromatic nuclei (strongly CK7 positive and vimentin negative).
- *Collecting duct: 1% of cases*, located in the medulla with irregular tubules in a desmoplastic stroma, hob-nail cells and nuclear stratification. Often infiltrates perirenal/renal sinus fat. It is aggressive (50% 2 year survival) although a rare low-grade variant exists.
- *Sarcomatoid: 2–3% of cases* with a pale solid/scirrhous appearance and fibrosarcomatous/malignant fibrous histiocytoma like spindle cell (± giant cell) morphology and usually with high nuclear grade. May occur either as a major or minor component with the other main subtypes, and also renal pelvic urothelial carcinoma. It is therefore regarded

as *an indication of disease progression* and *a poorly differentiated or high-grade form* of them with *poor prognosis (median survival 19 months)* rather than a specific entity in its own right. A sarcomatoid component >50% of the area of a renal cell carcinoma is adverse. A pale/scirrhous area within an otherwise usual renal cell carcinoma should be preferentially sampled for histology. Infiltrating urothelial carcinoma of the renal pelvis can have a similar appearance (GATA3, CK20 and p63 positive).

- *Mixed:* about *10% of cases* show mixed differentiation.
- *Unclassified: 5–10% of cases* do not fit into any distinctive category. Includes sarcomatoid carcinoma without an identifiable usual type of renal cell carcinoma, and carcinoma of undifferentiated morphology.
- *Renal medullary:* of collecting duct origin in young patients with sickle cell trait. Poorly differentiated cells in solid or cribriform clusters and intervening oedematous/collagenous stroma. Poor prognosis.
- Microphthalmia transcription factor *(MiT) gene family translocation renal cell carcinoma:* includes Xp11.2/TFE3 translocation RCC and t(6;11)/TFEB translocation RCC. TFE3 and TFEB both belong to the MiT gene family and constitute a higher proportion of renal cell carcinomas in children and young adults. Presentation is at an *advanced stage and is more aggressive in adults.* Alveolar and papillary patterns of clear to eosinophilic tumour cells of high nuclear grade. Psammomatous calcifications can be present. TFE3/TFEB protein and RCC antibody/CD10 positive, but only focally positive for cytokeratins and vimentin.
- *Mucinous tubular and spindle cell:* a low-grade biphasic carcinoma in females with a favourable prognosis. Macroscopically circumscribed, it comprises tubules and spindle cells in a myxoid stroma.
- *Hereditary leiomyomatosis and renal cell carcinoma (HLRCC) syndrome:* HLRCC is an autosomal dominant inherited cancer syndrome characterized by cutaneous leiomyomas, early onset uterine leiomyomas and renal tumours. Typically the cells have large nuclei with very prominent eosinophilic nucleoli surrounded by clear halos (CMV like inclusion).
- *Succinate dehydrogenase (SDH)-deficient renal carcinoma:* caused by germline mutations in one of the four subunits of SDH and associated with pheochromocytoma,
- Paraganglioma or gastrointestinal stromal tumour and RCC's. It is positive for PAX8 and AMACR, but negative for SDHB.
- *Tubulocystic carcinoma:* strong male predilection with various sized cysts lined by flat, cuboidal, columnar and hobnail cells with eosinophilic cytoplasm. Has a similar immunoprofile to papillary carcinoma which it is linked to genetically.
- *Acquired cystic disease-associated renal cell carcinoma:* tumour occurs exclusively in patients with acquired cystic disease and characterized by cells with
- Deeply eosinophilic cytoplasm forming microcystic or cribriform patterns with intratumoral oxalate crystal deposition.
- *Birt Hogg Dube syndrome (BHD)-associated renal tumour*: triad of skin lesions, pulmonary lesions and renal tumours. The renal tumours are hybrid oncocytic tumours, with chromophobe RCC and/or oncocytoma like features (hybrid oncocytic/chromophobe tumour—HOCT).
- *Tuberous sclerosis-associated renal cell carcinoma RCC:* occurs in 2–4% of tuberous sclerosis patients with a female preponderance, with a wide array of tumours, including renal angiomyoadenomatous tumour, chromophobe RCC-like and HOCT.

Neuroendocrine Carcinoma

- Either well differentiated/low-grade neuroendocrine (carcinoid) tumour, or, poorly differentiated/high-grade small cell or large cell neuroendocrine carcinoma. Rare.
- Variably chromogranin/synaptophysin/CD56 and Ki-67 positive.

- Small cell carcinoma may also arise from the *pelvic mucosa* as part of a *high-grade urothelial carcinoma* secondarily involving the kidney parenchyma. It is also important to exclude clinically and radiologically a metastasis from a lung small cell carcinoma. Both primary renal and pulmonary small cell cancers can be TTF-1 positive.

Renal Pelvis/Ureter Carcinoma

Urothelial Carcinoma (Transitional Cell Carcinoma)

- Generally in patients over 65 years of age and with a male to female ratio of 3:1.
- ±Calculi or a history of analgaesic nephropathy with renal papillary necrosis.
- *Single/multifocal*: renal pelvis 58%, ureter 35%, pelvis and ureter 7%, bilateral 2–5%. About 30–75% also develop synchronous or metachronous *urinary bladder tumours*.
- *10% of renal neoplasms* and *5% of urothelial tumours*.
- *30% of cases are low-grade* and papillary giving hydroureter/hydronephrosis with a non-functioning kidney and a radiological filling defect in the pelvis/ureter. The other *70% of cases are high-grade* (particularly renal pelvis) and a mixture of papillary and sessile lesions. The latter can show "cancerisation" of renal medullary tubules, and infiltrate the medulla and cortex with a scirrhous gross appearance and squamoid or spindle cell morphology. *Invasive micropapillary urothelial carcinoma* is high-grade with aggressive behaviour. Other variants include clear cell, plasmacytoid cells, rhabdoid cells and giant cells (see Chap. 30—WHO 2016 classification of bladder tumours).

Squamous Cell Carcinoma

- Calculi/infection/squamous cell metaplasia of the pelvic mucosa.
- Mostly *high-grade* and *locally advanced* or *metastatic* at presentation. Prognosis is poor.
- Mixed: as part of a high-grade transitional cell carcinoma *(20–40% of WHO III TCCs).*

Adenocarcinoma

- Pure: tubulovillous/mucinous/signet ring cell/papillary non-mucinous. Adjacent pyelitis cystica/glandularis secondary to chronic inflammation, e.g. calculi.
- Mixed: as part of a high-grade urothelial (transitional cell) carcinoma *(2–5% of WHO III TCCs).*

Sarcomatoid Carcinoma

- Spindle cell carcinoma with high nuclear grade and cytokeratin positive. It may be combined with foci of usual urothelial/transitional cell carcinoma, or show carcinosarcomatous homologous or heterologous components. Usually at an *advanced stage at diagnosis* and pursues an *aggressive course.*

Metastatic Carcinoma

- Often small and bilateral (50%).
- *Direct spread*: cervix, prostate, bladder (distal ureter), gastrointestinal tract, retroperitoneal metastases, e.g. lung and breast.
- *Distant spread*: lung, malignant melanoma (skin), breast, stomach, pancreas, ovary, testis.

Differentiation/Grade

Renal Cell Carcinoma

The four tiered WHO/ISUP grading system is now recommended by the WHO (this supersedes Fuhrman grading). This grading system has been validated only for clear cell RCC and papillary RCC.

Grade	
X	Cannot be assessed
1	Nucleoli absent or inconspicuous and basophilic at 400× magnification
2	Nucleoli conspicuous and eosinophilic at 400× magnification but inconspicuous at 100× magnification
3	Nucleoli conspicuous and eosinophilic at 100× magnification
4	Marked nuclear pleomorphism and/or multinucleate giant cells and/or rhabdoid and/or sarcomatoid differentiation

Grades 2 and 3 account for the majority of cases. Prognostic significance of nuclear grade also varies according to tumour type, e.g. metastatic papillary renal carcinoma is usually high-grade whereas metastatic clear cell renal carcinoma can be variably low to high nuclear grade.

Tumour Cell Necrosis

Coagulative tumour cell necrosis is an *adverse prognostic indicator*. It should be distinguished from the more usual degenerative changes such as hyalinization, haemorrhage and fibrosis. Any history of preoperative embolisation to reduce the risk of intraoperative haemorrhage should be borne in mind as this nullifies any prognostic significance of tumour necrosis.

Urothelial Carcinoma

- WHO I/II/III (WHO 1973).
- WHO 2004/2016—Low-grade (WHO I/II) or high-grade (WHO II/III).
- For further discussion of classification of urothelial neoplasms see Chap. 30.
- For non-urothelial pelviureteric cancers: well/moderate/poor/undifferentiated, or, Grade 1/2/3/4.

Extent of Local Tumour Spread

- Border: pushing/infiltrative.
- Lymphocytic reaction: prominent/sparse.

Capsule, Perirenal Fat, Medulla

The capsule is often elevated and compressed by the pushing and lobulated margin of renal cell adenocarcinoma and this must be distinguished from actual histologically proven invasion of perirenal fat by tumour cells (pT3a). In this respect the capsule and fat should not be stripped from the kidney prior to sectioning perpendicular to it otherwise the cortex/capsule/fat interface is lost. *Extension to the renal sinus* (peripelvicalyceal fat) should be actively examined for as it is particularly susceptible to *small vessel vascular invasion*. Invasion of the medullary collecting system is infrequent but confers a worse prognosis.

Adrenal Gland

Involvement of the ipsilateral adrenal gland (5% of cases) is either by direct spread (pT4) or metastasis (M1). Risk is related to tumour grade, size and location with upper pole tumours particularly prone.

Renal Vein

Renal cell adenocarcinoma has a propensity for *venous invasion*, and involvement of the *renal vein or its segmental branches* should be *identified grossly at specimen dissection* with subsequent histological confirmation. In partial nephrectomy specimens presumptive absence of renal vein involvement is dependent on clinical imaging studies as it is usually not submitted in these specimens.

Pelvis, Ureter

Renal pelvic urothelial carcinoma is not infrequently *multifocal (40%)* with concurrent ureteric lesions ± bladder tumour. The adjacent urothelium is often abnormal ranging from hyperplasia through dysplasia to carcinoma in situ.

Renal Cell Carcinoma

pTx	Primary tumour cannot be assessed
pT0	No evidence of primary tumour
pT1	Tumour 7 cm or less in greatest dimension, limited to kidney
pT1a	Tumour 4 cm or less
pT1b	Tumour more than 4 cm but not more than 7 cm
pT2	Tumour more than 7 cm in greatest dimension, limited to the kidney
pT2a	Tumour more than 7 cm but not more than 10 cm

pT2b	Tumour more than 10 cm, limited to the kidney
pT3	Tumour extends into major veins or perinephric tissues but not into the ipsilateral adrenal gland and not beyond Gerota's fascia
pT3a	Tumour extends into the renal vein or its segmental branches, or tumour invades the pelvicalyceal system or tumour invades perirenal and/or renal sinus (peripelvic) fat but not beyond Gerota's fascia
pT3b	Tumour extends into the vena cava below the diaphragm
pT3c	Tumour extends into the vena cava above the diaphragm or invades the wall of the vena cava
pT4	Tumour invades beyond Gerota's fascia (including contiguous extension into the ipsilateral adrenal gland)

Contralateral adrenal gland involvement is rare (pM1). Gerota's (renal) fascia is retrorenal and prerenal with invasion beyond the latter sometimes resulting in peritoneal involvement (pT4). *Renal sinus involvement* has been noted to be the *commonest site of extrarenal extension (pT3a) and vascular involvement*, and, correlates with tumour type, grade and size. Its evaluation can upstage disease if involved. Careful scrutiny is required (Fig. 29.1).

Involvement of *renal vein* and *ipsilateral adrenal gland* is seen in *10%* and *5%* of cases respectively. *Extrarenal tumour* is present in up to *30–45%* of cases at presentation, its frequency varying with tumour type, e.g. 85% of chromophobe carcinomas are organ confined at diagnosis. *Metastases* occur in the lung, pleura, skeleton, soft tissues and skin, and to almost any site where they can *mimic* primary clear cell tumour in the involved organ e.g. kidney, thyroid, ovary. *Preferential metastatic sites* are seen with various subtypes of carcinoma: papillary carcinoma has fewer lung metastases and more lymph node deposits than clear cell carcinoma; chromophobe carcinoma tends to spread to liver.

Pelvis/Ureter Carcinoma

pTX	Primary tumour cannot be assessed
pT0	No evidence of primary tumour
pTa	Non-invasive papillary carcinoma
pTis	Carcinoma in situ
pT1	Tumour invades subepithelial connective tissue
pT2	Tumour invades muscularis
pT3	Renal pelvis: tumour invades beyond muscularis into peripelvic fat or renal parenchyma
	Ureter: tumour invades beyond muscularis into periureteric fat
pT4	Tumour invades adjacent organs or through the kidney into perinephric fat

For ureter adjacent organs include parietal peritoneum. Ureteric pT3 disease is prognostically equivalent to pT4 renal pelvis tumour (Fig. 29.2).

Pelvis/ureter carcinoma: single/multifocal lesion(s); hydroureter/hydronephrosis.

For the purposes of TNM8 the pelviureteric system is considered as a single organ and synchronous pelviureteric lesions are classified according to the highest pT category e.g. pT2 (m). In contrast synchronous renal pelvis and bladder cancers are classified independently.

50% present as *superficial disease* and 50% are *deeply invasive* (pT2 or beyond).

Lymphovascular Invasion

- Present/absent.
- Intra−/extratumoural.
- Renal cell carcinoma has a tendency to *involve the main renal vein* while infiltrating pelvic transitional cell carcinoma often shows invasion of *small lymphovascular channels* in the medulla and cortex. However, it can also subsequently involve the renal vein. In renal cell carcinoma, prognostically adverse *renal sinus (peripelvicalyceal) fat macro- and microvascular invasion* should also be sought and identified.

Lymph Nodes

- Site/number/size/number involved/limit node/extracapsular spread.
- Regional nodes: hilar, abdominal para-aortic, paracaval (ureter-intrapelvic). A regional lymphadenectomy will ordinarily include a

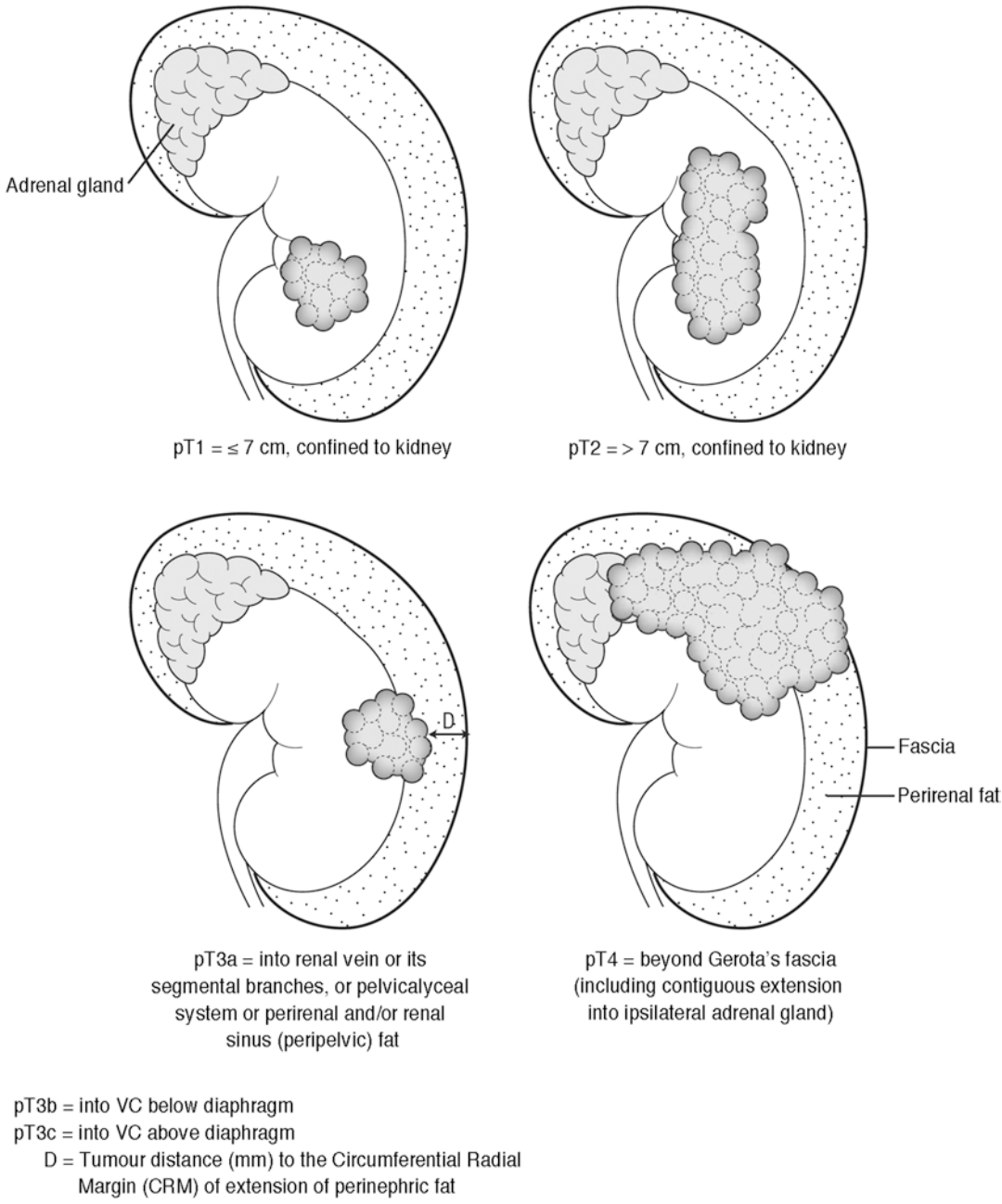

Fig. 29.1 Renal cell carcinoma. Reproduced, with permission, from *Histopathology Reporting: Guidelines for Surgical Reporting*, *2nd ed.,* © 2006, Springer

minimum of eight lymph nodes although in UK surgical practice lymph nodes are found in <5% of nephrectomy specimens with an overall positive metastasis rate of 4%. A few lymph nodes may be found at the renal hilum and occasionally individual operatively suspicious nodes will be submitted. Sometimes coincidental pathology, e.g. sarcoidosis is found as a basis for the lymphadenopathy. *Separate dissection of the paraaortic and*

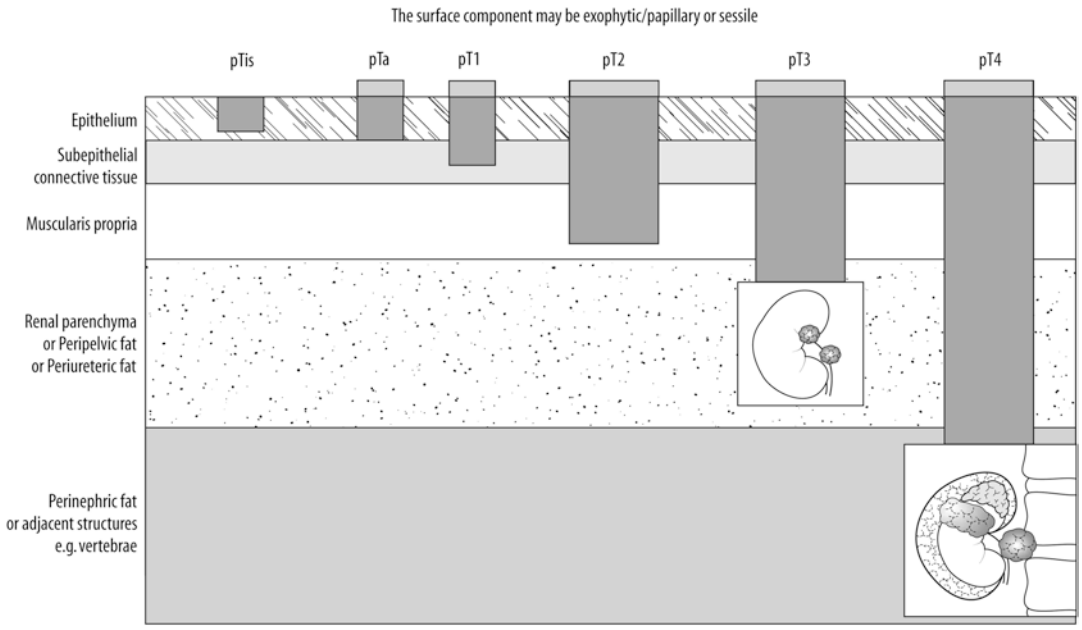

Fig. 29.2 Renal pelvis and ureter carcinoma. Reproduced, with permission, from *Histopathology Reporting: Guidelines for Surgical Reporting*, *2nd ed.,* © 2006, Springer

paracaval lymph nodes gives optimal staging information in that a pNX specimen has worse 5 year survival (61%) than a pN0 nephrectomy (74%) implying inaccurate downstaging.

Renal Cell Carcinoma

pNx	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Metastasis in regional lymph node(s)

The regional lymph nodes are hilar, abdominal para-aortic, and paracaval nodes (TNM8). Regional lymph node metastases occur in *10–15% of cases* and are associated with an adverse prognosis.

Pelvis/Ureter Carcinoma

Regional lymph nodes are hilar, abdominal para-aortic and paracaval nodes and, for the ureter, intrapelvic nodes (TNM8).

pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis[a]
pN1	Metastasis to a single lymph node 2 cm or less in greatest dimension (refers to the size of the largest metastasis, not the size of the largest lymph node)[b]
pN2	Metastasis in a single lymph node more than 2 cm, or multiple lymph nodes

[a]pN0(i+): Isolated tumour cells (defined: single tumour cells or small clusters of tumour cells (in a regional lymph node) not more than 0.2 mm in greatest extent that can be detected by routine H&E stains or immunohistochemistry)
[b]pN1(mi): Micrometastasis (defined as a metastasis in a regional lymph node >0.2 mm but ≤2.0 mm)

Regional lymph node metastases occur in *5–10%* of renal pelvic urothelial.

carcinomas.

Excision Margins

Distances (mm) to the distal ureteric limit, renal vein limit, perirenal fat and periureteric resection margins. Also the renal parenchyma resection limit in partial nephrectomy specimens.

Other Pathology

Macro−/micro morphology of renal carcinoma: there is a degree of correlation between gross morphology, cell type and architectural patterns in renal cell carcinoma, e.g. clear cell is lobulated, yellow with areas of haemorrhage and non-papillary. Chromophobe and papillary tumours are circumscribed and tan in colour. Sarcomatoid carcinoma is usually pale and scirrhous in appearance with a differential diagnosis of invasive pelvic transitional cell carcinoma. Renal clear cell adenocarcinoma may become totally cystic with only residual mural nodules of viable tumour. *Multilocular cystic renal cell carcinoma* has a very good prognosis with no evidence of progression. It comprises numerous cysts with fibrous septa lined by clear cells with grade 1 nuclei.

Chromosomal analysis: characterises various morphological subtypes, e.g. *papillary* versus *non-papillary renal carcinoma.* Cytogenetically papillary tumours show a trisomic gain on chromosomes 7, 16 and 17 rather than the 3p13 deletion of usual renal clear cell carcinoma. Chromophobe carcinomas can be hypodiploid with multiple monosomies. Renal cell carcinoma with familial Xp11 translocations are identified by fluorescent in situ hybridization (FISH). Primary pelviureteric carcinoma is associated with HNPCC.

Von Hippel-Lindau (VHL) disease: has an increased incidence *(40–50% overall and a 70% risk by 70 years of age)* of renal cell carcinoma, as do *acquired cystic disease* (e.g. long term dialysis) and *autosomal dominant polycystic kidney disease (ADPKD*: *5–10%)*. VHL has cysts with variable single cell, hyperplastic or solid clear cell epithelial lining. The renal clear cell carcinoma tends to be multiple and there are cysts elsewhere in kidney, liver and pancreas, and also phaeochromocytoma and cerebellar haemangioblastoma. ADPKD shows similar precancerous and established clear cell foci.

Miscellaneous renal and systemic features: occasionally renal cell carcinoma can spontaneously *regress* and also be host to *cancer metastasis to within cancer*, particularly from lung carcinoma. Other associated characteristics are *fever, hepatic dysfunction, hypercalcaemia, hypertension (secretion of renin), polycythaemia (secretion of erythropoietin)* and *hormonal effects (secretion of ACTH like substance). Amyloid* can be present in the renal interstitium adjacent to renal cell carcinoma and systemic in distribution. *Glomerulonephritis* is also an association. Examination of adjacent non-neoplastic parenchyma shows pathological abnormalities in 60% of nephrectomies for renal tumour, commonly related to *coincidental vascular disease (arteriosclerosis, atherosclerosis, hypertension)* or *diabetes.* Severe changes can be associated with progressive deterioration in postoperative renal function.

Xanthogranulomatous pyelonephritis (XGP), malakoplakia: both can mimic pelvic and renal cell carcinoma grossly and on needle biopsy. XGP comprises CD68 positive macrophages lacking cytokeratin positive epithelial cells, while malakoplakia shows eosinophilic macrophages with von Kossa or PAS stainable Michaelis-Gutmann bodies. Other differential diagnoses of a renal nodule or mass can be

- *Renal papillary adenoma*: circumscribed, <15 mm, papillae with fine fibrovascular cores ± tubules and a single layer of uniform cuboidal, eosinophilic or basophilic cells. Associated with renal cell carcinoma, papillary renal cell carcinoma, oncocytoma, long term haemodialysis, tubulo-interstitial scarring and acquired renal cystic disease, and may be multiple. They were previously defined as tumours measuring ≤5 mm but unencapsulated grade 1–2 tumours ≤15 mm have no capacity to metastasize (WHO 2016 classification). Caution should be shown on needle cores as capsule may not be visualized.
- *Metanephric adenoma/adenofibroma*: circumscribed, small uniform cells in crowded tubules and papillae forming glomeruloid bodies. Scanty intervening stroma with psammoma bodies but areas of fibrosis in the adenofibroma variant. It is EMA/CK7 negative unlike its differential diagnosis papillary renal carcinoma.

- *Oncocytoma:* on a spectrum with and forming a differential diagnosis for the eosinophilic variants of well differentiated (grade 1) renal cell carcinoma and chromophobe carcinoma. A circumscribed, tan/brown lesion with a central radial scar comprising sheets, tubules and small nests of cells with abundant eosinophilic cytoplasm and a central, small round nucleus, set in a variably oedematous stroma. It forms *3–5% of renal neoplasms* and is usually an asymptomatic incidental finding in male patients over 50 years of age. Its cytoplasm is rich in mitochondria. It is *excluded in favour of a designation of renal cell carcinoma* by: necrosis, mitoses, clear cells, spindle cells, papillary areas, gross vascular invasion or gross extension into perirenal fat (focally CK7 positive and vimentin negative). It is occasionally multifocal and bilateral with associated microscopic oncocytomatosis.
- *Angiomyolipoma*: epithelioid variant (HMB-45 positive). See Sect. 10.
- *Cystic nephroma*: simple cysts lined by flat cuboidal to columnar hob-nail cells and intervening fibrous (and ovarian type) stroma ± muscle, cartilage and focal blastematous elements (cystic partially differentiated nephroblastoma). The International Society of Urological Pathology (ISUP) (2014) recommended that the lesions cystic nephroma (CN) and mixed epithelial stromal tumour (MEST) be considered under one category.

Clear cell morphology: a solid clear cell lesion of any size should be regarded as a renal carcinoma with a 3 cm dimension considered a threshold for metastatic potential. Adenoma is reserved for small papillary, or, metanephric lesions as defined above.

Percutaneous FNAC: usually avoided but can give a diagnosis of simple renal cysts versus cystic or solid renal cell carcinoma. Carcinoma is cellular, shows nuclear atypia with a low nuclear/cytoplasmic ratio, nucleolar enlargement and variable fat/glycogen positive cytoplasmic vacuolation. Pelvic carcinoma also shows cytological features of malignancy. *FNAC* has a more defined role in the *investigation of suspected metastatic disease.*

Immunophenotype

Renal clear cell carcinoma: typically cytokeratin (AE1/AE3, CK8, CK18)/EMA/vimentin/CD10/N-cadherin positive, variable for RCC antibody/PAX-2/PAX-8, and negative for CK7/E-cadherin/BerEP4/MOC 31 and CD117.

Papillary renal cell carcinoma: CK7/RCC antibody/BerEP4/EMA/AMACR positive, and variable for CD 10/MOC 31/PAX-2/PAX-8, and CD117 negative.

Chromophobe carcinoma: CK7/EMA/E--cadherin/MOC 31/BerEP4 and CD117 positive, and CD10/RCC antibody/vimentin/N-cadherin negative.

Collecting duct carcinoma: CK7, EMA, CEA, 34βE12, PAX8, CK19 positive and p63/GATA3 negative.

Sarcomatoid carcinoma: retains vimentin and focal keratin positivity.

Oncocytoma: CD117 positive, CK7 focal, and vimentin, CD10/MOC 31 negative.

Thus the various combinations of immunophenotype can aid morphology in distinguishing the histological subtypes although there can be a marked overlap of expression between categories. A recent survey of European uropathologists showed that the most commonly used antibodies in nephrectomy surgical pathology are:

CK7 (95%), CD10 (93%), vimentin (86%), HMB-45 (68%) and CD117 (61%).

A useful limited diagnostic panel is:

Oncocytoma	CD117/CK7(focal) positive, vimentin negative
Chromophobe carcinoma	CD117/CK7 positive (diffuse), vimentin negative
Papillary carcinoma	CK7 and AMACR positive and CD117 negative
Clear cell carcinoma	CK7/CD117 negative and vimentin, EMA/CD10 positive.

Renal clear cell carcinoma (PAX8, CD10 positive, melan-A/inhibin negative) may also be discriminated from *adrenal gland carcinoma* which has a converse immunophenotype. Immunophenotype is of use in determining a renal origin for clear cell carcinoma metastatic to various body sites, e.g. skin, liver, lung, pleura.

RCC antibody and CD10 mark proximal tubules, MOC 31 and BerEP4 distal tubules. CD10 is also positive in renal pelvic urothelial carcinoma particularly high-grade and stage tumours but its expression is inversely related to tumour grade in renal clear cell carcinoma. *High-grade urothelial cancer* invading the medulla may mimic renal parenchymal cancers of various types. Use of p63, 34βE12 (positive in urothelial carcinoma) and PAX-8 (variably positive in renal carcinomas) may help make the distinction.

Treatment

Renal cell carcinoma: is treated by *surgical excision*, either partial or heminephrectomy (nephron sparing surgery), or radical nephrectomy depending on the size and location of the tumour. *Partial nephrectomy* ranges from tumour enucleation to heminephrectomy with part of the pelvicalyceal system and related overlying perirenal fat. *Radical nephrectomy* removes an entire kidney, en bloc adrenal gland, perirenal fat out to Gerota's fascia, and variable lengths of hilar vessels and ureter. Indications for nephron sparing surgery are: tumour <4 cm diameter, location at a renal pole and of non-papillary type, and compromised function in the contralateral kidney. However, the increasing use of imaging has led to an increase in incidental small renal masses (SRMs) and 20% of these are benign. Management includes active surveillance, ablation and partial nephrectomy. Multiple ablative techniques exist including radiofrequency ablation (RFA), microwave ablation, and cryoablation with the goal of each to achieve necrosis of the entire SRM. A biopsy is usually done prior to ablation for histological confirmation of tumour type.

Renal pelvis/ureter carcinoma: requires *laparoscopic nephrectomy with ureterectomy*, often including resection of the *ureteric orifice* because of multifocality and involvement of its *terminal, vesical (intramural) portion*. Solitary distal ureteric lesions may be treated by *segmental ureterectomy with ureteral reimplantation. Ureterectomy with endoscopic resection* of small low-grade, superficial pelvic lesions may be used as a renal sparing procedure when there is a solitary kidney or poor renal function. Occasionally a pelvic carcinoma may be found in a radical nephrectomy supposedly removed for a diagnosis of renal cell carcinoma. This leaves the issue of potential metachronous disease in the residual ureter and secondary ureterectomy may be considered. *Resection of solitary pulmonary metastases* can be of benefit for renal cell and renal pelvic cancers.

Prognosis

Renal cell carcinoma: up to 35% of cases present with *spread beyond the kidney* and *10% involve renal vein* with a tendency to solitary *distant haematogenous metastases*, e.g. lung, skin and bone with pathological fracture, e.g. neck of femur. *Recurrence develops in 40% of patients* treated for localized tumour. Recurrence is an end point, the likelihood of which is risk stratified in clinical algorithms (Memorial Sloan Kettering or Mayo Clinic) based on clinical presentation, performance status and various pathological factors. Chromophobe (mortality <10%) and papillary carcinomas have a better prognosis than equivalent grade and stage renal clear cell carcinoma while sarcomatoid and unclassified cancers are worse (94%, 86%, 76%, 35% and 24% 5 year survivals, respectively). *Overall 5 year survival is 70%* relating to *tumour grade, type (e.g. grade III/IV and sarcomatoid lesions are aggressive), necrosis, vascular invasion* and *stage* (particularly extrarenal disease), although cure is possible even with main vessel involvement. Targeted therapy in eligible patients improves outcome and has survival benefit (with Tyrosine kinase and mTOR inhibitors). *Chemotherapy* is largely ineffective. Potentially beneficial checkpoint inhibitor drugs are being introduced in clinical trials and may have a role to play in lymph node positive or widespread disease. Radiation is used for symptomatic and palliative treatment. Five year survival ranges from around 80% at stage 1 to 5% at stage 4 with little difference between the sexes.

Carcinoma of the renal pelvis/ureter: is predominantly *urothelial* in type with occasional

squamous cell, adenocarcinoma and sarcomatoid carcinomas. Typically there are *multifocal*, synchronous pelviureteric (25%) and bladder (15%) lesions with a *50% risk of subsequent metachronous tumours* at these sites. About *30% are low-grade* and *70% high-grade* with *50% representing superficial disease* and *50% deeply invasive* (pT2 or beyond). The latter can form poorly differentiated nests, sheets and cords of tumour in a desmoplastic stroma often assuming a squamoid appearance. Retrograde involvement of medullary collecting ducts (mimicking adenocarcinoma) and lymphovascular invasion are not uncommon. *Low-grade superficial lesions are of good prognosis but critical invasion of or beyond ureteric muscle coat, renal pelvic wall or parenchyma results in 5 year survival rates of 35% (pT1: 91%, pT2: 23%).* Chemotherapy and targeted therapies have an evolving role to play.

Other Malignancy

Malignant Lymphoma/Leukaemia

- Usually *secondary to systemic/nodal disease* and present in up to 50% of cases, and potentially bilateral. If established on clinical and radiological grounds as a primary lymphoma it is usually *diffuse large B cell* in type. Diffuse parenchymal tumour cell permeation or discrete tumour masses.
- Malignant lymphoma is occasionally associated with renal cell carcinoma.
- Post-transplant lymphoproliferative disorder.

Leiomyosarcoma, Liposarcoma, Malignant Fibrous Histiocytoma, Rhabdomyosarcoma, Synovial Sarcoma, Ewing's Sarcoma/Peripheral Neuroectodermal Tumour (PNET)

- *All are rare,* and important to exclude more common diagnoses, i.e. sarcomatoid renal cell carcinoma, or, primary retroperitoneal sarcoma with secondary renal involvement.
- *Leiomyosarcoma:* is the commonest primary sarcoma (50–60% of cases) arising from the renal capsule, parenchyma, pelvic muscularis or renal vein. It is *aggressive* with a majority of patients dead from disease within one year of diagnosis.

Angiomyolipoma with Malignant Transformation

- A perivascular epithelioid cell tumour (PEComa) forming 1% of surgically resected renal tumours and is *usually benign*.
- There is an association with *tuberous sclerosis in 30% of cases* (more frequent in the epithelioid variant), and PEComa may be *multifocal (30%)* or *bilateral (15%).*
- *Classic triphasic histology* comprising: HMB-45/melan-A/CD117/desmin positive *spindle cells* with variable cellularity and pleomorphism (giant/epithelioid cells) ± mitoses, *mature fat*, and *dystrophic thick walled vessels* in varying proportions. This gives a distinctive radiological appearance on CT scan due to its fat content.
- Capsular invasion and lymph node disease may be seen and often regarded as multicentricity rather than malignancy. *Rarely true malignant change can occur* (epithelioid variant) with a 30% risk of metastases.
- Prone to *catastrophic/potentially fatal retroperitoneal haemorrhage* particularly if >4 cm.
- Rarely associated with concurrent renal cell carcinoma.

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Bladder Carcinoma

30

Declan O'Rourke

Introduction

Bladder cancer is the seventh (tenth in UK) commonest cancer worldwide (3.2% of cases) with a male to female ratio of 3.5:1 and geographical variation with highest incidence in the Western hemisphere. Tobacco use is the most common predisposing factor to bladder cancer. Other causes include exposure to industrial aniline dyes (aromatic amines), petrochemicals, cyclophosphamide, and the analgaesic phenacetin. A minority of cases have a positive family history. Advances in early detection and treatment have improved prognosis with 5 year survival rates of 60–80%. In terms of local recurrence rates, disease free survival and mortality, bladder cancer comprises two distinct groups: either genetically stable low-grade, non-invasive papillary tumours, or, genetically unstable high-grade and invasive cancers (including pTaG3 lesions and CIS (carcinoma in situ)).

Bladder cancer commonly presents as an exophytic papillary, solid, or ulcerated endophytic mass at cystoscopy with symptoms of painless haematuria. Terminal haematuria at the end of micturition points to bladder neck pathology. Rarely, advanced bladder cancer may present with a pelvic mass, lower limb oedema due to lymphatic obstruction, or distant metastases. Investigation is by urinary cytology, cystoscopy and biopsy. Cytology is good at designating high-grade papillary, in situ and invasive urothelial neoplasia but poor at separating low-grade papillary lesions from reactive atypia and cellular changes associated with calculi, in-dwelling catheters, recent instrumentation and post therapy changes. Other roles for cytology are in the clinical follow up and surveillance of high-grade urothelial carcinoma and CIS. Biopsy is with "cold" cup forceps or a small diathermy loop, the advantage of the former being good preservation of histological detail. Flexible cystoscopy is easier for the patient and allows a wide field of vision but rigid cystoscopy with a larger lumen allows instrument access for transurethral resection of superficial bladder tumours (TURBT) and diathermy to the base. However, this can be associated with extensive distorting diathermy artifact that compounds accurate histological assessment of tumour grade and stage. Deep biopsy of the muscularis propria is important for staging information in invasive tumours and may be submitted separately by the urologist. Further staging is by a combination of endoluminal ultrasound, CT and MRI scans. Diagnostic accuracy for PET scan in invasive disease is poor. CIS can present with irritative bladder symptoms and appears as multifocal red patches on cystoscopy, although it can be endoscopically inapparent and require multiple random biopsies. It can occur in isolation

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_30

(primary CIS) but more usually in association with prior (secondary CIS), synchronous (concomitant CIS) or subsequent bladder cancer.

CIS is usually treated by intravesical topical chemotherapy (mitomycin) or immunotherapy (Bacille Calmette Guerin (BCG) therapy), or if localized in extent resected by TURB. Widespread disease determined by multiple site biopsy of the urothelium (bladder, ureters, urethra, prostatic ducts, seminal vesicles) may necessitate radical surgery. Superficial urothelial cancer (i.e. that confined to the mucous membrane) is resected transurethrally with submission of multiple fragments and follow up by cystoscopy. Adjuvant intravesical chemo−/immunotherapy and/or radiotherapy are used for high-grade or recurrent disease. Recurrent superficial cancer refractory to local resection and adjuvant therapy, and muscle invasive tumours, require radical surgery with cystectomy ± in continuity prostatectomy/urethrectomy and regional lymphadenectomy (robot-assisted radical cystectomy in many centres). In the female this entails cystourethrectomy or an anterior exenteration. Partial cystectomy is reserved for solitary, dome or urachal tumours with no previous bladder tumour, CIS, bladder neck or trigone involvement. Pelvic lymph node dissection imparts staging information and is of potential therapeutic benefit. Neoadjuvant chemoradiation has a role to play. Radiotherapy is also used as a palliative measure. Cystoscopy requires alternative urinary drainage. Options include: urinary diversion and intestinal ileal conduit, continent cutaneous diversion, continent orthotopic reservoir ('neobladder'), or, ureterosigmoidostomy. Agents that inhibit programmed cell death 1 (PD-1) protein and its ligands PD-L1 and PD-L2, which are part of immune checkpoint pathways that regulate T-cell activation to escape antitumour immunity, are now approved (cancer drugs fund UK) as first and second line (pembrolizumab and atezolizumab) therapy for locally advanced or metastatic urothelial cancer, and are also beginning to establish a role as first-line agents in patients who are not candidates for cisplatin chemotherapy.

Gross Description

Specimen

- Urine cytology/bladder washings/cystoscopic biopsy/transurethral resection bladder (TURB)/cystectomy/cystourethrectomy/cystoprostatectomy (including seminal vesicles)/cystoprostatourethrectomy/anterior or total exenteration (including uterus and adnexae ± rectum).
- Weight (g) and size (mm).
- Length (mm) of ureters and urethra.

Tumour

Site
- Fundus/body/trigone/neck/ureteric orifices.
- Anterior/posterior/lateral (right or left).
- Single/multifocal.
- Diverticulum.

Size
- Length × width × depth (mm) or maximum dimension (mm).

Appearance
- Papillary/sessile/ulcerated/mucoid/keratotic/calcification.
- Bladder mucosa: erythematous/oedematous (CIS).

Edge
- Circumscribed/irregular.

Histological Type

Urothelial Carcinoma

- Formerly transitional cell carcinoma
- *90% of cases*.
- Usual type: papillary or sessile (solid).
- Urothelial carcinoma has a propensity for *divergent differentiation* (squamous cell and

glandular are the commonest) usually in association with high-grade, high stage disease.
- Variants with deceptively benign features:
- *Microcystic*: intraurothelial microcysts containing protein secretions mimicking cystitis cystica and adenocarcinoma. It is of no particular prognostic significance.
- *Inverted*: architecturally similar to inverted papilloma but has WHO II/III cytology (WHO 2016 high grade). Look for stromal and muscle invasion. It is of relatively indolent behaviour.
- *Nested*: uniform cell nests in the lamina propria mimicking florid von Brunn's nests/cystitis glandularis/cystica, or paraganglioma. The cell nests are crowded with an irregular margin. Look for muscle and lymphovascular invasion. It is *potentially aggressive* with bladder wall invasion disproportionate to its histological grade.
- *Micropapillary*: resembles ovarian serous papillary carcinoma. It comprises fine papillary, filiform epithelial processes, sometimes with a central core and a surrounding clear space. This stromal retraction artifact mimics lymphovascular invasion. It also often shows true *lymphovascular invasion* and is a *high-grade tumour* with frequent presentation at an *advanced stage*. Some studies have observed that intravesical therapy is ineffective for micropapillary variant. Early cystectomy should be considered even if only superficially invasive disease is apparent. ERBB2 amplification by FISH is more frequently observed in micropapillary urothelial carcinoma.
- *Plasmacytoid/signet ring*: rare but aggressive variant of urothelial carcinoma that is characterized by the presence of dyscohesive individual cells that resemble plasma cells. 50% of cases also show conventional urothelial carcinoma or urothelial carcinoma in situ. The urothelial markers CK7, CK20, p63, GATA3, and uroplakin III are usually positive although CD138 (marker of plasma cells) is positive in a third of cases. Loss of E-cadherin is seen. Presentation is more often at an advanced stage but they are chemotherapy responsive.
- *Lymphoepithelioma-like urothelial carcinoma*: resemble lymphoepithelioma like tumour from the head and neck region, more common in men. The epithelial component is composed of nests, sheets, and cords of undifferentiated cells with a prominent lymphoid stroma that includes T and B lymphocytes, plasma cells and histiocytes. EBV has not been identified in lymphoepithelioma-like carcinoma of the bladder. It has been found to have similar prognosis, chemo-sensitivity, and response to immunotherapy to the observed in conventional urothelial carcinoma and there is recent evidence of benefit of using immunotherapy.
- *Others:* variants include clear cell, lipid rich, sarcomatoid and giant cell.

Squamous Cell Carcinoma

- *2–5% of cases*.
- *Keratinising squamous cell metaplasia* of the urothelium (to be distinguished from physiological trigonal vaginal type squamous epithelium) due to chronic irritation (catheter, calculi, parasitic infection) is a *risk factor for the development of carcinoma.* It is present adjacent to 20–60% of squamous cell cancers and potentially progresses to dysplasia and carcinoma. Its presence is also a predictor for local tumour recurrence post radical cystectomy.
- *Classical/verrucous/basaloid/sarcomatoid*
- Old age and associated with calculi, schistosomiasis (prevalent in Egypt and Africa forming 75% of bladder cancers in endemic areas), diverticulum, non-functioning bladders, renal transplantation, chronic infection and long term cyclophosphamide treatment. *Prognosis is poor* with a *13–35% 5 year survival* and two thirds are stage pT3/pT4 at presentation.
- Designated as a primary lesion only after exclusion of high-grade urothelial carcinoma with squamous cell differentiation (usually GATA3/Uroplakin negative), and secondary squamous cell carcinoma, e.g. cervix.

Adenocarcinoma

- The common cloacal embryological origin of bladder and rectum highlights the *range of glandular differentiation* that can be seen in the bladder mucosa.
- *2% of bladder malignancy*. A gland forming carcinoma devoid of urothelial or squamous cell carcinoma components. *Direct spread or metastasis from elsewhere is commoner* and should always be excluded clinically particularly in the absence of mucosal glandular intestinal metaplasia, dysplasia or in situ changes. Usually secondary adenocarcinoma is from a *colorectal (35%), prostatic (19%) or cervical (11%) primary.* Distinction must also be made from various *benign mimics* of adenocarcinoma: nephrogenic adenoma, endometriosis, endocervicosis, Mullerian inclusions, or, urachal remnants.
- *Enteric, mucinous (colloid), signet ring cell, clear cell (mesonephroid), hepatoid, ex villous adenoma,* or, *adenocarcinoma of no special type (NST),* enteric, mucinous and NST each comprising 20–30% of cases.
- *Urachal* or *non-urachal in origin, bladder adenocarcinoma* can arise out of chronic irritation associated with intestinal metaplasia/cystitis glandularis (60%), neurogenic bladder, exstrophy, diverticulum, endometriosis or bladder dome wall urachal remnants (30%). Usually muscle invasive and of poor prognosis (particularly signet ring cell carcinoma) due to *advanced stage at presentation* and an *18–47% 5 year survival.*
- Infrequently there is adjacent adenocarcinoma in situ. Histology cannot reliably distinguish between urachal or non-urachal origin. This is determined more by the anatomical site of origin, urachal lesions arising either in the bladder dome or high anterior aspect with an epicentre in the bladder wall. Adenocarcinoma complicating exstrophy tends to be localised in the anterior abdominal wall.
- Immunophenotype is often enteric (CK20, CDX-2, CK7±) in character.

Urothelial Carcinoma with Divergent Differentiation

- *Squamous cell carcinoma or adenocarcinoma* components are seen in *20–30%* and *5–10%,* respectively, of *high-grade invasive urothelial cell carcinomas* emphasising a capacity for *divergent differentiation* and carrying *a worse prognosis*. An adenocarcinoma component is reported to confer an increased resistance to chemotherapy, presentation at a more advanced stage and likelihood of progression.

Sarcomatoid Carcinoma

- *"spindle cell carcinoma"* or *carcinosarcoma*.
- Old age. Large and polypoid with a *poor prognosis (50% dead within 1 year).* There may be a recognisable in situ or invasive epithelial component (transitional cell, glandular, squamous cell or undifferentiated), and cytokeratin/vimentin positive spindle cells (34βE12, CK5/6 in 25% of cases, usually only focal) with varying stromal differentiation. This ranges from non-specific fibrosarcoma/MFH like to specific heterologous, mesenchymal differentiation, e.g. rhabdomyosarcoma, chondrosarcoma, osteosarcoma.

Small Cell Carcinoma

- A poorly differentiated/high-grade neuroendocrine carcinoma either primary or secondary from lung. Tumours presenting with predominantly urological symptoms and signs are usually primary in nature. It is CAM 5.2/synaptophysin/CD56 positive and often TTF1 positive (not discriminatory between primary and secondary disease) with a high Ki-67 index, and, *aggressive with early metastases at presentation in 56% of cases* to lymph nodes, liver, bone and peritoneum. *5 year survival is <10%.* It is treated with 'small cell type' chemoradiotherapy. Small cell carcinoma may be *pure or mixed* with other in situ

or invasive bladder cancer subtypes and coexistent prostatic disease, both in up to 50% of cases.
- Large cell neuroendocrine carcinoma also occurs rarely.

Undifferentiated Carcinoma

- No specific differentiation and an *aggressive high-grade* tumour.

Malignant Melanoma

- Primary or secondary (commoner).
- Note there can be spread to bladder from a primary urethral lesion.

Metastatic Carcinoma

- *Metastases should be considered in any bladder tumour with unusual histology,* e.g. adenocarcinoma or squamous cell carcinoma. Knowledge of a *previous positive history* and *comparison of morphology* are crucial. Metastatic disease in the bladder is usually solitary.
- *Direct spread:* from adjacent pelvic organs (>70% of cases)—prostate, cervix, uterus, anus, rectum, colon. To distinguish primary adenocarcinoma from secondary colorectal carcinoma, look for an origin at the dome from urachal remnants, or areas of adjacent mucosal intestinal metaplasia/cystitis glandularis in a primary lesion. Bladder adenocarcinoma can be of enteric morphology and immunophenotype and this is not necessarily indicative of the organ of origin. Strong nuclear staining for βcatenin may indicate a colorectal primary. Knowledge of a prior positive colorectal biopsy or resection is obviously helpful. A previous positive history on cervical smear/biopsy or endometrial sampling raises the possibility of a primary gynaecological tract malignancy. Oestrogen receptor positivity may also help. Prostatic cancer is PSA/PSAP/androgen receptor positive but CK7/CK20/34βE12/P63/GATA3 negative which is the converse of bladder neck urothelial cancer. Note that some poorly differentiated or metastatic prostate cancers stain more strongly with polyclonal rather than monoclonal PSA with the potential for a false negative result using only the latter.
- *Distant spread*: breast, malignant melanoma, lung, stomach.

Differentiation/Cytological Grade

Flat, papillary and invasive urothelial neoplasia are graded separately. There are several classification options although in the UK the *WHO 1973* scheme remains in favour. This is preferred over the WHO 2004/2016 classification due to concerns about reproducibility. Current European Association of Urology (EAU) and the Royal College of Pathologists recommendations for grading of non-muscle invasive bladder cancer indicate that both classifications should be used in the bladder (and collecting system) pending further prospective audits of comparative patient outcomes (most recent 2017). In general, these classification schemes are based on the *degree of cytoarchitectural abnormality* characterised by increasing nuclear atypia, hyperchromasia and crowding with upregulated proliferative and mitotic activity.

Flat urothelial neoplasia: comprises a spectrum of flat and non-invasive lesions, with *urothelial proliferation of uncertain malignant potential* (a newly introduced term for urothelial hyperplasia), *urothelial dysplasia* (defined as cytologically and architecturally falling short of carcinoma in situ) *and urothelial carcinoma in situ (CIS).* Patterns of CIS include: pleomorphic, 'monomorphic', pagetoid, clinging (denuding), 'small-cell' and undermining types. Urologists will follow up and may not treat a diagnosis of dysplasia, but, *initiate treatment for CIS* which may entail BCG immunotherapy, intravesical chemotherapy or surgery. Dysplasia and CIS (syn: *low-* and *high-grade intraurothelial neo-*

plasia respectively) are to be distinguished from *urothelial hyperplasia* (thickening of the urothelium without cytological atypia), and *regenerative/reactive atypia* which can be encountered in a range of conditions, e.g. cystitis, calculi, an indwelling urinary catheter or post chemo−/radiotherapy. In addition to morphological clues dysplasia/CIS over expresses *p53* and *Ki-67* with strong, diffuse *pan-epithelial CK20 staining*. Non-neoplastic urothelium tends to show only basal layer proliferative activity, CK20 staining of surface umbrella cells and increased staining for *CD44s*. A Ki-67 index ≥16% in flat mucosa favours a diagnosis of CIS over non-CIS conditions. Nuclear inclusions due to Polyoma virus infection are another diagnostic pitfall. A further consideration is atypical urothelium adjacent to a carcinoma, i.e. non-representative sampling of the lesion, or, residual papillary carcinoma post treatment.

Papillary urothelial neoplasia: papillary urothelial lesions with a spectrum of minimal to marked cytoarchitectural abnormalities. This ranges from the very rare benign transitional cell papilloma covered by normal urothelium to papillary urothelial proliferation of low malignant potential (PUNLMP) in the 2004/2016 classifications to the common place urothelial carcinomas cytological grades WHO 1973 I/II/III (G1/G2/G3)/WHO 2016 low/high grade. A *WHO I/G1(low grade WHO 2016) carcinoma* is the least disordered being a *low-grade* lesion with *<5% risk of progression* to invasion. A *WHO III/G3 (high grade WHO 2016) tumour* is the most anaplastic being *high-grade* with a *30–60% risk of progression*. Thus a papillary neoplasm is *typed, graded and staged* by assessing the degree of cytoarchitectural changes and the presence of underlying connective tissue invasion. Comment is also made on the absence or presence of dysplasia or CIS in the represented flat mucosa and whether muscularis propria has been sampled. A comparison of the classifications is given in Table 30.1.

Invasive urothelial neoplasia: *WHO (1973) I/II/III (G1/2/3) or lowgrade/high grade (WHO 2016)*. Note that WHO III invasive urothelial carcinoma often assumes a squamoid appearance in addition to actual mixed squamous cell or glandular differentiation (20–30% of cases). High-grade often correlates with advanced stage of disease.

Non-urothelial invasive neoplasia: well/moderate/poor/undifferentiated, or, Grade 1/2/3/4. For squamous cell carcinoma based on keratinisation, cellular atypia and intercellular bridges, and, for adenocarcinoma, on the tumour percentage gland formation (well/G1: >95%, moderate/G2: 50–95%, poor/G3: <50%). Signet ring cell adenocarcinoma is high-grade (G3).

Extent of Local Tumour Spread

- Border: pushing/infiltrative.
- Lymphocytic reaction: prominent/sparse.

The TNM8 classification applies to urinary bladder carcinomas (non-invasive and invasive).

Table 30.1 Classification of papillary urothelial neoplasms

WHO 1973	Transitional cell papilloma	Transitional cell carcinoma		
	WHO 0/G0	WHO I/G1[a]	WHO II/G2[a]	WHO III/G3[b]
WHO/ISUP 1998	Urothelial papilloma	Papillary urothelial neoplasm of low malignant potential (PUNLMP)	Low-grade urothelial carcinoma	High-grade urothelial carcinoma
WHO 2004/2016	Urothelial papilloma	Papillary urothelial neoplasm of low malignant potential (PUNLMP)	Low-grade urothelial carcinoma	High-grade urothelial carcinoma

Note that the columns are not discrete categories or directly transferable but represent a spectrum of cytoarchitectural abnormalities

Urothelial (Transitional cell) papilloma can be exophytic or inverted in type

[a]Low-grade disease. Includes most (but not all) WHO II/G2 tumours

[b]High-grade disease

pTX	Primary tumour cannot be assessed
pT0	No evidence of primary tumour
pTa	Non-invasive papillary carcinoma
pTis	Carcinoma in situ
pT1	Tumour invades subepithelial connective tissue
pT2	Tumour invades muscularis propria
pT2a	Tumour invades superficial muscularis propria (inner half)
pT2b	Tumour invades deep muscle (outer half)
pT3	Tumour invades perivesical tissue
pT3a	Microscopically
pT3b	Macroscopically (extravesical mass)
pT4	Tumour invades any of the following: prostate stroma, seminal vesicles, uterus, vagina, pelvic wall or abdominal wall
pT4a	Tumour invades prostate stroma, seminal vesicles, uterus or vagina
pT4b	Tumour invades pelvic wall or abdominal wall

Direct invasion of distal ureter is classified by the depth of greatest invasion in any of the involved organs (Fig. 30.1).

The suffix (is) may be added to any stage to indicate presence of associated carcinoma in situ.

Involvement of prostatic stroma by invasive bladder disease is pT4a, as is small or large intestine, peritoneum covering the bladder and seminal vesicles.

Superficial tumours: are regarded as either pTa or pT1 and are often histological grade WHO I or II (low grade). Formal substaging of pT1 bladder tumours is not usually done but if feasible, comment is made on the degree of invasion, e.g. focal or extensive, above/at/below muscularis mucosae (pT1a: cores of papillae, pT1b: lamina propria, pT1c: below muscularis mucosae).

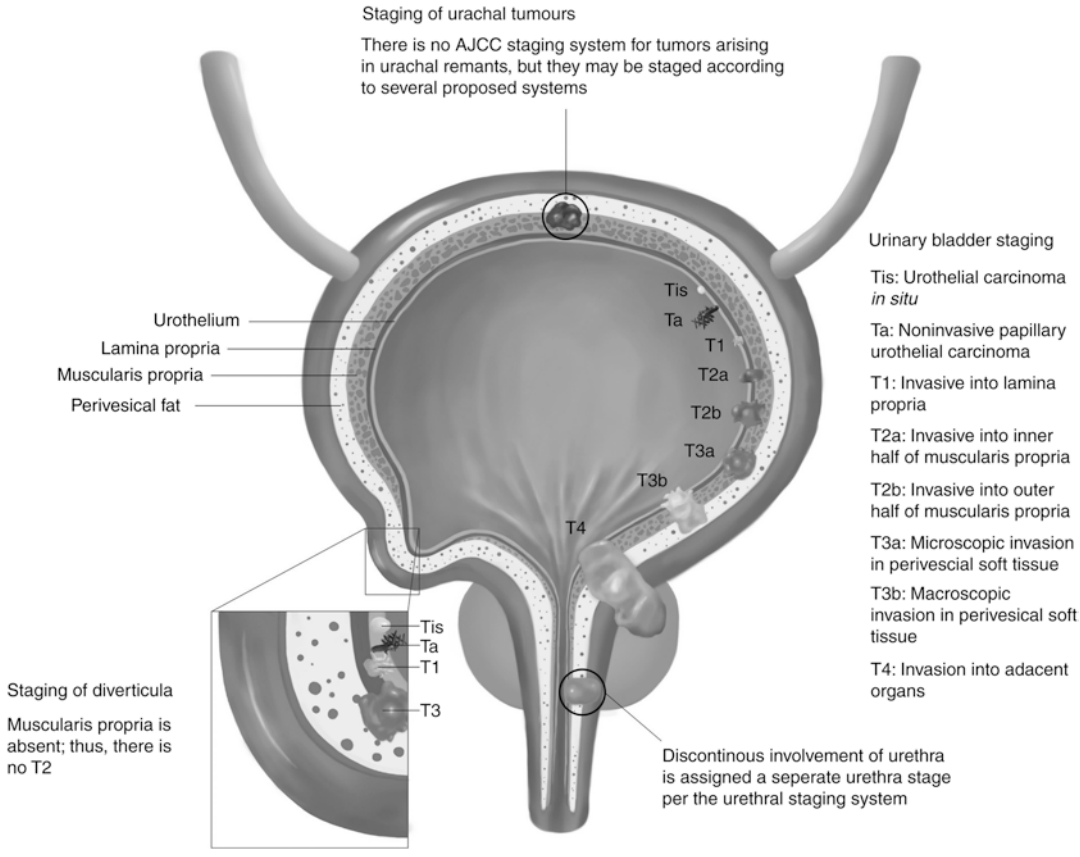

Fig. 30.1 Staging of tumours arising from the urinary bladder, diverticulum and urachal remnants. Similarly worded AJCC staging is shown. *Reproduced, with permission, from* Magers et al. (2019)*, John Wiley & Sons*

Notably, the *extent of invasion* (≤ or >1 high power field) *stratifies risk for local recurrence*.

Deep (muscle invasive) tumours: are pT2 or pT3 and more often non-papillary and histological grade WHO III. They have an adverse prognosis requiring more radical treatment and assessment of *invasion of the detrusor layer of the muscularis propria* is of crucial importance in their designation. Unless submitted separately by the urologist, superficial and deep muscle cannot be distinguished on TURBT samples and the stage is reported as at least pT2a. As well as invasion of the bladder wall urothelial cancer can extend into the bladder neck, prostate, urethra and seminal vesicles. Because of this urethral biopsy is done in staging patients with CIS or high-grade invasive disease and if positive, en bloc prostatourethrectomy is favoured at the time of cystectomy.

Lymphovascular Invasion

- Present/absent.
- Intra-/extratumoural.
- Invasion into the lamina propria may result in prominent *retraction artifact* spaces around tumour cells and nests *mimicking lymphovascular invasion*. This phenomenon is particularly prominent in invasive micropapillary carcinoma which also does show a propensity for lymphovascular and lymph node disease. For true vascular involvement identify an endothelial lining with adherent tumour and red blood cells. Endothelial markers (CD31, CD34) may be of use. *Vascular invasion* is associated with an *increased rate of recurrence.*

Lymph Nodes

- Site/number/size/number involved/limit node/extracapsular spread.
- Regional lymph nodes: pelvic lymph nodes below the bifurcation of the common iliac arteries—hypogastric, obturator, external iliac, presacral but include the lymph nodes along the common iliac artery too.

pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Metastasis in a single lymph node in the true pelvis (hypogastric, obturator, external iliac or presacral)
pN2	Metastasis in multiple lymph nodes in the true pelvis (hypogastric, obturator, external iliac or presacral)
pN3	Metastasis in a common iliac lymph node(s)

Lymph node metastases are present in *25% of invasive urothelial carcinomas.* The total number and proportion of involved lymph nodes, and the presence of extracapsular spread are prognostic indicators. Common sites of *distant metastases* are lungs, liver, bone and central nervous system.

Excision Margins

Distances (mm) to the limits of the urethra, ureters, circumferential perivesical fat margin and the inferior resection limit of the bladder wall in a partial cystectomy. Note also the presence of any dysplasia/CIS at the ureteric and urethral limits. Intraoperative frozen section is sometimes required to assess tumour clearance of the urethra and ureters during cystectomy. A *positive margin* post cystectomy is an *adverse indicator*. Great care must be taken not to miss a subtle pattern of invasion in the muscle coat or external connective tissue adventitia.

Other Pathology

Diagnostic Criteria for Urothelial Carcinoma

Carcinoma In Situ (CIS)

- Flat urothelium of variable thickness (3–20 layers).
- *Marked cytological abnormality of usually (but not always) the whole epithelial thickness of either large cell (± pleomorphism) or small cell types.*
- Note unusual patterns such as the Pagetoid variant, undermining (lepidic) growth, or

clinging ('denuding') CIS resulting from dyscohesion and shedding of cells.
- CIS equates to severe dysplasia and is by its nature a *high-grade lesion*.
- CIS comprises *1–3% of urothelial neoplasms* and is present adjacent to invasive carcinoma in *50–60% of cases*. It is a strong marker for subsequent tumour relapse, disease progression, decreased survival and increased risk of developing upper urothelial tract cancer.
- *CIS shows strong, diffuse panepithelial positivity for CK20, Ki-67 and p53 but is negative for CD44s. There is progression to invasive malignancy in up to 30% of cases.*
- Beware of overcalling dysplasia or in situ change, as normal urothelium often partially denudes on biopsy leaving a thin covering of basal cells which can then appear hyperchromatic.
- Note that a biopsy diagnosis of *dysplasia* with no previous history may *progress* to CIS or invasive malignancy in up to *19%* of cases over the course of several years.

Papillary Urothelial Carcinoma
- >7 cell layers thick.
- *Papillae with fine stromal cores* which are not true lamina propria (a distinguishing feature from the broad oedematous cores of polypoid cystitis).
- Variable grade of *nuclear atypia*.

Growth Pattern

Papillary, Exophytic
- The vast majority of cases.

Sessile/Solid
- Tends to be associated with high-grade lesions.

Endophytic and Non-Invasive, or Invasive
- Differentiate from *inverted papilloma* which occurs at a younger age and at the bladder trigone. It is characterized by a covering of normal urothelium, no atypia or mitoses, inversion of the epithelial layers, and an endophytic nested and anastomosing jig-saw like pattern.
- Non-invasive lesions have an intact, round basement membrane and no desmoplastic or inflammatory stromal response. They often represent a complex crypt pattern to the lesion base or extension of malignant epithelium into Brunn's nests. Invasive endophytic lesions can have a rounded deep border with no inflammatory reaction making assessment of invasion difficult—look for atypical urothelium present in relation to surrounding fibres of muscularis propria.

Microinvasion
- Terminology used more as a descriptive term in uropathology practice, as may relate to incomplete sampling (incomplete TURBT or diagnostic biopsy). Characterised by *single cells, irregular spicules or nests of atypical urothelium budding into or lying separately in the superficial lamina propria.* They are often associated with stromal oedema, retraction artifact mimicking lymphovascular invasion, or a loose fibroblastic response. Its *extent is defined as ≤2 mm* from the nearest basement membrane but represents pT1 stage (also dependent on the size and nature of the biopsy). Its identification can be facilitated by use of cytokeratin immunohistochemistry. It is to be distinguished from CIS inhabiting von Brunn's nests. *Extent of CIS >25%* of the bladder is strongly associated with the presence of *microinvasion (34%)* and *progression (5.8%)* to lymph node metastases and death. *Deeper invasion* comprises irregular cell nests separated by variably desmoplastic submucosa or detrusor muscle bundles. Histological interpretation can be partially compromised by TURBT diathermy distortion.

Pathological Predictors of Prognosis

- *Number of tumours/multifocality*: both within the bladder and extravesical, e.g. ureters and renal pelvis is a strong marker for subsequent *tumour recurrence.*

- *Size* of tumour.
- *Depth* of invasion.
- Histological *grade.*
- *Coexistent CIS/dysplasia* adjacent to or away from the tumour are markers of higher risk for recurrence and progression.
- *Progression* of grade and stage with time is more likely if the index histology shows: high-grade, CIS, pT1 disease, lymphovascular invasion and micropapillary, sarcomatoid or plasmacytoid pattern.
- *Poor initial response* to chemo−/radiotherapy
- Current international research is focusing on the search for biomarkers predictive for tumour BCG response, recurrence, disease progression and survival. Some of these include over expression of ezrin, p53, FGFR3 and methylated myopodin and PMF1. The commercial Urovysion system for the detection of tumour recurrence is based on the FISH identification of aneuploidy in chromosomes 3, 7 and 17, and, loss of the 9p21 locus.

Additional Comments

Biopsies

Stage and field change: assess material from the *base (for pT stage),* and *adjacent and distant mucosa (for pTis).* Clear distinction between superficial and deep muscle coat cannot be made on biopsy material (unless submitted separately by the clinician), so that muscle invasive carcinoma should be reported as *at least stage pT2a*. The muscle bundles should be coarse indicating the detrusor layer or muscularis propria rather than the fine fibres of the poorly defined lamina propria. Smoothelin immunohistochemistry may be helpful in distinguishing muscularis mucosa from muscularis propria. There is weak, patchy staining in muscularis mucosa and strong diffuse reactivity in the muscularis propria. This selective staining may be dependent on the staining conditions and occasionally a false positive staining may be found in the muscularis mucosae. *Note that fat may also be present in the lamina propria and between muscle coat bundles and does not necessarily mean invasion of perivesical tissue*. This designation is reserved for complete assessment of the cystectomy specimen. A European uropathologist network survey has shown that there is an educational and training need for standardization of various reporting parameters with regard to pathological staging in relation to invasion of muscle, perivesical fat and prostate.

Mimics of bladder cancer: biopsies from other lesions that can *mimic a papillary neoplasm* are polypoid cystitis, nephrogenic adenoma/metaplasia, follicular cystitis, cystitis cystica, malakoplakia, prostatic adenocarcinoma, villous adenoma and inverted transitional cell papilloma. Although *inverted papilloma* is benign a small minority can be multifocal and recurrent and difficult to distinguish from urothelial carcinoma with an inverted pattern. *Polypoid cystitis* has broad oedematous connective tissue cores covered by reactive urothelium and there may be a history of an indwelling urinary catheter. *Nephrogenic metaplasia (adenoma)* is often associated with prior instrumentation, treatment, calculi, cystitis or in 8% of cases renal transplantation. Its distinctive tubulopapillary, polypoid and sometimes cystic pattern of cuboidal to variably atypical epithelial cells (CK7, AMACR, PAX-2, PAX-8 positive) is distinguished from adenocarcinoma and clear cell adenocarcinoma by its low mitotic and proliferative (Ki-67, p53) activity. It is considered either metaplastic or in transplant patients of renal tubular stem cell origin. *Villous adenoma* is analogous to its intestinal counterpart and can be benign, associated with urachal adenocarcinoma, or progress (20–30%) to non-urachal adenocarcinoma. *Prostatic adenocarcinoma* (PSA/PSAP/androgen receptor positive) is a consideration in material derived from the bladder base or prostatic urethra. *Mimics of a solid urinary neoplasm* are previous biopsy or local ablation site reaction, myofibroblastic proliferations, amyloid, endometriosis, sarcoma, extrinsic and metastatic carcinoma.

Resection Specimen Blocks

- Urethral limit: transverse section.
- Ureteric limits: transverse section.
- Prostate, seminal vesicles: in *30-50% of radical cystoprostatectomy specimens for urothe-*

lial cancer, systematic sampling shows that there is concurrent, undiagnosed prostatic adenocarcinoma which requires separate typing, Gleason score (and Grade group), TNM8 staging and assessment of margin status.
- Normal bladder: lateral walls, dome, trigone.
- Tumour and wall: one section per cm of tumour diameter to show the deepest point of mural invasion.

Post-Operative Necrobiotic Granuloma

- *Post-TURP fibrinoid necrosis with palisading histiocytes*. Biopsy site reaction to previous TURB often shows prominent granulation tissue, focal dystrophic calcification and a foreign body giant cell response which may be associated with diathermy coagulum. The giant cells can be epithelioid and "pseudocarcinomatous" in appearance and cytokeratin/ CD68 staining may be necessary to help distinguish from residual invasive urothelial carcinoma.

Myofibroblastic proliferations: post-operative spindle cell nodule (PSCN), inflammatory myofibroblastic tumour (IMFT/pseudosarcomatous fibromyxoid tumour/ inflammatory pseudotumour / atypical fibromyxoid tumor / Plasma cell granuloma).

A range of myofibroproliferative processes histologically resembling sarcoma. *PSCN* is small (5–9 mm) and has a history of recent genitourinary tract instrumentation. It comprises a proliferation of cytologically bland spindle cells in which normal mitoses are readily seen and it occurs at the operative site. *IMFT* occurs de novo, can be several centimetres in diameter and forms a polypoid mass. The atypical myofibroblastic proliferation is associated with prominent inflammation and granulation tissue type vasculature. Mitoses can be seen, but are not prominent or abnormal. *Both lesions are usually benign* and must be distinguished from sarcoma although some cases of IMFT have been reported to recur and even progress with local infiltration of muscularis propria. Recent evidence supports a neoplastic process of low-grade nature because of its often deep infiltration, occasional coexistence with urothelial carcinoma, chromosomal translocation involving chromosome 2p23 and cytogenetic monoclonality. Cytokeratin is positive in PSCN but can be negative in IMFT (a useful discriminator from spindle cell carcinoma): actin and desmin/h-caldesmon are variably positive (40–80%). IMFT can be ALK 1 positive but is p53 negative in contradistinction to sarcoma. Rearrangement of ALK gene on chromosome 2p23 has been noted in these tumours.

Immunophenotype

Urothelial carcinoma is positive for cytokeratins 7, 8, 18 and 20, CEA, GATA3, uroplakin III, CK5/6, 34βE12, p63, thrombomodulin, CA19-9, Leu M1 and Lewis X antigen. Over expression of p53 correlates with the likelihood of progression in superficial disease. A useful panel is 34βE12/ CK7/CK20/p63/GATA3/p53 positive: PSA/ PSAP negative with the converse for poorly differentiated prostatic carcinoma which helps to clarify the nature of poorly differentiated carcinoma at the bladder base. Positive marking with uroplakin III can be of help in assessing metastatic carcinoma of unknown primary site particularly in pelvic and retroperitoneal lymph nodes.

Treatment

Treatment of bladder urothelial carcinoma is usually by *transurethral resection* and *cystoscopic follow up.* High-grade or refractory superficial disease (pT1G3 i.e. confined to the mucosa or submucosa) is in addition given a course of *intravesical chemotherapy/BCG*. The latter is also useful for urothelial CIS. In follow up, *intravesical agents* such as mitomycin can result in *urothelial atypia* which must not be confused with dysplasia or CIS. Due to the slow turnover of urothelial cells these changes can persist for long periods of time. The nuclei are focally enlarged and have a "smudged" chromatin appearance

rather than the angular, ink blank hyperchromasia of CIS. Post radio−/chemotherapy pseudocarcinomatous and atypical stromal cells are also encountered. BCG often results in inflammation and superficial non-caseating granulomas. A range of other post therapy changes can be seen with systemic chemotherapy, radiotherapy, laser and photodynamic therapy. Urachal related adenocarcinoma at the bladder dome may be considered for a *partial cystectomy* with resultant preservation of urinary function. Non-responsive superficial or deep (muscle invasive) cancer necessitates *radical cystectomy.* Checkpoint inhibitor drugs (PD-L1) may be offered to patients with locally advanced or metastatic bladder cancer who are not eligible for or refractory to cisplatin-containing chemotherapy.

Prognosis

Muscle invasive cancer often starts as CIS or a flat/sessile rather than a papillary lesion and relates strongly to histological grade (WHO I = 2% invasive; WHO III = 40% invasive). Invasive cancer will develop in up to 30–50% (or more) of patients with untreated CIS but 85–90% 5 year survival rates can be achieved by radical surgery which is also targeted at multifocal field change in the urothelium (bladder, prostatic ducts, urethra, ureters, seminal vesicles). Up to *80% of urothelial carcinomas are non-invasive at the time of presentation* and although *tumour recurrence* is common (single lesion 30–45%, multiple 60–90%, <5% risk after a disease free interval of 5 years), *tumour progression (10%)* relates strongly to histological grade, tumour size, multifocality, short disease free interval between local recurrences, non-tumour dysplasia/CIS of bladder mucosa, and depth of invasion. Over expression (>20% of tumour cells) of Ki-67, p53 and HER2 may also be another indicator. *Five year survival rates* also vary according to these parameters:

Urothelial carcinoma	Superficial invasion	5 year survival
	Grade I	70%
	Grade III	60%
	Deep muscle invasion	40–55%
Squamous cell carcinoma		15%
Adenocarcinoma		15–35%

As can be seen, *squamous cell carcinoma* and *adenocarcinoma* are of *worse prognosis.* Other *adverse histological types* are: nested and invasive micropapillary urothelial carcinoma, urothelial carcinoma with rhabdoid or plasmacytoid features, sarcomatoid carcinoma, undifferentiated carcinoma and small cell carcinoma.

Other Malignancy

Malignant Lymphoma/Leukaemia

- *Usually secondary* to advanced stage systemic disease.
- *Primary malignant lymphoma* comprises *<1% of bladder neoplasms* and varies from low-grade MALToma with indolent behavior, to *diffuse large B cell malignant lymphoma (commoner).* It can be a solitary mass (70%), multifocal (20%) or diffuse in distribution. Leukaemic involvement is seen in 15–30% of cases especially chronic lymphocytic leukaemia (CLL).

Carcinoid Tumour

- Rare. A well differentiated/low-grade neuroendocrine tumour: 25% have regional lymph node or distant metastases but most are treated adequately by local excision. Chromogranin/synaptophysin/CD56 positive with a low Ki-67 index (≤2%).

Paraganglioma (Phaeochromocytoma)

- Young women, classically with paroxysmal hypertension, intermittent haematuria, and 'micturition attacks' due to spasmodic increased catecholamine levels in the blood. It forms a paragangliomatous nested pattern of

cells with eosinophilic cytoplasm and variable nuclear features. Chromogranin/synaptophysin positive with S100 positive sustentacular cells.

- *Local recurrence* and *metastases* can occur in about *10% of cases.* Histology does not reliably predict behaviour, although higher stage tumours are more prone to metastases which can occur up to many years later.

Leiomyosarcoma

- Commonest sarcoma but *<1% of bladder malignancy.* In adults, at the bladder dome and infiltrates muscle. It is an *aggressive tumour* with 70% developing metastases, recurrent or fatal disease.
- Nuclear atypia, mitoses and tumour necrosis: some are extensively myxoid.
- Desmin/h-caldesmon positive.
- Mucosal leiomyoma is small, cytologically bland and lacks mitotic activity.

Other Sarcomas

- Rhabdomyosarcoma, malignant fibrous histiocytoma, angiosarcoma, osteosarcoma: all rare and must exclude bladder sarcomatoid carcinoma (carcinosarcoma). Other rarely occurring sarcomas are Ewing's/PNET, malignant peripheral nerve sheath tumour and alveolar soft part sarcoma.

Rhabdomyosarcoma

- Embryonal variant in children, sarcoma botryoides. There are also low-grade spindle cell, and aggressive alveolar subtypes. Rare in adults and must exclude bladder sarcomatoid carcinoma with rhabdomyoblastic differentiation.
- Cellular subepithelial cambium layer with a loose myxoid zone and cellular deep zone ± rhabdomyoblasts. Desmin/myo D1/myogenin positive.

Choriocarcinoma and Yolk sac Tumour

- Choriocarcinoma: exclude urothelial carcinoma with trophoblastic differentiation.
- Yolk sac tumour: rare; childhood (<2 years of age).

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Prostate Carcinoma

31

Declan O'Rourke

Prostatic adenocarcinoma is a common visceral malignancy with a 1 in 6 lifetime risk and crude mortality figure of 22%. It is androgen dependent, its incidence increasing with age and is present in 80% of men by the age of 80 years. Some 5–10% have a strong family history (BRCA2, HOXB13—first hereditary prostate cancer gene). Racial (black) ethnicity, a high calorie diet and lack of physical exercise are contributory. Many men die with their disease due to its indolent behaviour rather than because of it, although a significant minority show aggressive behaviour with metastases. It is the sixth leading cause of cancer death in men and 1 in 27 will die of prostate cancer.

Prostatic cancer is symptomatic in 25% of cases and this is often indicative of widespread disease with lumbar pain as a result of bone metastases. It may also present with symptoms of prostatism (nocturia, urinary frequency, hesitancy and dribbling). However, it is often asymptomatic and detected because of an elevated serum prostate specific antigen (PSA) with digital rectal examination (DRE), either as part of a screening programme or a family practitioner's well man checkup clinic. Recent NICE guidance suggest that PSA, DRE, co-morbidities, risk factors and history of previous biopsy performed, should all be taken into consideration prior to MRI and possible subsequent biopsy. Further investigation comprises transrectal ultrasound (TRUS) to identify classical tumour related hypoechoic areas. As 70% of prostatic cancer is present posteriorly and peripherally, this is coupled to per rectum clinical or TRUS directed needle core biopsies. Studies have now indicated that a 12-core systematic biopsy (TRUS and transperineal template (TTP)) incorporating apical and lateral cores in a template distribution allows for maximum cancer detection, avoids repeat biopsies and minimises the detection of insignificant prostate cancers. The use of TTP biopsy of the prostate has been introduced in many centres. The advantage of this procedure is that it facilitates a relatively large number of tissue samples from across the prostate, in three dimensions, to increase detection of small lesions. This may improve the detection of small cancers (detection rate of 39% in total) compared with other biopsy methods. Disadvantages include the impact on laboratory services relating to additional workload. However, both TRUS and TTP approaches to prostate biopsy have been found to have similar cancer detection rates and complications. Saturation or extended biopsies (sampling the transition zone) have been shown to offer no benefit for initial diagnostic biopsies. However, such approaches might be useful for resampling following a negative biopsy, when

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_31

indicated, and for planning the use of novel therapeutic approaches. Many biopsies now are performed following MRI: increasingly, MRI guided prostate biopsy is being used in both initial and repeat prostate biopsies, potentially improving sampling efficiency, increasing detection of clinically significant cancers, and reducing detection of insignificant cancers. TRUS biopsies are targeted with sextant core samples (six each lobe) aimed at the apex, mid-zone and base regions of the gland. The resultant fine biopsy cores need careful handling, wrapping and painting with alcian blue prior to processing to allow their visualisation at the block cutting stage. This minimises initial block trimming which may result in loss of diagnostic tissue. Blocks are cut through at least three histological levels and the intervening ribbons kept pending any subsequent need for immunohistochemistry which may be required in a minority of cases. Microscopic assessment is at low power looking for abnormalities of glandular architecture and medium to high power to confirm cytological features of malignancy. The biopsy report should indicate which biopsy site(s) is/are positive, the modified Gleason tumour grade, the percentage of differing modified Gleason patterns (e.g. in Gleason 7 cases in particular), the number of positive cores and percentage of involved tissue, and the maximum linear dimension of carcinoma. It may be possible to comment on other features such as perineural or lymphovascular invasion, and, staging information, e.g. spread into extracapsular fat or neurovascular bundles, or, involvement of seminal vesicles. Another indication for prostatic biopsy is a rising serum PSA after radiotherapy or brachytherapy for a previously proven cancer. Reasons for a repeat biopsy are an insufficient index biopsy, features suspicious but not diagnostic of malignancy, high-grade PIN (Prostatic Intraepithelial Neoplasia), and a rising serum PSA after a negative biopsy.

Treatment of prostatic cancer is age, fitness, Gleason grade and tumour stage dependent. This ranges from active surveillance, to radiotherapy and hormonal therapy (androgen deprivation) for focal, and, locally advanced or metastatic disease (chemotherapy an option), respectively. Androgen deprivation is effected by use of luteinizing hormone releasing hormone agonists or oestrogen preparations. Metastatic disease is assessed by radioisotope bone scan. CT scan has limited sensitivity for local spread. Pre-biopsy multiparametric MRI scan should be standard practice as it is much better at identifying significant prostate cancer (compared to TRUS), reduces unnecessary biopsies by 27% and can improve the accuracy of biopsy sampling. It is particularly helpful in detection of biopsy negative central or anteriorly sited tumours (20–25% of cases). Radical robotic prostatectomy is aimed at younger patients (age 56–60 years) with low to modest elevations in serum PSA who are more likely to have gland confined disease and negative surgical margins. It is an operation with significant morbidity and side effects, e.g. incontinence, impotence. Some of these may be avoided by a selective nerve sparing procedure, although this can have implications for the completeness of excision and tumour clearance of the margins. An equivalent alternative with fewer complications is radical radiotherapy, and there is also increasing use of brachytherapy (radioactive seed implants), or cryotherapy of the tumour and its bed. Preoperative combination therapy can downsize tumour while use of post-operative radiotherapy and/or chemotherapy are based on the Gleason component and sum scores, margin status and the presence of extracapsular disease. Prostatic chippings piece-meal resect the periurethral and central zones and transurethral resection of the prostate (TURP) is performed in two main situations

(a) In patients with benign hypertrophy of the medial aspect of the gland who have persistent troublesome prostatism that is refractory to medical therapy, or who develop acute urinary retention
(b) TURP channel re-do in a patient with known cancer and significant prostatic symptoms.

In the former incidental cancer may be detected by microscopy in up to 8% of cases, and the significance of this is then interpreted in light

of the patient's serum PSA and clinical staging. It may either be an incidental low volume, low-grade cancer arising from the transitional zone, or, contiguous spread from a larger, often high-grade, peripherally sited tumour.

Gross Description

Specimen

- Fine needle aspirate cytology (FNAC)/needle core biopsy (18 gauge)/transurethral resection (TUR) chippings/ radical prostatectomy (including seminal vesicles) and regional lymphadenectomy.
- Weight (g) and size (mm).
- Number and length of cores (mm).

Tumour

Site

- Inner (transitional)/outer (central and peripheral) zones. The transitional zone surrounds the proximal urethra and the central zone is posterior to it. The peripheral zone occupies 70% of the gland in a horseshoe shape around its posterior and lateral aspects.
- Medial/lateral (right or left) lobes. These are not defined anatomical structures but relate to clinically palpable masses on per rectum examination. For the purposes of TNM staging the gland is notionally divided into right and left lobes about a mid-point sagittal plane.
- Posterior/subcapsular.
- The majority of carcinomas are *posterior* and peripheral with *multicentricity* present *in up to 75% of cases*.

Size

- Length × width × depth (mm) or maximum dimension (mm).
- *Tumour volume (mm/cm^3)*. Derived by outlining and calculating the area of tumour in each slide and then multiplying by the mean block thickness (the anteroposterior (AP) diameter divided by the number of coronal slices). The volume is the sum total for all the blocks/slides. Alternatively, a quicker method is to outline the tumour in each slide, lay them out on the bench and estimate the total percentage area involved, e.g. 60%. Knowing the gland volume (AP × width × depth) the tumour volume is presumed to be 60% of it assuming even tumour distribution throughout each block. The ratio of positive to negative blocks on microscopy is an alternative time efficient index of tumour volume. Clinicians vary in the use of these data but pragmatically they give an indication of low or high volume tumour and the consequent *risk of extraprostatic disease* being present. It is also useful for correlation with serum PSA and TRUS assessment (maximum dimension of any dominant tumour nodule) along with tumour site location. However, its importance is far outweighed by that of consistent Gleason scoring, and assessment of extraprostatic extension and margin status, as even small volume cancers (<10 mm maximum dimension) can show extraprostatic extension and margin positivity depending on their location within the gland. Given the heterogeneity of tumour distribution within the prostate, the inapparent macroscopic features of prostate cancer, and increasingly early stage detection, volume estimates are more accurate based on whole gland serial slices and processing. This can mean 40–50 slides for a small gland using routine blocks. Two alternatives are
  - Whole mount sections that reduce the block numbers and allow easier assessment of anatomical tumour distribution, or,
  - A sampling strategy to include the base and apical margins, seminal vesicles, all posterior sections and a mid-slice of the anterior prostate on either side.

The latter may result in a loss of prognostic data, e.g. extraprostatic extension or positive margins, the significance of which varies in the literature. A European uropathologist network (ENUP) survey found that 71.6% of respondents embedded the entire prostate, with variation in other parameters such as specimen inking,

Gleason grading, stratifying the degree of extraprostatic extension, reporting TNM stage and the location of positive margins. Whole mounts and standard blocks were used in 37.5% and 55.5% of laboratories respectively. In needle biopsy and TURP specimens the number of cores, linear millimeters of carcinoma and percentage of chippings involved are routinely given in the surgical histopathology report. A combination of *millimeters of cancer* and *Gleason sum score* in a core biopsy is a *predictor* of the *presence of extraprostatic disease*.

Appearance

- Soft/firm
- Pale/yellow/granular

Similar changes are seen in tuberculosis, infarction, granulomatous prostatitis and acute and chronic prostatitis, i.e. tumour is difficult to define macroscopically and histological assessment is necessary.

Edge

- Circumscribed/irregular.

Histological Type

The *vast majority of prostate cancers* are of *acinar origin* including and coexisting with a minority of variants, namely, atrophic, pseudohyperplastic, microcystic, foamy cell, mucinous (colloid), signet ring cell, pleomorphic giant cell, and sarcomatoid.

About *5–10% of prostate cancers* are of *non-acinar origin*—sarcomatoid, prostatic ductal adenocarcinoma, squamous cell, adenosquamous, urothelial, small cell, basal cell and clear cell carcinomas.

Prostatic Acinar Adenocarcinoma

- *>90–95% of cases* with a range of acinar, papillary, cribriform, comedo and single cell patterns of infiltration.
- Acini are usually small to medium in size and can be rounded or angular in contour. The cell cytoplasm is amphophilic (clear to light eosinophilia) in character. Diagnosis is made at low power magnification on the basis of a *gland rich haphazard infiltrative pattern* in comparison to, and between adjacent benign prostatic ducts and acini. Medium to high power can then confirm the *lack of an epithelial bilayer* and *nuclear characteristics of malignancy* (enlargement/angularity/membrane folds/nucleolar prominence). Luminal crystalloids, glomerulations, mucinous fibroplasia or mucin extrusion may also be present. Cribriform and comedo patterns resemble intraductal carcinoma and can be difficult to distinguish from high-grade PIN, but again are architecturally too ductal rich compared to adjacent tissues.
- *Diagnostic pitfalls* are large gland, atrophic and pseudohyperplastic variants of carcinoma, which can resemble post atrophic or benign hyperplastic prostate. Occasionally foamy gland adenocarcinoma is encountered to be distinguished from cytokeratin negative/CD68 positive aggregates of xanthomatous histiocytes. Notably these well differentiated carcinoma variants are negative for the *basal layer cytokeratin markers 34βE12 and p63,* and, are variably positive for *AMACR* (alpha methylacyl coenzyme A racemase). In radical prostatectomy specimens they are often admixed with usual acinar pattern adenocarcinoma. Patient outcomes for these variants are not significantly different from usual prostate cancer.

Histologic Variants (Acinar Adenocarcinoma)

Pseudohyperplastic Adenocarcinoma

- Rare carcinoma that resembles benign hyperplastic glands: difficult to grade and may be best to defer grading to the associated usual type adenocarcinoma if in a radical prostatectomy specimen.
- Easily overlooked, particularly in TURP specimens and despite its benign appearance, may be associated with intermediate grade cancer. Can exhibit aggressive behavior.

Atrophic Adenocarcinoma

- Typically co-exists with acinar prostate carcinoma and can mimic benign atrophy or postatrophic hyperplasia. It does show the typical diagnostic morphological features of cancer. However, has infiltrative growth pattern, macronucleoli and enlarged nuclei.

Microcystic Adenocarcinoma

- Dilated glands x tenfold in comparison with usual acinar type and seen in up to 11% of radical prostatectomy specimens. Distinguish from benign cystic glands with basal marker immunohistochemistry.

Mucinous (Colloid) Adenocarcinoma

- Distinguish from *secondary colorectal or bladder cancer* by a relevant past history, and usually CK7/CK20 negative with variable PSA/PSAP expression. Indicators to a bladder/urethral origin are CK7/CK20 positivity, and for colorectum CK20/CDX-2/βcatenin positivity.
- ≥25% of the tumour area is intra−/extracellular mucin.
- More frequent bone metastases and *less hormone/radioresponsive* than usual prostatic carcinoma, although a difference in biological behaviour and outcome from usual prostate cancer is uncertain.

Signet Ring Cell Adenocarcinoma

- Rare, distinguish from secondary gastric or colorectal cancer by a relevant past history, and usually CK7/CK20 negative with variable PSA/PSAP expression.
- ≥25% of the tumour area is signet ring cells, usually coexisting with other poorly differentiated carcinoma and of *poor prognosis* presenting at an *advanced stage.*

Pleomorphic Giant Cell Adenocarcinoma

- Rare. Usually admixed with Gleason 9 or 10 tumour. Some cases are seen post hormone therapy or radiation treatment. Outcome is poor.

Sarcomatoid Carcinoma

- Rare, predominately older men with obstructive symptoms, variable serum PSA levels, an enlarged hard gland and may represent sarcomatoid transformation of prior adenocarcinoma or be related to radiotherapy/hormonal therapy. It follows an aggressive clinical course.
- Cytokeratin positive spindle cells with variable malignant stromal mesenchymal differentiation with usually homologous ± heterologous elements (bone, cartilage, striated muscle).

Non-Acinar Adenocarcinoma

Prostatic Ductal Adenocarcinoma

- Older patients with haematuria or obstructive symptoms. Polypoid/villous or infiltrative on cystoscopy. Has a higher volume and more *advanced stage at presentation* (12–20% have metastases to testis, penis, lung or bone). *Aggressive.* May be associated with a diverticulum and can also be sited in the gland periphery. Serum PSA *may not* be elevated. May have normal DRE.
- Variable papillary, cribriform and endometrioid patterns (uterine carcinoma analogue), and may coexist with usual pattern prostate adenocarcinoma. PSA/PSAP positive, ± androgen deprivation/oestrogen sensitive. AMACR can be reduced in intensity and 34βE12 focally present.
- There may be associated Paget's disease of the prostatic urethra.
- Exclude secondary renal clear cell carcinoma if mesonephroid or hobnail clear cell in type.

Adenosquamous/Squamous Cell Carcinoma

- Rare and *poor prognosis*. Exclude squamous cell metaplasia due to infarction or hormone therapy, or, spread from an anal cancer.
- Up to 50% may arise *in patients with previous usual prostate cancer treated by endocrine or radiotherapy* at an interval of several months

to years. Squamous cell carcinoma is also associated with schistosomiasis.
- *Mean survival of 6–24 months*, direct pelvic spread and metastases to lymph nodes and bone (osteolytic rather than the usual osteosclerotic prostate cancer deposits).

Urothelial (Transitional Cell) Carcinoma

- *2% of prostatic cancers.*
- Arises from the urothelial cell lining of the prostatic urethra or proximal periurethral ducts.
- Usually *high-grade* with extension into ducts, central comedonecrosis ± adjacent *stromal invasion* the presence of which is the *strongest prognostic indicator*. Survival for urothelial CIS (carcinoma in situ) is good but for invasive carcinoma is 17–29 months.
- Rarely primary, and usually represents spread from a bladder/urethral urothelial carcinoma, or, multifocal urothelial field change. This association is *present in up to 40% of locally extensive high-grade bladder cancer cases.*
- CK7, CK20, 34βE12, GATA3, p63 positive: PSA/PSAP negative.

Neuroendocrine Tumors of the Prostate

- Classified as adenocarcinoma with neuroendocrine differentiation, well differentiated neuroendocrine tumour (carcinoid), small cell neuroendocrine carcinoma and large cell neuroendocrine carcinoma (recent addition).
  - ***Carcinoid (well differentiated/low-grade neuroendocrine) tumour:*** rare. Up to 33% of usual prostatic carcinomas can show neuroendocrine differentiation on immunohistochemistry and this is usually of no prognostic significance.
  - ***Small cell neuroendocrine carcinoma:*** a poorly differentiated/high-grade neuroendocrine carcinoma which is CAM5.2/synaptophysin/CD56 positive ±TTF-1 with a high Ki-67 index. Immunonegative cases are classified as poorly/undifferentiated prostatic carcinoma. Usually PSA/PSAP negative.
  - Lung small cell carcinoma analogue.
  - In the vast majority of cases primary, either pure or mixed (25–50%) with usual prostatic carcinoma ± an associated bladder component. Gleason grading is not applied to pure small cell neuroendocrine carcinoma. Usual prostate cancer may precede it or patient may have received prior hormonal therapy. Rarely, secondary from lung.
  - *Aggressive* often *presenting with disseminated disease*, typically low volume osseous involvement in the presence of visceral metastases. It is sometimes associated with inappropriate ACTH/ADH secretion. *Survival is 9–17 month*s and it requires different chemotherapy to usual prostate cancer. It cannot be followed up clinically by serum PSA levels.
  - ***Large cell neuroendocrine carcinoma***
  - Rare with a mean survival of 7 months. It was not recognized until more recently (WHO 2016) and almost all cases arise after hormonal treatment of adenocarcinoma of the prostate.

Basal Cell Carcinoma and Clear Cell Carcinoma

- A morphological continuum from typical through florid basal cell hyperplasia/adenoma to carcinoma with infiltrative edges, stromal desmoplasia, comedonecrosis and adenoid cystic like differentiation. 34βE12 positive and a tumour of variable malignant potential, *mostly indolent*, but 14% show local recurrence and distant metastases.
- Clear cell carcinoma typically arises from the anterior or transitional zone of the prostate.

Metastatic Carcinoma

- *Direct spread*: bladder (present in 40% of radical cystoprostatectomies for bladder cancer), colorectum, anus, retroperitoneal sarcoma.
- *Distant spread*: kidney, lung (squamous cell carcinoma), malignant melanoma.

Differentiation/Grade

Gleason Score for Prostatic Adenocarcinoma

Gleason score remains the standard approach and single most powerful predictor of prostate cancer prognosis and plays a significant role in clinical management. The correct diagnosis and grading of prostate cancer is crucial for prognosis and therapeutic options. The 2005 and 2014 ISUP (International Society of Urological Pathology) grading consensus conferences have improved the overall Gleason grading system. There have been modifications to the Gleason grading system, now incorporated into WHO 2016 guidance. Major changes are:

- Cribriform glands should be assigned a Gleason pattern 4, regardless of morphology
- Glomeruloid glands should be assigned a Gleason pattern 4, regardless of morphology
- Grading of mucinous carcinoma of the prostate should be based on its underlying growth pattern rather than grading them all as pattern 4
- Intraductal carcinoma of the prostate without invasive carcinoma should not be assigned a Gleason grade
- Regarding Gleason patterns, there is clear consensus that Gleason pattern 4 includes cribriform, fused, and poorly formed glands seen at 10x objective magnification.
  - Occasional poorly formed/fused glands between well-formed glands is insufficient for a diagnosis of pattern 4.
  - In borderline morphology between Gleason pattern 3 and pattern 4 and crush artefacts, the lower grade should be favoured.
  - Branched glands are allowed in Gleason pattern 3.
  - Gleason pattern 5 includes small solid cylinders, medium to large nests with rosette-like spaces or unequivocal comedonecrosis (Fig. 31.1).

Fig. 31.1 Modified Gleason grading schematic diagram, according to the International Society of Urological Pathology. Reproduced with permission from Indiana University

The Gleason system proposes that any given prostate carcinoma may show one or several of five histological glandular architectural patterns ranging from the lowest grade (pattern 1) to the highest grade (pattern 5). Taking the two predominant patterns one can arrive at a *sum score* (e.g. 3 + 4 = 7) which has *prognostic significance correlating with biochemical evidence of treatment failure, development of distant metastases, survival post radiotherapy, progression free survival and overall survival*. The following rules apply to reporting patterns in biopsies and radical prostatectomy specimens:

(a) On needle biopsy, the Gleason score is based on the predominant pattern + the highest grade pattern (the tertiary pattern is not included), e.g. in a biopsy core with 70% pattern 3, 25% pattern 4, and 5% pattern 5, the cancer would be considered as Gleason score 3 + 5 = 8.

(b) When there is only one pattern, double it, e.g. 3 + 3 = 6. 4 + 4 = 8.
(c) Scores 2–5 are currently no longer assigned.
(d) Individual Gleason scores are assigned to separate biopsy cores as long as the cores are sent to the pathology laboratory in separate containers, or the cores are in the same container.

In clinical practice the highest Gleason score is used for prognosis and treatment. The Gleason scores of multiple cores in the same specimen pot from a certain location (e.g. based on multiparametric MRI) are averaged together: the rationale being that they are from the same location in the prostate. It is not uncommon for a set of prostate biopsies to show different Gleason scores in individual cores and it can be difficult to determine whether this variation reflects sampling from multiple tumours or intratumoral heterogeneity. In such cases, pathologists should use their judgement to determine the most appropriate Gleason score.

There may be multiple separate tumour nodules in radical prostatectomy specimens and these may show variation in Gleason patterns. A smaller nodule could exhibit high grade patterns (e.g. 4 + 4 = 8,) with a larger nodule showing a 3 + 3 = 6 pattern. The prognosis of the tumor in the overall radical prostatectomy would be expected to be based on the higher grade nodule, and the pathologist should list separate tumour nodules with individual Gleason scores. ISUP and International Collaboration on Cancer Reporting (ICCR) recommend reporting the Gleason score of the dominant nodule.

If pattern 5 is to be considered as a tertiary pattern it should represent less than 5% of the tumor in a radical prostatectomy. A tumour with a score 3 + 4 = 7 with less than 5% pattern 5 is 3 + 4 = 7 with tertiary 5 (whereas a tumour with an otherwise 3 + 4 = 7 score but with more than 5% of pattern 5 is 3 + 5 = 8).

Grade Groups

A new set of grade groups was recently developed at the 2014 ISUP consensus conference. These grade groups are as follows:

Grade group 1: Gleason score 6
Grade group 2: Gleason score 3 + 4 = 7
Grade group 3: Gleason score 4 + 3 = 7
Grade group 4: Gleason score 4 + 4 = 8; 3 + 5 = 8; 5 + 3 = 8
Grade group 5: Gleason scores 9–10

Gleason scores 2–5 are rarely used and do not feature in this grading system. Gleason score 6 is the lowest possible grade (Grade group 1) rather than an intermediate grade (i.e. score 6 is not intermediate in terms of a maximum score of 10). Many patients with grade group 1 tumours, in the correct clinical context with consideration of other parameters (e.g. serum PSA level, clinical stage, and volume of cancer in needle core tissue), could be candidates for active surveillance. The prognostic impact of these five grade groups has been validated in a large multi-institutional study. The five-year biochemical recurrence-free progression probabilities for radical prostatectomy Grade Groups 1–5 are 96%, 88%, 63%, 48%, and 26% respectively.

Extent of Local Tumour Spread

- Border: pushing/infiltrative.
- Lymphocytic reaction: prominent/sparse.
- Weight of chippings or length of cores, and the *proportion (%) of the chippings*, or, *number of cores involved*. Give a *maximum linear dimension (mm)* of carcinoma.
- Apex of gland, urethral limit, proximal bladder limit, capsule and margins, seminal vesicles.
- TNM8 classification applies only to prostatic adenocarcinomas. Urothelial carcinoma of the prostate is classified as a urethral tumour.

Tx	Primary tumour cannot be assessed
T0	No evidence of primary tumour
T1	Clinically inapparent tumour not palpable or visible by imaging
T1a	Tumour incidental histological finding in 5% or less of tissue resected
T1b	Tumour incidental histological finding in more than 5% of tissue resected
T1c	Tumour identified by needle biopsy (e.g. because of elevated PSA)[a]
T2	Tumour confined within prostate
T2a	Tumour involves one half of one lobe or less

T2b	Tumour involves more than half of one lobe, but not both lobes
T2c	Tumour involves both lobes
T3	Tumour extends through the prostate capsule
T3a	Extraprostatic extension (unilateral or bilateral) including microscopic bladder neck involvement
T3b	Tumour invades seminal vesicle(s)
T4	Tumour is fixed or invades adjacent structures other than seminal vesicles external sphincter, rectum, levator muscles or pelvic wall

[a]Tumour found in one or both lobes by needle biopsy, but not palpable or visible by imaging, is classified as T1c

The subdivision of pT2 based on lobar distribution is somewhat subjective and non-anatomical. It has been suggested that stratification of pT2 by tumour size with dimension thresholds of 5 mm and 16 mm would give more clarity. Invasion into but not beyond prostatic apex or capsule is pT2 (Figs. 31.2, 31.3, and 31.4).

The capsule: is a condensation of smooth muscle and collagen rich soft tissue around the prostate but with no clear fascia. In the lateral and posterior parts of the gland, it consists of a band of fibromuscular connective tissue that blends with the prostatic stroma, but at the apex and the bladder neck, the capsule is not present so that definitions of extraprostatic spread in a prostatectomy specimen are important. Extraprostatic extension (EPE) is defined as: tumour in fat or large nerve bundles in the neurovascular bundles beyond the contour of the prostate; tumour that is beyond the normal contour of the prostatic edge involving connective tissue that is typically looser than prostatic stroma; bulging tumours

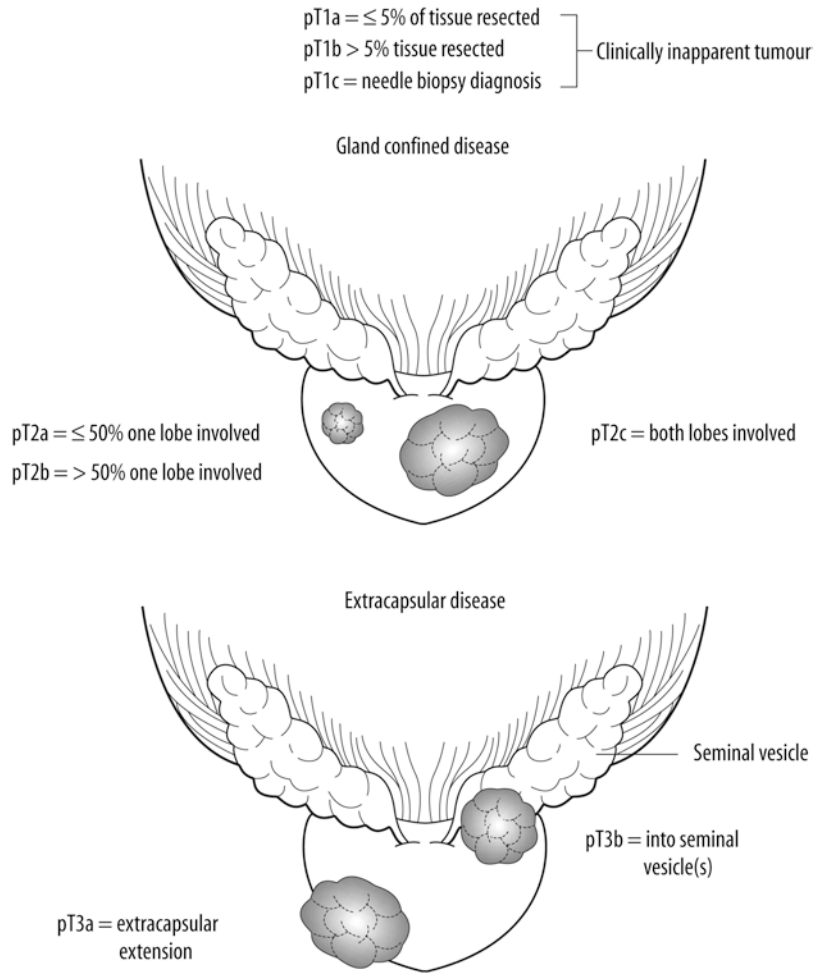

Fig. 31.2 Prostatic carcinoma. Reproduced, with permission, from *Histopathology Reporting: Guidelines for Surgical Reporting, 2nd ed.,* © 2006, Springer

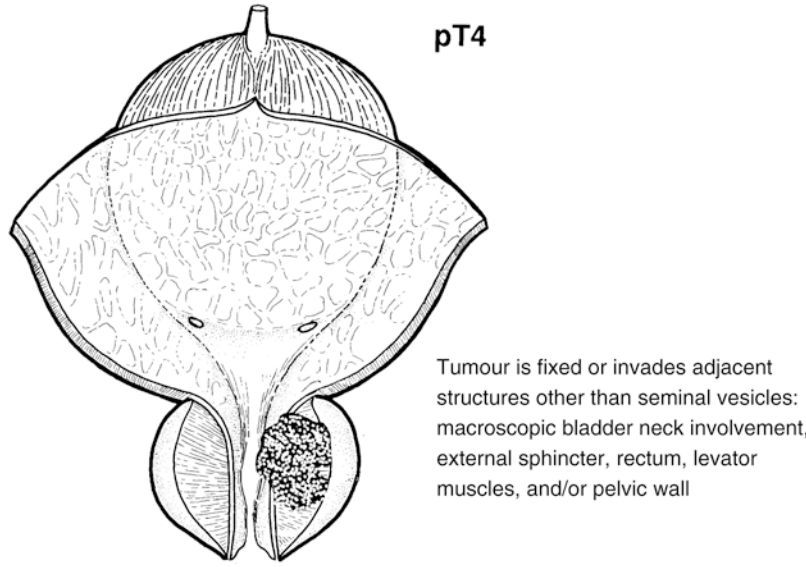

Fig. 31.3 Prostatic carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

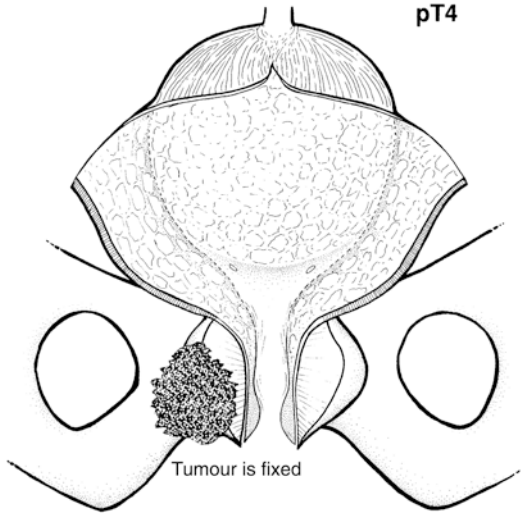

Fig. 31.4 Prostatic carcinoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

associated with a desmoplastic stromal response that extends beyond the confines of the normal glandular portion of the prostate. EPE is most commonly seen in peripheral zone tumours posterolaterally. Completeness of the capsule depends on patient anatomy, surgical expertise and choice of operative technique, e.g. a nerve sparing procedure. EPE can be described as *focal (<1 high power field in no more than 2 sections)* or *extensive (i.e. >focal)* with an adverse prognosis. Its quantitation is a predictor for *biochemical recurrence* after radical prostatectomy and of potential *disease progression*.

Due to the inferoposterior approach of per rectal needle biopsy there may be representation of *extracapsular* and *seminal vesicle tissues* which should be assessed for invasion (=pT3). Note that fat and muscle can also be, albeit rarely, intraprostatic, and involvement on its own in a needle biopsy, while suspicious of, does not necessarily imply extracapsular disease. However, *tumour in perineural spaces in neurovascular bundles in fat is an indicator of extraprostatic spread,* as is *infiltration around ganglion cells.* Tumour in fibrous tissue in the same anatomical plane as fat is another helpful clue. These features are most commonly encountered at the posterolateral aspect of the gland. Occasionally, benign acini may be present in perineural spaces but they do not show the circumferential or intraneural disposition of malignancy. Seminal vesicle involvement (pT3b) occurs in 4% of cases indicative of high stage disease and adverse prognosis. It is defined as invasion of the seminal vesicle smooth muscle wall and not just the surrounding connective tissue between it and the prostate. Seminal vesicle must not be confused with similar looking intraprostatic ejaculatory duct type epithelium,

which is orientated to loose, vascular connective tissue and surrounded by prostate gland. Microscopic invasion into the bladder neck is staged as pT3a. This may be difficult to assess but if neoplastic glands are seen in thick muscle bundles with no associated benign glands, then the possibility of pT3a disease must be considered.

Advanced disease: manifests spread into seminal vesicle, prostatic urethra and bladder. Presentation can be by an anterior rectal mass or stricture and PSA staining of rectal biopsy material is diagnostically useful. *"Frozen pelvis"* is a clinical term meaning tumour extension to the pelvic wall(s) with fixation and is designated pT4. Note that the normal prostatic apex may incorporate some striated muscle fibres and cancer lying in relation to these does not necessarily imply extraprostatic disease.

Histological cancer in TURPs performed within 2 months of each other should have a pT1a or pT1b designation based on the sum total of carcinoma over both specimens. In a prostatectomy specimen where part of the capsule is missing the pT designation can only be accurately assigned if the tumour is clearly surrounded by non-malignant prostatic tissue.

Lymphovascular Invasion

Perineural and lymphovascular space: present in *75–90% of radical prostatectomy specimens.* While its positive predictive value is low, *perineural invasion* is an indicator of *potential extraprostatic extension*, and is associated with prostatic carcinoma of higher Gleason score and volume. Microvascular invasion is also an independent predictor of biochemical recurrence after radical prostatectomy and tumour progression.

- Present/absent.
- Intra-/extratumoural.

Lymph Nodes

Site/number/size/number involved/limit node/extracapsular spread/diameter of the largest metastasis.

Regional nodes: pelvic lymph nodes below the bifurcation of the common iliac arteries. These are present in <5% of radical prostatectomy specimens but may be submitted as separate pelvic lymph node specimens.

pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Metastasis in regional lymph node(s)

Lymph node metastases are present in up to *10–30% of prostate cancer patients,* relating to tumour stage (in 50% of pT3 patients at diagnosis), volume, differentiation or grade, and serum PSA levels. However, they are only present in some *1–2% of radical prostatectomy specimens.* This is related to preferred surgical practice, PSA screening and better clinical imaging with patient selection for surgery. Some patients with high risk disease defined by serum PSA level and biopsy tumour grade may have frozen section assessment of regional lymph nodes prior to carrying out radical surgery. Even with positive pelvic lymph nodes, radical prostatectomy with regional lymphadenectomy followed by adjuvant therapy is a treatment option. *The presence of lymph node metastasis, the proportion of involved nodes, and nodal tumour volume or maximum diameter (more predictive of cancer-specific survival than the number of positive nodes), are indices of disease free survival and metastatic potential*. The commonest sites of *distant metastases* are bone (osteoblastic in character), lung, and occasionally testis. Visceral metastases often represent end stage disease. Occult primary disease can present with metastases to unusual sites, e.g. pleura, bronchus, mediastinal and supraclavicular lymph nodes. Bronchial biopsy and aspiration cytology coupled with PSA/PSAP/AR/NKX3.1 immunoreactivity can be useful in these circumstances particularly as the tumour can mimic other cancers, e.g. secondary colonic adenocarcinoma. Lymph node metastases of prostate cancer can also be encountered incidentally in other contexts, e.g. anterior resection with total mesorectal excision for a rectal cancer. If not recognized as such the rectal cancer may be erroneously over staged and receive inappropriate adjuvant therapy.

Excision Margins

Distances (mm) to the circumferential surgical resection margins, proximal bladder and distal urethral limits.

A margin is involved or positive if *tumour is present histologically at the inked edge.* If close to but not touching the ink, it is negative. Occasionally assessment is not clear cut and margin status is deemed to be equivocal. Interpretation of the benign or malignant nature of crushed or diathermied glands at a margin is in the context of adjacent preserved glands, and can be helped with use of the standard immunohistochemical panel (34βE12, p63, AMACR). A positive margin does not necessarily alter the stage or mean pT3a disease as the gland may have been excised at or internal to the capsule at this particular point. The designation of stage "pT2+" disease may be used, indicating that the tumour is essentially organ confined where examinable, but EPE cannot be assessed. The apex and posterolateral margins are unduly susceptible to positive margins, and proximally a pT4 designation generally requires gross tumour rather than just microscopic involvement (pT3a) of the bladder neck. The proximal and distal limits are transverse sectioned and then serially sliced perpendicular to this to satisfactorily demonstrate their actual outer surfaces.

Capsular and *marginal invasion* are strong *indicators of extraprostatic disease* and *potential progression.* Note that the prostatic capsule is poorly defined particularly at the base and apex of the gland where there is a paucity of fat and extraprostatic extension is hard to ascertain. *A positive margin* may be designated R1 by TNM, i.e. incomplete resection with residual microscopic disease (e.g. pT3 (R1)) and its *linear extent (< or >3 mm or 10 mm)* is significantly associated with PSA relapse (see below). Extensive positive margins demonstrate a higher risk of relapse and there is evidence that the 5-year PSA recurrence risk appears to be significantly greater when the length of the involved margin is ≥3 mm. A higher Gleason grade at the surgical margin has a positive correlation with recurrence.

Other Pathology

High-grade prostatic intraepithelial neoplasia (HGPIN): extent and location which is usually peripheral and multifocal as in carcinoma. HGPIN is a *precancerous condition* and has *malignant cytology* (i.e. nuclear/nucleolar enlargement) within *preserved ducts* and *acini.* It can show focal disruption of the basal cell layer with high molecular weight 34βE12 cytokeratin and p63 stains. Its intraductal architectural patterns are tufting, micropapillary, cribriform and flat although in addition, other less common patterns, such as hobnail, signet-ring cell, small-cell neuroendocrine, foamy, mucinous, squamous differentiation and inverted patterns have been reported. It must be distinguished from intraductal carcinoma (IDC-P) of the prostate (see below). *It is present in 5–10% of needle core biopsies and about 40–50% are associated with either concurrent, or subsequent adenocarcinoma developing within 10 years.* It is most strongly linked to intermediate grade cancer many of which are diagnosed on rebiopsy following a core biopsy finding of HGPIN. Its extent correlates with cancer stage, Gleason grade, margin involvement and perineural invasion. *Its presence in biopsy cores or chippings indicates the need to process more tissue and for clinical reassessment and follow up.* One focus requires rebiopsy within a year, and multiple foci, short to intermediate term rebiopsy. *HGPIN present in 1, 3 or more than 3 cores* has a *30%, 40% and 75% cancer risk, respectively*. HGPIN does not cause a significant rise in serum PSA or form a palpable mass. *It is a histological finding* and is not detected by ultrasound. Its prevalence and extent are decreased by androgen deprivation and radiation therapy. A differential diagnosis is with invasive ductal cribriform adenocarcinoma. Low-grade PIN is not reported due to variation in its observer reproducibility and uncertainty as to its significance as a precursor lesion.

Intraductal carcinoma of the prostate (IDC-P) refers specifically to prostatic adenocarcinoma extending into and proliferating within preexisting prostatic ducts. The association of IDC-P

with aggressive high-volume, high-grade and high-stage prostate cancer has been confirmed by several studies. Greater standardisation of the diagnosis and reporting of IDC-P is required and confident exclusion of IDC-P requires the use of immunohistochemistry for basal markers. IDC-P is usually seen with high grade prostate cancer but may also be encountered alone in needle biopsies. Diagnostic criteria for IDC-P with and without invasive prostate carcinoma on needle biopsy have been proposed: the presence of marked nuclear atypia at least six times larger than adjacent benign nuclei and non-focal comedonecrosis are the most reliable diagnostic criteria. Reporting of IDC-P is critical in needle biopsy specimens for appropriate treatment and has significant clinical implications (also to predict outcomes in radical prostatectomy) with radical therapy recommended by most. Recent proposals (ISUP Nice, 2019) for grading IDC-P have been approved.

Ancillary immunohistochemistry and differential diagnoses: may be used in up to 20–25% of diagnostic prostatic specimens according to local pathological practice, although this decreases considerably with acquired morphological experience. *High molecular weight cytokeratin antibody 34βE12* and *p63* antibodies react with the *basal cells* of prostatic glands in *benign conditions,* but are *negative in adenocarcinoma.* Important *morphological markers of adenocarcinoma* are: nuclear/nucleolar enlargement, cytoplasmic amphophilia, absence of the basal cell layer, perineural invasion (especially if circumferential and intraneural) and loss of gland architecture. This comprises small, crowded round to angular, amphophilic glands infiltrating between benign glands. A stromal desmoplastic reaction is usually not present. Luminal crystalloids, mucinous fibroplasia (collagenous micronodules), glomerulations and extracellular mucin extension may also be seen. A putatively *positive immunomarker* for *prostatic adenocarcinoma* is *(AMACR/P504S).* The basal layer can be difficult to identify on morphology and is best identified by 34βE12 and p63 staining. A cocktail of all three antibodies can be used and the glands in question assessed on one slide instead of three.

A *34βE12, p63, AMACR/P504S panel* is of use in *difficult differential diagnoses of low-grade prostatic adenocarcinoma.* These conditions include: HGPIN, atrophy/partial atrophy, postatrophic hyperplasia, sclerosing adenosis, atypical adenomatous hyperplasia (benign cytology within an abnormal glandular architecture at the edge of hyperplastic nodules), nephrogenic adenoma, verumontanum mucosal gland hyperplasia, central zone epithelium, mesonephric remnants, various hyperplasias (transitional/squamous cell/cribriform), and Cowper's glands. *Overdiagnosis* of entrapped distorted rectal glands, seminal vesicle and ejaculatory duct epithelium as malignant should also be borne in mind: look for cytoplasmic lipofuscin pigment and characteristic cytoarchitectural appearances in the latter two contexts. Basal cell hyperplasia/adenoma are also strongly 34βE12/p63 positive, and, can *mimic high-grade prostatic carcinoma* as can other benign pathology such as: florid clear cell cribriform hyperplasia, healed infarcts, reactive epithelial atypia (e.g. radiation induced), granulomatous prostatitis, malakoplakia and prostatic xanthoma. *False positive diagnoses* can be avoided by an awareness of these various benign mimics, and other local malignancy, e.g. bladder neck urothelial cancer. *False negative diagnosis* should also be borne in mind due to focal distribution of cancer within the gland and individual specimens, the deceptively bland appearances of some adenocarcinoma variants, and prior effects of adjuvant treatments. About 3% of needle biopsy specimens show *atypical small acinar proliferation (ASAP) suspicious but not diagnostic of malignancy.* This can be due either to the presence of small atypical glands without fully developed cytoarchitectural features of malignancy, or, a very limited number (e.g. <2–3) of glands with overtly malignant features, i.e. quality and quantity. There may be associated adjacent HGPIN. Interpretation must be viewed in light of the clinical findings, e.g. a rising serum PSA. Further biopsy may be necessary.

Effects of non-surgical therapies: radiotherapy (external beam or interstitial brachytherapy seeds) and *hormonal treatment* (androgen deprivation therapy) produce a range of changes. This includes glandular atrophy with nuclear

apoptosis and smudging, cytoplasmic vacuolation, mucinous change, squamous cell metaplasia and stromal fibrosis. These may lead to underestimation of tumour volume in post-treatment resection specimens and difficulty in deriving and in validating the Gleason score. Current recommendations are to not assign a Gleason grade following androgen deprivation treatment. Other modalities include high intensity focused ultrasound (HIFU), photodynamic and interstitial laser thermotherapy, and gamma knife radiosurgery. These treatment related changes along with better diagnosis of early disease can result, in 0.5% of cases, in the *minimal residual* or *"vanishing cancer" phenomenon* in prostatectomy specimens. In such cases the needle biopsy material should be reviewed to verify the primary diagnosis and future management based on its prognostic indicators. Conversely, lack of morphological response to neoadjuvant therapy has adverse prognosis. Note that treatment related changes may persist for up to several years, and may be complicated by secondary cancer, e.g. squamous cell carcinoma and urothelial carcinoma.

Secondary carcinoma: mucinous and signet ring cell carcinoma have to be distinguished from *secondary colorectal carcinoma.* Immunomarkers PSA, androgen receptor, NKX3.1 and PSAP will be positive in 95% of primary prostatic carcinomas but may be negative in mucinous prostatic cancers. If immunomarkers are negative, absence of any obvious primary elsewhere is important in designation as a primary prostate lesion. Note that PSAP can also be positive in rectal carcinoid tumours and anal carcinoma. PSA can also stain some ovarian, salivary gland, skin and breast cancers.

Prostatic ductal carcinoma: has a worse prognosis than prostatic carcinoma of usual type but may also be androgen deprivation/oestrogen sensitive. It has cribriform or papillary patterns and is PSA positive. Many coexist with typical acinar prostatic carcinoma and may be related.

Postoperative necrobiotic granuloma: post-TURP ductocentric fibrinoid necrosis with palisading histiocytes. Also granulomatous prostatitis (idiopathic, BCG).

Myofibroblastic proliferations: postoperative spindle cell nodule/inflammatory myofibroblasitic tumour (IMFT/pseudosarcomatous fibromyxoid tumour) (see Chap. 30).

Cancer distribution and extent: prostatic carcinoma has a tendency to be *peripheral and posterior in distribution* allowing a diagnosis to be made in a significant number of cases by *multiple per rectal needle biopsies.* Multiple sextant biopsies can also act as a guide to the distribution and extent of the lesion. Biopsies should be examined histologically through multiple levels (at least 3) to detect focal lesions. Measurement of the *maximum tumour dimension (mm)* should be given. Small foci (<3 mm) in one core may represent true focal cancer, or, a sampling issue and not representative of the whole lesion. Small areas of tumour in a biopsy may indicate small volume disease in the radical prostatectomy but the considerable overlap that exists in individual patients poses considerable limitations on this as a predictor. Conversely *high percentage tumour involvement and measurable linear dimension in needle core biopsies generally correlate with high volume, potentially advanced stage disease.* The weight of prostatic chippings determines the number of blocks processed for histology but it is estimated that with selection 5–8 blocks will detect 90–98% of the prostatic carcinomas that are represented in a specimen.

Prognosis

Prognosis in prostate cancer is related to the *volume of tumour, Gleason score (and Grade Group) and presence of extracapsular extension.* Tumour volume can only really be derived by systematic measurement of serial slices of prostatectomy specimens. However, *the proportion or percentage of TURP chippings or needle biopsy cores involved, and the maximum linear dimension of cancer, give a reasonable estimate of disease extent. Overall 10 year survival is 50%* and up to *30% can be regarded as cured*. Gland-confined disease (pT1/pT2) shows 80–95% 10 year survival depending on tumour volume whereas *extraprostatic extension (pT3/pT4)* decreases

10 year survival to 60% and a "cure" rate of only 25%. *Other prognostic factors* are positive surgical margins, perineural and lymphovascular invasion and serum PSA levels (an indirect indicator of tumour volume and extension). The *risk of tumour recurrence* can be stratified on the basis of stage, Gleason score and serum PSA at diagnosis as:

(a) Low risk: pT1 – pT2a, Gleason ≤6, PSA < 10 ng/mL
(b) Intermediate risk: pT2b – pT2c, Gleason 7, PSA 10–20 ng/mL, or,
(c) High risk: pT3 – pT4, Gleason 8–10, PSA > 20 ng/mL.

Treatment (active surveillance only for localised disease, radical prostatectomy, radiotherapy including brachytherapy, hormonal manipulation, chemotherapy) is tailored to the patient's *age, general level of health, grade* and *clinicopathological stage of disease. Non-surgical modalities* are of use in localised disease and as palliation in locally advanced (pT3/pT4) or metastatic disease. Indications for *radical prostatectomy* are a younger patient (up to the sixth/seventh decade) with persistent but modestly elevated serum PSA (less than 10–15 ng/mL), needle biopsy proven adenocarcinoma, and an absence of extraprostatic spread on bone scan. Serum PSA > 15 ng/mL is associated with a greater likelihood of the tumour not being gland confined and subsequent positive surgical margins. *Gleason score ≥ 4 + 3 = 7, extraprostatic disease or positive surgical margins* may necessitate *post operative radiotherapy* as they are predictors of poor prognosis and treatment failure.

Serum PSA Level

Serum PSA is a biochemical marker used as a screening, diagnostic, prognostic and clinical follow up tool. High levels in relation to the patient's age (>3 ng/mL at ≥50 years: >2.5 ng/mL at <50 years: free to total ratio <15%), and *changing levels over time* (previously a velocity >0.75 ng/mL per year: however, evidence suggests in men <50 years, a PSA velocity of 0.6 ng/mL per year may be more appropriate) of PSA should prompt processing of further tissue and/or multiple levels as there is a strong correlation with the presence of adenocarcinoma (elevated in 64% of cases). Levels above 4 ng/mL and 10 ng/mL confer *cancer risks of 25% and 50%* respectively. There is also a significant positive correlation with Gleason grade as poorly differentiated tumours are usually of high volume. Serum levels >0.2 ng/mL post radical prostatectomy can represent biochemical failure or relapse, local recurrence or metastatic disease. Elevated levels in inflammatory conditions (prostatitis, infarct, benign hypertrophy, post catheterisation) are usually of lesser magnitude, transitory and resolve with time and treatment. Screening is based on DRE, TRUS and serum PSA levels. Measurement of PSA density (serum PSA/prostatic volume) may also prove useful: negative multiparametric MRI can reliably exclude significant prostate cancer when used in combination with low PSA density (0.1 ng/mL/mL). There are now also a number of blood-based ancillary tests available such as PCA3, PHI, and 4 K score which may become useful in helping to stratify patients.

Immunophenotype

The vast majority of prostatic adenocarcinomas are strongly *PSA, PSAP and PSMA* positive although a small minority of poorly differentiated tumours may show only focal staining for monoclonal PSA and are serum PSA negative. This is strengthened by use of polyclonal PSA with PSAP, the caveat being the cross reaction of rectal carcinoid and anal tumours with the latter. In addition, NKX3.1 (prostate specific androgen regulated homeobox gene) is the most specific prostatic marker for distinguishing prostate from urothelial carcinoma. Loss of the basal layer and *lack of 34βE12* has become a standard tool in diagnosing prostatic adenocarcinoma. This can be equally well demonstrated with other basal layer markers such as *CK5/6* or *p63. AMACR/P504S* is a good diagnostic counterpart to

34βE12/p63 showing cytoplasmic granular positivity in up to 80% of malignant prostatic glands or atypical glands suspicious of malignancy. It arose as a product of high throughput microarray technology and although it has sensitivity and specificity limitations, it is routinely used in tandem with 34βE12/p63 in the assessment of atypical glandular foci. Note that it can also be positive in HGPIN, adenosis/atypical adenomatous hyperplasia, nephrogenic adenoma and prostatic periurethral ducts emphasising the importance of using these *markers in the context of appropriate morphology*. A combination of PSA/PSAP/NKX3.1 with AMACR/P504S and basal layer immunohistochemistry also remains relatively robust for the identification of residual tumour after radiotherapy, hormonal treatment or HIFU. Tissue expression of PSA/PSAP/AR/NKX3.1 is useful in identifying a prostatic origin for metastatic carcinoma and distinguishing it from poorly differentiated urothelial carcinoma (PSA/PSAP/NKX3.1 negative, CK7/CK20/34βE12/p63/GATA3 positive) particularly in TURP specimens derived from the bladder neck, or, in prostatic needle biopsies. A potential marker for prostatic malignancy and prostatic tissue of origin is over expression of ERG protein—a product of the TMPRSS2-ERG gene fusion present in 50% of prostatic carcinomas.

Other Malignancy

Malignant Lymphoma/Leukaemia

- Especially chronic lymphocytic leukaemia (20% of cases at autopsy).
- Primary MALToma (rare), diffuse large B cell malignant lymphoma, or secondary to systemic/nodal lymphoma; prognosis is poor; 33% 5 year survival.

Leiomyosarcoma

- Rare, with *local recurrence* and *metastases common* and a survival of 3–4 years.

Other rare sarcomas, e.g. prostatic stromal sarcoma or synovial sarcoma, must be distinguished from sarcomatoid carcinoma with homologous or heterologous differentiation, GIST or solitary fibrous tumour. Direct spread from a retroperitoneal or pelvic primary sarcoma must also be considered.

Embryonal Rhabdomyosarcoma

- <20 years age with an average age of 5 years.
- Second commonest site after head and neck.
- Usually extensive tumour of prostate, bladder and surrounding soft tissues with a botryoid (grape-like) appearance.
- Cellular subepithelial cambium layer, loose myxoid zone, cellular deep zone ± rhabdomyoblasts.
- Vimentin, desmin, myo D1, myogenin positive. Many are cured or remain disease free for long periods of time. About 15–20% die at presentation with high stage disease. A minority are alveolar and more aggressive. Adult rhabdomyosarcoma does not respond to multimodal therapy and has a poor prognosis.

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Urethral Carcinoma

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Declan O'Rourke

Primary urethral cancer is relatively rare compared to secondary involvement of the urethra by high-grade bladder cancer, in which there is a reported incidence of up to 10–20%. Recent studies (RARECARE project and SEER study) have found an almost threefold (age-adjusted) incidence in males compared with females with an increasing incidence with age. Risk factors include urethral strictures, chronic irritation, HPV (Human Papilloma virus) and diverticula. Proximal lesions are urothelial in type and distal lesions squamous cell in character. A small minority are adenocarcinoma associated with a diverticulum, periurethral glands, or, the prostate gland.

Urethral cancers can present with haematuria, urinary hesitancy or retention mimicking benign prostatism. Proximal lesions can be relatively asymptomatic and present at a late stage. Investigation is by urethroscopy and biopsy, often combined with cytoscopy. CT/MRI scans determine tumour stage and spread into local soft tissues that might require en bloc resection. CT/PET scan has a role in detecting distant metastatic disease.

Treatment is by surgical excision, the extent of which depends on the location and stage of disease: e.g. local excision for cancer of the distal or meatal male urethra, with or without partial or radical penectomy. Radiotherapy can preserve the penis but results in troublesome strictures and higher local recurrence rates. Advanced proximal tumours may require a combination of radical surgery and radiotherapy for palliative control. Surgery may comprise radical cystoprostatectomy, penectomy and pelvic lymph node dissection. Brachytherapy and radiosensitising chemotherapy are other options. Secondary urethral cancers from the penis or bladder are excised as part of a penectomy (see Chap. 34) or cysto(prostato)urethrectomy, respectively. In women urethrectomy is usually in continuity as part of a radical cystectomy, although with careful preoperative biopsy staging it may be preserved for orthotopic functional reconstruction using a neobladder. In men, preoperative biopsies are carried out to determine the presence of urethral disease (either *in situ* or invasive). If positive, the procedure is then carried out in two stages: cystoprostatectomy down to the level of the urogenital diaphragm and then a perineal urethrectomy for the residual urethra. Follow up may require cytological washings or biopsy from the urethral stump. Recurrent disease may necessitate secondary urethrectomy. Intraoperative frozen section may be required to confirm negative surgical margins.

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_32

Gross Description

Specimen

- Biopsy/urethrectomy or as part of cysto (prostato)urethrectomy.
- Weight (g) and size/length (mm), number of fragments.

Tumour

Site
- Prostatic/bulbomembranous/pendulous urethras/meatus.

Size
- Length × width × depth (mm) or maximum dimension (mm).

Appearance
- Polypoid/verrucous/papillary/sessile/ulcerated/pigmented.

Edge
- Circumscribed/irregular.

Histological Type

Primary urethral carcinoma is rare and a urethral cancer is much more likely to represent *secondary involvement* from adjacent structures, e.g. penis or urinary bladder.

Squamous Cell Carcinoma

- *60–70% of cases.*
- *Distal in location.*
- Keratinising/non-keratinising, well to moderately differentiated.
- Large cell/small cell.
- *Typical/usual, variant, verrucous carcinoma, basaloid squamous cell carcinoma, Sarcomatoid carcinoma*. These may coexist with usual squamous cell carcinoma.

Urothelial Carcinoma

- *20–30% of cases.*
- *Proximal in location*: either high-grade in situ or invasive disease.

Adenocarcinoma

- *10% of cases.*
- Female > male; arising in strictures, diverticula or fistulae.
- Either *non-clear cell* (60%: glandular, enteric, mucinous, signet ring cell, papillary), or *clear cell* (40%) patterns, with or without urethritis cystica/glandularis. Of variable low to high cytological grades.
- Prostatic urethra: 'endometrioid' carcinoma (PSA positive), also known as carcinoma of the prostatic periurethral ducts. See Chap. 31.

Adenosquamous Carcinoma

- Rare.

Small Cell Carcinoma

- A poorly differentiated/high-grade neuroendocrine carcinoma either primary, or secondary from lung.
- CAM 5.2/synaptophysin/CD56 positive with a high Ki-67 index.

Malignant Melanoma

- *4% of urethral malignancy.*
- Extensive radial growth is usual leading to *local recurrence. Metastatic spread is common* to regional lymph nodes, liver, lungs and brain. The *poor prognosis* relates to the tumour thickness. Mucosal junctional activity may indicate a primary lesion.

Metastatic Carcinoma

- *Multifocal/direct spread:* urothelial cancer from bladder is commoner than a primary lesion. Other cancers that spread directly are penis, rectum, vagina, cervix and endometrium.
- *Distant spread:* ovary, kidney (distinguish from primary clear cell carcinoma).

Differentiation/Grade

- Well/moderate/poor/undifferentiated, or, Grade 1/2/3/4.
  - For squamous cell carcinoma grading is based on cellular atypia, keratinisation and intercellular bridges, and, for adenocarcinoma on the tumour percentage gland formation (well/G1: >95%, moderate/G2: 50–95%, poor/G3: <50%).
- WHO grades I–III (based on WHO 1973), low/high grade (WHO 2004/2016) (see Chap. 30).

Extent of Local Tumour Spread

- Border: pushing/infiltrative.
- Lymphocytic reaction: prominent/sparse.
- The TNM8 classification applies to carcinomas of the urethra and urothelial carcinoma of the prostate and prostatic urethra.

Urethra (Male and Female)

pTa	Non-invasive papillary, polypoid or verrucous carcinoma
pTis	Carcinoma in situ
pT1	Tumour invades subepithelial connective tissue
pT2	Tumour invades any of the following: corpus spongiosum, prostate or periurethral muscle
pT3	Tumour invades any of the following: corpus cavernosum, beyond prostatic capsule, anterior vagina or bladder neck (extraprostatic extension)
pT4	Tumour invades other adjacent organs (invasion of the bladder)

Urothelial Carcinoma of Prostatic Urethra

pTis pu	Carcinoma in situ, involving prostatic urethra, periurethral or prostatic ducts without stromal invasion
pT1	Tumour invades subepithelial connective tissue (for tumours involving prostatic urethra only)
pT2	Tumour invades any of the following: corpus spongiosum, prostate or periurethral muscle
pT3	Tumour invades any of the following: corpus cavernosum, beyond prostatic capsule or bladder neck (extraprostatic extension)
pT4	Tumour invades other adjacent organs (invasion of bladder or rectum)

Distinction must be made between periurethral duct involvement by tumour (pTis pu) and *invasion into periurethral or prostatic stroma* (pT2) as the latter worsens the prognosis.

In urethral diverticular carcinoma, a differentiation between T2 and T3 is not possible and it is classified as T2.

Lymphovascular Invasion

- Present/absent.
- Intra−/extratumoural.

Lymph Nodes

- Site/number/size/number involved/limit node/extracapsular spread.
- Regional lymph nodes are the inguinal and the pelvic nodes.

pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis[a]
pN1	Metastasis in a single lymph node[b]
pN2	Metastasis multiple lymph nodes

[a]pN0 (i+) Isolated tumour cells: single tumour cells or small clusters of tumour cells (in a regional lymph node) not more than 0.2 mm in greatest extent that can be detected by routine H&E stains or immunohistochemistry

[b]pN1(mi) Micrometastasis: a metastasis in a regional lymph node >0.2 mm but ≤2.0 mm

Excision Margins

Distances (mm) to the proximal and distal longitudinal and deep circumferential resection limits.

Other Pathology

Bladder cancer: urethral *involvement by bladder carcinoma* is much commoner than primary urethral carcinoma which is a relatively rare disease. In the female the urethra is short (4 cm) and removed by cystectomy, which involves total urethrectomy. However, there is potential for local recurrence in the residual male urethra. The histological status of the prostatic urethra is therefore assessed by biopsy prior to definitive surgical resection and consideration of cytoprostatourethrectomy.

Urinary tract field change: multifocal urothelial carcinoma of urethra, bladder, ureter, and renal pelvis can occur either as papillary carcinoma, carcinoma *in situ* or Pagetoid urethral spread from a bladder lesion.

Gender and site disposition: the male to female ratio is 3:1 for urethral carcinoma. Carcinomas arising *proximally* (proximal one third in women; prostatic, bulbomembranous urethra in men) are generally *urothelial carcinoma* while *distal* lesions (distal two thirds in women; penile urethra in men) are usually *squamous cell carcinoma*. In the distal one third they are often well differentiated squamous cell or verrucous in type. *Clear cell (mesonephroid) adenocarcinoma* is rare, arising in either the female or prostatic urethra where it may be associated with a stricture or diverticulum. It should be distinguished from similar lesions arising in the female genital tract and metastatic renal cell adenocarcinoma by clinical history and anatomical distribution of disease. Another differential diagnosis is nephrogenic metaplasia (adenoma), which is usually small and lacks significant cellular atypia and mitoses. *Prostatic duct ('endometrioid') adenocarcinoma* arises from periurethral prostatic ducts (androgen receptor, PSA, PSAP positive) and may be oestrogen sensitive.

Benign mimics of carcinoma: nephrogenic metaplasia/adenoma is a reactive proliferative lesion associated with recent instrumentation, calculi, trauma, post renal transplant and cystitis. It is benign and does not predispose to but rarely may be associated with concurrent carcinoma, e.g. with adenocarcinoma in a urethral diverticulum. It usually has a distinctive exophytic, polypoid or papillary growth pattern with a covering tubulopapillary proliferation of cuboidal epithelium also present in the underlying lamina propria. The cells may show tubule formation and cystic change with degenerative atypia but are not mitotically active. The cells are CK7, AMACR/P504S, PAX2, PAX8 positive, and an origin from renal tubular stem cells in renal transplant patients has been postulated. Other *protuberant urethral lesions that can mimic carcinoma* at cystoscopy are benign prostatic urethral polyp, prominent verumontanum, fibrovascular polyp, villous adenoma and inverted transitional cell papilloma. Condyloma accuminatum associated with "high-risk" HPV types (16, 18, 31, 33, 35) may rarely undergo malignant transformation to verrucous or infiltrating squamous cell carcinoma.

Prognosis

Distal urethral carcinoma (well differentiated squamous cell/verrucous) presents early with a reasonable prognosis. *Overall prognosis of urethral carcinoma (40% 5 year survival)* relates to the *anatomical site and stage of disease*, e.g. pendulous urethral carcinomas have *60–70% 5 year survival* while the figure for bulbomembranous/prostatic lesions is *20%*. Proximal cancers also present at a more advanced stage and with high-grade (poorly differentiated) histology in which it may be difficult to distinguish squamous cell from urothelial carcinoma.

Other Malignancy

Malignant Lymphoma/Leukaemia

- As a manifestation of systemic disease.

Embryonal Rhabdomyosarcoma

- Sarcoma botryoides in children.
- Superficial subepithelial cambium layer, intermediate myxoid zone, deep cellular zone, desmin/myo D1/myogenin positive.

Aggressive Angiomyxoma

- Myxoid stroma/thick vessels with desmin, ER and HMGA2 positive stellate/spindle mesenchmyal cells.
- Locally recurrent/infiltrative.

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Testicular Cancer

33

Declan O'Rourke

Testicular cancer represents less than 1% of malignancy in men although it is the commonest cancer in those under 45 years of age, and its incidence is increasing by 2–3% per annum. About 95% are germ cell tumours and predisposing factors include cryptorchidism, infertility, genetic factors, testicular dysgenesis, prior testicular tumour and germ cell neoplasia in situ (GCNIS: previously referred to as ITGCN). Organochlorine compounds have been associated with a risk of developing testicular cancer. Patient age and a testicular or paratesticular location are important indicators to likely tumour type (Table 33.1).

Testicular cancer usually presents with a painless unilateral lump or swelling of some duration, and any non-resolving lump lasting more than 2–3 weeks should be referred to a urologist. Occasionally there is scrotal pain, backache or endocrine effects such as gynaecomastia. Torsion or incidental trauma to a fully descended or undescended testis may draw attention to underlying tumour. Delay in presentation correlates with the development of metastases. Some cases may present with malignant axial or cervical lymphadenopathy of unknown primary site, and up to 10–30% of patients have metastatic disease at diagnosis. Investigation involves careful clinical examination, serum tumour markers (AFP: alphafetoprotein, ß-HCG: human chorionic gonadotrophin, LDH: lactate dehydrogenase), and ultrasound assessment to detect hypoechoic areas of tumour. Ultrasound scan is 95–100% sensitive for detecting a scrotal abnormality and determining an intra- or extratesticular location. Tumour staging is by CT scan for pulmonary involvement, and CT/PET scan for abdominopelvic and mediastinal lymph node

Table 33.1 Testicular cancer diagnosis by age and site

Age	Testis	Paratesticular
Neonate	Juvenile granulosa cell tumour	
Infant	Yolk sac tumour	Rhabdomyosarcoma
Young adult	Germ cell tumours- non-seminoma (2nd/3rd decade) or seminoma (3rd/4th decade) Sex cord stromal tumours	Rhabdomyosarcoma Adenomatoid tumour Desmoplastic small blue cell tumour
Older adult (>60 years)	Spermatocytic tumour Sex cord stromal tumours Malignant lymphoma Metastatic cancer	Adenomatoid tumour Malignant lymphoma Metastatic cancer Liposarcoma, and other sarcomas

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_33

disease. FNAC (fine needle aspiration cytology) and needle biopsy are avoided due to the potential risk of iatrogenic tumour dissemination, and because a testis should always be excised if there is a suspicion of tumour. Exceptions to this would be patients with known disseminated leukaemia, malignant lymphoma or carcinoma in whom FNAC/core biopsy would provide a relatively accessible and non-invasive tissue diagnosis of relapse as a basis for further systemic treatment.

Serum AFP and ß-HCG are significantly raised in >80% of non-seminomatous germ cell tumours, specifically embryonal carcinoma, yolk sac tumour and choriocarcinoma. ß-HCG may be modestly elevated in pure seminoma, and LDH is an indicator of bulky advanced or metastatic germ cell tumour.

In the radical orchidectomy specimen the pathologist must determine:

(a) the extent or stage of tumour spread
(b) distinguish between seminoma (radiosensitive), teratoma (chemosensitive) and sex cord stromal tumours (surgery alone)
(c) identify if there is direct extension into the rete testis, epididymis, tunica vaginalis, hilar soft tissue and spermatic cord
(d) establish if blood or lymphatic vascular invasion is present as this is an indicator for chemotherapy in stage I malignant germ cell tumours

Resection is by radical inguinal orchidectomy as a scrotal approach would then in addition incorporate pelvic lymph nodes as regional. The patient is offered a testicular prosthesis and sperm storage if chemoradiation is anticipated. For patients with proven metastases and high serum tumour marker levels, orchidectomy may be delayed until after chemotherapy is completed or other surgery is performed: e.g. excision of residual retroperitoneal masses. Some specialist centres occasionally offer partial orchidectomy for a tumour <2 cm in size, where there are bilateral tumours, a metachronous tumour or tumour in a solitary testis. The residual testis is treated with adjuvant chemotherapy or radiotherapy. Overall, oncological therapeutic options and prognosis are based on histological tumour type (seminoma versus non-seminoma), the presence or absence of lymphovascular invasion, levels of serum tumour markers, and radiological determination of the clinical stage of disease. Management should be by a specialist multidisciplinary testicular cancer team and cure rates of 95% are achieved. Central review of pathology has noted discrepancy rates of up to 4% and 10% in tumour type (seminoma changed to non-seminoma) and presence of vascular invasion, respectively, both of which are important criteria for treatment decisions.

Gross Description

Specimen

- FNAC/biopsy (open or needle)/radical orchidectomy (testis, tunica vaginalis, coverings and spermatic cord).
- Weight (g) and size (mm): overall and testicular.
- Length of spermatic cord (mm).

Tumour

Site

- Testicular/paratesticular.
- Bilateral: 1–3% of cases, synchronous or metachronous, similar or dissimilar types. The commonest bilateral tumours are seminoma or spermatocytic tumour but beware malignant lymphoma in the older age group.

Size

- Length × width × depth (mm) or maximum dimension (mm).
- Embryonal carcinoma tends to present at a smaller size than seminoma.

Appearance

- Pale/fleshy/nodular ± necrosis: seminoma/malignant lymphoma.
- Cysts/cartilage ± necrosis: teratoma.

- Haemorrhage: choriocarcinoma, yolk sac tumour.
- Fibrous/calcific scar: regression.
- Pale or tan/lobulated, often small and circumscribed: Leydig cell/stromal tumours.
- Note that some inflammatory conditions, e.g. granulomatous orchitis or malakoplakia, can mimic germ cell tumour clinically and macroscopically.

Edge

- Circumscribed/irregular.

Histological Type

Germ cell tumours comprise 95% of testicular neoplasms (of which 40–50% are seminoma), and *sex cord stromal lesions* 4% of cases.

Therapeutic distinction is drawn between *seminomatous* and *non-seminomatous germ cell tumours* due to adjunctive radiotherapy and chemotherapy approaches, respectively. List and semi-quantify the *percentage of tumour types* present in a mixed germ cell tumour. Histopathological grading is not applicable, biological behaviour being determined by the constituent tumour types present.

Seminoma

- *40–50% of testicular tumours* occurring in patients of mean age 40 years and 70% present with stage I disease.
- *Classical (93% of cases)*, or, anaplastic with the same behaviour despite different mitotic rates and the term anaplastic is not justified. However, evidence that such anaplasia correlates with behaviour and outcome in pure seminoma is currently largely mixed and inconclusive. Apparent anaplastic morphology may indicate poor fixation of a seminoma or possibly a solid pattern embryonal carcinoma.
- Seminoma typically comprises large, polygonal cells with clear to eosinophilic cytoplasm and an intervening stroma with aggregates of lymphocytes. Granulomas and ß-HCG positive syncytiotrophoblastic giant cells (7% of cases) may also be present.
- Usually sheets of cells but subtle interstitial or intertubular infiltration, corded growth, microcystic or tubular structures and signet ring-like features also occur. There can also be "cystic "spaces due to oedema, sclerotic stroma, and pagetoid spread into the rete.
- Seminoma cells are: PLAP, CD117, OCT3/4, SALL4, D2–40 (podoplanin) positive, cytokeratin focal or negative.
- *Spermatocytic*: benign with three cell types, SALL4 positive and OCT3/4, PLAP negative presenting usually in old age (see section "Other Pathology").

Non-Seminomatous Germ Cell Tumours

- A range of tumours showing either
  - Embryonic differentiation: *teratoma*—differentiated ± a malignant somatic component,
  - *Embryonal carcinoma*, or,
  - Extraembryonic differentiation: *yolk sac (endodermal sinus) tumour*, *choriocarcinoma (malignant teratoma trophoblastic).*

They are generally *more aggressive*, metastasise earlier, and have a greater tendency for *haematogenous spread* than seminomas with a cure rate of 95%, dropping to 40–95% if metastases are present. *Extensive pulmonary disease* is an *adverse prognostic indicator.* Metastases may also *change differentiation* and are *radioresistant.*

There has been variability in use of classifications in the past between the British Testicular Tumour Panel (BTTP) in the UK and WHO 2004 in Europe/US. However the WHO 2016 classification (Table 33.2) system for testicular tumours is now used by the majority of pathologists and use of BTTP classification is discouraged. For management and prognostic purposes, the most important distinction is between seminomatous and non-seminomatous tumors. Pathological staging has minor clinical significance as therapy is largely dependent on clinical

Table 33.2 World health organization (who 2016) histological classification of testicular germ cell tumours

Germ cell tumours derived from germ cell neoplasia in situ
Non-invasive germ cell neoplasia
Germ cell neoplasia in situ
Specific forms of intratubular germ cell neoplasia in situ
• *Tumours of a single histological type*
Seminoma
Seminoma with syncytiotrophoblastic giant cells
• *Non-seminomatous germ cell tumours*
Embryonal carcinoma
Yolk sac tumor, post-pubertal
Trophoblastic tumours
Choriocarcinoma
• *Non-choriocarcinomatous trophoblastic tumours*
Placental site trophoblastic tumour
Epithelioid trophoblastic tumour
Cystic trophoblastic tumour
Teratoma, post-pubertal
Teratoma with somatic malignancy
• *Non-seminomatous germ cell tumours of more than one histological type*
Mixed germ cell tumour
Germ cell tumours of unknown type
Regressed germ cell tumour
Germ cell tumours of derivation unrelated to germ cell neoplasia in situ
Spermatocytic tumour
Teratoma, prepubertal-type
Dermoid cyst
Epidermoid cyst
Well differentiated neuroendocrine tumour (monodermal teratoma)
Mixed teratoma and yolk sac tumour, prepubertal-type
Yolk sac tumour, prepubertal-type

staging (TNM and the modified Royal Marsden systems) based on imaging techniques (for abdominal/pulmonary/cerebral metastases) and levels of serum tumour markers.

Teratoma

- *5–10% of cases* with cellular components from 2 to 3 germ cell layers (endoderm, mesoderm, ectoderm).
- There has been a major shift in the restructuring of germ cell tumour classification with the discrimination of prepubertal and postpubertal type germ cell tumours of the testis.
- Prepubertal teratomas include dermoid and epidermoid cysts, and monodermal teratomas. Prepubertal teratomas (in contrast with their postpubertal counterparts) are associated with a normal genotype, absence of GCNIS and behave in a benign fashion. Dermoid/epidermoid cysts in adults are classified within the prepubertal teratoma category provided they are correctly classified with the absence of GCNIS, tubular atrophy, scarring or microliths and an absence of cytological atypia in the lesion.
- With postpubertal teratomas there is no longer a need to divide into *mature and immature* as there is no prognostic impact associated with making such distinctions. However, cases with an overgrowth (defined as a nodule of a single cytologically malignant element that occupies an area larger than a 4× magnification field) of frankly malignant elements (epithelial or mesenchymal) are considered diagnostic of teratoma with somatic-type malignancy and should be clearly identified. Overgrowth of primitive neuroectodermal elements is the principal criterion for the diagnosis of primitive neuroectodermal tumour.
- Sarcoma represents the most common (>50%) malignant somatic transformation in teratoma but others such as adenocarcinoma and squamous cell carcinoma occur. Distinct sarcomatous differentiation may manifest: e.g. leiomyosarcoma, rhabdomyosarcoma, PNET. Prognosis is adversely affected with metastatic spread, e.g. in the retroperitoneum, as deposits are non-responsive to germ cell chemotherapy and require surgical resection.

Embryonal Carcinoma (EC/MTU: Malignant Teratoma Undifferentiated)

- Present in *87%* of non-seminomatous germ cell tumours but usually as part of a mixed germ cell tumour, and rarely in 2% of cases in pure form. With the *poorest prognosis* of the

germ cell tumours it comprises primitive anaplastic epithelial cells in solid, glandular or tubulopapillary patterns. *Vascular invasion* in the adjacent testicular parenchyma and overlying tunica is relatively common. Intratubular embryonal carcinoma is present adjacent to the invasive tumour in about 25% of cases. The intratubular component is often necrotic and it may show calcification. Syncytiotrophoblastic giant cells (STGC) are a common finding and associated with increasedserumß-HCG.Immunohistochemistry shows positive staining for cytokeratin, CD30, OCT3/4, D2–40, SALL4, LIN28, NANOG, SOX2 and PLAP.

Yolk Sac Tumour (YST)

- Prepubertal-type yolk sac tumour is the most common testicular tumour of children, accounting for 50–60% of such tumours. 75% are found in children less than 2 years old. It is biologically different from postpubertal-type YST, despite having a generally similar range of histological features. Association with GCNIS is lacking. There is a low incidence of extratesticular involvement compared to postpubertal germ cell tumours, and when advanced, chemotherapy is very effective.
- Postpubertal-type: pure form is rare in adults where it usually presents as a component of mixed germ cell tumours. More than 95% of 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 include endodermal sinus (Schiller Duval bodies: distinct perivascular arrangement of tumour cells surrounded by a cystic space in an attempt to form yolk sacs), solid, papillary, and glandular. The cells can look very pleomorphic and difficult to separate from embryonal carcinoma. They are positive for cytokeratin, AFP, PLAP (variable), SALL4, glypican-3, LIN28 and negative for CD30, D2–40, OCT3/4, and ß-HCG.

Choriocarcinoma (MTT: Malignant Teratoma Trophoblastic)

- 0.3–1% of germ cell tumours are pure choriocarcinoma, but mixed tumours are more common. Requires *biphasic syncytiotrophoblastic and cytotrophoblastic differentiation* for diagnosis, although the "syncytio" element may be inconspicuous. *Angioinvasion* with *tumour haemorrhage* is common. Designation is dependent on the morphology and not the serum hormone levels. It is positive for cytokeratins, ß-HCG, HPL, EMA (only syncytiotrophoblast), and SALL4. Treatment is radical orchidectomy and systemic chemotherapy. Given its propensity for *angioinvasion* it can present with advanced stage disease and *early haematogenous metastases* to liver, lung, mediastinum and retroperitoneum. This produces diverse symptomatology: dyspnoea, haemoptysis, haematemesis, central nervous system dysfunction, with potential *haemorrhage* at multiple visceral sites. The level of ß-HCG correlates with prognosis, reflecting tumour load. In the new WHO classification trophoblastic tumours are divided into choriocarcinoma and non-choriocarcinomatous trophoblastic tumours. Monophasic choriocarcinoma is considered a morphological variant of choriocarcinoma. The non-choriocarcinomatous group has been expanded to recognise placental site trophoblastic tumour (PSTT), epithelioid trophoblastic tumour (ETT) and cystic trophoblastic tumour (CTT).

Mixed Germ Cell Tumour

- More than one germ cell type in any combination occurs in *30–40% of cases overall* and *70%* of non-seminomatous tumours, e.g. seminoma and EC, EC and teratoma, EC with YST and teratoma *(MTI: malignant teratoma intermediate).* Sample extensively (1 block/cm tumour diameter) and target block any unusual gross appearance, e.g. the association of choriocarcinoma with haemorrhage, to allow for this tumour heterogeneity particularly if there are significantly elevated serum AFP and

ß-HCG levels. Clinical presentation and management are the same as non-seminomatous germ cell tumour, and the prognosis is usually that of the worst component.

Mixed Germ Cell and Sex Cord Stromal Tumour

- *Gonadoblastoma:* a mixture of seminoma type cells and sex cord (Sertoli/granulosa) cells in a nested arrangement progressing, in 30% of cases, to invasive germ cell tumour, usually seminoma. Mostly found in dysgenetic gonads of intersex patients with a Y chromosome. Germ cell-sex cord/gonadal stromal tumour, unclassified is removed from recent classification as its existence is debatable.

Sex Cord Stromal Tumours

- Sex cord stromal tumours represent 4% of testicular neoplasms (increased in the paediatric population), 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. Almost all are immunoreactive for inhibin.
- *Leydig cell tumour*: 1–3% of testicular neoplasms and 30% bilateral or hormonally active with gynaecomastia or precocious puberty. Well circumscribed, yellow to tan in colour with eosinophilic or clear cells and Reinke's crystalloids (25%). They are positive for inhibin-α, calretinin, melan-A (MART-1), WT1, androgenic hormones, SF-1, FOXL2, and vimentin. They are typically negative for cytokeratins, OCT3/4, SALL4, S100, PLAP, and HMB-45. Lack of nuclear β-catenin expression and strong inhibin staining supports the diagnosis of Leydig cell tumour over Sertoli cell tumour. 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.
- *Sertoli cell tumours*: <1% of testicular neoplasms. One-third present with gynaecomastia without virilism and there is an association with Peutz-Jeghers and Carney's (large cell variant) syndromes. Around 10% are malignant (to local lymph nodes): indicators of malignancy include 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), β-catenin, SF-1, FOXL2, and synaptophysin. They are typically negative for PLAP, OCT3/4, SALL4, α-fetoprotein and CD30. Sclerosing sertoli cell tumour is considered to be a morphological variant of sertoli cell tumour based on similar genetic mutations (CTNNB1). Treatment is orchidectomy: radiation and chemotherapy have little effect.
- *Granulosa cell tumour*: Resembles the analogous ovarian tumour. The adult form is rare with an age range 20–53 years, usually non-functional and rarely associated with gynaecomastia. It is usually benign but metastasis occur in 10% (associated with size >7 cm, haemorrhage, necrosis, lymphovascular invasion). Inhibin, calretinin, SF-1, FOXL2, WT1and melan-A variably positive. Cytokeratin ± but EMA negative. The juvenile form is the most common neonatal testicular tumour with an average age of onset less than 1 month and sometimes congenital. There is an association with trisomy 12 and sex chromosome mosaicism if abnormal external genitalia. There is no association with endocrine manifestations. Benign behaviour following orchidectomy is expected.
- *Undifferentiated or mixed types:* thecoma/fibroma and other gonadal stromal tumours are rare.

Malignancy (10% of adult sex cord stromal tumours): cannot be reliably predicted but relates to size (>5–7 cm), cellular atypia, mitoses (>3/10

high-power fields), infiltrative margins, vascular invasion and a high Ki-67 proliferation index.

A diagnostic pitfall is sex cord stromal tumour positivity for S100 and melan-A mimicking metastatic malignant melanoma. However, they are usually HMB-45 negative.

Other Tumours

- *Adenocarcinoma of the rete* (rare, poor prognosis).
- *Cystadenocarcinoma of the epididymis* (very rare). Papillary cystadenoma is associated with von Hippel-Lindau syndrome.
- *Metastatic carcinoma: 2–3% of testicular neoplasms* with a mean age of 50 years. Prostate, lung, malignant melanoma, Merkel cell carcinoma, colon, kidney, stomach, pancreas. Bilaterality, vascular involvement and absence of GCNIS favour metastatic disease. Malignant melanoma, renal cell carcinoma and malignant lymphoma are particular mimics of germ cell tumour.
- *Carcinoid tumour:* Good prognosis, a well differentiated/low-grade neuroendocrine tumour or monodermal teratoma, but 20% have other teratomatous elements. Exclude metastatic carcinoid, e.g. from ileum (vascular invasion, extratesticular extension, bilateral).
- *Primitive neuroectodermal tumour:* Can be primary but more frequently arises within a testicular teratoma. Particularly look for its presence with adverse prognosis in retroperitoneal metastases.

Extent of Local Tumour Spread

- Border: pushing/infiltrative.
- Lymphocytic reaction: prominent/sparse.

Lymphocytic reaction: a consistent (80%) feature of seminoma, and *granulomas* can also be present in up to 50% of cases. Sometimes the inflammatory infiltrate can be so intense that it partially obscures the germ cells and immunohistochemical markers are necessary. The intensity of inflammation and presence of granulomas are not prognostically significant.

GCNIS: sample the adjacent testis.

Intratubular spread: seminoma/EC.

It can be difficult to distinguish between GCNIS and intratubular spread although in EC GCNIS will be PLAP/CD117 positive, and intratubular EC PLAP negative/CD30 positive. Intratubular EC has been suggested as an intermediate step between GCNIS and established EC. Intratubular spread of seminoma and GCNIS into the rete can also mimic EC or carcinoma of the rete.

Rete testis: Pagetoid or luminal spread of seminoma/EC. Distinguish from true *invasion of the rete stroma* which is an indicator for *postoperative adjuvant therapy*. Extratesticular extension of germ cell tumours is commoner at the rete/hilum.

The TNM8 classification applies only to germ cell tumours of the testis.

pTx	Primary tumour cannot be assessed (used if no radical orchidectomy has been performed, except for pTis and pT4, where radical orchidectomy is not always necessary for classification purposes)
pT0	No evidence of primary tumour (e.g. histological scar in testis)
pTis	Germ cell neoplasia in situ (GCNIS)
pT1[a]	Tumour limited to testis and epididymis[b] without vascular/lymphatic invasion; tumour may invade tunica albuginea but not tunica vaginalis
pT2[a]	Tumour limited to testis and epididymis with vascular/lymphatic invasion, or tumour extending through tunica albuginea with involvement of tunica vaginalis
pT3[c]	Tumour invades spermatic cord with or without vascular/lymphatic invasion
pT4	Tumour invades scrotum with or without vascular/lymphatic invasion

[a]Soft tissue invasion is not specifically mentioned in UICC TNM 8, but should be interpreted as pT2 (hilar soft fat also lacks tunica albuginea)

[b]Epididymal invasion in the absence of soft tissue or vascular invasion is very unusual. In these circumstances, extension into the epididymis is likely to represent occult soft tissue extension/vascular invasion and should be recorded as pT2

[c]Direct invasion of the spermatic cord soft tissue (with or without lymphatic/vascular invasion) is considered pT3. However, the finding of intravascular tumour in an *en face* section of the spermatic cord margin does not constitute a positive surgical margin

In mixed germ cell tumours, pathologic staging is determined by the highest stage component. Synchronous bilateral tumours are staged as independent primary tumours (Fig. 33.1).

Lymphovascular Invasion

- Present/absent.
- Intra−/extratumoural.

Lymphovascular invasion correlates with a significantly elevated *risk of distant metastasis* (40–50% versus 15% if absent) and is an *indication for chemotherapy*. Consequently strict criteria for its identification are necessary: an endothelial lined space with tumour conformed to its shape ± thrombosis or a point of attachment to the endothelium. Sample particularly the tumour/parenchyma interface and the overlying tunica looking for vasculocentric nodular deposits. As testicular cancers are

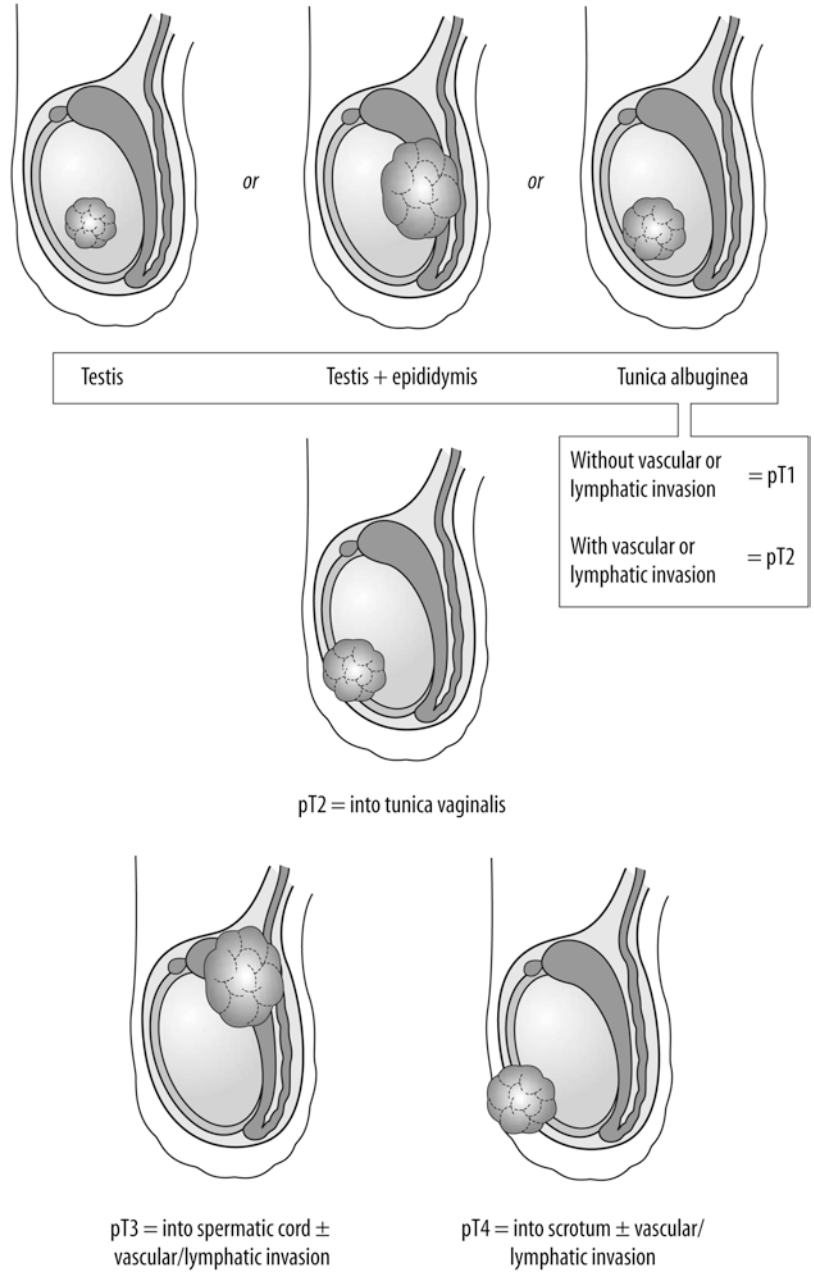

Fig. 33.1 Testicular germ cell tumours**.** Reproduced, with permission, from *Histopathology Reporting: Guidelines for Surgical Reporting, 2nd ed.,* © 2006, Springer

cellular tumours with delayed fixation due to the relatively impervious tunica, beware of knife carry-in misinterpreted as vascular invasion and characterized as loose floating tumour cells in vascular spaces. This can be particularly prominent in seminomas.

Lymph Nodes

Site/number/size/number involved/limit node/extracapsular spread.

Regional nodes: abdominal para-aortic (periaortic), pre-aortic, interaortocaval precaval, paracaval, retrocaval and retro-aortic nodes. Nodes along the spermatic vein should be considered regional. The intrapelvic and inguinal nodes are considered regional after scrotal or inguinal surgery.

pNx	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Metastasis with a lymph node mass 2 cm or less in greatest dimension and five or fewer positive nodes, none more than 2 cm in greatest dimension
pN2	Metastasis with a lymph node mass more than 2 cm but not more than 5 cm in greatest dimension; or more than five nodes positive, none more than 5 cm; or evidence of extranodal extension of tumour
pN3	Metastasis with a lymph node mass more than 5 cm in greatest dimension

Seminoma tends to metastasise through lymphatics while *choriocarcinoma shows haematogenous spread* with presentation from metastatic disease to lung, liver, brain, bone and gastrointestinal tract. *EC spreads by a combination of these mechanisms*. Lymph node involvement depends on the stage of disease and laterality of the primary tumour. Initial spread is periaortic but external iliac and inguinal node involvement may be seen if the tumour spreads to the epididymis and scrotal skin respectively. Mediastinal and left supraclavicular lymph node metastases occur late in the disease course. YST spreads in a similar manner to other non-seminomatous germ cell tumours, although it can present with haematogenous deposits, e.g. lungs (Fig. 33.2).

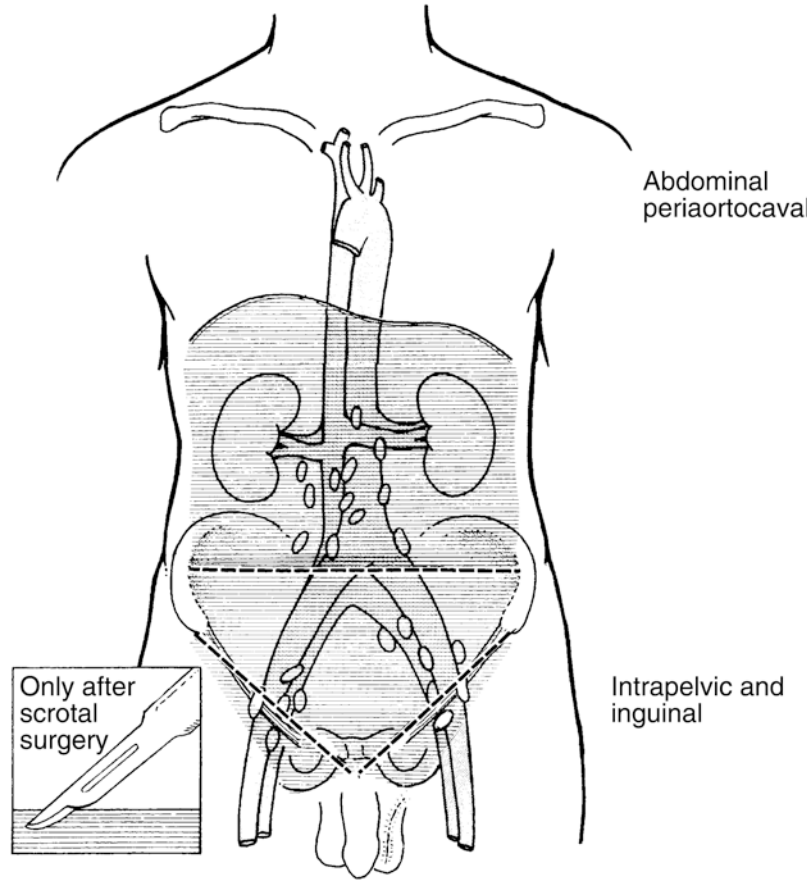

Fig. 33.2 Testicular germ cell tumours: regional lymph nodes. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

Clinical Stage

Modified Royal Marsden Staging System.

I	Tumour confined to the testis
II	Lymph nodes involved below the diaphragm
III	Lymph nodes involved above the diaphragm—supraclavicular or mediastinal
IV	Extranodal metastases—lung or brain.

Up to 10–15% of patients with seminoma have *metastases at the time of diagnosis*, 30–60% with EC, and the majority with choriocarcinoma.

Clinical staging: is based on the radiological determination of the anatomical extent of disease and the assessment of post-orchidectomy serum markers LDH, ß-HCG and AFP. *High levels* (AFP > 10,000 ng/mL, ß-HCG > 50,000 IU/l, LDH > 10× normal) indicate *worse prognosis* and usually a diagnosis of *non-seminomatous germ cell tumour.*

Metastatic germ cell tumour: is an important diagnostic consideration in a *young male with evidence of extensive visceral disease but no known primary somatic site carcinoma.* This is particularly so if there is cervical, mediastinal or retroperitoneal lymphadenopathy or lung metastases. Ultrasound may show a small or scarred testicular lesion. Tumour regression is characterized by an irregular or nodular scar with adjacent ghosted tubules showing GCNIS or intratubular microcalcifications. Immunohistochemistry can be relatively robust in the presence of tumour necrosis and helpful in subtyping residual tumour—particularly cytokeratins, OCT3/4, CD117, glypican-3, ß-HCG and CD30. Biopsy of metastases may only show undifferentiated tumour and chemotherapy is instigated empirically on the basis of elevated serum markers. Occasionally poorly differentiated carcinoma of stomach, lung, breast or bladder may cause a modest elevation in serum ß-HCG. AFP is raised in hepatocellular carcinomas and some gastrointestinal cancers. Crucially the pathologist has to think of the possibility of germ cell tumour and look for the distinctive morphological and immunohistochemical clues with a panel of germ cell tumour markers (SALL4, PLAP, CD117, OCT3/4, CD30, SOX2, AFP, Glypican3, ß-HCG).

Excision Margins

Distances (mm) to the proximal limit of the spermatic cord.

Other Pathology

GCNIS: the *precursor lesion* of all germ cell tumours except spermatocytic tumour, prepubertal teratoma and YSTs. It is usually seen in a patchy distribution within the testicular tubules away from and adjacent to the tumour in up to 90% of seminomas and malignant teratomas. It may also be detected by needle biopsy as a *risk factor* for tumour development in the *contralateral testis* particularly if the testis is soft, atrophic or of low volume. It can be managed by *surveillance, orchidectomy* or *low-dose irradiation* as *50–90% of untreated GCNIS will progress to germ cell tumour over a five year period*. Chemotherapeutic agents do not cross the blood/testis barrier. It comprises a proliferation of seminoma like cells (clear cytoplasm, PAS/PLAP/CD117(c-kit)/p53/OCT3/4/D2–40/SALL4 positive) at the base of the tubules which often have a hyalinised and thickened basement membrane and absence of spermatogenesis. Tubular microcalcifications may be present. It can be associated with intratubular or extratubular (microinvasive) interstitial extension as either seminoma or EC and usually accompanied by a herald lymphocytic infiltrate.

Predisposing conditions: prior testicular tumour on the contralateral side confers an increased malignancy risk ×5–10. *Maldescent/cryptorchidism* and *infertility* confer an increased malignancy risk ×3–5 and 1% incidence respectively, correlating with GCNIS rates of 2–4% and 1%. Various *genetic conditions* may be associated with testicular tumours, e.g. mediastinal and testicular germ cell tumours in Klinefelter's syndrome, Sertoli cell proliferation and tumour in Peutz-Jeghers syndrome, and Carney complex (cardiac myxomas, Cushing's syndrome, hyperpigmentation).

Tumour regression: "scar" cancer (fibrosis, haemosiderin-laden macrophages, intratubular calcification) with retroperitoneal secondaries is

regression of the primary and *presentation with metastatic disease*, especially EC or choriocarcinoma.

Age incidence: age is also a helpful indicator in that malignant germ cell tumours and sex cord stromal tumours present in the *third and fourth decades* but malignant lymphoma and spermatocytic tumour occur in an older age group. Seminoma is usually in patients 10 years older than those with non-seminomatous germ cell tumours. YST is the commonest testicular neoplasm in children but also a common component of adult germ cell tumours.

Clinical mimics: other scrotal swellings mimicking testicular cancer include epididymo-orchitis, granulomatous orchitis, malakoplakia and peritesticular hydrocoele. Ultrasound examination is useful in delineating the latter and intratesticular lesions. This, coupled with increased male health awareness, has led to an increasing proportion of small and unusual tumours being detected, e.g. sex cord stromal lesions, epidermoid inclusion cyst.

FNAC: can be of use in those patients suspected of having *metastatic carcinoma* in the testes, or *testicular relapse in malignant lymphoma and leukaemia*. It is usually limited in germ cell tumours to those patients who are medically unfit for orchidectomy but in whom a tissue diagnosis is necessary for further management. Due to the considerable heterogeneity of germ cell tumours it can be subject to marked sampling error. Abdominal and thoracic FNAC (± core biopsy) are useful for the assessment of germ cell tumour metastases which should be categorised as seminomatous (requiring radiotherapy ± chemotherapy depending on the bulk of disease) or non-seminomatous. The latter is either pure teratomatous (requiring surgery) or other, e.g. EC or YST (requiring chemotherapy). Serum ß-HCG and AFP levels are also useful in making these management decisions.

Spermatocytic tumour (formerly spermatocytic seminoma): 1–2% of germ cell tumours, *indolent behaviour* and treated by *orchidectomy alone*. It presents in the *older age group* (50–70 years) and shows *no evidence of adjacent GCNIS*. It is lobulated ± microcystic with stromal oedema and comprises a *tripartite population* of small, intermediate and large glycogen negative cells with indistinct cell boundaries, a "spireme" (filamentous) chromatin pattern and scattered mitoses. It lacks a stromal lymphocytic component, is *germ cell marker negative* (PLAP/OCT3/4/CD117/D2–40/ß-HCG/EMA/CD30), but SALL4 positive and variably CAM 5.2 positive. Differential diagnosis is seminoma of usual type (distinct cell boundaries, uniform polygonal cells with clear cytoplasm, an enlarged nucleus with nucleoli and clumped nuclear chromatin, lymphocytic stroma, PLAP/OCT3/4/CD 117/SALL4/D2–40 positive), and malignant lymphoma (CD 45, CD 20, κ/λ light chain restriction, interstitial/peritubular infiltration, can be bilateral). Rarely spermatocytic tumour undergoes highly malignant (rhabdomyo-) sarcomatous change.

Immunophenotype

The reactions of different tumour types with immunohistochemical markers are summarised as follows:

GCNIS	SALL4, OCT3/4, CD117, PLAP +, and CAM5.2 ±
Seminoma	SALL4,OCT3/4,CD117,PLAP+,andCAM5.2–
YST	AFP, CAM5.2, glypican3 +, CD117 and SALL4±, OCT3/4–
EC	SALL4, OCT3/4, CAM5.2, CD30, SOX2 +, and CD117, PLAP, ß-HCG, AFP ±
MTT	ß-HCG +, CAM5.2 and SALL4±, OCT3/4, CD117, PLAP–

A simple algorithm for the use immunohistochemistry in subtyping testicular germ cell tumours is given in Table 33.3.

PLAP positivity in seminoma/GCNIS is membranous and not cytoplasmic as seen in some non-small cell lung carcinomas and malignant melanomas. OCT3/4, SALL4 and SOX2 are nuclear epitopes. Markers may also help distinguish metastatic EC (CAM 5.2+, CD30+, SALL4+, OCT3/4+, SOX2, PLAP±, EMA–) from metastatic carcinoma (CAM+, CD30–, SALL4–, OCT3/4–, SOX2–, EMA+). Seminoma is generally CAM 5.2–, CD30– and EMA- but can show focal positivity for these markers as well as CK7 and AE1/AE3. EC is

Table 33.3 Germ cell tumour immunohistochemistry

Pangerm cell marker SALL 4	→	YST AFP GLYPICAN 3 ±PLAP/CD117
↓		
Seminoma/EC CD117 OCT3/4 ± PLAP, - EMA	→	EC CD30 CAM5.2
↓		
Seminoma PLAP		
GCNIS OCT3/4, CD117, PLAP SALL 4 stains normal **and** neoplastic germ cells		

EC embryonal carcinoma, *YST* yolk sac tumour, *GCNIS* germ cell neoplasia in situ

strongly positive for various cytokeratin markers, CD30, SALL4, OCT3/4 and SOX2 but variable (often only focal) for CD117. YST is AFP+, glypican3+, CD117± and SALL4±, OCT3/4−. SALL4 is a robust pluripotential pan germ cell marker staining tumour and normal germ cells. Due to the latter, careful correlation with morphology is required in designation of GCNIS.

Serum markers: often do not show good correlation with their tumour tissue expression but are good for *monitoring disease treatment response and relapse* as they are raised in >80% of patients with non-seminomatous germ cell tumours. Seminoma may have mildly elevated high ß-HCG levels (15%) and increased serum PLAP levels (40% of cases). Seminoma and teratoma rarely give elevated AFP levels. If present, other elements, e.g. YST or EC are identified by taking extra blocks to confirm their presence.

Apparently aberrant tissue expression is acceptable without changing the diagnosis or prognosis, e.g. seminoma with ß-HCG positive syncytiotrophoblastic cells (10–20% of cases) or EC with elevated serum ß-HCG.

Prognosis

With modern oncological treatment regimens the prognosis of even metastatic germ cell tumour is excellent with *more than 90% cure*. It relates to *serum tumour marker levels, stage of disease, histological type and lymphovascular invasion.* Stage I disease and stage II with non-bulky (<5–10 cm) retroperitoneal secondaries have 5 year survival rates of 90–95% for both seminoma and EC, whereas the rate for bulky stage II tumour is 70–80%. YST presents as stage I (90%) in childhood with >90% 5 year survivals however it can exhibit chemoresistance in adults with metastatic disease. The presence of YST elements in an immature teratoma of childhood is also an indicator for potential recurrence of disease. *Extensive pulmonary disease in EC is a poor prognostic indicator.*

Prognosis of seminoma worsens with:

1. Tumour diameter ≥ 4 cm.
2. Age < 34 years.
3. Vascular invasion.
4. Rete testis invasion.

Prognosis of teratoma worsens with:

1. Increasing stage.
2. Presence of EC.
3. Absence of YST.
4. Lymphovascular invasion.

The Medical Research Council scheme scores 1 for each of: presence of EC,

absence of YST, lymphatic invasion, blood vessel invasion.

Tumour score:

0–2	Surgery with follow-up only
3–4	Surgery with adjuvant chemotherapy.

Low volume/percentage tumour area of EC and a low Ki-67 index are beneficial. *Relapse rates are 15–20% for seminoma* (80% in the retroperitoneum) and *30–35% for teratoma* (66% in the retroperitoneum, 33% in the lung or mediastinum). *Vascular invasion* is a strong determinant of *postoperative chemotherapy* in stage I disease. Stage I *seminoma* is treated by *orchidectomy* and then either *surveillance* (serum tumour markers, chest x-ray, CT scan) for low risk tumour (<4 cm dimension, no rete invasion), *single dose carboplatin chemotherapy,* or *radiation* to regional lymph

node sites. Stage II and more advanced disease require chemotherapy and/or radiotherapy based on the bulk of retroperitoneal disease. *Non-seminomatous germ cell tumours* require *orchidectomy* alone with *surveillance* for stage I disease with no high risk factors (particularly lymphovascular invasion). Otherwise this is combined with *platinum based chemotherapy*. This is supplemented by *retroperitoneal lymph node dissection (RPLND)* for post-treatment residual disease or recurrent disease refractory to chemotherapy. Postchemotherapy cytoreduction of metastases results in necrosis, xanthomatous inflammation, fibrosis and variably viable tumour tissue. *Ominously, carcinomatous* or *sarcomatous (e.g. rhabdomyosarcoma, primitive neuroectodermal tumour) differentiation* may occasionally occur. Metastatic disease not infrequently *changes differentiation* with treatment leaving residual masses of cystic, mature tissues in the lung or para-aortic lymph nodes. They can be insensitive to adjuvant therapy, press on local structures (the growing teratoma syndrome), and may require surgical resection. Alternatively they can be monitored by serum tumour marker levels and CT scan +/− PET scan and further investigated for malignant change if growth recurs. *Prognosis of metastatic disease* relates to the size, site and number of metastases, the extent of tumour mass shrinkage during chemotherapy, completeness of excision, nature of the resected masses and serum ß-HCG and AFP levels. Metastases comprising total necrosis or fully mature tissue correlate with better prognosis. Fibrosis, necrosis and differentiated teratoma are present in 20–70% of RPLNDs; and in 5–25% of cases of EC, choriocarcinoma and YST. A minority of seminomas may develop non-seminomatous germ cell tumour metastases. This may relate either to true transformation of seminoma, or a focus of non-seminomatous germ cell tumour in the primary lesion which was not identified. In orchidectomy and RPLND specimens sufficient numbers of blocks should be sampled to establish the presence of any malignant components as a basis for further chemotherapy, e.g. germ cell, carcinomatous, sarcomatous or primitive neuroectodermal elements.

The number of chemotherapy cycles is minimised by titrating against normalisation of the serum tumour marker levels. This is done to *decrease the risk of developing a second malignancy* in later life, e.g. sarcoma or malignant lymphoma, predisposition to which is greatest in those treated before the age of 30 years. *Relapse* following complete remission after chemotherapy for metastatic testicular cancer is only seen in *10% of patients* and is more likely if there has been advanced disease.

Other Malignancy

Malignant Lymphoma

- *Diffuse large B cell non-Hodgkin's malignant lymphoma* (70–80% of cases) in older men (60–80 years of age). Uni−/bilateral (20%), comprising *2% of testicular neoplasms,* and 5% of extranodal malignant lymphomas in men. Primary (60% of cases) or secondary to systemic/nodal disease, and often associated with disease elsewhere, e.g. central nervous system, skin, soft tissues, liver, kidney, lung, bone, orbit and Waldeyer's ring. Classically shows an interstitial/peritubular pattern of infiltration. Prognosis is stage dependent with a *70–80% overall survival*.

In children: *Burkitt's lymphoma* ("starry sky" pattern, CD10/Ki-67 positive), *lymphoblastic lymphoma* or diffuse large B cell lymphoma.

Leukaemia

ALL: children, site of relapse in 5–10% and predictive of systemic relapse.

CLL: the testis is involved in 20–35% of patients.

Leukaemia can be bilateral and testicular disease the presenting feature in a minority of cases. Granulocytic or myeloid sarcoma is CD34, CD43, CD68, CD117, myeloperoxidase and chloroacetate esterase positive.

Plasmacytoma

- Rare, usually secondary to an established myeloma. Differential diagnosis is spermatocytic tumour. Plasmacytoma expresses CD79a, CD38 and CD138.

Paratesticular Tumours: Sarcomas (Liposarcoma, Rhabdomyosarcoma, Leiomyosarcoma), Mesothelioma of the Tunica Vaginalis, Desmoplastic Small Round Cell Tumour, Mullerian Tumours

- Treatment of sarcomas is orchidectomy with high ligation of the cord and post-operative radiation therapy. Presentation is with a large rapidly growing inguinoscrotal mass.
- *Liposarcoma:* adults and well differentiated adipocytic/sclerotic with variation in adipocyte size and scattered lipoblasts. Local excision and a 23% local recurrence rate. Occasionally represents an extension from a retroperitoneal neoplasm.
- *Rhabdomyosarcoma*: in children (peak age 7 years) of embryonal round cell (± spindle cells) type with mitoses and necrosis. Desmin and myogenin/myo D1 positive. Excision and adjuvant therapy give *80% long term survival* with localized and disseminated tumours having 95% and 60% 5 year survival rates, respectively. There is also a low-grade fascicular spindle cell variant of good prognosis, but alveolar rhabdomyosarcoma (6%) has an adverse outlook (translocation t(2;13)(q35;q14)).
- *Leiomyosarcoma:* adults with atypia, necrosis, mitoses.
- *Mesothelioma*: cystic/solid/nodular masses lining a hydrocoele/hernia sac and a variably aggressive clinical course. There may be associated asbestos exposure. Epithelial (75%), sarcomatoid or biphasic patterns. Distinguish from *benign cystic adenomatoid tumour* of mesothelial origin forming >30% of testicular adnexal tumours: circumscribed, glands, cysts, cords of CK5/6, calretinin, WT1 positive cells. Excision is curative. Sites of occurrence are spermatic cord, epididymis, tunica, rete.
- *Desmoplastic small round cell tumour:* lower abdomen, pelvi-inguinal and scrotal area of young men. It comprises nests of small cells in a fibrous stroma, and is polyimmunophenotypic—cytokeratin, desmin (dot reactivity), synaptophysin, WT1 positive. It is characterized by a recurrent t(11;22)(p13;q12) translocation. Most develop peritoneal or retroperitoneal disease within 2 years and die in 3–4 years with metastases to liver and lungs.
- *Papillary serous carcinoma, Mullerian subtype*: rare, ovarian tumour analogue.
- *Malignant lymphoma*: usually represents secondary spread from the adjacent testis, diffuse large B cell in type.

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Penile Carcinoma

34

Declan O'Rourke

Introduction

Penile cancer is relatively uncommon in the UK with an incidence of 1 in 100,000 in Western countries and representing less than 1% of all cancers in men, indicating the need for clinical assessment and treatment by supra-regional specialist teams. There is worldwide variation in incidence being highest in South America, Asia and Africa. Predisposing factors are human papilloma virus (HPV) infection, phimosis, psoriasis, smoking, and chronic non-viral infection associated with poor hygiene and lack of circumcision. Obesity may also play a role.

Penile cancer can present as a warty/nodular lesion, plaque or bleeding ulcer commonly on the foreskin, glans penis or in the coronal sulcus. Investigation is by diagnostic punch or wedge biopsy, although in well differentiated exophytic lesions definite invasive malignancy may be hard to demonstrate in a limited sample and difficult to distinguish from wart virus infection or pseudoepitheliomatous hyperplasia. The clinical impression is then important in designation and planning of management. Fine needle aspiration cytology (FNAC) of inguinal lymphadenopathy may demonstrate metastases (10–15% of cases at diagnosis) as a prequel to radical surgery and regional ilioinguinal lymphadenectomy. Alternatively lymph node enlargement may be solely on the basis of inflammation or infection. CT scan can demonstrate the presence of any ilioinguinal lymphadenopathy that is subclinical in extent. CT/PET scan can help to detect distant metastases.

A majority of penile cancers are superficial and well to moderately differentiated with metastases uncommon. They can be treated by penile sparing limited resection (wedge resection, wide local excision with circumcision, glansectomy, glans resurfacing) with reconstruction of the glans rather than amputation or primary radiotherapy. Accurate assessment of the proximal extent and depth of invasion (e.g. corporal or urethral involvement) by MRI scan is important to avoid incomplete excision or unnecessarily extensive resection. In some specialist centres this may be determined intraoperatively by frozen section. The treatment goal is complete local excision with adequate margins and choice of therapy is related to tumour size, extent of infiltration and destruction of normal tissues. Radiotherapy is reserved for high stage tumours, recurrences, metastatic disease and patients unfit for surgery. It may be followed by salvage surgery. Localised tumours of the prepuce are treated by circumcision. Glansectomy removes the foreskin and glans for penile intraepithelial neoplasia (PeIN) or localised cancer (with primary closure or grafting and reconstruction), but there is a higher risk of incomplete removal and local

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_34

recurrence. Glans resurfacing can be offered to patients with predominant or significant disease of the glans. Partial penectomy relies on transection of the penis 2 cm proximal to the gross tumour edge but may be precluded in favour of total penectomy because of tumour size, site and destruction. Ilioinguinal lymphadenectomy is for known metastases, or clinically negative nodes but poorly differentiated high risk carcinomas. In some specialist centres dynamic sentinel lymph node biopsy (DSNB) is used as a guide to whether inguinal lymph node block dissection is necessary. This is a minimally invasive surgical staging technique with excellent performance. In medically unfit patients radiotherapy to the groin is an option, and it may also decrease inguinal recurrences in the adjuvant setting. In patients with bulky inguinal lymph nodes or enlarged pelvic lymph nodes, triple regimen chemotherapy has a response rate of 50%, but long-term survival rates are very poor. Targeted therapies (against the EGFR pathway) have shown promising results in patients after chemotherapy failure.

Gross Description

Specimen

- Wedge biopsy/wide local excision with circumcision/penile sparing resection (glansectomy/partial penectomy)/total penile amputation/radical penectomy (including scrotum, testes, spermatic cords, groin lymph node dissection).
- Size (mm) and weight (g).

Tumour

Site
- Urethral meatus/glans/prepuce/coronal sulcus/penile urethra/shaft (dorsal/ventral/lateral).
- Multicentric: particularly foreskin.

Size
- Length × width × depth (mm) or maximum dimension (mm).
- Tumour thickness (mm) is a gauge of depth of invasion and prognosis.

Appearance
- Exophytic (warty, verrucous, papillary, fungating)
- Superficial spreading (plaque)
- Endophytic (sessile, ulcerated, infiltrative)
- Pale/pigmented.

Edge
- Circumscribed/irregular.

Histological Type

Squamous Cell Carcinoma

- *95% of penile malignancies*, 70–80 years of age.
- *Usual type (70% of cases):*
- Exophytic or endophytic.
- Large cell/small cell.
- Keratinising/non-keratinising.

Variants (In Order of Frequency)
- *Warty* and *papillary:* exophytic and well differentiated, the former with HPV related surface koilocytic atypia, and the latter irregular, complex papillae and stromal cores.
- *Basaloid:* comprises *5–10% of cases* and is a poorly differentiated *aggressive high-grade tumour* 50% of which present with lymph node metastases. It is usually ulcerated and endophytic with nests of basaloid cells showing abrupt central keratinisation or comedonecrosis. It is HPV related.
- *Verrucous:* on the glans and foreskin, *5–16% of cases* and exophytic with a deep pushing margin of cytologically bland bulbous processes. Prone to multifocality and local recurrence if incompletely excised, and may dedifferentiate with radiotherapy. It generally has a good prognosis. It can coexist with usual squamous cell carcinoma.
- *Papillary*: exophytic tumours with a complex pattern, and usually have an irregular interface with the underlying stroma. The deep

border of papillary carcinoma is jagged compared to the broad and pushing border of verrucous carcinoma. They are low grade and not HPV related and may have coexistent differentiated PeIN.

- *Spindle cell (sarcomatoid):* arises de novo or post radiotherapy. It comprises cytokeratin positive spindle cells associated with a surface epithelial origin or more recognisable in situ or invasive squamous cell component. Areas of heterologous dedifferentiation (muscle, cartilage, bone) can occur. It is a high-grade endophytic cancer with *poor prognosis* and a *high rate of local recurrence.*
- *Pseudohyperplastic:* rare, foreskin, associated with lichen sclerosis, good prognosis.
- *Carcinoma cuniculatum:* recently described entity consisting of multiple sinuses and fistulas mimicking a rabbit's burrow. It is a low-grade lesion with no metastases reported to date.
- *Adenosquamous*: rare surface tumour and tends to originate centrally in the glans with a predominance of squamous areas intermixed with glandular foci.
- *Pseudoglandular*: aggressive with acantholysis and pseudoglandular spaces. Most are poorly differentiated, high-grade tumours. Lymph node metastases occur in more than two-thirds and the mortality rate is high.
- *Clear-cell carcinoma*: aggressive, HPV related (p16 positive immunochemistry) and involving glans and foreskin. Tumour-related mortality is approximately 20%.
- *Lymphoepithelioma-like carcinoma*: poorly differentiated, resembling the lymphoepithelioma like carcinoma of the nasopharynx with sheets of lymphocytic or plasmacytic cells mixed with tumour cells (p16 positive). Prognosis is poor.
- *Mixed types: 25% of cases*. Adequate tumour sampling is necessary to find less differentiated components.

Basal Cell Carcinoma

- A local carcinoma of penile shaft skin.

Urothelial Carcinoma

- Either as a primary lesion of the proximal urethra, or secondary to bladder cancer, both of which can show Pagetoid urethral spread

Malignant Melanoma

- <1% of cases and primary or secondary situated on the glans penis. 50% have lymph node metastases at presentation and *poor prognosis*, being related to tumour thickness and stage with 2 and 5 year survivals of 61% and 20%, respectively.

Metastatic Carcinoma

- Characteristic multinodular growth pattern in the corpora cavernosa.
- Rare; originating in prostate, bladder, kidney, gut, testis.
- Usually as a late manifestation of systemic disease and *poor prognosis* with a *71% 6 month mortality*. Can present with priapism, or as extramammary Paget's disease from an underlying adnexal tumour or distant spread e.g. bladder.

Differentiation

The new WHO classification utilises grades 1–3 in squamous cell carcinoma. The grade allocated is the highest grade identified, irrespective of the predominant grade present. Sarcomatoid differentiation should also be reported separately.

Many are exophytic and well to moderately differentiated with variable keratinisation.

Ulcerated, infiltrating cancers of the glans penis tend to be moderately to poorly differentiated and non-keratinising. About 50% of shaft cancers are poorly differentiated and only 10% of prepuce tumours. *Grading* based on the degree of keratinisation, intercellular bridges, mitoses, cellular atypia and inflammatory infiltrate *correlates with prognosis*. Sarcomatoid change is regarded as undifferentiated. Over expression of p53 and Ki-67 correlate with tumour grade but not cancer specific and overall survivals.

Extent of Local Tumour Spread

- Border: pushing/infiltrative.
- Lymphocytic reaction: prominent/sparse.

Microscopic growth patterns are: verruciform, superficial spreading (horizontal with superficial invasion), vertical with deep penetration, or multicentric. Superficial spreading and vertical growth patterns have 10% and 67% mortality rates, respectively.

Anatomical levels are: epithelium (1 mm thick), lamina propria (2 mm thick), corpus spongiosum (periurethral and limited inferiorly by tunica albuginea), and corpus cavernosum (surrounded by tunica albuginea with its distal tapered end within the glans). A suggested *threshold value for metastatic potential is 4–6 mm invasion into the corpus spongiosum.*

TNM classification of penile and distal urethral tumours.

TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ (Penile intraepithelial neoplasia—PeIN)
Ta[a]	Noninvasive localised squamous cell carcinoma[a]
T1a	Tumour invades subepithelial connective tissue[b] without lymphovascular invasion or perineural invasion and is not poorly differentiated (i.e. grade 3 or sarcomatoid)
T1b	Tumour invades subepithelial connective tissue[b] with lymphovascular invasion or perineural invasion or is poorly differentiated
T2	Tumour invades corpus spongiosum with or without invasion of the urethra
T3	Tumour invades corpus cavernosum with or without invasion of the urethra
T4	Tumour invades other adjacent structures

[a]Including verrucous carcinoma. The category Ta is to be used with care as these tumours are exceptionally rare and are not evidence based

[b]Glans: Tumour invades lamina propria. Foreskin: Tumour invades dermis, lamina propria or dartos fascia
Shaft: Tumour invades connective tissue between epidermis and corpora and regardless of location

In the case of multiple tumours, the tumour with the highest T category should be classified and the multiplicity or number of tumours should be indicated in parentheses, e.g. pT2 (m). Urethral involvement is no longer regarded as a defining feature of staging in TNM8.

The *depth or extent and the pattern of infiltrative spread* correlate with the incidence of *lymph node metastases*. Satellite nodules are not unusual and occasionally there is Pagetoid spread along the urethra to involve its proximal margin. Despite the vascularity of the structures, *haematogenous spread* to the liver, heart, lung and bone is *rare* (2%) (Figs. 34.1, 34.2, and 34.3).

Lymphovascular Invasion

- Present/absent.
- Intra-/extratumoural.
- Vascular invasion is an important adverse factor and predictor of lymph node metastases. Perineural invasion should also be noted.

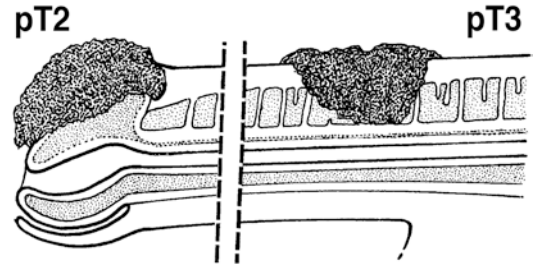

Fig. 34.1 Penile carcinoma. Invasion of corpus spongiosum is pT2 while invasion of corpus cavernosum is pT3. Invasion of the urethra does not affect staging. Adapted, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

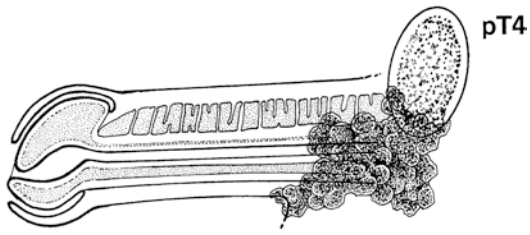

Fig. 34.2 Penile carcinoma: tumour invades other adjacent structures. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

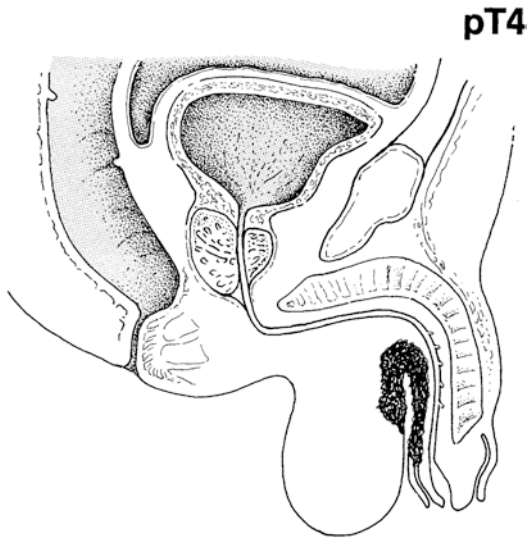

Fig. 34.3 Penile carcinoma: tumour invades other adjacent structures. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed*., © 2005, Springer-Verlag

Lymph Nodes

- Site/number/size/number involved/limit node/extracapsular spread.
- Regional nodes: superficial and deep inguinal and pelvic.

pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Metastasis in up to two regional lymph nodes
pN2	Metastases in three or more unilateral lymph nodes or bilateral inguinal lymph nodes
pN3	Extranodal extension of lymph node metastasis or pelvic lymph node(s), unilateral or bilateral

The incidence of lymph node metastases is greater (>80%) in deeply invasive than superficially spreading carcinomas (30%). Pattern of spread is initially to superficial then deep inguinal lymph nodes, pelvic and lastly retroperitoneal lymph nodes. Rarely, skip metastases can occur, e.g. direct to deep inguinal lymph nodes. *Survival is influenced by the number of positive lymph nodes, the presence of extracapsular invasion, and the level of lymph node involvement (inguinal versus pelvic).* Lymphadenectomy improves prognosis but is only carried out when there are known metastases, lymphovascular invasion, or in high-grade disease, e.g. basaloid, sarcomatoid or undifferentiated carcinoma. Low-grade tumours such as verrucous carcinoma seldom result in lymph node disease although there may be lymphadenopathy due to inflammation or infection. Nodal involvement is the most important prognostic indicator in patients with penile SCC. Regional node staging with CT or MRI has a role in patients with clinically node positive disease. However, these are unreliable in detecting occult metastasis and DSNB has shown excellent performance for clinically negative nodes.

Excision Margins

- Distance (mm) to the proximal limit of excision in a penectomy specimen.
- Distances (mm) to the deep corporal and lateral glans or cutaneous margins in a local resection specimen.

Local recurrence is rare if margins are tumour free. Traditionally a margin clearance of 15–25 mm was required but this has been reduced with the use of organ sparing surgery in an effort to achieve better cosmetic and functional outcomes. Margin clearance may require checking by intraoperative frozen section examination.

Other Pathology

Predisposing Factors and Lesions

Predisposing factors for penile carcinoma are older age (rare <40 years of age), lack of circumcision, poor hygiene and phimosis. Cases can be non-HPV

or HPV related. *Predisposing lesions* are *squamous hyperplasia* (leukoplakia), *lichen sclerosus (LS)/ Balanoposthitis xerotica obliterans (BXO)*, and *penile intraepithelial neoplasia (PeIN)/squamous intraepithelial lesion (SIL)*. PeIN is of two types, either *differentiated* (simplex: non-HPV related) or *classical* (basaloid/warty: HPV related). These precursor lesions can be multifocal. Treatments are local excision, Moh's surgery with reconstruction, laser therapy, electrodessication and curettage, cryosurgery and topical 5-fluorouracil.

Squamous Hyperplasia (Leukoplakia)

Commonly identified in association with LS, differentiated PeIN and low-grade keratinising penile SCC and may be difficult to separate from differentiated PeIN. May mimic a neoplasm as whilst typically flat, it may also have a verrucous/ papillary appearance.

Lichen Sclerosus (LS)

Also referred to as Balanitis xerotica obliterans (BXO), more frequent in the inner foreskin, but coronal sulcus, glans and even urethra may be affected and may cause narrowing of the urethral meatus or phimosis. LS may have autoimmune aetiology but there is no strong association with HPV. There is a weak association with low grade keratinizing squamous cell carcinoma (non HPV variants—squamous cell NOS, pseudohyperplastic, verrucous and papillary carcinoma of the foreskin).

Histologically, the affected areas show epidermal atrophy with an underlying "band like" chronic inflammatory infiltrate that is gradually replaced by eosinophilic hyalinisation in more advanced cases.

PeIN/SIL (Erythroplasia de Queyrat/ Bowen's Disease/Bowenoid Papulosis)

- Terms such as eythroplasia of Queyrat and Bowen's disease have been abandoned with adoption of the encompassing term PeIN. Two forms are noted: undifferentiated PeIN (previously designated severe dysplasia/carcinoma in situ and associated with HPV) and differentiated PeIN (involving only the basal layers and associated with architectural atypia and unrelated to HPV). PeIN does not need to be graded and is regarded as high grade by definition.

Condyloma Accuminatum

- Coronal sulcus, inner foreskin.
- *HPV 16, 18* are particularly associated with warty and basaloid PeIN and their respective cancer types, but not typical keratinizing squamous cell carcinoma.
- HPV 6, 11 are not associated with PeIN and penile cancer.

Extramammary Paget's Disease

- *Secondary* to concurrent urothelial neoplasia ± Pagetoid spread of transitional cell carcinoma into the penile urethra (CK7/CK20 positive), or, *primary* and limited to the glans penis (CK7 positive, CK20 negative).

Clinical Mimics of Penile Cancer

- Beware Zoon's plasma cell balanitis, nicorandil (an anti-angina drug) induced penile ulceration, sexually transmitted diseases (e.g. syphilis), Wegener's granulomatosis.

Prognosis

More than 95% of penile carcinomas are squamous cell carcinoma. At presentation about 40% are exophytic and superficially invasive with extensive in situ change, 30% endophytic and deeply invasive, 10–20% verrucous and 5–10% multifocal. *Inguinal lymph node metastases are present in 15–45% of cases.* Prognosis relates to tumour site, size, infiltrative growth pattern,

depth of invasion, stage, histological grade, and vascular invasion with on average *70–80% 5 year survival rates*. Adverse factors are lymphovascular invasion, vertical growth pattern, and, basaloid, sarcomatoid, solid, undifferentiated and pseudoglandular subtypes.

Other Malignancy

Sarcoma

- More often affecting the penile shaft than the distal or glans penis and forming *<5% of penile malignancy*, especially:

Kaposi's sarcoma: about 20% of HIV/AIDS male patients on the skin of the shaft or glans and usually associated with other systemic lesions. It is HHV8 positive.

Leiomyosarcoma: 50–70 years of age. Superficial and subcutaneous has a good prognosis, whereas corporal and deep in location with early metastases has a poor prognosis.

Epithelioid haemangioendothelioma: varying grade and outlook (CD 31/CD34 positive epithelioid cells with intracytoplasmic vacuoles).

Others: angiosarcoma, rhabdomyosarcoma, fibrosarcoma, epithelioid sarcoma.

Malignant Lymphoma

- Primary lesions are very rare and malignant lymphoma is usually secondary to systemic disease.

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Part VIII

Lymph Node Cancer

- Nodal Malignant Lymphoma (With Comments on Extranodal Malignant Lymphoma and Metastatic Cancer)

35

Nodal Malignant Lymphoma (With Comments on Extranodal Malignant Lymphoma and Metastatic Cancer)

Lakshmi Venkatraman

Introduction

Representing 5–7% of all cancers and 55% of haematological malignancies with an increasing incidence, malignant lymphoma presents as persistent, mobile, rubbery and non-tender lymphadenopathy with or without associated systemic symptoms such as weight loss, itch or night sweats. Investigation is by full blood picture (infections/leukaemias), serology (infections/autoimmune diseases), fine needle aspiration cytology (FNAC: to exclude metastatic cancer) and biopsy. Clinical staging is by CT/PET/MRI scans appropriate to the clinical context, bone marrow aspirate and trephine biopsy.

In the UK contemporary guidance from the Royal College of Pathologists and the NHS National Cancer Action Team indicates concordance of diagnosis for malignant lymphomas of less than 85%. It is advised that diagnosis should be in accordance with the WHO 2016 classification of haematopoietic neoplasms and within the remit of a specialist integrated haematological malignancy diagnostic service covering a catchment population of at least two million. There should be a single integrated report encompassing the requisite specialist morphological expertise, immunohistochemistry, flow cytometry, cytogenetics, in situ hybridization and molecular diagnostics. Local arrangements will require at least prompt referral through the cancer network to the appropriate haematological malignancy diagnostic team. Underpinning this will be the ongoing need for general diagnostic pathologists to competently recognize the wide range of haematolymphoid pathology so that relevant and expeditious clinicopathological referrals are made.

Gross Description and Morphological Recognition

Specimen

- FNAC/needle biopsy core/excisional biopsy/regional lymphadenectomy.
- Regional lymphadenectomy comprises part of a formal cancer resection operation. This can either be for removal of a primary malignant lymphoma, e.g. gastrectomy, or, where malignant lymphoma is found incidentally in a resection for a primary carcinoma, e.g. in the mesorectal nodes of an anterior resection for rectal cancer.
- Size (mm) and weight (g)
- Colour, consistency, necrosis.

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_35

The *preferred specimen* for *diagnosis, subtyping and grading of nodal malignant lymphoma* is an *excisional lymph node biopsy* carefully taken by an experienced surgeon to ensure representation of disease and avoidance of traumatic artifact. Submission of the specimen fresh to the laboratory allows material to be collected for flow cytometry, cytogenetics, and imprints to be made, to which a wide panel of immunohistochemical antibodies or FISH probes can be applied some of which are more effective than on formalin fixed paraffin processed tissue sections. E.g. the demonstration of light chain restriction. Tissue can also be harvested for molecular and genetic techniques. Triaging of FNA and core biopsy samples for morphology, and ancillary studies is helped by rapid on site evaluation of the specimen (ROSE). Needle core samples should be blocked in individual cassettes and spare serial sections requested to allow selective panels of immunohistochemistry or FISH to be performed. Knowledge of the broad diagnostic categories of haematological diseases is helpful in maximizing information from small samples. *Morphological classification* is generally based on well fixed, thin slices, processed through to paraffin with high quality 4 μm H&E sections. *Core biopsy* may be the only option if the patient is unwell or the lesion is relatively inaccessible, e.g. mediastinal or para-aortic lymphadenopathy. Allowances must be made in interpretation for underestimation of nuclear size, sampling error and artifact. Confirmation of lymphomatous (or other) malignancy is the prime objective and further comments on subtyping and grading given with care and only if definitely demonstrable. Despite these considerations a positive diagnosis can be given in a significant percentage of cases. Importantly, interpretation should be in light of the clinical context, i.e. the presence of palpable or radiologically proven significant regional or systemic lymphadenopathy, and the absence of any obvious carcinoma primary site. Tumour heterogeneity must also be borne in mind. The same principles apply to *FNAC*, which is excellent at excluding inflammatory lymphadenopathy, e.g. abscess or sarcoidosis, and non-lymphomatous cancer (e.g. metastatic squamous cell carcinoma, breast carcinoma or malignant melanoma). It is also reasonably robust at designating Hodgkin's and high-grade non-Hodgkin's malignant lymphoma. *Morphology is the starting point and principal diagnostic criterion* when assessing excisional lymph node biopsies, core biopsies and FNAC but is supplemented by *immunohistochemical antibody panels* targeted at the various diagnostic options, e.g. a small lymphoid cell proliferation (lymphocytic lymphoma versus mantle cell lymphoma etc.). In addition, *flow cytometry* and *molecular tests including PCR for gene rearrangements, FISH for chromosomal abnormalities and mutation tests (single/multiplex or next generation sequencing)* can be important in determining a *diagnosis* and its attendant *treatment* and *prognosis.* Limited needle sampling techniques can also be used in patients with a previous biopsy proven tissue diagnosis of malignant lymphoma and in whom recurrence is suspected. However, possible *transformation* of grade must be considered and even change of malignant lymphoma type, e.g. small lymphocytic lymphoma to Hodgkin's malignant lymphoma, or Richter's transformation to diffuse large B cell malignant lymphoma. A range of inflammatory and neoplastic lymph node pathology may also be encountered secondary to chemotherapy and *immunosuppression*, e.g. tuberculosis, EBV (Epstein Barr Virus) driven lymphoproliferation, and various malignant lymphomas.

A systematic approach to excisional lymph node biopsies will allow the majority to be categorised as specific inflammatory pathology, benign or malignant, and the latter as haematolymphoid or non-haematolymphoid in character. *Diagnostic morphological clues* to malignant lymphoma are *architectural* and *cytological.*

Architectural descriptors are: diffuse, follicular, nodular, marginal zone, sinusoidal, paracortical, and angiocentric/angioinvasive distributions.

Cytological descriptors are: cell size (small, medium (the size of a histiocyte nucleus), large), the relative proportions of the cell populations, specific cytomorphological features, and cellular proliferative activity (mitoses, apoptosis, Ki-67 index). Various malignant lymphomas are also characterized by a typical host connective tissue and/or cellular inflammatory response.

Low Power Magnification

- Capsular/extracapsular spillage of lymphoid tissue
- Capsular thickening and banded septal fibrosis or hyaline sclerosis
- Loss of sinusoids with either compression or filling due to a cellular infiltrate (preservation of sinuses is occasionally seen)
- Alteration in follicular architecture with changes in
  - Distribution: proliferation in the medulla
  - Size and shape: relative uniformity of appearance
  - Definition: loss of the mantle zone- germinal centre interface/"filling up" of the germinal centre/loss of tingible body macrophages
  - Absence: the architecture may be completely effaced by a diffuse infiltrate
- Prominent post capillary venules.

High Power Magnification

- Presence of a background polymorphous inflammatory cellular infiltrate, e.g. eosinophils, plasma cells and histiocytes (epithelioid in character ± granulomas)
- Alterations in the proportions of the normal cellular constituents
- Dominance of any mono- or dual cell populations
- Presence of atypical lymphoid cells
  - Nuclei: enlargement/irregularity/hyperchromasia/bi- or polylobation/mummification/apoptosis
  - Nucleoli: single/multiple/central/peripheral/eosinophilic/basophilic/Dutcher inclusions
  - Cytoplasm: clear/vacuolar/eosinophilic/scant/plentiful/paranuclear hof.

A *morphological diagnostic short list* should be created, e.g. mixed cellularity Hodgkin's lymphoma versus T cell malignant lymphoma versus T cell rich large B cell malignant lymphoma, and a *targeted immunohistochemical antibody panel* used. The determination of *cell lineage* is a prerequisite for diagnosis. In the majority of cases immunohistochemistry will confirm the preliminary diagnosis, but will in a minority lead to its modification and either a refinement within or revision of diagnostic category. A pitfall for the unwary is *aberrant expression* of T cell antigens by a B cell malignant lymphoma and vice versa, e.g. expression of CD5 in small lymphocytic lymphoma/ chronic lymphocytic leukaemia. *Clonality* and *gene rearrangement studies*, either by immunohistochemistry, flow cytometry or molecular techniques can be important in confirming the neoplastic character of the B and T cell populations. Another relevant ancillary technique in various clinical settings is in situ hybridization for *EBERs* (Epstein Barr virus Encoded RNAs). *Patient age, disease site and distribution* also contribute to making a correct diagnosis, e.g. nodular lymphocyte predominant Hodgkin lymphoma usually presents in younger patients and as solitary or localized lymphadenopathy rather than extensive disease.

Histological Type and Differentiation/Grade

Therapeutic and prognostic distinction is made between *Hodgkin* and *non-Hodgkin malignant lymphomas (HL/NHLs)*, with a significant proportion of the former being reclassified as variants of the latter on the basis of improved immunophenotyping. Within classical Hodgkin malignant lymphoma there are several subtypes from nodular sclerosis and lymphocyte rich through mixed cellularity to lymphocyte depleted, with nodular sclerosis divided into two subtypes that are of prognostic significance in limited stage disease. In non-Hodgkin lymphoma, indolent tumours often have a follicular pattern and are composed of small cells while aggressive lesions are usually diffuse with proliferation of medium to large cells or blasts. The morphological spectrum can predict the likelihood of untreated disease progression from indolence to aggressive behaviour, but paradoxically often does not correlate with extent of disease stage, chemoresponsiveness, long term disease free survival and potential for cure. For example, grade 1/2 follicular lymphoma is often of extensive distribution (stage IV), indolent in behaviour, yet incurable and ultimately fatal at 5–10 years after diagnosis. Morphology and immunophenotyping

can identify most low and high-grade lymphomas, e.g. mantle cell lymphoma, mediastinal large B cell lymphoma and lymphoblastic lymphoma. In recent years, cell of origin as identified by immunohistochemistry or gene expression profiling and elucidation of the genetic and mutational landscape of lymphomas has led to upfront molecular testing for accurate diagnosis, treatment and prognosis.

Non-Hodgkin's Malignant Lymphoma

Non-Hodgkin's malignant lymphoma (NHL) is classified according to the WHO 2016 system (Table 35.1) which defines each disease by its *morphology, immunophenotype, genetic characteristics, proposed normal counterpart* and *clinical features.* It is *reproducible,* with *prognostic* and *therapeutic implications*. Broad categories are malignant lymphomas of B, T or NK (natural killer) cell types. Malignant lymphomas and leukaemias are both included as many haematolymphoid neoplasms have both solid and fluid circulatory phases. *Prognosis* relates to *stage of disease, treatment protocols* and *individual disease biology.*

Table 35.1 The 2016 WHO classification of lymphoid neoplasms

B cell neoplasms
Precursor B cell neoplasms
Precursor B lymphoblastic lymphoma/leukaemia
Mature B cell neoplasms
Chronic lymphocytic leukaemia/small lymphocytic lymphoma
Monoclonal B-cell lymphocytosis
B prolymphocytic leukaemia
Lymphoplasmacytic lymphoma/Waldenströms macroglobulinaemia
Heavy chain disease (α, γ, μ)
Splenic marginal zone lymphoma
Hairy cell leukaemia
Splenic B-cell lymphoma/leukemia unclassifiable
Splenic diffuse red pulp small B-cell lymphoma
Hairy cell leukemia-variant
Plasma cell myeloma
Solitary plasmacytoma of bone
Extra osseous plasmacytoma
Extranodal marginal zone B cell lymphoma of mucosa associated lymphoid tissue (MALT lymphoma)
Nodal marginal zone B cell lymphoma
Paediatric nodal marginal zone lymphoma
Follicular lymphoma
Grading:
Grade 1: 0 to 5 centroblasts per high power field[a]
Grade 2: 6 to 15 centroblasts per high power field[a]
Grade 3: greater than 15 centroblasts per high power field[a]
Grade 3a: centrocytes are still present
Grade 3b: centroblasts form solid sheets with no residual centrocytes
Reporting of pattern:
Follicular: greater than 75% follicular
Follicular and diffuse: 25% to 75% follicular
Focally follicular: less than 25% follicular
Diffuse
In situ follicular neoplasia
Duodenal-type follicular lymphoma
Paediatric follicular lymphoma
Large B-cell lymphoma with IRF4 rearrangement
Primary cutaneous follicle centre cell lymphoma
Mantle cell lymphoma
In situ mantle cell neoplasia
Diffuse large B cell lymphoma (DLBCL)
Germinal centre B-cell type
Activated B-cell type
T cell/histiocyte rich, DLBCL
Primary DLBCL of CNS
Primary cutaneous DLBCL leg type
EBV+ DLBCL
EBV + mucocutaneous ulcer
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Primary Mediastinal (thymic) B cell lymphoma
Intravascular large B cell lymphoma
ALK positive large B cell lymphoma
Plasmablastic lymphoma
Primary effusion lymphoma
HHV8+ DLBCL
Burkitt lymphoma
Burkitt-like lymphoma with 11q aberration
High grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements
High grade B-cell lymphoma, nos
B-cell lymphoma, unclassifiable with features intermediate between DLBCL and Classic Hodgkin lymphoma
T cell neoplasms
Precursor T cell lymphomas
Precursor T lymphoblastic lymphoma/leukaemia
Blastic NK cell lymphoma

Table 35.1 (continued)

B cell neoplasms
Mature T cell and NK cell neoplasms
T cell prolymphocytic leukaemia
T-cell large granular cell lymphocyte leukaemia
Aggressive NK cell leukaemia
Systemic EBV + T-cell lymphoma of childhood
Hydroa vacciniforme-like lymphoproliferative disorder
Adult T cell lymphoma/leukaemia
Extranodal NK/T cell lymphoma, nasal type
Enteropathy associated T cell lymphoma
Monomorphic epitheliotropic intestinal T-cell lymphoma
Indolent T-cell lymphoproliferative disorders of the GI tract
Hepatosplenic T cell lymphoma
Subcutaneous panniculitis like T cell lymphoma
Mycosis fungoides (MF) and Sézary syndrome
Variants:
Pagetoid reticulosis
MF associated follicular mucinous
Granulomatous slack skin disease
Sezary syndrome
Primary cutaneous CD30+ T-cell lymphoid proliferative disorders
Lymphomatoid papulosis
Primary cutaneous anaplastic large cell lymphoma (C-ALCL)
Primary cutaneous γδ T cell lymphoma
Primary cutaneous $CD8^+$ aggressive epidermotropic cytotoxic T-cell lymphoma
Primary cutaneous acral $CD8^+$ T-cell lymphoma
Primary cutaneous CD4+ small/medium T-cell lymphoproliferative disorder
Peripheral T cell lymphoma, unspecified
Angioimmunoblastic T cell lymphoma
Anaplastic large cell lymphoma, ALK positive,
Anaplastic large cell lymphoma, ALK negative
Breast implant associated anaplastic large cell lymphoma
Hodgkin lymphoma
Nodular lymphocyte predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
Nodular sclerosis classical Hodgkin lymphoma
Lymphocyte rich classical Hodgkin lymphoma
Mixed cellularity classical Hodgkin lymphoma
Lymphocyte-depleted classical Hodgkin lymphoma
Post transplant lymphoproliferative disorders (PTLD)
Plasmacytic hyperplasia PTLD
Infectious mononucleosis like PTLD
Florid follicular hyperplasia PTLD
Polymorphic PTLD
Monomorphic PTLD (B, T/ NK cell types)
Classical Hodgkin lymphoma PTLD

Table 35.1 (continued)

B cell neoplasms
Histiocytic and dendritic cell neoplasms
Histiocytic sarcoma
Langerhans cell histiocytosis
Langehans cell sarcoma
Indeterminate dendritic cell tumour
Interdigitating dendritic cell sarcoma
Follicular dendritic cell sarcoma
Fibroblastic reticular cell tumour
Disseminated juvenile xanthogranuloma
Erdheim Chester disease

[a]Average over 10 high power fields

Hodgkin's Malignant Lymphoma

WHO Classification

Comprising *nodular lymphocyte predominant Hodgkin lymphoma* (NLPHL—a B cell lymphoma), and *classic Hodgkin lymphomas* encompassing nodular sclerosis, lymphocyte rich, mixed cellularity and lymphocyte depleted variants. Hodgkin lymphoma is *a tumour of abnormal B-cell lineage*.

Lymphocyte and Histiocyte (L and H) Predominant: Multilobated "Popcorn" Cell

- Nodular:a B cell lymphoma of early stage (cervical, axilla, groin), predominantly in young men and low-grade indolent behaviour (80% 10 year survival) with a 4% risk of diffuse large B cell change. Some association with progressive transformation of germinal centres.
- Diffuse: a controversial category with overlap between lymphocyte rich classic Hodgkin lymphoma, vaguely nodular lymphocyte predominant Hodgkin, and exclusion of other entities such as T cell/histiocyte rich large B cell NHL.

Overlap of nodular and diffuse patterns exists; diffuseness is a poor prognostic feature.

Classic Hodgkin lymphoma includes nodular sclerosis, lymphocyte rich, mixed cellularity and lymphocyte depleted categories. They vary in their clinical features, growth pattern, degree of

fibrosis, background cells, tumour cell numbers and atypia, and, frequency of EBV infection.

Nodular Sclerosis: Lacunar Cell

- Female adolescents, young adult preponderance. Mediastinal or cervical involvement and either localised disease or high stage at presentation. Moderately aggressive but curable.
- Birefringent fibrous bands (capsular and intranodal septa) with mixed inflammatory cell nodules containing lacunar cells, or, cellular phase (rich in lacunar cells, scant fibrosis)
- ∗Type 1.
- ∗Type 2: lymphocyte depletion or pleomorphism of R-S (Reed Sternberg) cells in more than 25% of nodules. An alternative descriptor is syncytial variant (sheets/clusters of R-S cells with central necrosis and a polymorph infiltrate).
  - ∗Grade 1/grade 2 British National Lymphoma Investigation (BNLI). These types and grades are not prognostically relevant or independent of stage.

Mixed Cellularity: Reed Sternberg Cell

- Male adults, high stage disease at presentation: lymph nodes, spleen, liver ± bone marrow. Moderately aggressive but curable.
- R-S cells of classic type in a mixed inflammatory background. A category of exclusion in that no specific features of other subtypes are present.

Lymphocyte Rich Classic

- Scattered R-S cells against a nodular or diffuse background of small lymphocytes but no polymorphs. Reactive lymphoid follicles are present.

Lymphocyte Depleted

- Older patients, high stage disease at presentation, aggressive, association with HIV.
- R-S cells ± pleomorphism; diffuse fibrosis (fibroblasts obscure scattered R-S cells) and reticular variants (cellular, pleomorphic R-S cells).

Other Features

- Follicular and interfollicular Hodgkin cell with a high epithelioid cell content (granulomas).
- R-S cells: classic mirror image, binucleated cell with prominent, inclusion -like eosinophilic nucleolus ("owl's eye" appearance) characteristic of the mixed cellularity and lymphocyte depleted categories. Mononuclear, polylobated and necrobiotic (mummified) forms are also common. Lacunar cells (nodular sclerosis) can be mono-, bi- or polylobated (± necrobiotic), with characteristic perinuclear artifactual cytoplasmic retraction and clarity. Mononuclear cells tend to be termed Hodgkin cells. Hodgkin/R-S cells are derived from germinal centre B cells with monoclonal, nonfunctional immunoglobulin gene rearrangements.

Immunophenotype

Nodular lymphocyte predominant Hodgkin lymphoma

- Popcorn cells: CD45/CD20/CD79a/EMA/J chain/bcl-6 positive, CD30 weak or negative, CD15 negative, EBV negative. Nuclear transcription factors Oct2/BoB1 positive.
- Small lymphocytes: nodules of B cells (CD20) and intervening T cells (CD3).
- Rosettes: CD57/CD4 positive T cell rosettes around the popcorn cells.

Classic Hodgkin lymphoma

- R-S cells: CD15/CD30 positive in 75%/90% of cases respectively, EBV (variable-10–70% 60–70% of cases), CD20/79a ±, CD45/ALK negative. MUM1/PAX5 positive. CD15 positivity can be weak and focal, limited to the Golgi apparatus in 15% of cases.
- Small lymphocytes: T cells (CD3/CD4).

In Hodgkin lymphoma the *heterogeneous cellular background (comprising 90% of the tissue) is an important part of the diagnosis:* small lymphocytes, eosinophils, neutrophils, fibroblasts, histiocytes and follicular dendritic cells. Note

that this cytokinetic diathesis is also seen in T cell NHLs and T cell rich B cell NHLs. Another differential diagnosis with which there can be overlap is anaplastic large cell lymphoma. Other features are progressive transformation of germinal centres (particularly associated with NLPHL), granulomas, necrosis, interfollicular plasma cells and reactive follicular hyperplasia, all of which should prompt a careful search for R-S cells.

Differential Diagnoses in Haematolymphoid Pathology

Relatively common diagnostic difficulties in lymph node assessment are:

- Follicular hyperplasia vs. follicular lymphoma
- Follicular hyperplasia vs. partial nodal involvement by in situ follicular lymphoma
- Progressive transformation of germinal centres vs. NLPHL
- T cell hyperplasia vs. dermatopathic lymphadenopathy vs. T cell lymphoma
- Histological underestimation of aggressiveness in small lymphoid cell infiltrates, e.g. mantle cell or lymphoblastic lymphomas
- Burkitt lymphoma vs. Burkitt-like diffuse large B cell lymphoma
- Hodgkin lymphoma vs. anaplastic large cell lymphoma, and subtle infiltration of sinusoids by the latter
- Interfollicular Hodgkin lymphoma
- Anaplastic large cell lymphoma vs. metastatic carcinoma, malignant melanoma or germ cell tumour
- Post immunosuppression lymphoproliferative disorders.

Important *non-malignant differential diagnoses* for malignant lymphoma are Castleman's disease (hyaline vascular and plasma cell variants), drug induced (e.g. phenytoin) and viral reactive hyperplasia with paracortical transformation (e.g. herpesvirus, infectious mononucleosis), and necrotising and granulomatous lymphadenitis (Kikuchi's, toxoplasmosis, tuberculosis, sarcoidosis). A clinical history of immunosuppression in EBV driven lymphoid proliferations must always be borne in mind.

Extent of Local Tumour Spread

Part of node or whole node.

Extracapsular into adjacent soft tissues or organ parenchyma.

A TNM classification is not used as the primary site of origin is often uncertain and attribution of N and M stages would therefore be arbitrary.

Staging often requires extensive imaging including CT/PET scan as well as bone marrow examination.

Stage: Modified Ann Arbor system
I Single lymph node region or localised extralymphatic site/organ
II Two or more lymph node regions on same side of the diaphragm or single localised extralymphatic site/organ and its regional lymph nodes ± other lymph node regions on the same side of the diaphragm
III Lymph node regions on both sides of the diaphragm ± a localised extralymphatic site/organ or spleen
IV Disseminated (multifocal) involvement of one or more extralymphatic organs ± regional lymph node involvement, or single extralymphatic organ and non-regional nodes. Includes any involvement of liver, bone marrow, lungs or cerebrospinal fluid
A without weight loss/fever/sweats
B with weight loss/fever/sweats:
Fever >38 °C
Night sweats
Weight loss >10% of body weight within the previous 6 months.
Subscripts e.g. III_E denotes stage III with Extranodal disease
III_S denotes stage III with splenic involvement
III_3 denotes stage III with involvement of 3 lymph node regions: > 2 is prognostically adverse.
Lymph node regions: head, neck, face
Intrathoracic
Intraabdominal
Axilla/arm
Groin/leg
Pelvis

Other major structures of the lymphatic system are the spleen, thymus, Waldeyer's ring (palatine, lingual and pharyngeal tonsils), vermiform appendix and ileal Peyer's patches. Bone marrow, skin, liver, lung, pleura and gonads are sites with relatively low volumes of lymphoid tissue.

Bilateral involvement of axilla/arm or inguinal/leg regions is considered as involvement of two separate regions.

Direct spread of lymphoma into adjacent tissues or organs does not alter the classification, e.g. gastric lymphoma into pancreas and with involved perigastric lymph nodes is stage II_E.

Involvement of two or more discontinuous segments of gastrointestinal tract is multifocal and classified as stage IV, e.g. stomach and ileum. However multifocal involvement of a single extralymphatic organ is I_E.

Involvement of both organs of a paired site, e.g. lungs is also I_E. Regional nodes for an extranodal lymphoma are those relevant to that particular site, e.g. gastric lymphoma—perigastric, left gastric, common hepatic, splenic and coeliac nodes (Figs. 35.1, 35.2, 35.3, 35.4, and 35.5).

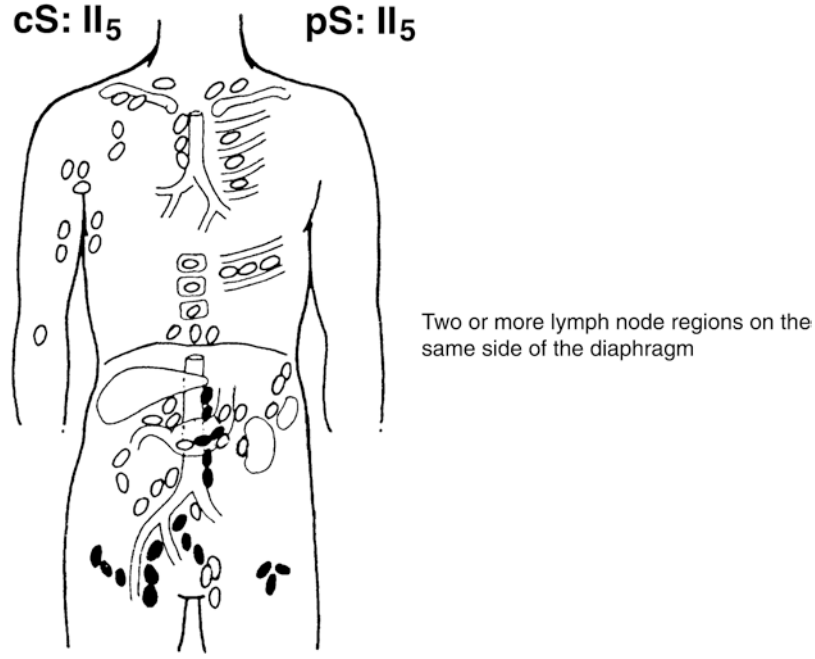

Fig. 35.1 Malignant lymphoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

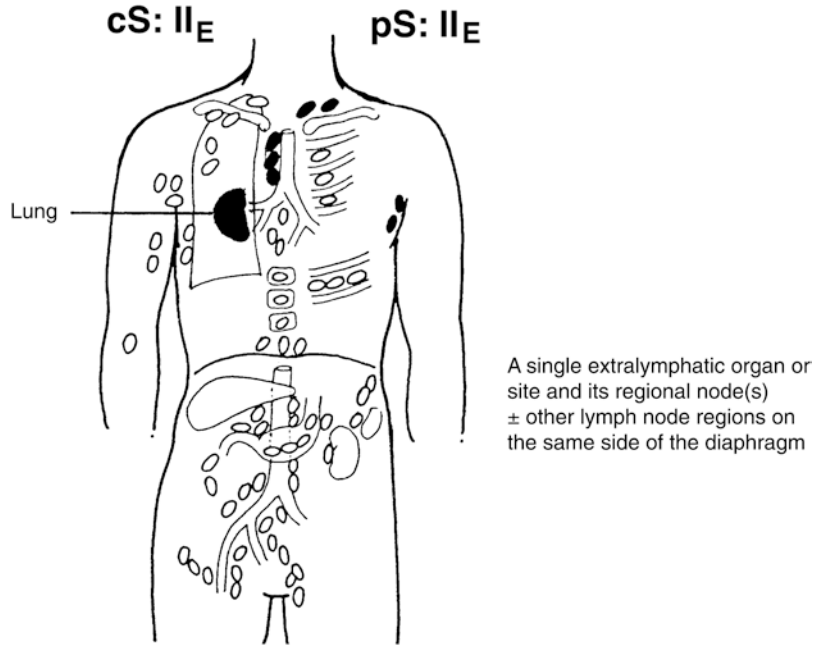

Fig. 35.2 Malignant lymphoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

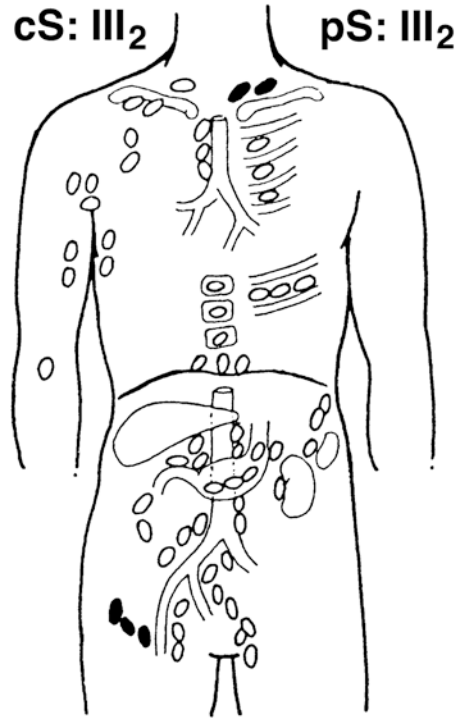

Fig. 35.3 Malignant lymphoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

Involvement of lymph node regions on both sides of the diaphragm (III) (Fig. 35.3), which may also be accompanied by localized involvement of an associated extralymphatic organ or site (III_E) (Fig. 35.4), or by involvement of the spleen (III_S), or both (III_{E+S}) (Fig. 35.5)

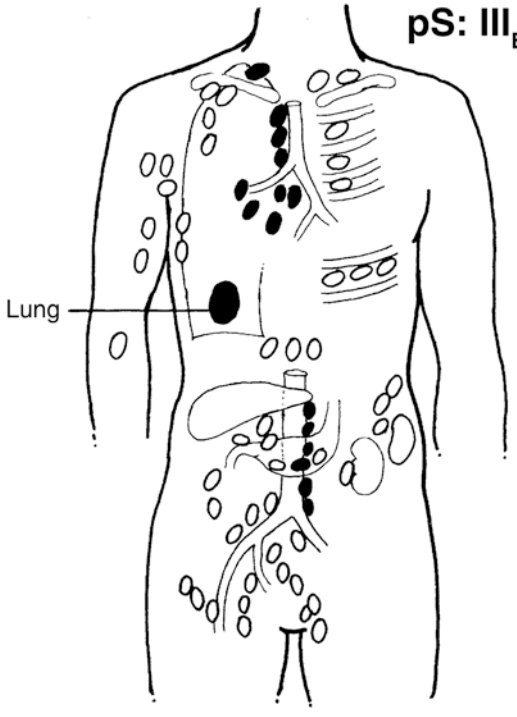

Fig. 35.4 Malignant lymphoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

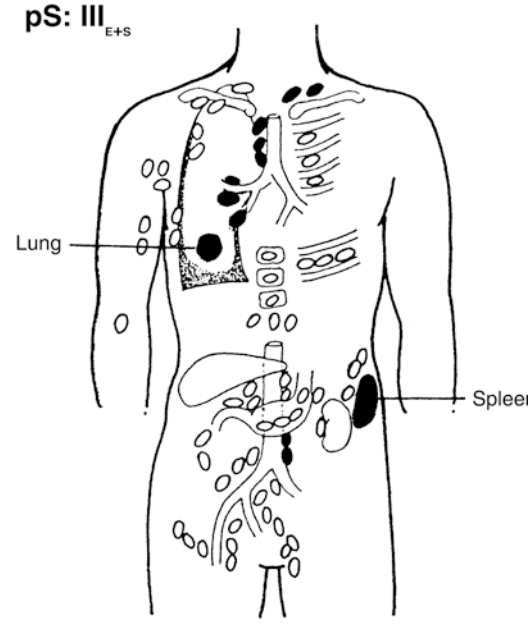

Fig. 35.5 Malignant lymphoma. Reproduced, with permission, from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed.*, © 2005, Springer-Verlag

Staging laparotomy has been replaced by assessment of *clinical* and *radiological parameters*, e.g. peripheral blood differential cell counts, clinical chemistry including LDH, and imaging for hepatosplenomegaly, involved lymph node sites and other organ involvement. Bone marrow biopsy remains part of normal staging. Bone marrow involvement in Hodgkin lymphoma is characterized by the presence of Hodgkin cells in a typical fibroinflammatory stroma; nodular lymphoid aggregates or granulomas per se are not sufficient for a positive diagnosis of Hodgkin lymphoma. Bone marrow involvement by NHL can be diffuse, nodular or focal, and paratrabecular infiltration is a characteristic site of distribution.

Lymphovascular Invasion

Vessel wall invasion and destructive angiocentricity can be a useful indicator of malignancy in NHL and in specific subtypes, e.g. nasal angiocentric T/NK cell lymphoma.

Immunophenotype

In general an *antibody panel* is used with both expected positive and negative antibodies, and, *two antibodies per lineage*. *Select combinations* are targeted at determining the nature of the various lymphoid proliferations, e.g. *follicular* (follicular lymphoma vs. reactive hyperplasia), *small cell* (CLL/SLL vs. mantle cell lymphoma), and *large cell* (HL vs. NHL vs. ALCL). Interpretation must take into account artifacts such as *poor fixation* or lymph node *necrosis* following FNAC. In the latter, expression of nuclear antigens is affected first and T cell stains can give false positive results in inflammatory debris. Some antigens such as CD20 retain remarkably robust expression. *Expression must also be appropriate* to the antibody concerned, e.g. localization to the nucleus, cytoplasm or cell membrane. Suitable in built positive, and, external positive and negative *controls* are used. Interpretation must also account for expression in physiological cell populations in the test tissue. High quality thin sections are required to aid visualisation and interpretation of applied antibodies. Commonly used antibodies available for formalin fixed, paraffin embedded sections are:

CD45	Pan lymphoid marker (Leucocyte Common Antigen) and excellent in the characterisation of a poorly differentiated malignant tumour, e.g. malignant lymphoma vs. carcinoma vs. malignant melanoma.
CD20	Mature B cell marker (and some cases of plasma cell myeloma).Lymphoma cell positivity is a marker for specific anti-CD20 immunotherapy.
CD79a	As for CD20 but also less mature (pre B: lymphoblastic) cells and plasma cell tumours.
CD3	Pan T cell marker.
CD5	T cell marker and aberrant consistent expression in some B cell lymphoma (small lymphocytic, mantle cell lymphomas).
CD4, CD8:	T cell subsets of use in some T cell proliferations e.g. mycosis fungoides. Other T cell markers include CD45R0 (UCHL1), CD43 (MT1), CD2, CD7, CD28, CD57 and TIA-1.
CD10	Lymphoblastic lymphoma (CALLA), Burkitt lymphoma and follicular lymphoma. Also with CD5 and CD23 in the diagnosis of small B cell lymphomas (lymphocytic CD5/CD23+: mantle cell CD5+: follicle centre cell CD10+). 40-50% of DLBCL are CD10 positive and referred to as germinal centre phenotype.
CD15, CD30	Classic Hodgkin lymphoma R-S cells. CD30 is also positive in ALCL, a proportion of DLBCLs, embryonal carcinoma and malignant melanoma. CD15 is positive in some large T cell lymphomas.
CD56, CD57	Natural killer (NK) cells e.g. angiocentric sinonasal lymphoma. Also T cells and plasma cell myeloma/AML (CD56).
CD68	Macrophages, cells of granulocytic lineage.

CD 38/138	Plasma cells and multiple myeloma (usually CD45/20 negative; CD79a±). Other low grade B cell lymphomas (CD38+ CLL has a worse prognosis).
CD123	Hairy cell leukaemia, AML.
κ, λ light chains	Immunoglobulin light chain restriction is difficult to demonstrate satisfactorily in paraffin sections but more easily shown on fresh imprint preparations or by in situ hybridisation.
CD21, CD23	Follicular dendritic cells. CD23 also stains most B cell lymphocytic lymphomas, some follicular and DLBCLs.
bcl-1/cyclin D1	Mantle cell lymphoma with the t(11:14) translocation. A nuclear epitope. Also variably positive in hairy cell leukaemia and plasma cell myeloma cases. $p27^{kip1}$ is positive in cyclin D1 negative mantle cell lymphoma.
bcl-2	An apoptosis regulator: positive in many B cell lymphomas, strongly expressed in 80–85% follicular lymphoma. Also normal B, T cells and negative in reactive germinal centres. Strong expression is adverse in DLBCLs.
bcl-6	Follicular lymphoma, Burkitt lymphoma and a large proportion of DLBCLs. A transcription factor in the nuclei of germinal centre cells.
Ki-67/MIB-1	Nuclear proliferation marker useful for identifying high-grade lymphomas e.g. Burkitt (98–100%), lymphoblastic lymphoma (also TdT positive), and DLBCL with a high proliferation fraction. Its distribution in the germinal centre is helpful in distinguishing reactive lymphoid hyperplasia (shows polarity with staining in the dark zone) from follicular lymphoma (lower level of staining than reactive hyperplasia and lacks polarity).
EBV	LMP-1 (latent membrane protein) antibody is positive in a proportion of R-S cells in HL. In situ hybridisation is more sensitive with a higher rate (60–70%) of positivity.
TdT	Terminal deoxynucleotidyltransferase is positive in precursor B and T cell lymphoblastic lymphomas. A nuclear epitope.
CD1a	Langerhans cell histiocytosis, cortical thymic T cells.
ALK-1/ (CD246)	Nucleophosmin-anaplastic lymphoma kinase fusion protein associated with the ALK-1 gene t(2:5) translocation and good prognosis ALCLs.
C-MYC	Identifies an aggressive subset of DLBCLs that are less responsive to usual chemotherapy agents.
MUM1	A nuclear proliferation/differentiation epitope positive in post germinal centre B cells. It is of prognostic value in DLBCLs and stains classic R-S cells.
PAX5	A nuclear B-cell transcription factor expressed in B lymphoctyes and weakly in R-S cells; absent in plasma cells.
Oct2/BoB1	B cell transcription factors positive in the nuclei of NLPHL L and H popcorn cells. Also in DLBCL.
EMA	Plasma cells, ALCL, NLPHL L and H cells.
Lysozyme	(muramidase/myeloperoxidase)—granulocytic and myeloid cell lineages. AML: also CD34, CD43, CD68, CD117, neutrophil elastase and chloroacetate esterase.
p53	A prognostic marker in various lymphoid neoplasms and MDS/AML.
p21	A prognostic marker in multiple myeloma.
PD1, CXCL13, ICOS & bcl6	Valuable in identification of follicular T-cell helper cells
S100	Interdigitating reticulum cells, Langerhans cells.
Factor VIII/ CD61	Markers of megakaryocytes.

Molecular Techniques

Immunoglobulin receptor (heavy and light chain restriction) and *T cell receptor (TCR) gene rearrangements* using polymerase chain reaction are of use in difficult diagnostic cases, e.g. follicular hyperplasia vs. follicular lymphoma, or T zone reactive hyperplasia vs. malignant lymphoma. Also, where immunohistochemistry has been equivocal (e.g. dubious cyclin D1 staining in mantle cell lymphoma) chromosomal studies for specific translocations have a role to play. These techniques vary in their applicability to fresh tissue and routine paraffin sections and suitable arrangements for prompt tissue transportation and *referral on a regional network basis* should

be put in place. A majority of malignant lymphomas can be provisionally diagnosed without these techniques but their role is rapidly evolving in importance with respect to diagnostic confirmation, prognostication and therapy.

The evolution of new generation robust antibodies applicable to paraffin sections with unmasking of antigenic sites by antigen retrieval methods and more sensitive visualization techniques has led to considerable reclassification of malignant lymphomas and emergence of new entities. The full spectrum of NHL widens viz. T cell NHL, anaplastic large cell NHL, T cell rich B cell lymphoma (<10% CD20 positive large cells on a background of CD3 small lymphocytes). Unusually composite (HL/NHL) and gray zone (HL/DLBCL and HL/ALCL) cases also occur. It is important that a panel of antibodies is used and markers assessed in combination. The WHO 2016 classification includes several established lymphomas (e.g. lymphoplasmacytic lymphoma) and provisional entities such as Burkitt-like lymphoma with 11q aberration in which the molecular abnormality is a disease defining characteristic feature. There is an explicit recognition of the need to incorporate genetic findings in diagnosis and management of diffuse large B-cell lymphoma. Thus *morphology and immunohistochemistry are routinely supplemented by molecular studies such as FISH, clonality assessment and mutation tests.* Gene expression profiling and next generation sequencing (NGS) are currently restricted to large tertiary referral centres. Molecular test results need careful correlation with morphology and immunophenotype. Clonality does not always correlate with malignant lymphoma; it has been demonstrated in some inflammatory skin (e.g. eczema vs. mycosis fungoides), salivary and gastric biopsies. Pathogenic mutations and chromosomal abnormalities are sometimes found in normal people and in patients lacking firm clinical evidence of disease.

Formalin fixation and high quality, thin (2–4 μm) paraffin sections are adequate for morphological characterisation in most cases. Fixation should be sufficient (24–36 h) but not excessive as this may mask antigenic sites.

The majority (60–70%) of NHLs are diffuse large B cell lymphomas and follicular lymphoma.

Characteristic Lymphomas

Precursor B Cell Lymphoblastic Lymphoma/Leukaemia

- Presents as childhood leukaemia or occasionally solid tumour (skin, bone, lymph node) and relapses in the central nervous system or testis.
- 75% survival in childhood but <50% in adults. Aggressive but potentially curable by multiagent chemotherapy.
- Medium sized round, mitotically active lymphoid cells with small nucleolus.
- CD79a, CD10, TdT, CD99±, Ki-67 > 95%, CD20±.

Small Lymphocytic Lymphoma/ Chronic Lymphocytic Leukaemia (CLL)

- *5–10% of lymphomas* occurring in older adults with diffuse lymph node, bone marrow and blood involvement, and hepatosplenomegaly.
- Indolent and incurable with 5–10 year survival even without treatment but ultimately fatal.
- Small lymphocytes with pale proliferation centres (para-immunoblasts/prolymphocytes)
- CD45, CD20, CD5, CD23, LEF1, bcl-2, Ki-67 < 20%. Cyclin D1 and CD10 negative.
- Richter's transformation to large cell lymphoma in 3–5% of cases. Worse prognosis cases are CD38 positive.
- occasional cases have Hodgkin like cells (CD30/15+) and < 1% develop classic HL.

Lymphoplasmacytic Lymphoma

- *1–2% of cases* and in the elderly involving bone marrow, nodes and spleen. Indolent course with a median survival of 5–10 years.

- Monoclonal IgM serum paraprotein with hyperviscosity symptoms and autoimmune/cryoglobulinemia phenomena.
- Small lymphocytes, plasmacytoid cells and plasma cells.
- Intranuclear Dutcher and cytoplasmic Russell bodies.
- CD45, CD20, VS38 positive and CD5/CD10/CD23 negative.
- MYD88 L265P mutation positive by PCR in more than 90% cases.

Marginal Zone Lymphoma of MALT (Mucosa Associated Lymphoid Tissue)

- *8% of NHLs*, stomach 50% of cases, also salivary gland, lung, thyroid, orbit and skin. Multiple extranodal sites in 25–45% of cases. 80% are stage I or II disease and indolent. Many are cured by local excision, or antibiotic therapy in gastric MALToma.
- Usually extranodal associated with chronic autoimmune or antigenic stimulation.
- Centrocyte like cells, lymphocytes, plasma cells (scattered immunoblast and centroblast like cells).
- Destructive lymphoepithelial lesions, reactive germinal centres and follicular colonisation by the lymphoma cells.
- CD45, CD20 positive but CD5/10/23 negative.
- Lymph node variant is monocytoid B cell lymphoma: indolent (60–80% 5 year survival) but has potential for large cell transformation.
- Gastric MALT with t(11:18) confers resistance to anti-*Helicobacter* treatment.
- Splenic marginal zone lymphoma: splenomegaly, lymphocytosis, stage III/IV disease.

Hairy Cell Leukaemia

- Rare, elderly in the bone marrow, spleen and lymph nodes. Typically marked splenomegaly with pancytopaenia. 10 year survival >90%.
- "fried egg" perinuclear cytoplasmic clarity with prominent cell boundaries.
- CD45, CD20, CD72(DBA44), CD123, tartrate resistant acid phosphatase (TRAP) positive, cyclin D1 ± and BRAF.
- BRAFV600E mutation positive.

Mantle Cell Lymphoma

- *6% of NHLs* predominantly in older adult males (75%).
- Extensive disease including spleen, bone marrow, Waldeyer's ring ± bowel involvement (multiple lymphomatous polyposis).
- Monomorphic small to medium sized irregular nuclei (centrocytic). Rare blastoid and pleomorphic variants.
- Diffuse with vague architectural nodularity.
- CD45, CD20, and typically CD5, cyclin D1, SOX11 and CD43 positive (t11;14 positive).
- CD10, CD23, bcl-6 negative. Cyclin D1 negative (also t11;14 negative) cases can be $p27^{kip1}$ positive; these are usually CD5 and SOX11 positive.
- Aggressive with mean survival of 3–5 years. A high Ki-67 index (>30%) is prognostically adverse.
- An indolent variant of mantle cell lymphoma is recognized; these patients have peripheral blood and bone marrow or spleen involvement with no evidence of lymphadenopathy. Some patients have overlapping features with conventional mantle cell lymphoma.

Follicular Lymphoma

- *30% of adult NHLs* and transformation to DLBCL is relatively common.
- Patterns: follicular, follicular and diffuse, diffuse (see Table 35.1)
- Cell types: *centroblasts* with large open nuclei, multiple small peripheral basophilic nucleoli, variable cytoplasm; *Centrocytes* with medium sized irregular nuclei.
- Grade: 1/2/3 according to the number of centroblasts per high power field (see Table 35.1). Grade 3 has a high Ki-67 and 50% may be bcl-2 negative—it is high-grade requiring

immunochemotherapy and is to be distinguished from low-grade (grade 1/2) disease.
- CD45, CD20, CD10 (60%), bcl-2 (t14:18; 70–95%), bcl-6.
- Usually CD21/23 positive and CD5 negative (20% positive).
- High stage disease at presentation (splenomegaly and bone marrow involvement in 40% of cases), and indolent time course, but late relapse (5–10 years) with large cell transformation in 25–35% of cases to DLBCL.
- Pattern and grade can vary within a lymph node necessitating adequate sampling.

Diffuse Large B Cell Lymphoma (DLBCL)

- *30% of adult NHLs*, *40% are extranodal* (especially stomach, skin, central nervous system, bone, testis, etc.). Forms a rapidly growing mass in older patients which usually arises de-novo, or, occasionally from low-grade B cell NHL. Aggressive but potentially curable—immunochemotherapy has improved outlook considerably.
- Centroblasts, immunoblasts (prominent central nucleolus), bi−/polylobated, cleaved, anaplastic large cell (ALK+), plasmablastic (HIV+) forms, basophilic cytoplasm.
- Aggressive variants: T cell/histocyte rich, mediastinal/thymic, intravascular, primary effusion (chronic inflammation associated), primary central nervous system.
- CD45, CD20, CD79a, Ki-67 40–90%, CD10 (30–60%), bcl-2 (30%), bcl-6 (60–90%), CD5/23/CD30/CD43 ±. MUM1(35–65%).
- Strong bcl-2 expression is adverse.
- Gene expression profiling (GEP) identifies two types: germinal centre (52%); non germinal centre (activated B-cell phenotype and unclassifiable 48%). Immunohistochemistry is a surrogate and demonstrates up to 85% concordance with the classification based on GEP. Approximately 10% DLBCL demonstrate C-MYC gene rearrangement and poorer prognosis.
- *Hans algorithm*: DLBCLs of germinal centre origin (CD10+, or, CD10−/bcl-6 +/MUM1-) are of better prognosis (76% 5 year survival) than those of non-germinal centre origin (CD10−/bcl-6- or, CD10−/bcl-6+/MUM1+: 34% 5 year survival). "Germinal centre markers" include CD10, bcl-6, CD21 and CD23 while MUM-1 (Interferon Regulating Factor 4: IRF4) is expressed by post germinal centre destined B cells.
- Strong and diffuse MYC protein overexpression correlates with MYC gene translocation.
- Primary testicular and CNS DLBCL are usually of non germinal centre phenotype and often demonstrate MYD88 L265P mutations.

Burkitt Lymphoma

- *1–2.5% of NHLs.*
- Childhood or young adult: endemic/sporadic/HIV related (EBV: 95%/15–20%/30–40% of cases respectively).
- Jaw and orbit (early childhood/endemic), or abdomen (ileocaecal/late childhood or ovaries/young adult/sporadic) and breasts with risk of central nervous system involvement.
- Monomorphic, medium-sized lymphoid cells, multiple small central nucleoli, basophilic cytoplasm.
- Mitoses, apoptosis, "starry-sky" pattern.
- CD79a, CD20, CD10, bcl-6, Ki-67 98–100%, and bcl-2/TdT/CD5/23 negative.
- t(8:14) and t(2:8)/t (8:22) variants by FISH in paraffin sections or karyotyping in fresh tissue.
- Requires aggressive polychemotherapy and is potentially curable: 90% in low stage disease, 60–80% with advanced disease, children better than adults.
- Burkitt like morphology and the C-MYC rearrangement are not specific to this lymphoma. Aggressive B-cell lymphoma with MYC rearrangement, transformed follicular lymphoma with MYC and bcl2 rearrangments (so called 'double hit' lymphoma, 'triple hit' if MYC, bcl6, and bcl2 rearrangements identified) and Burkitt-like lymphoma with 11q aberration require FISH and/or additional molecular testing for diagnosis.

Precursor T Lymphoblastic Lymphoma/Leukaemia

- Expresses CD3, Ki-67 > 95%, TdT.
- Presents in childhood/adolescence as leukaemia or a mediastinal mass (also lymph nodes, skin, liver, spleen, central nervous system, gonads).
- Aggressive with 20–30% 5 year survival but potentially curable.

Angioimmunoblastic T Cell Lymphoma

- Neoplasm of follicular T-helper cells and constitutes up to 25% of all T-NHL in adults.
- Clinical features include fever, skin rash and generalized lymphadenopathy associated with laboratory abnormalities such as raised ESR, polyclonal hypergammaglobulinemia and anaemia.
- Tumour cells are CD3, CD5 and CD7 positive with variable expression of follicular T-helper cell antigens such as CD4, CD10, CXCL13, ICOS and PD1.
- Expanded extra-follicular dendritic meshwork, high endothelial venules and EBV positive B immunoblasts are other characteristic findings.
- Clonal TCR rearrangement is present in the majority of cases. Expansion of the EBV positive B-cell population with clonal immunoglobulin receptor rearrangements may be seen in a subset of cases and result in a B-cell lymphoma.
- Aggressive disease with median survival of 2 years.

Mycosis Fungoides

- Cutaneous patch, plaque and tumour stages with or without lymph node involvement.
- Indolent but stage related and can transform to high-grade NHL of large cell type.
- Several biopsies over time may be required before diagnosis is made
- Usually CD3, CD5, TCRαβ and CD4+/CD8−; loss of CD7 is common and large cell transformation is associated with CD30 positivity.

Sézary Syndrome

- Defined by the triad of erythroderma, lymphadenopathy and peripheral blood involvement by clonally related neoplastic T cells with cerebriform nuclei (Sézary cells). Histologic features can be similar to mycosis fungoides. The tumour cells are typically PD-1 positive.

Subcutaneous Panniculitis Like T Cell Lymphoma

- Nodules trunk/extremities, CD3, CD8, TCRαβ.
- 80% 5 year survival but sometimes haemophagocytic syndrome supervenes with poor prognosis.

Enteropathy Associated T Cell Lymphoma

- Type 1—pleomorphic, small to medium or large and anaplastic cells
- CD3 positive, associated gluten enteropathy or ulcerative jejunitis.
- Aggressive and in adults presents with abdominal pain, mass, ulceration or perforation, or a change in responsiveness to a gluten free diet.
- Type 2—monomorphic, CD8, CD56 and CD3 positive cells; not associated with celiac disease.

Hepatosplenic Gamma Delta T Cell Lymphoma

- Male adolescents and young adults (some following immunosuppressive treatment for inflammatory bowel disease, transplant, etc.).
- Aggressive and relapses despite treatment with survival <2 years.

- Liver, spleen, bone marrow and lymph node sinus involvement.
- CD3+, CD4/8−, γδ TCR, often CD56 positive.

Peripheral T Cell Lymphoma, Unspecified

- *10% of NHLs and 30% of T cell NHLs*. Diagnosed if cases are not classifiable as one of the previously described specific entities (i.e. Precursor T lymphoblastic lymphoma/leukaemia, Angioimmunoblastic T Cell Lymphoma, Mycosis Fungoides, Sézary syndrome, Subcutaneous Panniculitis Like T Cell Lymphoma, Enteropathy Associated T Cell Lymphoma, Hepatosplenic Gamma Delta T Cell Lymphoma).
- Adults. Generalised lymph node or extranodal disease at presentation with involvement of skin, subcutaneous tissue, viscera and spleen. Aggressive with relapses and median survival of 2–3 years.
- Interfollicular/paracortical or diffuse infiltrate.
- Variable nuclear morphology from medium sized cells with minimal atypia to large blast-like pleomorphic cells, "crows-feet" appearance with irregular nuclear contours.
- Cytoplasmic clearing.
- Accompanying eosinophils, histiocytes and vascularity with prominent post capillary venules.
- Lymphoepithelioid variant (Lennert lymphoma).
- Usually CD3, CD4, CD5 and TCR gene rearrangement positive, variable expression of CD7/CD8/CD15/CD30/CD56/TIA-1.
- *Worse prognosis than B cell lymphomas*.

Extranodal NK/T Cell Lymphoma, Nasal Type

- Nasal type is the prototypic presentation. Also seen in any organ system including skin and soft tissues. Aggressive with 30–40% survival.
- Polymorphic inflammatory infiltrate of granulocytes which may obscure the tumour cells.
- Variably sized minimally atypical to markedly pleomorphic or blastic lymphoid cells.
- Angiocentric and destructive.
- Usually CD2, CD56, CD16, CD3 (cytoplasmic) and EBER-ISH positive, TCR germline, STAT3 and STAT5 mutations in 40% cases. Cytoplasmic but not surface CD3. Also ± CD57, perforin, granzyme B, TIA-1.

Anaplastic Large Cell Lymphoma (ALCL)

- *2.5% of NHLs* in adults and *10–20% of childhood malignant lymphomas*.
- Elderly and young (25% < 20 years): ALK negative and ALK positive/male predominance.
- May also follow mycosis fungoides, lymphomatous papulosis or HL.
- Cohesive, sinusoidal growth pattern of "epithelioid" cells mimicking carcinoma, malignant melanoma and germ cell tumour.
- Large pleomorphic nuclei, multiple nucleoli, polylobated forms, "hallmark cells" with horseshoe or reniform nucleus; rarely small cell variant.
- CD30, and, EMA/CD45/CD3 ±, CD2+, CD4 more often positive than CD8.
- Mainly T (60–70% TCR), and null (20–30%) cell types. 90% have clonal TCR rearrangements.
- 12–50% of adult cases are t(2:5) and ALK-1 positivity confers a good prognosis (80% 5 year survival) despite presentation with stage III/IV disease. ALK-1 negative cases have 40% 5 year survival.
- Recently, breast implant associated ALCL has been described. This presents as a seroma a number of years following breast implant/reconstruction. The tumour cells are mostly confined to the seroma fluid and rarely penetrate the capsule, involve regional lymph nodes or spread to distant sites. Seroma cytology and imaging are critical for this diagnosis.

Granulocytic (Myeloid) Sarcoma

- Myelomonocytic markers are CD68, myeloperoxidase, chloroactetate esterase, neutrophil elastase, lysozmye, CD15, CD34, CD43, CD117.
- Megakaryocytic component: CD61, factor VIII.
- If a tumour looks like a malignant lymphoma but does not show appropriate immunohistochemical marking, think of granulocytic (myeloid) sarcoma.

Extranodal Lymphoma

Of *NHLs, 25–40% are extranodal*, defined as when a NHL presents with the main bulk of disease at an extranodal site usually necessitating the direction of treatment primarily to that site. In order of decreasing frequency sites of occurrence are

- Gastrointestinal tract (especially stomach then small intestine)
- Skin
- Waldeyer's ring
- Salivary gland
- Thymus
- Orbit
- Thyroid
- Lung
- Testis
- Breast
- Bone

A majority are *aggressive large B cell lymphomas* although *T cell lesions* also occur (cutaneous T cell lymphoma, enteropathy associated T cell lymphoma, subcutaneous panniculitis like T cell lymphoma, NK/T cell nasal lymphoma). Their incidence is rising partly due to increased recognition and abandonment of terms such as pseudolymphoma, but also because of aetiological factors, e.g. HIV, immunosuppression after transplantation or chemotherapy, autoimmune diseases (e.g. systemic lupus erythematosis), and chronic infections (*H. pylorii*, EBV, hepatitis C virus).

Many remain localised to the extranodal site. However a significant proportion present as or undergo high-grade transformation and when they metastasise typically do so to other extranodal sites. This site homing can be explained by the embryological development and circulation of mucosa associated lymphoid tissue (MALT). The *low-grade MALTomas* often arise from a background of chronic antigenic stimulation:

Gastric MALT lymphoma	*H. pylori* gastritis
Thyroid MALT lymphoma	Hashimoto's thyroiditis
Salivary gland MALT lymphoma	Lympho(myo-)epithelial sialadenitis/Sjögren's syndrome

They normally comprise a sheeted or nodular infiltrate of centrocyte like cells, destructive lymphoepithelial lesions and sometimes monotypic plasma cell immunoglobulin expression. Interfollicular infiltration or follicular colonisation of reactive follicles by the neoplastic cells is characteristic. There is often a component of blast cells and the *immunophenotype is one of exclusion* in that they are CD5 and cyclin-D1 negative ruling out mantle cell lymphoma and other small B lymphoproliferative disorders. Other extranodal lymphomas have diverse morphology and immunophenotype correlating with the full spectrum of the WHO classification, although the lymph node based categories are not consistently transferable to extranodal sites.

Immunosuppressed post transplant (solid organs or bone marrow) patients are prone to a wide spectrum of nodal/extranodal polyclonal and monoclonal B/T cell *lymphoproliferative disorders (PTLD: post-transplant lymphoproliferative disorder).* The latter is either non-destructive: plasmacytic hyperplasia/infectious mononucleosis like, or destructive: polymorphic B cell hyperplasia/polymorphic B cell lymphoma. Early PTLD is more likely to be EBV positive while late PTLD is usually EBV negative. Monomorphic PTLD resembles conventional B or T-cell lymphoma and Hodgkin lymphoma. In general monomorphic/monoclonal lesions require multi-modality therapy while polymorphic/polyclonal lesions may regress without therapy or following elimination of EBV positive B-cells. However, even apparent

high-grade lymphoma *may potentially regress if immunosuppressant therapy is decreased.* Whole blood and/or tissue expression of EBV are ascertained and clinical response to reduction of immunosuppressants is assessed prior to escalation of therapy. PTLD like findings can also be present in patients receiving chronic immunosuppression therapy for autoimmune diseases.

Prognosis

For some malignant lymphomas *watchful waiting* is the initial course of action and treatment is only instigated once the patient is symptomatic. Otherwise, immuno-*chemotherapy* and *radiotherapy, autologous or allogeneic bone marrow transplant* are the principal treatment modalities for malignant lymphoma. However, *surgical excision* is often involved for definitive subtyping in primary lymph node disease or for removal of a bleeding or obstructing tumour mass and primary diagnosis of extranodal malignant lymphoma, e.g. gastric lymphoma. *Prognosis* relates to *lymphoma type/grade* (small cell and nodular are better than large cell and diffuse), and *stage of disease*. *Low-grade or indolent nodal malignant lymphomas* have a high frequency (>80% at presentation) of bone marrow and peripheral blood involvement. They are incurable pursuing a protracted time course and multiple relapses with potential for blastic or high grade transformation (e.g. CLL: 23% risk at 8 years). *High-grade or aggressive lymphomas* develop bone marrow or peripheral involvement as an indication of advanced disease and the latter are known to have high fatality rates. Overall, four broad prognostic categories are identified in NHL, although outlook does vary within individual types e.g.:

NHL type	*5 year survival*
1. Anaplastic large cell/MALT/follicular	>80%
2. Nodal marginal zone/small lymphocytic/lymphoplasmacytoid	60–80%
3. Mediastinal B cell/large B cell/ Burkitt's	30–70%
4. T lymphoblastic/peripheral T cell/ mantle cell	<50%

Hodgkin lymphoma (HL) is relatively *radiotherapy and chemotherapy responsive. Prognosis* relates to *stage of disease* more than histological subtype. Average 5 year survival and cure rates for HL are 75% with worse outcome for older patients (>40–50 years), disease of advanced stage (i.e. more than one anatomical site), involvement of the mediastinum by a large mass (>1/3 of the widest thoracic diameter), spleen or extranodal sites.

There is evidence that early (confined to the mucosa), *gastric MALToma* is potentially reversible on removal of the ongoing antigenic stimulus, i.e. *antibiotic treatment of H. pylori.* However, lesions with deep submucosal or muscle invasion require *chemotherapy supplemented by surgery* if there are local mass effects, e.g. bleeding or pyloric outlet stenosis. High grade transformation of MALT lymphoma us usually treated by immunochemotherapy.

T cell lymphomas form a minority of NHL (10–15%) and tend to have a *worse prognosis* than B cell lesions. Their cytological features are not particularly reliable at defining disease entities or clinical course, which is more dependent on tumour site and clinical setting. *Involvement of extranodal sites* and *relapse* is not infrequent with typically an *aggressive disease course,* e.g. enteropathy associated T cell lymphoma and extra nodal T/NK lymphoma. Cutaneous ALCL has a favourable prognosis while that of systemic ALCL with skin involvement is poor: 50% present with stage III/IV disease and there is a 65–85% 5 year survival rate but relapse is high (30–60%).

Similarly some B cell lymphomas have site specific characteristics and clinical features, e.g. *mantle cell lymphoma* in the gut (lymphomatous polyposis) or *mediastinal diffuse large B cell lymphoma* – young females with a rapidly enlarging mediastinal mass associated with superior vena cava syndrome. A large (>10 cm) mass and extramediastinal spread indicate poor prognosis. *Generally adverse prognostic factors in NHLs are:*

- Age > 60 years.
- Male gender.
- Systemic symptoms (fever >38 °C, weight loss >10%, night sweats).

- Poor performance status.
- Anaemia
- Elevated serum LDH.
- Tumour bulk:
  - 5–10 cm (stage I/II); >10 cm (stage III/IV)
  - Large mediastinal mass
  - Palpable abdominal mass
  - Combined paraortic and pelvic nodal disease.

Combinations of these parameters can be scored clinically using the international prognostic index (IPI) for various NHLs (e.g. FLIPI for follicular lymphoma) for risk stratification and 5 year survival figures.

Other Malignancy

Carcinoma, germ cell tumours and *malignant melanoma* frequently *metastasise to lymph nodes* and are seen either in diagnostic biopsies (or FNAC) in patients with lymphadenopathy, or in regional lymph node resections in patients with known cancer. Spread of *malignant mesothelioma* or *sarcoma* to lymph nodes is *unusual* although it does occur, e.g. alveolar rhabdomyosarcoma, epithelioid sarcoma, synovial sarcoma. Assessment is by *routine morphology supplemented by ancillary techniques, e.g. immunohistochemistry* and *molecular methods,* although it should be noted that the significance of nodal micrometastases in a number of cancers is still not resolved. Metastases are initially in the subcapsular sinus network expanding to partial or complete nodal effacement with potential for extracapsular spread. *Anatomical site of involvement* can be a clue as to the *origin of the cancer,* e.g. neck (cancer of the upper aerodigestive tract, lung, breast, salivary glands or thyroid gland), supraclavicular fossa (lung, stomach, prostate, testis, ovary or breast cancer), axilla (breast, lung cancer or malignant melanoma), groin (cancer of the perineum or perianal area, cutaneous melanoma and rarely the pelvis) and retroperitoneum (germ cell tumour, genitourinary cancers). The metastatic deposit may be necrotic or cystic (e.g. squamous cell carcinoma of the head and neck, germ cell tumour in the retroperitoneum), resemble the primary lesion or be more or less well differentiated. Cell cohesion with nesting, necrosis, focal or sinusoidal distribution, solid lymphatic plugs of tumour and plentiful cytoplasm favour non-lymphomatous neoplasia although this is not always the case, e.g. ALCL or DLBCL. In this respect a *broad but basic panel of antibodies* is crucial for accurate designation (e.g. cytokeratins, CD45, CD30, OCT3/4, S100, melan-A, chromogranin) occasionally supplemented by histochemistry (e.g. PAS diastase resistant mucin positivity, an organoid pattern of reticulin fibres). Some metastases also induce characteristic inflammatory responses, e.g. squamous cell carcinoma of head and neck, large cell lung cancer and nasopharyngeal carcinoma (lymphocytes, leukaemoid reaction, eosinophils, granulomas) even mimicking HL. Some diagnostic clues are:

Malignant melanoma	Cell nests, eosinophilic nucleolus, spindle/epithelioid cells, melanin pigment, S100, HMB-45, melan-A, SOX10.
Germ cell tumour	Midline (mediastinum or retroperitoneum), elevated serum βHCG or AFP (± tissue expression), PLAP/CD117 (seminoma), cytokeratins/CD30 (embryonal carcinoma). Also SALL4 and OCT3/4 (except yolk sac tumour).
Lobular breast cancer	Sinusoidal infiltrate of sheeted, non-cohesive small cells, intracytoplasmic lumina, cytokeratins (CAM 5.2, AE1/AE3, CK7), GCDFP-15 and ER positive. Metastatic ductal cancer often has a nested pattern of larger cells with variable ER/HER2 positivity (Grade 1 or 2 tumours will have a tubular component).
Small cell carcinoma	Small (×2–3 the size of a lymphocyte), round to fusiform cells, granular chromatin, inconspicuous nucleolus, moulding, crush and DNA artifact, ± paranuclear dot CAM 5.2, and chromogranin/synaptophysin/CD56/TTF-1 (Merkel cell carcinoma is CK 20 positive). In addition to positivity with the above markers other metastatic neuroendocrine tumours (e.g. carcinoid, large cell neuroendocrine carcinoma) show stronger chromogranin and cytokeratin expression than small cell carcinoma.

Lung adenocarcinoma	Variably glandular or tubulopapillary, CK7/TTF-1/napsin-A/CEA/BerEP4/MOC31.
Thyroid carcinoma	Papillae, characteristic nuclei (overlapping, optically clear, grooves), psammoma bodies, CK7/TTF-1 and thyroglobulin/CK19.
Colorectal adenocarcinoma	Glandular with segmental and dirty necrosis, CK20/CEA/CDX-2/βcatenin.
Upper gastrointestinal and pancreaticobiliary adenocarcinoma	Tubuloacinar, tall columnar cells with clear cytoplasm, CK7/CEA/CA19-9/± CK20/CDX-2).
Ovarian carcinoma	Serous (tubulopapillary, psammoma bodies, CK7/CA125/WT-1/p16), or mucinous (glandular, CK7, ± CK20/CA125).
Uterine adenocarcinoma	Endometrioid (CK7/vimentin/ER) or serous (CK7/p53/Ki-67/HMGA2/PTEN/AMACR).
Prostate adenocarcinoma	Acinar or cribriform, PSA m/p, PSAP.
Bladder carcinoma	Nested (squamoid) or micropapillary, CK7/CK20/34βE12/CK5/6/uroplakin III.

m monoclonal, *p* polyclonal

The reader is referred to the Introduction for further discussion of the use of immunohistochemistry. Regardless, there are approximately 3–5% cancers of unknown primary origin wherein despite extensive imaging and pathological examination, a primary site is not found. These require expert review and discussion at specialised MDT.

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Part IX

Bone and Soft Tissue Cancer

- Bone and Soft Tissue Sarcomas (With Comments on Retroperitoneum)

36 Bone and Soft Tissue Sarcomas (With Comments on Retroperitoneum)

Oisin Houghton

Bone and soft tissue sarcomas are relatively uncommon and more appropriately managed by a regional multidisciplinary clinical team inclusive of specialist pathology review. Histological tumour type is a strong indicator of likely biological behaviour and prognosis, and, along with tumour grade, size, depth, stage and completeness of surgical excision helps determine selection of patients for neoadjuvant, adjuvant and targeted therapies.

Bone tumours often present as severe continuous pain unrelieved by anti-inflammatory agents, swelling, or sometimes as a pathological fracture following low impact trauma. Investigation is by plain x-ray, CT scan and MRI scan, looking particularly for tumour location, size, type of matrix, interface with adjacent bone and signs of periosteal reaction. A tissue diagnosis is obtained by needle core biopsy under radiological control. Osteosarcoma and Ewing's sarcoma are treated by a combination of chemotherapy and surgery, chondrosarcoma by surgery. Where feasible, surgery is limb salvage and curative in intent. Most primary bone tumours arise de-novo but a minority of bone sarcomas occur in association with genetic syndromes such as retinoblastoma, Li-Fraumeni and Rothmund-Thomson syndrome. Other precursors of primary bone sarcomas include Paget's disease, prior radiation therapy and pre-existing benign neoplasms. Metastatic bone disease due to secondary carcinoma can cause hypercalcaemia and is detected by isotope bone, CT or MRI scans. A past medical history of cancer is important in raising awareness of this possibility. Typical primary sites are breast, lung, thyroid, kidney and prostate.

Benign soft tissue tumours far outnumber malignant cases by a ratio of 50–100:1. Clinical ultrasound examination can rapidly triage benign from more suspicious lesions. Soft tissue sarcomas form 1–2% of cancers with an incidence of 25 per million in the UK, and, occur mainly in the extremities (often thigh) but also the retroperitoneum and trunk wall and various other locations. They are usually deep seated and progressively increase in size causing a lump or swelling, and sometimes pain with a loss of function in the limb or adjacent organs. Plain x-ray may show focal calcification (e.g. synovial sarcoma), but MRI is the investigation of choice in defining the nature of the mass, its extent and involvement of adjacent structures. CT scan is used for lesions of the chest, for detection of bone involvement, and as for bone sarcomas, for pretreatment staging of pulmonary metastases. After full clinical and radiological assessment most centres use needle core biopsy to obtain a tissue diagnosis and open biopsy only when needle core biopsy has proven inconclusive (in 5–10% of cases only). Fine

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_36

needle aspiration cytology (FNAC) has a limited role but can be used to confirm recurrence or metastasis in a patient with a previously diagnosed sarcoma. Surgery is the mainstay of treatment with the aim of complete excision, limb salvage and retention of function. Postoperative radiotherapy is indicated for deep seated tumours, intermediate to high-grade tumours, or those with a close or involved surgical margin. Large pelvic or retroperitoneal sarcomas impacting on several organ systems may require a specialist multidisciplinary surgical team, e.g. gynaecological, urological, gastrointestinal and vascular surgeons as appropriate. Chemotherapy is indicated for Ewing's sarcoma and rhabdomyosarcoma.

A small minority of bone and soft tissue sarcomas (osteosarcoma/angiosarcoma) can arise in the vicinity and several years after use of a metallic orthopaedic or Dacron smooth surface vascular surgical implant. Immunosuppression after cancer therapy or organ transplantation can result in a range of cutaneous and soft tissue lesions, e.g. squamous cell carcinoma, lymphoproliferative disorders, low-grade smooth muscle proliferations and vascular endothelial tumours.

Gross Description

Specimen

- FNAC/needle core biopsy/open biopsy (incisional/curettings/reamings)/enucleation (marginal excision)/wide local excision/compartmentectomy/segmental resection/en bloc resection/pelvic exenteration/amputation (limb (below/above knee, etc.))/complex resection (forequarter/hindquarter/hemipelvectomy).
- Right or left
- Size (mm) and weight (g)

Tumour

Site

- Osseous: Diaphysis, metaphysis or epiphysis. Medulla, cortex, periosteal surface, soft tissue or joint.
- Soft tissue: Dermal/Subcutaneous/Suprafascial/Fascial/Subfascial/Intramuscular/Intra-abdominal/Retroperitoneal/Mediastinal/Paratesticular/Head and neck.
- Satellite nodules: size (mm) and distance (mm) from the main tumour.
- Location: may be indicative, e.g. pelvis—Ewing's sarcoma or chondrosarcoma; chest wall—Askin (thoracopulmonary neuroectodermal) tumour, alveolar rhabdomyosarcoma.

Size

- Length × width × depth (mm) or maximum dimension (mm).
- It is generally recommended that 1 block per 1 cm dimension of tumour (up to a maximum of 12) be sampled to account for the variation in grade and differentiation that is encountered in some sarcomas. Tumour regression after neoadjuvant therapy must also be accounted for.

Appearance

- Solid/cystic/necrotic/lobulated/fatty/myxoid/cartilaginous/osseous.
- The percentage tumour necrosis after neoadjuvant therapy is a prognostic indicator.

Edge

- Circumscribed/irregular.

Vessels

- Relationship of tumour to vessels.

Histological Type

Prior to histological evaluation of any bone or soft tissue sarcoma, knowledge of patient demographics, anatomical site of the lesion, subsite (e.g. epiphysis, metaphysis or diaphysis of bone) and radiologic appearances, provides essential context. For example, a rapidly growing chest wall lesion in a young male may be nodular fasciitis rather than a sarcoma; peripheral chondroid lesions are usually benign whereas proximal/axial cartilaginous lesions are more likely to be malignant; an epiphyseal lesion is likely to be a giant cell tumour (adult) or chondroblastoma (child) rather than an osteosarcoma (young/

metaphysis). Age also closely correlates with the type of soft tissue sarcoma: embryonal rhabdomyosarcoma (infants), synovial sarcoma (young adult), liposarcoma (middle aged to elderly) and undifferentiated sarcoma (previously designated as malignant fibrous histiocytoma (MFH)) (elderly). *Close clinicopathological correlation* is therefore fundamental to the diagnosis.

Osteosarcoma, chondrosarcoma, Ewing's sarcoma, liposarcoma, synovial sarcoma, fibrosarcoma, rhabdomyosarcoma, leiomyosarcoma, angiosarcoma, malignant peripheral nerve sheath tumour, and undifferentiated sarcoma are amongst the main categories of sarcoma and each comprises variable numbers of subtypes (Tables 36.1 and 36.2).

Although morphology and immunohistochemistry have traditionally been the mainstay of diagnosis, *cytogenetic and molecular analysis* is of increasing importance in diagnosis and prediction of behaviour and are essential in pediatric small round cell sarcomas. This approach allows *subclassification regarding histogenetic type in the majority of lesions* with, by exclusion, a minority of undifferentiated/pleomorphic sarcomas (electron microscopy has a limited role to play in the diagnosis of some peripheral nerve sheath tumours, marker negative synovial sarcomas and pleomorphic sarcomas). Most soft tissue sarcomas arise from primitive multipotential mesenchymal cells which can differentiate along one of several lines resulting in histological overlap. One morphological approach is to categorise lesions based on their cell type (pleomorphic/spindle cell/round cell/rhabdoid/epithelioid) and/or pattern (fascicular/storiform/myxoid), or a combination, e.g.

(a) Predominantly spindle cell with various patterns: e.g. monomorphic fibrosarcomatous (synovial sarcoma) or pleomorphic MFH-like (pleomorphic liposarcoma).
(b) Spindle cells mixed with other mesenchymal elements: e.g. liposarcoma (fat), synovial sarcoma (epithelial component) or myxoid liposarcoma (myxoid material, plexiform vasculature).

Table 36.1 Soft tissue tumours. Completely benign entities are not included

Adipocytic tumours
Intermediate (locally aggressive)
Atypical lipomatous tumour/well differentiated liposarcoma
Malignant
Dedifferentiated liposarcoma
Myxoid liposarcoma
Pleomorphic liposarcoma
Liposarcoma, not otherwise specified
Fibroblastic/myofibroblastic tumours
Intermediate (locally aggressive)
Palmar/plantar fibromatosis
Desmoids-type fibromatosis
Lipofibromatosis
Giant cell fibroblastoma
Intermediate (rarely metastasizing)
Dermatofibrosarcoma protuberans
Fibrosarcomatous dermatofibrosarcoma protuberans
Pigmented dermatofibrosarcoma protuberans
Solitary fibrous tumour
Solitary fibrous tumour, malignant
Inflammatory myofibroblastic tumour
Low grade myofibroblastic sarcoma
Myxoinflammatory fibroblastic sarcoma/
Atypical myxoinflammatory fibroblastic tumour
Infantile fibrosarcoma
Malignant
Adult fibrosarcoma
Myxofibrosarcoma
Low-grade fibromyxoid sarcoma
Sclerosing epithelioid fibrosarcoma
So-called fibrohistiocytic tumours
Intermediate (rarely metastasizing)
Plexiform fibrohistiocytic tumour
Giant cell tumour of soft tissue
Smooth-muscle tumours
Malignant
Leiomyosarcoma (excluding skin)
Skeletal-muscle tumours
Embryonal rhabdomyosarcoma
Alveolar rhabdomyosarcoma
Pleomorphic rhabdomyosarcoma
Spindle cell/Sclerosing rhabdomyosarcoma
Vascular tumours
Intermediate (locally aggressive)
Kaposiform haemangioendothelioma
Intermediate (rarely metastasizing)
Retiform haemangioendothelioma
Papillary intralymphatic angioendothelioma

(continued)

Table 36.1 (continued)

Composite haemangioendothelioma
Pseudomyogenic (epithelioid sarcoma-like) haemangioendothelioma
Kapsoi sarcoma
Malignant
Epithelioid haemangioendothelioma
Angiosarcoma of soft tissue
Gastrointestinal stromal tumours
Gastrointestinal stromal tumour, uncertain malignant potential
Gastrointestinal stromal tumour, malignant
Nerve sheath tumours
Malignant
Malignant peripheral nerve sheath tumour
Epithelioid malignant nerve sheath tumour
Malignant Triton tumour
Malignant granular cell tumour
Ectomesenchymoma
Tumours of uncertain differentiation
Intermediate (locally aggressive)
Haemosiderotic fibrolipomatous tumour
Intermediate (rarely metastasizing)
Atypical fibroxanthoma
Angiomatoid fibrous histiocytoma
Ossifying fibromyxoid tumour
Ossifying fibromyxoid tumour, malignant
Mixed tumour NOS
Mixed tumour NOS, malignant
Myoepithelioma
Myoepithelial carcinoma
Phosphaturic mesenchymal tumour, benign
Phosphaturic mesenchymal tumour, malignant
Malignant
Synovial sarcoma NOS
Synovial sarcoma, spindle cell
Synovial sarcoma, biphasic
Epithelioid sarcoma
Alveolar soft-part sarcoma
Clear cell sarcoma of soft tissue
Extraskeletal myxoid chondrosarcoma
Extraskeletal Ewing sarcoma
Desmoplastic small round cell tumour
Extra-renal rhabdoid tumour
Neoplasms with perivascular epithelioid cell differentiation (PEComa)
PEComa NOS, malignant
Intimal sarcoma
Undifferentiated/unclassified sarcomas (previously malignant fibrous histiocytomas)
Undifferentiated spindle cell sarcoma
Undifferentiated pleomorphic sarcoma
Undifferentiated round cell sarcoma
Undifferentiated epithelioid sarcoma
Undifferentiated sarcoma NOS

Table 36.2 Tumours of bone. Completely benign entities are not included

Chondrogenic tumours
Intermediate (locally aggressive)
Chondromyxoid fibroma
Atypical cartilaginous tumour/chondrosarcoma grade I
Intermediate (rarely metastasizing)
Chondroblastoma
Malignant
Chondrosarcoma
Grade II, grade III
Dedifferentiated chondrosarcoma
Mesenchymal chondrosarcoma
Clear cell chondrosarcoma
Osteogenic tumours
Intermediate (locally aggressive)
Osteoblastoma
Malignant
Low-grade central osteosarcoma
Conventional osteosarcoma
Chondroblastic osteosarcoma
Fibroblastic osteosarcoma
Osteoblastic osteosarcoma
Telangiectatic osteosarcoma
Small cell osteosarcoma
Secondary osteosarcoma
Parosteal osteosarcoma
Periosteal osteosarcoma
High-grade surface osteosarcoma
Fibrogenic tumours
Intermediate (locally aggressive)
Desmoplastic fibroma of bone
Malignant
Fibrosarcoma of bone
Haematopoietic neoplasms
Malignant
Plasma cell myeloma
Solitary plasmacytoma of bone
Primary non-Heodgekin lymphoma of bone
Osteoclastic giant cell rich tumours
Intermediate locally aggressive, rarely metastasizing
Giant cell tumour of bone
Malignant
Malignancy in giant cell tumor of bone
Notochordal tumours
Malignant
Chordoma
Vascular tumors
Intermediate locally aggressive rarely metastasizing
epithelioid hemangioma
Malignant
epithelioid hemangioendothelioma
angiosarcoma
Myogenic tumours
Malignant

Table 36.2 (continued)

Leiomyosarcoma of bone
Lipogenic tumours
Malignant
Liposarcoma of bone
Miscellaneous tumours
Ewing sarcoma
Adamantinoma
Undifferentiated high-grade pleomorphic sarcoma of bone

(c) Round cell morphology: e.g. Ewing's sarcoma, desmoplastic small round cell tumour and rhabdomyosarcoma (small cells), clear cell sarcoma (large cells) or extrarenal rhabdoid tumour (rhabdoid cells).

Diagnostic pointers to sarcoma: can be *general or distinctive*. The former include deep seated anatomical location, progressive increase in size with compression of adjacent structures, irregularity of margins, cellularity, cytological atypia, necrosis, mitotic activity and vascular invasion. More distinctive features of particular sarcomas are described below:

Liposarcoma

Forms a majority of deep limb, retroperitoneal and pelvic sarcomas and is prone to *local soft tissue recurrence.*

Well differentiated	Variation in adipocyte size and shape.
	Multivacuolar lipoblasts.
	Atypical mesenchymal nuclei in broad Connective tissue septa.
	May be MDM2, CDK4 positive.
Myxoid	Myxoid stroma with signet ring lipoblasts.
	Plexiform capillary network.
	± a high-grade round cell component (epithelioid cells/cords/trabeculae).
Dedifferentiated	Abrupt transition from well differentiated liposarcoma to non-lipogenic sarcoma. Associated with a *15–20% metastasis rate.*
	May be MDM2, CDK4 positive.
Pleomorphic	High grade sarcoma with pleomorphic lipoblasts.

Leiomyosarcoma

Usually a monomorphic spindle cell tumour and a fibrosarcomatous pattern of cells with tapering eosinophilic cytoplasm and blunt ended nuclei. Mitoses, atypia and necrosis may not be prominent so anatomical location and radiological findings are important. Forms a significant proportion of *retroperitoneal sarcomas* some of which arise from large blood vessels, e.g. inferior vena cava. Epithelioid and myxoid variants occur. Also primary cutaneous, peripheral soft tissue and visceral (e.g. uterine, vulvovaginal, pelvic, gastrointestinal) lesions. *Site of origin is a strong predictor of subsequent biological behaviour*—in general a deep seated lesion with any mitotic activity is considered to have metastatic potential. A proportion of retroperitoneal smooth muscle tumours are oestrogen receptor positive and potentially hormone responsive.

Synovial Sarcoma

Either *classical biphasic* with a glandular/epithelial component and background spindle cells, or *monophasic* with a fibrosarcomatous spindle cell pattern. Focal microcalcification may be present. Can be small and peripheral (e.g. tendons of the hand or foot), or large and central (e.g. head and neck area).

Undifferentiated Sarcoma

Elderly, lower limbs. Storiform (cartwheel/whorls) or fascicular spindle cell patterns. Mononuclear, osteoclast type and bizarre pleomorphic giant cells are not uncommon. *Excluded by histological identification of a more specific line of differentiation,* e.g. lipoblasts. Many retroperitoneal sarcomas resembling undifferentiated sarcoma are now considered to be dedifferentiated liposarcomas. Tumours with *myogenic differentiation* have a *worse prognosis*.

Pleomorphic rhabdomyosarcoma is a rare high-grade sarcoma in the deep soft tissues of the lower extremities of adults, or, a primary visceral lesion, e.g. uterus, which can be pure, or more commonly a component of a carcinosarcoma

(malignant mixed Mullerian tumour), or testicular germ cell tumour.

Dermatofibrosarcoma Protuberans (DFSP)

Uniform, moderately cellular dermal plaque of spindle cells in a storiform pattern with lace like honeycomb infiltration of subcutaneous fat. There is an uninvolved Grenz zone between overlying non-hyperplastic epidermis, elimination of dermal collagen and appendage structures, with diffuse CD34 positivity. These features help to distinguish it from usual dermatofibroma or benign fibrous histiocytoma. There is a *40% local recurrence rate* within 3 years if incompletely excised.

Malignant Peripheral Nerve Sheath Tumour (MPNST)

Monophasic fibrosarcomatous pattern with prominent nodularity and entrapped nerves at the tumour periphery. Can be epithelioid in character or include a biphasic glandular epithelial component or rhabdomyogenic foci (malignant Triton tumour). *50% arise in neurofibromatosis type 1 (von Recklinghausen's disease*). Shows only focal S100 positivity although epithelioid MPNST is diffusely S100 positive.

Angiosarcoma

Primary (e.g. scalp in the elderly) or secondary (e.g. post radiotherapy for breast carcinoma). It comprises a branching vasoformative network dissecting collagen and lined by abnormal endothelial cells which are CD31/CD34/ERG positive. Intracytoplasmic vacuoles containing red blood cells.

Epithelioid Haemangioendothelioma

A malignant vascular neoplasm which forms cords of small epithelioid cells with paranuclear intracytoplasmic lumina embedded in a myxohyaline stroma. It has a tendency for local recurrence and 20–30% of tumours metastasise.

Kaposi's Sarcoma

Cutaneous or visceral mucosal lesions with patch, plaque and tumour stages. Nodules of spindle cells (±hyaline globules) and slit like spaces containing red blood cells. Primary or immunosuppression related (e.g. HIV), and HHV8 positive.

Epithelioid Sarcoma

Nodular dermal/subcutaneous lesion in the hand/wrist of young adults with a "pseudonecrobiotic granulomatous" pattern of CAM 5.2/EMA/CD34 (50%) positive spindle/epithelioid cells. It can mimic granuloma annulare/rheumatoid nodule and squamous cell carcinoma. There is complete loss of nuclear expression of SMARCB1 (INI1).

Clear Cell Sarcoma of Tendon Sheath

Nests of S100 positive polygonal/fusiform cells in dense collagen septate stroma. Often in the foot or ankle of young adults.

Alveolar Soft Part Sarcoma

Organoid nests of large eosinophilic cells with central dyscohesion giving an alveolar pattern. Cytoplasmic PAS-diastase positive rhomboidal crystals. Found in the extremities in adults, and head and neck in children.

Pecomas

A family of tumours showing *Perivascular Epithelioid Cell differentiation*. It includes renal and hepatic angiomyolipomas (associated with tuberous sclerosis complex), pulmonary lymphangioleiomyomatosis and clear cell "sugar"

tumour, visceral (gastrointestinal/uterine), and soft tissue (retroperitoneal/abdominopelvic) lesions. Predominantly in females comprising nests of epithelioid and/or spindle cells with clear to granular cytoplasm and associated with a proliferation of thick walled dystrophic vessels and fat. The tumour cells are HMB-45 and melan-A positive and also express myogenic markers such as SMA and calponin. A minority (size >7 cm, cytological atypia, mitotically active, necrosis) behave in a malignant fashion. PEComas can be prone to *spontaneous life threatening retroperitoneal haemorrhage*.

Small Round Cell Tumours

More often in children and young adults including *Ewing's sarcoma* (pelvis, long bones, ribs), *rhabdomyosarcoma* (alveolar/chest wall, and, embryonal/bladder, biliary tract, head and neck subtypes) and *desmoplastic small round cell tumour* (abdominopelvic area/peritoneum with nests of cells in a prominent collagenous stroma), small cell osteosarcoma (small round cell sarcoma producing osteoid) and mesenchymal chondrosarcoma (small round cell sarcoma with well-differentiated hyaline cartilage and a myopericytomatous branching vascular pattern). Distinguished by characteristic anatomical locations, immunophenotype and cytogenetic/molecular analysis from other small cell malignancy, e.g. neuroblastoma, malignant lymphoma/leukaemia, small cell malignant melanoma or small cell carcinoma.

Chondrosarcoma

Axial location in older adults and hypercellular hyaline cartilage with atypical chondrocyte nests and nuclei. It shows an infiltrative margin into adjacent bone or soft tissues. Grading is prognostically important. There are also clear cell, mesenchymal and dedifferentiated variants. *Low-grade* chondrosarcoma tends to be prone to *local recurrence whereas high grade has significant metastatic potential.* Dedifferentiated lesions are *high-grade* and *metastasise early.*

Osteosarcoma

Metaphysis/diaphysis in long bones of children or young adults. Infiltrative and destructive with elevation of the periosteum. Variably pleomorphic spindle cells associated with formation of osteoid matrix. There are a number of variants including osteoblastic, chondroblastic, fibroblastic, giant cell, small cell and telangiectatic. Also low-grade central (medullary) and parosteal forms, and, intermediate periosteal and high-grade surface osteosarcomas.

Differential Diagnosis

A monomorphic or pleomorphic spindle cell tumour in breast, bowel, lung, kidney or other viscera is usually a sarcomatoid carcinoma, and, in skin or lymph node metastatic malignant melanoma. A similar soft tissue lesion is usually a sarcoma but *metastatic malignant melanoma, carcinoma and malignant lymphoma must be considered and excluded.* Also consider *myeloid sarcoma*, particularly if the tumour looks like it is a malignant lymphoma but does not show positive expression with the usual panel of lymphoid immunohistochemical markers.

Metastatic carcinoma: is the commonest malignant tumour of bone, typical primary sites being lung, kidney, breast, prostate and thyroid. It can be single or multiple, with 70% affecting axial skeleton, and metaphysis the preferred site. Metastatic carcinoma is rare distal to the elbow or knee.

Multiple myeloma: must always be borne in mind. In the elderly, multiple lytic bone lesions, serum monoclonal gammopathy, Bence Jones proteinuria with renal dysfunction, CD138 positive plasma cells with κ/λ light chain restriction.

Differentiation/Grade

Well/moderate/poor, or, Grade 1/2/3.

- Based on the degree of resemblance to adult mesenchymal tissues, cytological atypia, necrosis and mitotic activity with grade 1/well differentiated equating to low-grade, grade

2/moderate and grade 3/poor differentiation to high-grade.

Some lesions define their own grade by way of their inherent clinical behaviour:

- Well differentiated liposarcoma, dermatofibrosarcoma protuberans, infantile fibrosarcoma, angiomatoid fibrous histiocytoma are grade 1.
- Ewing's sarcoma, rhabdomyosarcoma (except spindle cell and botryoid types), angiosarcoma, pleomorphic liposarcoma, soft tissue osteosarcoma, mesenchymal chondrosarcoma and desmoplastic small round cell tumour are grade 3.

Others are allocated a grading score (synovial sarcoma (3), extraskeletal myxoid chondrosarcoma (2)), or, are not graded but are potentially metastatic and usually regarded as high-grade: clear cell sarcoma, alveolar soft part sarcoma, epithelioid sarcoma.

Grading can be prognostically useful in adult soft tissue spindle cell sarcomas: leiomyosarcoma, fibrosarcoma.

Grading (Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC))

Differentiation	Scores	
Well	1	Sarcoma closely resembling adult mesenchymal tissue, e.g. well differentiated liposarcoma.
Moderate	2	Sarcoma of certain histological type, e.g. myxoid liposaroma
Poor	3	Embryonal and undifferentiated sarcomas and sarcomas of uncertain type, e.g. synovial sarcoma.
Necrosis		
None	0	
≤50% tumour necrosis	1	
>50% tumour necrosis	2	
Mitoses		
0–9/10 HPF[a]	1	
10–19/10 HPF	2	
≥20/10 HPF	3	
Histological Grade		
Grade 1 = ≤3 Grade 2 = 4 or 5 Grade 3 = ≥6		

[a]HPF, high power field (×40 objective in the most proliferative area of the tumour)

Histological differentiation or grade can be heterogeneous within a tumour, e.g. juxtaposition of well differentiated and dedifferentiated chondrosarcoma or liposarcoma. The less differentiated component is chosen for grading purposes.

Preoperative adjuvant therapy can lead to quite extensive necrosis and changes in morphology potentially *invalidating grading criteria* on the resection specimen. In sarcomas the degree of *histological tumour response* can be graded from I (no effect identified) to IV (no viable tumour noted). A *90–97% response (III)* is a target standard of *favourable prognostic significance,* e.g. in osteosarcoma chemotherapy induced necrosis of >90% or <90% can result in disease free survival figures of >90% and <50% respectively. Note that treatment effects (necrosis or fibrosis) cannot be reliably distinguished from spontaneous tumour necrosis or degeneration. A majority of paediatric soft tissue sarcomas are treated chemotherapeutically while surgical excision is used in most adolescent and adult patients. High-grade tumours benefit from a combination of *local surgical control and systemic therapy*.

Extent of Local Tumour Spread

- Border: pushing/infiltrative.
- Lymphocytic reaction: prominent/sparse.
- Lymphatics/vessels/nerves including the proximal limit.
- Single/more than one anatomical compartment, e.g. skin/subcutaneous tissue, fascial/subfascial tissue, or more than one anatomical plane.

Soft Tissue Sarcoma

The TNM8 classification applies to all soft tissue sarcomas except angiosarcoma, Kaposi's sarcoma, dermatofibrosarcoma protuberans, fibromatosis and sarcomas arising from dura mater, brain, hollow viscera or parenchymatous organs except breast (See Fig. 36.1). Uterine sarcoma has a separate specific FIGO staging scheme.

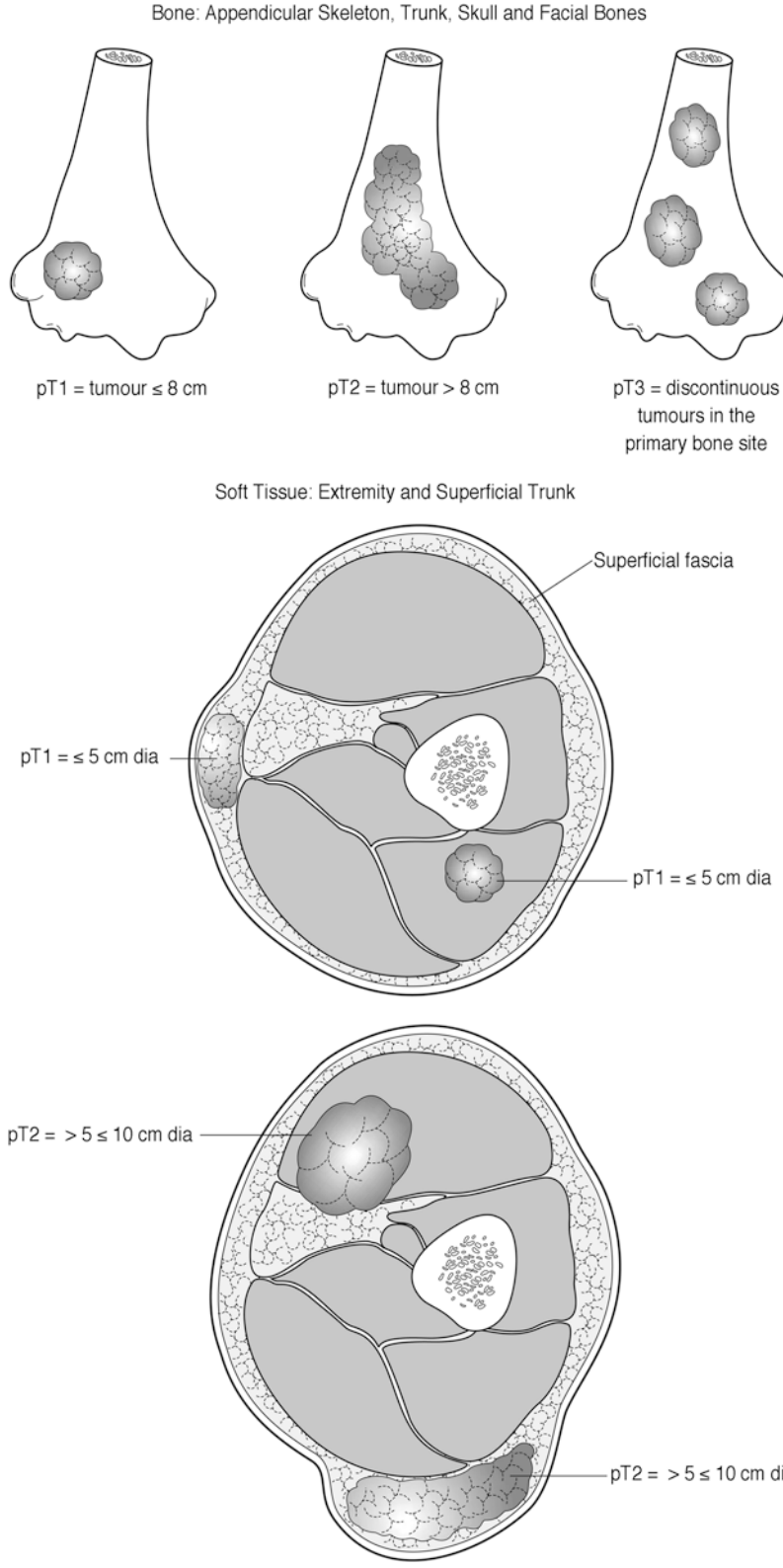

Fig. 36.1 Sarcoma. Adapted from *Histopathology Reporting: Guidelines for Surgical Reporting, 2nd ed.,* © 2006, Springer

Extremity and Superficial Trunk

T1	Tumour 50 mm or less in greatest dimension
T2	Tumour more than 50 mm but no more than 100 mm in greatest dimension
T3	Tumour more than 100 mm but no more than 150 mm in greatest dimension
T4	Tumour more than 150 mm in greatest dimension

Retroperitoneum

T1	Tumour 50 mm or less in greatest dimension
T2	Tumour more than 50 mm but no more than 100 mm in greatest dimension
T3	Tumour more than 100 mm but no more than 150 mm in greatest dimension
T4	Tumour more than 150 mm in greatest dimension

Head and Neck

T1	Tumour 20 mm or less in greatest dimension
T2	Tumour more than 20 mm but no more than 40 mm in greatest dimension
T3	Tumour more than 40 mm in greatest dimension
T4a	Tumour invades the orbit, skull base or dura, central compartment viscera, fascial skeleton and or pterygoid muscles
T4b	Tumour invades the brain parenchyma, encases the carotid artery, invades prevertebral muscle or involves the central nervous system by perineural spread

Thoracic and Abdominal Viscera

T1	Tumour confined to a single organ
T2a	Tumour invades serosa of visceral peritoneum
T2b	Tumour with microscopic extension beyond the serosa
T3	Tumour invades another organ or microscopic extension beyond the serosa
T4a	Multifocal tumour involving no more than two sites in one organ
T4b	Multifocal tumour involving more than two sites but not more than 5 sites
T4c	Multifocal tumour involving more than 5 sites

Stage grouping applies to extremity and superficial trunk and retroperitoneum tumours (but not head and neck and thoracic and abdominal viscera) and incorporates regional lymph node status (see below), distant metastasis (M0—no distant metastasis; M1—distant metastasis) and histological grade as follows:

Stage IA	T1	N0	M0	G1, GX low-grade
Stage IB	T2,3,4	N0	M0	G1, GX low-grade
Stage II	T1	N0	M0	G2, G3 high-grade
Stage III A	T2	N0	M0	G2, G3 high-grade
Stage III B	T3,4	N0	M0	G2, G3 high-grade
Stage III B	Any T	N1	M1	Any G
Stage IV	Any T	Any N	M1	Any G

AJCC classifies N1 as stage IV for extremity and superficial trunk

Bone Sarcoma

The TNM8 classification applies to all primary malignant bone tumours except malignant lymphomas, multiple myeloma, surface/juxtacortical osteosarcoma, and juxtacortical chondrosarcoma.

Appendicular Skeleton, Trunk, Skull and Facial Bones

T1	Tumour 8 cm or less in greatest dimension
T2	Tumour more than 8 cm in greatest dimension
T3	Discontinuous tumours in the primary bone site

Spine

T1	Tumour confined to a single vertebral segment or two adjacent vertebral segments[a]
T2	Tumour confined to three adjacent vertebral segments
T3	Tumour confined to four adjacent vertebral segments
T4a	Tumour invades into the spinal canal
T4b	Tumour invades the adjacent vessels or tumour thrombosis within the adjacent vessels

[a]*The 5 vertebral segments are the right pedicle, right body, left body, left pedicle and posterior element*

Pelvis

T1a	Tumour 8 cm or less in size and confined to a single pelvic segment[a] with no extraosseous extension
T1b	Tumour greater than 8 cm in size and confined to a single pelvic segment with no extraosseous extension

T2a	Tumour 8 cm or less in size and confined to a single pelvic segment with extraosseous extension or confined to two adjacent pelvic segments without extraosseous extension
T2b	Tumour greater than 8 cm in size confined to a single pelvic segment with extraosseous extension or confined to two adjacent pelvic segments without extraosseous extension
T3a	Tumour 8 cm or less in size and confined to two pelvic segments with extraosseous extension
T3b	Tumour greater than 8 cm in size and confined to two pelvic segments with extraosseous extension
T4a	Tumour involving three adjacent pelvic segments or crossing the sacroiliac joint to the sacral neuroforamen
T4b	Tumour encasing the external iliac vessels or gross tumour thrombus in major pelvic vessels

[a]*The four pelvic segments are the: sacrum lateral to the sacral foramen; iliac wing; acetabulum/periacetabulum; pelvic rami, symphysis and ischium*

Stage grouping incorporating regional lymph node status (see below) and distant metastasis (M0—no distant metastasis; M1a—distant metastasis to lung; M1b—distant metastasis to other sites) applies to appendicular skeleton, trunk, skull and facial bones. There is no stage grouping for bone sarcomas of the spine or pelvis.

Stage IA	T1	N0	M0	G1, GX low-grade
Stage IB	T2,3	N0	M0	G1, GX low-grade
Stage IIA	T1	N0	M0	G2, G3 high-grade
Stage IIB	T2	N0	M0	G2, G3 high-grade
Stage III	T3	N0	M0	G2, G3 high-grade
Stage IVA	Any T	N0	M1a	Any G
Stage IVB	Any T	N1	Any M	Any G
Stage IVB	Any T	N0	M1b	Any G

Lymphovascular Invasion

- Present/absent.
- Intra-/extratumoural.

Lymph Nodes

Site/number/size/number involved/limit node/extracapsular spread.

Regional nodes: those appropriate to the site of the primary tumour. Regional lymph node involvement is rare and cases in which lymph node status is not assessed may be considered N0 instead of NX.

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

Lymph node metastases are unusual with the commonest mode of spread being *haematogenous* resulting in pulmonary secondaries. Some sarcomas, e.g. angiosarcoma, epithelioid sarcoma and synovial sarcoma, may show lymph node spread. Alveolar rhabdomyosarcoma can present in lymph nodes or bone marrow as clinical lymphadenopathy or erythroleucocytopaenia, and mimicking haematological malignancy or disseminated carcinoma.

Excision Margins

Distance (mm) to the nearest painted excision margin.

Margins are superficial/deep, proximal/distal, medial/lateral and can be an anatomical structure, e.g. fascia or periosteum. Resection can be *intralesional or intracapsular* (within the tumour capsule and submitted in fragments), *marginal* (through the inflammatory tissue surrounding the tumour), *wide* (with a cuff of normal tissue) or *radical* in extent. The latter involves removal of the tumour and its related compartment of soft tissues, with or without the underlying bone, or amputation to include the joint proximal to the tumour. Neoadjuvant chemotherapy with limb salvage surgery is used increasingly for various bone sarcomas.

A surgical margin clearance of <15–20 mm in soft tissue sarcoma has an *increased risk of local recurrence* unless further surgery or radiotherapy is undertaken. This risk may be less if the margin is bound by a fascial plane. For high-grade tumour an even wider margin (20–50 mm) may be desirable.

Other Pathology

Prostheses: allied to limb salvage surgery following wide local excision with preoperative neoadjuvant therapy.

Radio−/chemotherapy changes: necrosis/inflammation and fibrosis in the primary tumour. Similar changes are seen in metastases and also tissue maturation phenomenon, e.g. pulmonary metastases of osteosarcoma resulting in nodules of paucicellular osteoid.

Predisposing factors to sarcoma: Paget's disease of bone, childhood chemotherapy, prior irradiation, and some metallic orthopaedic surgical implants can predispose to osteosarcoma.

Needle core biopsy: can allow categorization of soft tissue masses into benign and malignant lesions in a majority of cases. It can also exclude diagnoses such as *metastatic carcinoma, malignant lymphoma, multiple myeloma and malignant melanoma* allowing a more focused approach to the diagnosis of sarcoma. However, the pathologist must be aware of the potential for sampling error with regard to heterogeneity in tumour type and grade, and the latter should only be commented on if it is high-grade. The use of preoperative needle biopsy with neoadjuvant treatment can impose limitations on the prognostic information in the resection, e.g. necrosis induced by adjuvant therapy invalidates traditional grading criteria.

Immunophenotype

Immunohistochemical markers can be applied to formalin fixed paraffin embedded tissue to demonstrate a range of epithelial, neural, muscular, vascular and other mesenchymal antigens. They not only reflect *cell lineage* (e.g. cytokeratins, EMA, desmin, h-caldesmon, smooth muscle actin, S100, myoD1, myogenin, CD31, CD34, SATB2) but also protein expression as a marker of *molecular changes* (e.g. TLE-1, INI 1, MDM2, CDK4, STAT6, MUC4). None is totally specific or sensitive, indicating that an assimilation of results (including negative ones) from a *panel of antibodies* is necessary. However, for some soft tissue sarcomas a specific immunohistochemical profile is part of the definition of the tumour, e.g. rhabdomyosarcomas (positive with myogen and myoD1), epithelioid sarcoma (loss of SMARCB1), clear cell sarcoma (positive with S100, melanA and HMB45), desmoplastic small round cell tumour (expression of keratins and desmin, along with positively for WT1 (carboxy terminus)), and gastrointestinal stromal tumours (c-kit and DOG1). Metastatic carcinoma or malignant melanoma can be confidently excluded. Furthermore additional prognostic information can be given, e.g. myogenic differentiation in an undifferentiated sarcoma or a dedifferentiated liposarcoma, and strong myogenin expression in a alveolar rhabdomyosarcoma are adverse.

Antibody	Use
Cytokeratins	Synovial/epithelioid sarcoma
EMA	Synovial/epithelioid sarcoma
Desmin, myoD1, myogenin	Rhabdomyosarcoma
Desmin, h-caldesmon, smooth muscle actin	Leiomyosarcoma
Smooth muscle actin	Fibroblastic/myofibroblastic lesions
S100 protein	Malignant peripheral nerve sheath tumour, adipocytic and cartilaginous differentiation, clear cell sarcoma, synovial sarcoma
CD99 (MIC-2), Fli-1	Ewing's sarcoma (plus PAS for glycogen)
CD99, TLE-1	Synovial sarcoma
DOG-1, CD117	GIST
INI 1	Epithelioid sarcoma
Factor VIII, CD 31, CD 34, ERG	Angiosarcoma, epithelioid haemangioendothelioma
CD 34	Dermatofibrosarcoma, epithelioid sarcoma, solitary fibrous tumour (also CD99, bcl-2, STAT6)
HMB-45	Clear cell sarcoma, PEComa
HHV8	Kaposi's sarcoma
WT1	Desmoplastic small round cell tumour. It is also polyimmunophenotypic: cytokeratins/EMA/desmin and chromogranin/synaptophysin
MDM2, CDK4	Atypical lipoma/well-differentiated and dedifferentiated liposarcoma
βcatenin	Deep fibromatoses
ALK-1	Inflammatory myofibroblastic tumour

Cytogenetic and Molecular Analysis

Cytogenetic and molecular analysis is extremely important in the *classification, prognosis* and *choice of treatment for a range of sarcomas and is essential in pediatric small round cell sarcomas such as* Ewing's sarcoma, Ewing-like sarcomas (e.g. BCOR-CCNB3 fusion sarcoma and CIC-DUX4 fusion sarcoma), alveolar rhabdomyosarcoma and desmoplastic small round cell tumour. Distinct molecular changes can warrant specific targeted therapy in dermatofibrosarcoma protuberans. Fresh tissue in a suitable transport medium was previously required for tissue culture although *reverse transcriptase polymerase chain reaction (RT-PCR) techniques* have been developed and are routinely used on formalin fixed, paraffin embedded tissue for most investigations. Examples are given in the Introduction, Tables 4 and 5.

Prognosis

Prognosis in soft tissue sarcomas relates to:

- Tumour size: > 5 cm diameter.
- Grade: low-grade vs high-grade.
- Stage.
- Histological type.
- Site: superficial vs deep extremity vs retroperitoneum.
- Age: >50 years
- Adequacy of surgery.

The importance of excision margins is emphasised in soft tissue sarcomas where negative and positive margins in low-grade lesions are associated with 5 year recurrence rates of 2% and 28% respectively. Current treatment of soft tissue sarcomas is wide monobloc resection with postoperative adjuvant radiotherapy to the operative site of high-grade lesions. With modern surgical techniques and adjuvant chemo−/radiotherapy average 5 year survival figures for soft tissue and bone based sarcomas are 70–80%. *Prognosis varies with tumour stage, completeness of excision, histological type,* e.g. chondrosarcoma is better than osteosarcoma (60%), and *grade:* grade I chondrosarcoma (78%) vs grade III (22%), embryonal (botryoid/spindle cell: 85–95%) vs alveolar (53%) rhabdomyosarcoma. Grade of *response to preoperative chemotherapy* is also a very important indicator. Surgical excision of pulmonary metastases (20% of sarcomas) is also helpful.

Other Malignancy

Metastatic carcinoma, malignant melanoma, malignant lymphoma (primary or secondary), multiple myeloma and *leukaemia* can all *mimic soft tissue or bone sarcoma* and *immunohistochemical markers* will be required to make these distinctions. *Malignant lymphoma* is usually of high-grade B cell type, solitary but occasionally multifocal. The cells are CD45/CD20 positive with large irregular, multilobated nuclei. Fibrosis is present in 50% of cases giving spindle cell (mimicking sarcoma) or compartmentalised (mimicking metastatic carcinoma) appearances. A minority are CD30 positive with cytological features of anaplastic lymphoma and aggressive behaviour. *Metastatic carcinoma* to bone may be osteolytic (breast, lung, thyroid, renal) leading to pathological fracture or osteoblastic (prostate) in character. It can be focal or diffuse resulting in a leucoerythroblastic blood picture and extramedullary haemopoiesis. Rarely the bone marrow can show a granulomatous response as an indicator of micrometastasis (e.g. infiltrating lobular carcinoma of breast) which can be demonstrated by immunohistochemistry. Certain carcinomas tend to a preferred pattern of bone metastases, e.g. thyroid carcinoma goes to shoulder girdle, skull, ribs and sternum. Metastatic disease is usually to the axial skeleton and rarely to the hands and feet.

Immunochemical phenotype of other tumours in the differential diagnosis.

Carcinoma	cytokeratins, CEA, EMA.
Malignant melanoma	S100, HMB-45, melan-A.
Leukaemia/ myeloid sarcoma (chloroma)	CD34, CD43, CD68, CD117, myeloperoxidase, chloroacetate esterase, neutrophil elastase, tdt.
Multiple myeloma	CD138, κ/λ light chain restriction.

Specific markers: thyroglobulin/TTF-1 (thyroid), CK7/TTF-1/napsin-A (lung adenocarcinoma), PSA (polyclonal)/PSAP (prostate), CA125/PAX8 (ovary), ER/PR/GCDFP-15/GATA3 (breast), SALL4/OCT3/4/PLAP/AFP/βHCG/CD 30/glypican-3 (germ cell tumours)

Comments on Retroperitoneum

The retroperitoneum contains the kidneys, adrenal glands, ureters, aorta, inferior vena cava, vessel tributaries, lymph nodes, nerve plexuses and autonomic ganglia. Due to its inaccessible anatomical location *tumours can attain a considerable size* before clinical presentation with *vague symptomatology*, or because of *pressure effects on adjacent structures*, e.g. ureter. Investigation is by CT scan supplemented by ultrasound and MRI scan as appropriate. Arteriography may be used if resection of a large tumour is planned. Tissue diagnosis is by percutaneous CT guided needle core biopsy or FNAC. The commonest malignancies are periaortic lymphadenopathy due to nodal *malignant lymphoma* (diffuse large B cell lymphoma, follicular lymphoma or chronic lymphocytic leukaemia/lymphocytic lymphoma), or *metastatic disease* (testicular germ cell tumours, gastrointestinal, prostate, bladder, pancreatic or gynecological cancers). The need for a tissue diagnosis is determined by the availability of previous data, the nature and stage of the disease process at which the lymphadenopathy has arisen, and any further planned therapeutic management. Various clinical situations are: frozen section as a prequel to radical urological or gynaecological resection, needle core biopsy to establish a diagnosis, type and grade of malignant lymphoma, or, imaging with serum tumour markers to indicate surgery or radio−/chemotherapy in a patient with known prior testicular germ cell tumour. Kidney, germ cell and adrenal gland tumours are dealt with in their respective sections but primary tumours include:

Liposarcoma: especially well differentiated and dedifferentiated subtypes. Can attain a huge size encasing other structures and can be difficult to completely excise. Dedifferentiated elements are aggressive and potentially metastatic and comprise an abrupt junction between a well differentiated adipocytic tumour and high-grade spindle cell sarcoma.

Leiomyosarcoma: arising from the wall of the inferior vena cava or its tributaries and prone to cystic change when large. Tumour necrosis and size >10 cm are strong pointers to malignancy even if of low mitotic rate.

Undifferentiated sarcoma: exclude this pattern as an anaplastic component of other sarcomas (commonly dedifferentiated liposarcoma), and also sarcomatoid renal cell or pelviureteric urothelial carcinoma.

Peripheral nerve tumours: schwannoma, neurofibroma, malignant peripheral nerve sheath tumours, paraganglioma, ganglioneuroma, neuroblastoma. Schwannoma can be large, cystic and show significant cytological atypia mimicking a sarcoma. Nuclear palisading and thickened hyalinised vessels are useful diagnostic pointers.

Ewing's sarcoma, rhabdomyosarcoma, desmoplastic small round cell tumour: usually in the abdominopelvic regions of children and young adults. Initial chemotherapy is more appropriate but may be supplemented by surgery which is the main modality in the other sarcoma types.

Resection specimens: can be large and complex with structures such as the kidney enveloped by tumour. Due to the late presentation and difficulties in obtaining complete excision of retroperitoneal soft tissue sarcomas they have a *poor prognosis* with an *overall 25% 5 year survival.* An operative wedge biopsy may be taken from non-resectable tumour if needle core biopsy has been inconclusive. Paraaortic lymphadenectomy is commonly performed for cervical cancer, high-grade endometrial cancer, radical prostatectomy, and cystectomy for urothelial cancer. Retroperitoneal lymph node dissection (RPLND) in testicular germ cell tumour is discussed in Chap. 33.

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Part X

Ophthalmic Tumours

- Intraocular tumours
- Extraocular tumours

Intraocular Malignancy

37

Caroline Thaung

Intraocular malignancy may present as an alteration in vision, with a white pupil (hence retinoblastoma screening in children), with a secondarily blind painful eye in advanced disease, or it may be asymptomatic and detected on optometry examination.

As far as possible, diagnosis is made clinically, with methods including clinical examination, radiology, ultrasound and angiography. Diagnostic biopsies of intraocular lesions are technically possible although relatively rarely performed because of surgical risks and concern about possible tumour seeding. The commonest intraocular sample type is vitreous cytology for suspected lymphoma although sometimes chorioretinal biopsies are performed where the suspected diagnosis might be lymphoma, melanoma or a rarer tumour.

Local tumour resection is also rare, and it is unlikely to be performed outside of specialist centres. Most histology specimens for intraocular malignancy are enucleations (globes) and occasionally exenterations for extraocular spread. Macroscopic handling of exenteration specimens is covered in the chapter on extraocular malignancy.

The anatomy and histology of the eye is very different from other body sites, as is the pathology. In the United Kingdom, ophthalmic pathology is a subspecialty recognised by the Royal College of Pathologists. Even though some departments regard ophthalmic pathology as a sub-discipline of neuropathology, it is not a required component of histopathology or neuropathology training schemes, nor does it feature in the FRCPath examination. Therefore, histopathologists are advised not to report ophthalmic cases unless they have a specialist interest and participate in the Ophthalmic Pathology National EQA Scheme.

Gross Description

Enucleation

Specimen Description

- Globe—anteroposterior, horizontal and vertical dimensions (mm)
- Optic nerve length (mm)
- Cornea horizontal and vertical dimensions (mm)
- Any extraocular spread—dimensions and position
- Transilluminate to identify location of intraocular tumour

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_37

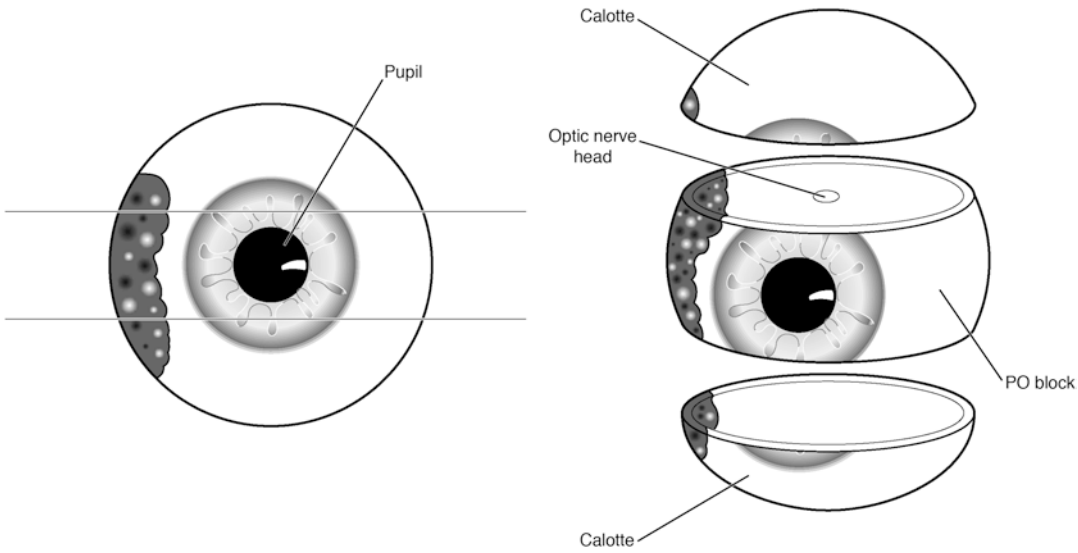

Fig. 37.1 Following localisation of an intraocular mass by transillumination or with clinical information, the calotte orientation may be chosen to best demonstrate the tumour and its anatomic relationships. Adapted from *Histopathology Specimens: Clinical, Pathological and Laboratory Aspects, 2nd ed,* 2012, Springer

- The globe is usually sliced by making two parallel slices at either edge of the limbus. This yields a central block (the "PO block", including cornea, pupil, optic nerve) and two calottes (caps). Ideally, the PO block will represent the tumour and its anatomical relationships. The lens can be hard (due to cataract) and care must be taken not to dislodge the retina (Fig. 37.1).

Tumour Description

- Site (iris, ciliary body, choroid, retina or vitreous). Any extraocular spread?
- For uveal (iris, ciliary body, choroid) tumours, measure across the base and into the eye. Beware ring melanoma. Colour, collar-stud profile (indicating breach of Bruch's membrane). Any extraocular spread.
- For retinoblastoma, note whether tumour is unifocal or multifocal. Measure. Endophytic, exophytic, seeding, calcification. Note presence of choroidal invasion, an adverse prognostic finding if >3 mm or full thickness. Any extraocular spread.
- Other findings might include retinal detachment, subretinal fluid, cataractous lens. If the patient has previously had cataract surgery, a synthetic intraocular lens implant (IOL) may be present. This need not be removed: it does not hamper microtomy.

Sampling

- Both uveal melanoma and retinoblastoma may be sampled fresh for genetics studies, either by the clinician at time of surgery or by the pathologist. Arrangements will depend on local agreement and facilities. The specimen may therefore not be intact on receipt, and this should be borne in mind when considering whether there is extraocular spread.
- Melanoma—optic nerve (if long enough to permit sampling), vortex veins, main block, calottes if they contain tumour.
- Retinoblastoma—optic nerve, main block, calottes. Documentation of optic nerve involvement at the resection margin is one of the most important prognostic details. Calottes should be bread-sliced and embedded on edge.
- Further information is available in the specific RCPath datasets for melanoma and retinoblastoma.

Exenterations

- Macroscopic handling is detailed in the chapter on extraocular malignancy. Additional comment will need to be made regarding the intraocular tumour as for the enucleation specimen, and any macroscopic evidence of extrascleral spread.

Intraocular Biopsies

- May be fragmented (e.g. choroidal cuttings) or discrete.

Histological Type

Melanoma

- Cell type (spindle, mixed, epithelioid). Epithelioid associated with worse prognosis
- Presence of PAS-positive loops and networks
- Extraocular spread
- Comment can also be made on pigmentation, presence of melanophages and mitoses
- TNM pathological staging is available for uveal melanoma, and there is an RCPath dataset
- pT staging is assigned depending on tumour size (across the base and into the eye), presence/absence of ciliary body involvement, and presence/size of extraocular spread.

Retinoblastoma

- Unifocal or multifocal (the latter supporting a germline mutation). Beware of seeding
- Optic nerve invasion—pre-laminar, laminar, retrolaminar, extension to optic nerve margin or involvement of meningeal space
- Choroidal invasion—massive/significant (3 mm singly or in aggregate or full thickness involvement) or focal
- Scleral infiltration and microscopic extraocular spread
- Anterior chamber involvement
- Rosettes (Homer-Wright or Flexner-Wintersteiner) and fleurettes, anaplasia
- Retinocytoma may be seen as a precursor lesion
- TNM pathological staging is available for retinoblastoma, and there is an RCPath dataset
- pT staging is assigned based on anatomical structures involved, especially the choroid and optic nerve, but also anterior segment structures (iris, trabecular meshwork). "Significant" choroidal invasion has a cut-off point of 3 mm in maximum dimension or aggregate, or any full thickness involvement. Broadly speaking, overall prognosis is worse with extraocular spread or optic nerve invasion to the surgical margin.

Lymphoma

- It is rare for an enucleation to be performed for intraocular lymphoma although intraocular biopsies (vitreous cytology, chorioretinal biopsies) might be performed for diagnosis.

Rarer Intraocular Primary Tumours

- There are other, rare, intraocular tumours. Whether they are benign or malignant, there may be clinical suspicion of malignancy leading to enucleation. Such tumours include medulloepithelioma, melanocytoma, retinal haemangioblastoma, ciliary body mesectodermal leiomyoma, pigmented or non-pigmented ciliary epithelial adenoma or adenocarcinoma.

Metastases to the Eye

- In metastatic disease, an enucleation might be performed for symptom control or in the absence of knowledge of the primary tumour. Tumours usually metastasise to the choroid in preference to the retina or vitreous.

Excision Margins

- Extrascleral spread of a tumour may be covered by a conjunctival cuff or orbital soft tissue (completely excised).
- If tumour extends into the optic nerve, note whether it is present at the surgical margin (cut end) or meningeal space.

Other Pathology

- There may be pre-existing pathology such as retinocytoma (in retinoblastoma).
- Pathology may arise secondarily to the tumour or its treatment. Examples include:
- Rubeosis iridis and secondary glaucoma
- Cataract
- Corneal vascularisation
- Chorioretinal adhesions and choroidal scarring

Prognosis

- Histological evaluation may be complemented by genetic information such as monosomy 3 in uveal melanoma.

Further Reading

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Extraocular Malignancy

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Extraocular cancers occur at a variety of sites, namely:

- Eyelid: skin, tarsus and/or conjunctiva
- Orbit: including orbital soft tissue, lacrimal gland, bone, and lacrimal sac
- Conjunctiva: including eyelid (palpebral), fornix, plica/caruncle and bulbar conjunctiva with possible extension on to cornea.

In the United Kingdom, ophthalmic pathology (including intraocular and extraocular pathology) is a subspecialty recognised by the Royal College of Pathologists. Pathology of extraocular specimens may overlap with other subspecialties such as dermatopathology, haematopathology or soft tissue. Otherwise, histopathologists are advised not to report ophthalmic cases unless they have a special interest and participate in the Ophthalmic Pathology National EQA Scheme.

Eyelid tumours may present as a mass, lash loss, ulceration, or other skin changes such as pigmentation or puckering.

Orbital tumours, whether benign or malignant, can present with proptosis, visual loss and (usually in malignant tumours) pain. Lacrimal sac tumours can present with watery eyes or a mass. Radiology and clinical evaluation need to be correlated.

Ocular surface tumours (conjunctiva and cornea) may present as a mass, plaque or discolouration.

Generally speaking, biopsies may be diagnostic or excisional. Because removal of eyelid or conjunctival tissue may compromise ocular surface integrity and vision, specimens and margins may be smaller than from other sites.

Gross Description

Eyelid Biopsies

- May be skin only, include eyelid-specific anatomy such as lash line or be full thickness (including skin, eyelashes, lid margin, tarsus and conjunctiva).
- Specimens may be irregularly shaped, particularly if taken from the canthal area.
- Skin biopsies—orientate if indicated, measure length × width × depth (mm) or maximum dimension.
- Partial or full thickness eyelid—orientate, measure specimen length × width × depth (mm) and lesion dimensions. Sagittal slicing allows easy assessment of the tumour in relation to anatomical structures.

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_38

- Eyelid specimens may be quite small, and macroscopic assessment may not clearly identify a lesion.

Conjunctiva

- Biopsies may be incisional or excisional. Multi-specimen mapping biopsies may be taken, particularly for suspected sebaceous carcinoma or melanoma. Orientate if indicated, measure specimen and any identified lesion. Can slice if large enough, but often need to embed whole on edge if small or a thin strip.

Orbital Biopsy

- Often not orientated. Measure in three dimensions or in aggregate if piecemeal (debulking).
- The specimen may include bone.

Exenteration

- May be performed for extensive eyelid or orbital tumours, or for intraocular tumours with extraocular extension. May be skin-sparing or include substantial eyelid and canthal skin. Rarely include orbital bone.
- Orientate. Caruncle, plica and upper and lower lid puncta are present medially. Upper lid usually has longer eyelashes and a squarer margin than lower lid although distortion may occur with tumour.
- Measure AP × horizontal × vertical (mm).
- Grossly identifiable pathology may include: eyelid distortion, mass, ulceration, discolouration; conjunctival adhesions, discolouration, mass; corneal ulceration, perforation or opacity; globe absence or disruption; mass in orbital soft tissue.
- Slicing is usually most informative sagittally. The cut surface may show tumour mass, haemorrhage, necrosis, distortion or breach of globe.

Microscopy

Eyelid Cancers

- Primary tumours may arise within the skin (basal cell carcinoma, squamous cell carcinoma, sebaceous carcinoma, Merkel cell carcinoma, melanoma), within the meibomian gland (sebaceous carcinoma) or within the conjunctiva (squamous cell carcinoma, sebaceous carcinoma, melanoma, lymphoma).
- Histological features of cutaneous eyelid malignancies are similar to other cutaneous tumours. Note that TNM staging for carcinoma of skin of eyelid differs from that for skin generally.
- Meibomian gland carcinoma (usually sebaceous) occupies an overlap area between cutaneous and conjunctival carcinoma. Generally, the TNM staging for carcinoma of skin of eyelid is used since the meibomian glands are specialised sebaceous glands. Note that there can be extensive intraepithelial (sometimes pagetoid) spread across the entirety of the conjunctiva, and that invasion may be multifocal. Depending on site, it may be more appropriate to stage as a conjunctival carcinoma.
- The posterior part of the eyelid (tarsus) is covered by conjunctiva (covered below).

Conjunctival Cancers

- These may occur in palpebral, forniceal or bulbar conjunctiva. The plica/caruncle includes skin adnexal-type structures, and accessory lacrimal gland may be found here or elsewhere in the conjunctiva. Corneal cancer is generally viewed as being secondary to spread from adjacent conjunctiva.
- Conjunctival pre-malignant changes include conjunctival intraepithelial neoplasia (CIN1/2/3), melanosis with atypia and in situ sebaceous carcinoma. Terminology may vary between institutions.
- Conjunctival cancers include squamous cell carcinoma, sebaceous carcinoma, melanoma and lymphoma.

- For melanocytic lesions, especially those with an intraepithelial component, it may be useful to perform immunohistochemistry for a pan-cytokeratin (in order to delineate the melanocytes in negative profile) as well as melanocytic markers.
- Immunohistochemistry for EMA and androgen receptor can help to differentiate sebaceous carcinoma from squamous carcinoma.

Orbital Cancers

- Lacrimal gland—lymphoma, adenocarcinoma NOS, adenoid cystic carcinoma, carcinoma ex pleomorphic adenoma are the commonest malignancies although other counterparts of salivary gland tumours may occur.
- Lacrimal sac—squamous cell carcinoma, transitional cell carcinoma. There may be morphological overlap.
- Orbital soft tissue—metastases, lymphoma, direct spread, e.g. SCC, melanoma (primary or secondary).
- Paediatric tumours—rhabdomyosarcoma, leukaemia, Burkitt's lymphoma.

Benign Pitfalls

- Eyelid—chalazion (lipogranulomatous reaction to a disrupted meibomian gland) is a common entity and may present with a tarsal mass or blepharitis, raising clinical suspicion of sebaceous carcinoma. Chalazion may also arise secondary to other pathology.
- Eyelid—Müller's muscle is a portion of smooth muscle that lies near the upper fornix. A similar muscle is present in the lower lid. For the unwary, it may be confused with a spindle cell tumour.
- Conjunctiva—melanocytic naevi may be difficult to differentiate from melanoma, particularly in children where the cytology may be alarming. Follicular conjunctivitis may be difficult to differentiate from lymphoma.
- Lacrimal gland—pleomorphic adenoma may harbour carcinoma, or a carcinoma may have arisen from previous pleomorphic adenoma.
- Orbital inflammation—even with immunohistochemistry, it can be challenging to differentiate between chronic inflammation and lymphoma.

Excision Margins

- Ophthalmic surgery may require smaller excision margins than conventionally used for other body sites in order to preserve visual function.

Staging

Ophthalmic-specific TNM staging is available for the following extraocular malignancies:

- Carcinoma of skin of eyelid. Site of sampling may overlap with skin of the face, and the pathologist may need to choose which site best reflects the specimen for staging
- Carcinoma of conjunctiva
- Malignant melanoma of conjunctiva. A Royal College of Pathologists dataset is also available for this tumour
- Carcinoma of lacrimal gland
- Sarcoma of orbit

Eyelid carcinoma pT staging depends on the tumour's maximum dimension

- pT1 is 10 mm or less
- pT2 is >10 mm but 20 mm or less
- pT3 is >20 mm
- pT4 invades adjacent ocular or orbital structures
- For pT1–3, suffix letters are given as follows (Fig. 38.1):
  - a—not invading the tarsal plate or lid margin
  - b—invading the tarsal plate or lid margin
  - c—involving full thickness eyelid

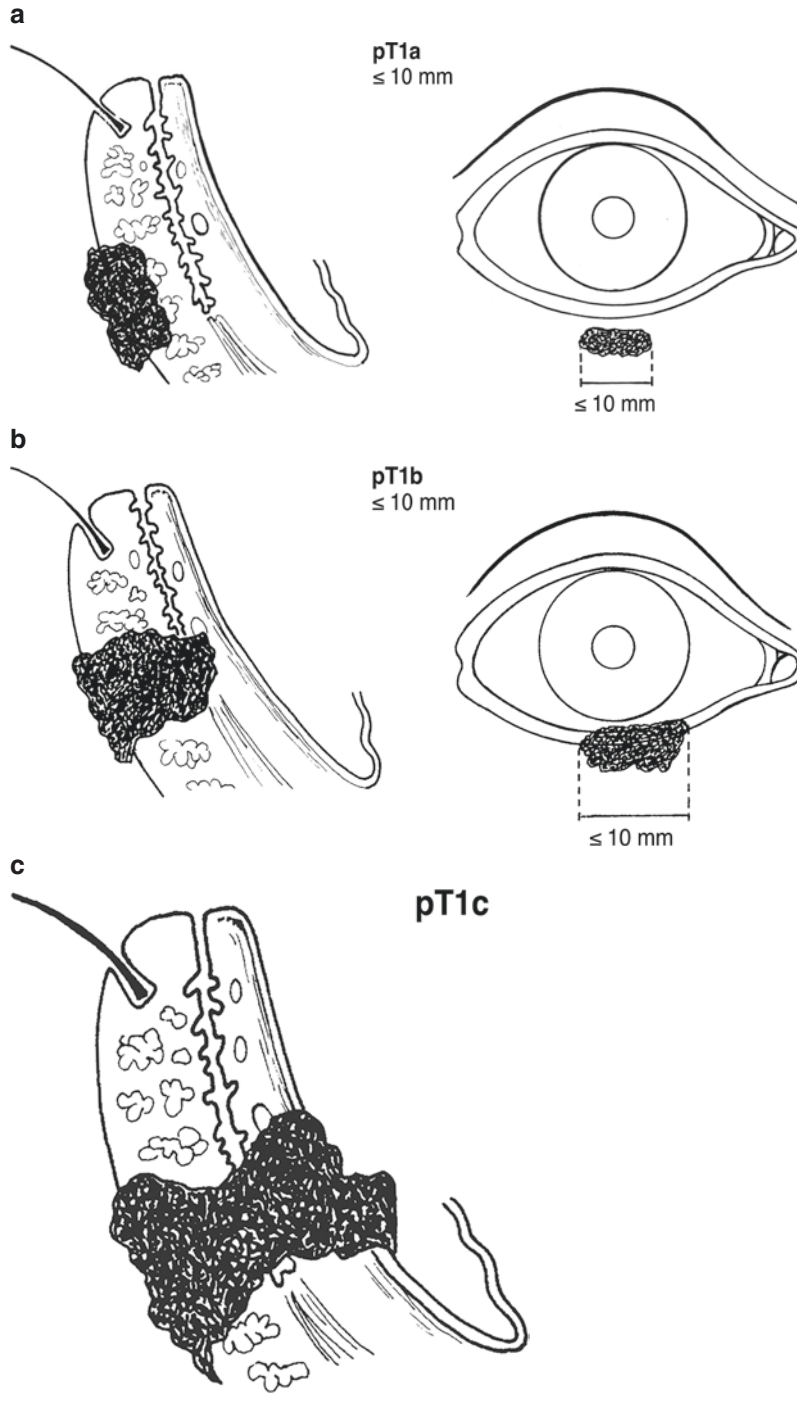

Fig. 38.1 Carcinoma in a full thickness eyelid, viewed sagittally. (**a**) Tumour may involve skin only (pT__a). Number depends on size. (**b**) Tumour may involve lid margin and/or tarsus (pT__b). Number depends on size. (**c**) Tumour may be full thickness (pT__c). Number depends on size. Adapted from *TNM Atlas: Illustrated guide to the TNM/pTNM classification of malignant tumours, 5th ed,* 2005, Springer-Verlag

Conjunctival carcinoma pT staging depends on tumour size (5 mm being a significant cut-off point) and whether the tumour invades adjacent structures.

Conjunctival melanoma pT staging depends on thickness of the tumour (2 mm being a significant cut-off point) and its location (bulbar vs other conjunctival regions) as well as

whether it invades other structures such as the globe or orbit.

Further Reading

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Part XI

Thyroid Gland Tumours

- Thyroid Gland Tumours (With Comments on Parathyroid and Adrenal gland)

39

Thyroid Gland Tumours (With Comments on Parathyroid and Adrenal Gland)

Caroline Coghlin

Introduction

Thyroid cancer comprises about 1% of human malignancies and is the most common cancer originating in the endocrine system. The majority are differentiated cancers; either papillary (60–70%) or follicular (15–20%) in type. They arise in young to middle-aged adults, females more commonly than males, and behave indolently, with 10 year survival rates in excess of 90%. Clinical features and histology identify those poorly differentiated and undifferentiated cancers that show aggressive local and metastatic spread. Benign tumours are common and cancers, relatively rare.

Thyroid gland tumours usually present with enlargement (goitre) due to a solitary nodule. This may be detected clinically by palpation, on ultrasound, or using other imaging modalities. About 5% of clinically detected thyroid nodules are malignant. Most thyroid cancers occur in euthyroid patients. There is a range of thyroid function tests including serum levels of thyroxine, thyroid stimulating hormone (TSH), triiodothyronine, and autoantibodies to thyroglobulin, microsomal antigen and the TSH receptor. These tests can help determine the functional status of the gland and aid in the diagnosis of autoimmune thyroid disease.

Differentiated cancers (papillary or follicular) may present with cervical lymph node or sclerotic bone metastases. Undifferentiated cancers are more often of rapid onset with symptoms due to infiltration or compression of local structures e.g. hoarseness, dysphagia or respiratory stridor. Fine needle aspiration cytology (FNAC) is the investigation of choice either of a clinically palpable lesion or under ultrasound guidance, with clinical follow up for benign cytology and surgery for a suspicious or malignant aspirate. Needle core biopsy may be used in specific contexts to distinguish between inflammatory/fibrotic lesions or widely infiltrative tumour, including anaplastic carcinoma or malignant lymphoma.

The extent of operative resection depends on the patient's age, tumour type and stage. The latter is assessed by MRI and CT scans of the neck and chest respectively. Intraoperative frozen section is occasionally used to confirm a diagnosis of papillary, medullary or anaplastic carcinoma, or of lymph node involvement. It is inappropriate for making the distinction between follicular adenoma and carcinoma as this requires full examination of the entirety of the tumour capsule in a well-fixed specimen. High stage undifferentiated and anaplastic cancers are often treated non-surgically with radiotherapy.

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1_39

Gross Description

Specimen

- FNAC/needle core biopsy/(sub)total thyroidectomy/leftorrightlobectomy/hemithyroidectomy/isthmusectomy/parathyroidectomy/selective neck dissection.
- Size (cm) and weight (g).

Tumour

Site
- Left/right lobe, isthmus, multifocal.

Size
- Length × width × depth (cm) or maximum dimension (cm).
- Size is a criterion for TNM staging.

Appearance
- Solid/cystic/calcified/haemorrhagic/pale/tan/papillary.
- From small foci to tumour replacing the entire gland.

Edge
- Circumscribed/irregular (encapsulated/non-encapsulated).

Gland
- Uniform, nodular, atrophic, pale in colour.

Histological Type

Follicular Adenoma

- *Usual type:* microfollicular, macrofollicular, or mixed patterns.
- *Variants:* oncocytic (Hürthle cell), clear cell, embryonal/fetal.

Hyalinizing Trabecular Tumour—HTT (or Hyalinizing Trabecular Adenoma—HTA): Most HTTs (organoid trabecular/nested pattern of spindled to epithelioid, eosinophilic cells and collagen) are benign and have an excellent prognosis but very rare cases show overlapping features with papillary carcinoma and/or capsular/vascular invasion and are regarded as indeterminate or (very rarely) malignant variants. Thyroglobulin and membranous Ki-67 positivity.

Papillary Carcinoma

- *Classical type:* psammomatous.
- *Variants with better prognosis:*
  - Encapsulated, including follicular variant
  - Papillary microcarcinoma (≤1 cm).
- *Variants with intermediate prognosis:*
  - Follicular (invasive)
  - Oncocytic (Hürthle cell).
- *Variants potentially more aggressive or with worse prognosis∗:*
  - Diffuse sclerosing
  - Solid
  - Tall cell∗
  - Columnar cell∗

Follicular Carcinoma

- *Widely invasive:* grossly apparent or extensive microscopic invasion of thyroid outside the lesion capsule and/or extrathyroidal soft tissue, often with prominent vascular invasion. Follicular/trabecular/solid patterns and vascular invasion.
- *Minimally invasive:* encapsulated—*angioinvasive* with potential for metastases, or, *capsular invasion* with equivocal potential for metastases. Note the number of foci of vascular and capsular invasion.
- *Formally a variant but now a separate group:* oncocytic (Hürthle cell: >75% of cells).

Undifferentiated (Anaplastic) Carcinoma

- Old age; *<5% of cases.* Clinically there is *rapid growth with involvement of vital neck structures and often death in 6 months.*

Treatment (decompressive surgery, external beam radiotherapy) is usually palliative.
- Spindle/squamoid/giant cells ± cartilage/osseous metaplasia ± a differentiated component i.e. evidence of origin from a more usual thyroid carcinoma e.g. papillary carcinoma. The tumour cells are cytokeratin positive/CD45 negative to distinguish from malignant lymphoma. TTF1 and thyroglobulin may be weak or absent. PAX 8 retained in some cases.

Poorly Differentiated Carcinoma

- "Insular" carcinoma: solid nests or trabeculae of small to medium sized uniform tumour cells with rounded or convoluted nuclei. Thyroglobulin/TTF-1 positive, calcitonin negative. In older age and grossly invasive with *aggressive behaviour*.
- Tumour shows limited evidence of thyroid follicular derivation (compared to differentiated tumours) and is associated with necrosis and increased mitoses (equal to or greater than 3 per 10 HPF), defined using the "Turin proposal": an internationally agreed algorithmic approach to the diagnosis of these tumours. Occurs either alone or as a dedifferentiated part of a carcinoma of more usual type. These tumours are intermediate between differentiated (papillary/follicular) and undifferentiated (anaplastic) thyroid cancers in terms of morphology and behavior. 5-year survival rate is 60–70%.

Non-Invasive Follicular Thyroid Neoplasm with Papillary-Like Nuclear Features (NIFTP)

- A well-circumscribed or entirely encapsulated tumour with a follicular growth pattern and papillary thyroid carcinoma-like nuclear features.
- Diagnosed carefully, NIFTP is a tumour of very low malignant potential. Prognosis is excellent.
- Entire capsule should be embedded and multiple sections examined to ensure no capsular or vascular invasion. No tumour necrosis or psammoma bodies allowed.

Follicular Patterned Tumours of Uncertain Malignant Potential (UMPs)

- Well-circumscribed or entirely encapsulated group of follicular-patterned thyroid tumours with equivocal capsular or vascular invasion.
- Two main types. Well-differentiated tumour of uncertain malignant potential (WDT-UMP) has well, or partially developed, nuclear papillary thyroid carcinoma (PTC) features with equivocal vascular or capsular invasion. Follicular tumour of uncertain malignant potential (FT-UMP) has no nuclear features of PTC with equivocal vascular or capsular invasion.
- UMPs are entirely follicular patterned lesions.
- Limited data on prognosis, likely to be low risk in both types.

Medullary Carcinoma

- *<5% of cases*. Malignant thyroid tumour with C-cell differentiation. Mixed medullary/follicular subtypes occur rarely. Cytoarchitectural features are variable, can mimic other tumour types.
- Often spindled to polygonal cells with low nuclear variability associated with abundant stroma, which may contain amyloid.
- Nodal metastases. 5 year survival 65–90%, stage dependent (see section "Other Pathology").

Miscellaneous

- Signet ring cell carcinoma, squamous cell carcinoma, mucoepidermoid carcinoma, spindle epithelial tumour with thymus-like differentiation (SETTLE), carcinoma showing thymus like differentiation (CASTLE), haematolymphoid tumours (see section "Other Malignancy").

Metastatic Carcinoma

- *Local spread:* upper aerodigestive tract, metastases in cervical lymph nodes.
- *Distant spread:* malignant melanoma, breast, kidney, lung carcinomas. Renal cell carcinoma can mimic primary clear cell carcinoma (papillary or follicular) of the thyroid. Renal cell carcinoma may have multiple nodules, shows vascular invasion and thyroglobulin/TTF-1 negative, but positive for renal carcinoma markers (RCC antibody/vimentin/EMA/CD10).

Differentiation

Differentiated thyroid tumours (papillary or follicular) comprise the vast majority of malignant neoplasms. These tumours recapitulate the cytoarchitectural features of thyroid follicular cells to variable degrees. Undifferentiated or anaplastic tumours occupy the opposite end of the spectrum of differentiation. Poorly differentiated tumours form an intermediate group between the differentiated malignancies and anaplastic carcinoma, in terms of morphology and biological behavior/prognosis. Tumour heterogeneity and a *minor undifferentiated component* within an otherwise differentiated tumour should be noted as this is an *adverse indicator*.

Extent of Local Tumour Spread

- Border: pushing/infiltrative.
- Lymphocytic reaction: prominent/sparse.
- Perineural involvement.
- Solitary/multifocal, one or two lobes.
- Involvement of lesion capsule, *presence or absence of capsular breach.*
- Involvement of other tissue or structures, *extrathyroidal spread.*

Specimen blocking must be generous to detect tumour heterogeneity, multifocality, tumour capsule and extrathyroidal involvement, vascular invasion and status of the excision margins. One suggestion is to process all of an encapsulated or well-defined tumour, or a minimum of 10 blocks. Some specific diagnoses (NIFTP) require the entire capsular interface to be embedded.

The TNM8 classification applies only to carcinomas.

pT1	Tumour ≤2 cm in greatest dimension, limited to thyroid
(a)	≤1 cm
(b)	Tumour >1 cm but ≤2 cm
pT2	Tumour >2 cm but ≤4 cm in greatest dimension, limited to thyroid
pT3	
(a)	Tumour > 4 cm in greatest dimension, limited to thyroid or
(b)	any tumour with gross extrathyroidal extension invading strap muscles (sternohyoid, sternothyroid, or omohyoid muscles).
pT4	
(a)	Tumour extends beyond capsule and involves any of: subcutaneous soft tissues, larynx, trachea, oesophagus, recurrent laryngeal nerve
(b)	Tumour invades prevertebral fascia, mediastinal vessels or encases carotid artery.

All anaplastic/undifferentiated thyroid carcinomas use the same T staging in TNM8 as differentiated thyroid cancers (Fig. 39.1).

Other pathological descriptors:

- pT1, 2, 3:
  - Grossly encapsulated tumour
  - Grossly non-encapsulated tumour

Separate *clinical stage groupings* are recommended for

1. Papillary or follicular carcinoma <55 yrs.
2. Papillary or follicular carcinoma ≥55 yrs.
3. Medullary carcinoma
4. Anaplastic/undifferentiated carcinoma (all stage IV) (Fig. 39.1).

Lymphovascular Invasion

- Present/absent, number of foci.
- Inside capsule or external to tumour.

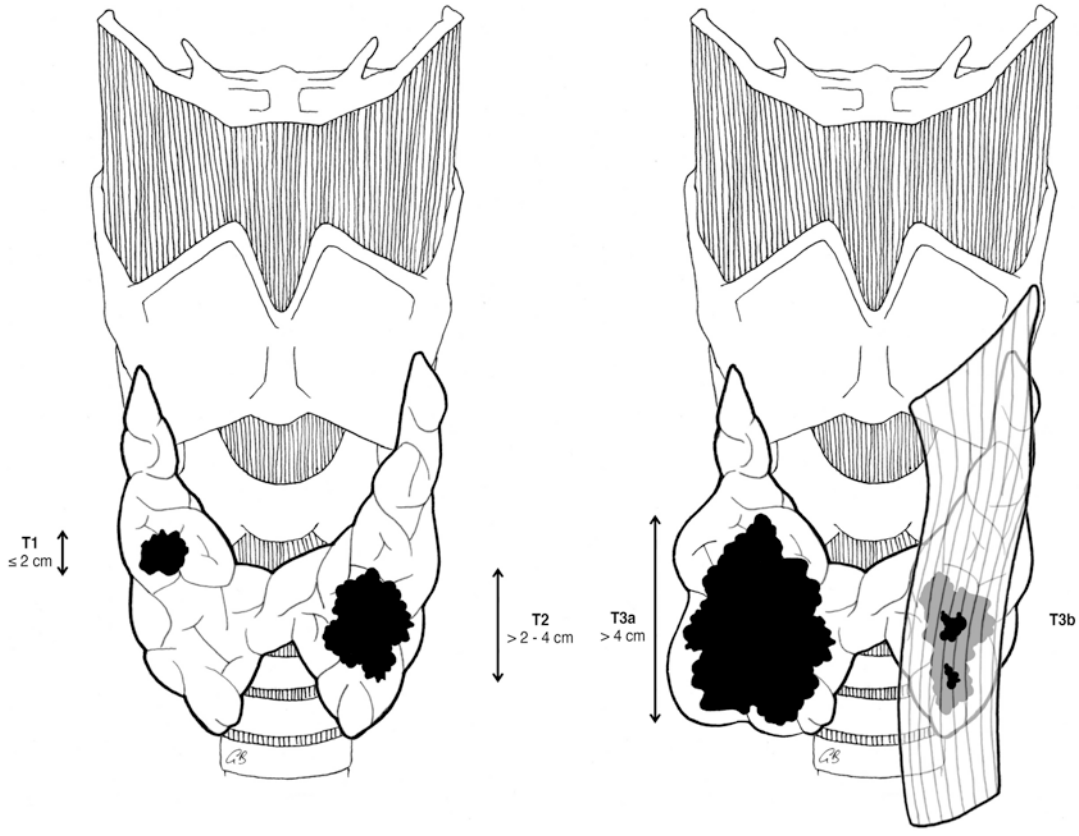

Fig. 39.1 Thyroid carcinoma. pT3b refers to tumour with gross extrathyroidal extension invading strap muscles (sternohyoid, sternothyroid, or omohyoid)

Papillary carcinoma tends to *lymphatic spread, follicular carcinoma* to *vascular spread.* Patient outcome may also relate to the *number of foci* of vascular invasion in follicular carcinoma, and the encapsulated follicular variant of papillary carcinoma (also foci of capsular invasion).

Lymph Nodes

Site/number/size/number involved/limit node/extracapsular spread.

Regional nodes: cervical, upper/superior mediastinal.

Level I	Submental, submandibular
Level II	Upper jugular
Level III	Middle jugular
Level IV	Lower jugular
Level V	Posterior triangle

A selective neck dissection will ordinarily include a minimum of six lymph nodes. Radical neck dissection is rarely performed for thyroid cancer.

pN0	No regional lymph nodes metastasis
pN1	Metastasis in regional lymph node(s)
	(a) level VI (including pre-/paratracheal and prelaryngeal/Delphian) or upper/superior mediastinal nodes.
	(b) metastasis in other unilateral, bilateral or contralateral cervical (Levels I, II, III, IV or V) or retropharyngeal nodes.

Excision Margins

Distances (mm) to the nearest surgical excision margins.

Other Pathology

Thyroid carcinoma: is commoner in females (3:1) with the most frequent subtypes being papillary carcinoma and follicular carcinoma. Previous irradiation predisposes to papillary carcinoma.

Papillary carcinoma: occurs in younger patients (age 30–60 years), is multifocal in a significant proportion of cases, and shows a tendency to *lymphatic vessel* and *lymph node spread.* Despite this, *prognosis is excellent* with a *low disease-related mortality of* < *5%.* Papillary carcinoma may undergo cystic change with only residual mural tumour—a potential pitfall on FNAC. Diagnostic cytological features are a combination of optically clear, irregular, overlapping nuclei with longitudinal grooves and nuclear pseudoinclusions. Architectural evidence of well-developed papillae is seen in the classical form of papillary carcinoma but several other growth patterns are recognized, including follicular and solid variants. Stromal (not intrafollicular) and tumour cell related calcified psammoma bodies are present (50% of cases) and the stroma may be hyalinised, calcified or ossified. The cells are thyroglobulin, TTF-1, CK7, CK19, galectin 3 and HBME-1 positive. RET/PTC is the most common chromosomal rearrangement in papillary carcinoma and tumours positive for *BRAF mutations* may be *widely infiltrative* and more *aggressive*. Papillary microcarcinoma (≤1 cm diameter) has an excellent prognosis if solitary. *Potentially more locally aggressive* are diffuse sclerosing (sclerosis, numerous psammoma bodies, solid foci, squamous metaplasia, heavy lymphocytic infiltrate) and solid types. There are *thought to be worse outcomes* for tall cell (older patients, papillary, eosinophilic cells with height two to three times the width) and columnar cell (nuclear stratification) variants with a higher risk of lymph node and distant metastases.

Follicular carcinoma: tends to be in older patients (50–60 years) than papillary carcinoma, unifocal and spreads via the blood stream to lung and bone. *Minimally invasive carcinoma has an excellent prognosis*, whereas *widely invasive carcinoma has a much higher mortality.* Multiple blocks (10 or more, or whole capsular interface) of the lesion and its capsule are required for the distinction between follicular adenoma and minimally invasive follicular carcinoma. *Diagnostic criteria are invasion of the capsule and/or its vessels.* Cytological appearances of the epithelium are not particularly helpful as adenomas may be markedly atypical, but the lesional nuclei are generally small and regular, and they lack characteristic nuclear features of papillary carcinoma. Carcinoma often has a thick, partly desmoplastic fibrous capsule and its full width must be traversed to qualify as invasion. The invasive tumour front may then form a second fibrotic interface (pseudocapsule) with the parenchyma giving a dumbbell or mushroom type appearance. *Vascular invasion, which is a more reliable indicator of malignancy,* requires invasion of vessels within or outside the tumour capsule. These vessels should be of venous calibre with an elastic lamina and an endothelial lining. Involved vessels should be partially or completely plugged by luminal tumour with an endothelial covering and a definite point of attachment to the vessel wall. Fibrin thrombus may also be present in association with the intravascular tumour. A cluster of follicular cells found free floating in a vascular lumen with an intact endothelium may represent artefactual displacement (pseudoangioinvasion). CD31/CD34 immunostains can help to identify true vascular structures.

Note that capsular invasion needs to be distinguished from rupture following FNAC which often shows organising haemorrhage and a fibrous reparative response. It can also cause tumour infarction and entrapment of cells within the capsule.

Ultrasound examination combined with FNAC: used in the investigation of a wide range of thyroid enlargement. Cytology can be helpful diagnostically in a range of thyroid conditions that may be encountered in determining the nature of a clinical or radiologically identified lesion: therefore briefly mentioned below

- *Inflammatory/autoimmune goitres:*
  - Hashimoto's thyroiditis (lymphocytes, Hürthle or Askanazy epithelial cells, colloid poor).

  - de Quervain's thyroiditis (lymphocytes, giant cells, degenerate follicular cells).
  - Riedel's thyroiditis (spindle cells, scant aspirate).
  - Abscess (polymorph rich).
- *Simple goitre*: multinodular colloid (characteristically *colloid rich/cell poor*) and forming the vast majority of thyroid nodules.
- *Simple thyroid cyst*: watery colloid and macrophages, scanty follicular cells.
- *Solitary, solid thyroid nodule*: follicular-patterned neoplasms, papillary carcinoma and medullary carcinoma (see above and below).

Adenoma and minimally invasive follicular carcinoma *cannot be distinguished on FNAC.* They are designated follicular lesion or neoplasm and are recognised by a variable *cell rich or microfollicular/colloid poor* pattern usually distinguishing them from simple goitres. *Surgical excision (hemithyroidectomy/diagnostic lobectomy) is often necessary for the histological assessment of capsular and vascular invasion.*

- *Malignant goitre:*
  - Widely invasive follicular carcinoma.
  - Malignant lymphoma (dispersed atypical lymphoid cells, lymphoglandular bodies).
  - Anaplastic carcinoma (spindle/giant cells with atypia).
  - Metastatic carcinoma.
- Care must be taken to closely *correlate imaging and FNAC* results, as well as in assessing *specimen adequacy* sufficient to constitute a safe diagnosis e.g. 6 groups of follicular cells with at least 10 well visualized cells per group.
- Diagnostic categories (Thy 1–5)
  - Non-diagnostic for cytological diagnosis (Thy1c denotes cystic lesion with mostly macrophages but little colloid and with insufficient follicular cells)
  - Non-neoplastic (nodular goitre, hyperplastic nodule or thyroiditis (Thy2c denotes cystic lesion, in appropriate clinical setting)
  - Neoplasm possible (Thy3a –cytological or nuclear atypia, or Thy3f—suggestive of follicular neoplasm)
  - Suspicious of malignancy
  - Malignant (papillary carcinoma, anaplastic carcinoma, etc.).

Hürthle cell neoplasms: oncocytic cell neoplasms, non-invasive are oncocytic or Hürthle cell adenomas. Once again capsular and vascular invasion are the hallmarks of malignancy. Typical oncocytic nuclear features with prominent nucleoli (lacking features of papillary carcinoma). Often encapsulated, minimally invasive Hürthle cell carcinomas have relatively good outcome but widely invasive (angioinvasive) tumours have a poor prognosis. Hürthle cell carcinomas can exhibit radioiodine resistance and treatment options may be fewer.

Medullary carcinoma: (C-cell differentiation—*<5% of thyroid cancers*) is in *the majority of cases sporadic (70%) and unifocal.* The *hereditary forms* occur in *younger patients*, can be *multifocal and bilateral*, associated with diffuse C-cell hyperplasia, C-cell tumourlets and the multiple endocrine neoplasia (MEN) syndromes types II and III. Its morphology is heterogeneous, but usually comprises polygonal or plump spindle cells with a nested, trabecular or solid pattern. The hyalinised stroma can be calcified and is typically positive for amyloid. The tumour cells are *CEA, calcitonin and chromogranin/synaptophysin positive.* Staining for TTF-1 and thyroglobulin is variable. Loss of calcitonin staining is adverse and in its absence CEA positivity acts as a surrogate marker. Lymph node metastases are seen in up to 75% of cases but *5 year survival is >80%.* High serum calcitonin levels, soft tissue and regional lymph node involvement are associated with an adverse prognosis. Elevated serum calcitonin following ablative therapy can indicate recurrence or metastases. Family members should be *genetically screened* for the *RET mutation* to establish the hereditary cases (autosomal dominant), and *prophylactic thyroidectomy* may be offered to affected children. Serial blocking of the gland is often required to look for C cell hyperplasia. *Treatment is surgical* with radio−/chemotherapy of limited use.

Poorly differentiated carcinoma: 5 year survival of 60–70% with a prognosis intermediate

between that of usual thyroid carcinoma and anaplastic carcinoma. It is more frequently associated with older age, lymph node metastases and extrathyroidal extension.

Anaplastic or undifferentiated carcinoma: requires immunohistochemistry to distinguish it from high-grade malignant lymphoma, malignant melanoma and angiosarcoma:

Immunophenotype

Carcinoma	Low molecular weight cytokeratin positive, thyroglobulin, CK19, TTF-1. Undifferentiated carcinoma may be negative or weakly positive for thyroglobulin and TTF-1 but cytokeratin and PAX-8 positive.
Malignant lymphoma	CD 45, CD 20 (B), CD 3 (T)
Malignant melanoma	S100, SOX10, HMB45, melanA
Angiosarcoma	CD 31, CD 34, factor VIII positive.

Prognosis

Prognosis is worse in:

- Male patients.
- Patients >55 years of age.
- Large tumours: <1 cm, excellent; >3.5–5 cm, poor.
- Multicentric tumours.
- Unencapsulated tumours.
- Widely invasive (angioinvasive) tumours with extrathyroidal extension.

Differentiated Malignancy

- Papillary carcinoma (and variants).
- Minimally invasive follicular carcinoma.
- Widely invasive follicular carcinoma.

Malignancy of Intermediate Differentiation

- Poorly differentiated carcinoma.

Undifferentiated Malignancy

- Anaplastic or undifferentiated carcinoma

Other Malignant Lesions

- Angiosarcoma.
- Malignant lymphoma

Thus, patients with differentiated cancer can be managed based on risk stratification according to their TNM stage or using other specific prognostic systems such as MACIS, based on Metastasis, Age at presentation, Completeness of surgical resection, Invasion (extrathyoid) and Size. This enables patients to be assigned to low or high risk categories.

Treatment: most solitary thyroid neoplasms are treated *surgically* by hemithyroidectomy. Total or completion thyroidectomy tends to be reserved for larger tumours (>4 cm) worse prognosis subtypes of differentiated thyroid cancer, bilateral or multifocal disease, medullary carcinoma (particularly if familial) and tumours with clinically or radiologically involved nodes. Central and selective lateral neck dissection is performed for clinically palpable lymph node metastases or those detected using imaging modalities. Additional treatment options are tumour and TSH suppression by administration of *levothyroxine or radioactive iodine* (papillary and follicular carcinoma), and *radiotherapy* (anaplastic/undifferentiated carcinoma). Treatment is therefore tailored to the patient's age, tumour type and stage.

Other Malignancy

Malignant Lymphoma

- Background lymphocytic/Hashimoto's thyroiditis.
- Low-grade: centrocyte-like cells, lymphoepithelial lesions, follicle loss/destruction. MALT lymphoma (extra-nodal marginal zone B cell lymphoma of mucosa-associated lymphoid tissue).
- High-grade: blast cells, usually *diffuse large B cell lymphoma.*

The majority of lesions are *diffuse large B cell lymphoma (DLBCL)* and there is some evidence for progression from Hashimoto's/lymphocytic thyroiditis through low-grade lymphoma to high-grade DLBCL. *Clinical onset can be rapid* with compression of neck structures and presentation is usually in elderly patients with an enlarging mass, sometimes associated with stridor and dysphagia. *Overall 5 year survival is 50–80%*. Advanced age, size (>10 cm), high-grade and stage of disease (particularly with extrathyroidal soft tissue extension) can decrease *5 year survival rates to 40% or lower.* Malignant lymphoma responds to *chemoradiotherapy.*

Primary Hodgkin's lymphoma is extremely rare.

Angiosarcoma

- Overlaps morphologically with undifferentiated thyroid carcinoma and is a pleomorphic tumour with vasoformative areas in elderly patients. Endothelial markers (CD31, CD34) are required for confirmation.

Parathyroid

Hyperparathyroidism is due to over secretion of parathyroid hormone or parathormone and results in *hypercalcaemia.* It arises in three main contexts.

1. *Primary hyperparathyroidism*: over-secretion by one or more parathyroid glands sometimes due to *diffuse hyperplasia* (10–25% of cases, 25% of which are associated with MEN I and IIa syndromes) but more commonly (70–85% of cases) an *adenoma.*
2. *Secondary hyperparathyroidism*: as a physiological response of all four glands to chronic hypocalcaemia, due to renal failure (most often), malabsorption or vitamin D deficiency.
3. *Tertiary hyperparathyroidism*: autonomous hypersecretion in long-standing secondary hyperparathyroidism (even after correction of the hypocalcaemia).

Investigation: is by serum calcium, phosphate, parathyroid hormone and alkaline phosphate levels. *Gland localisation* is by technetium labelled isotope scintigraphy (Technetium 99 sestamibi) with CT scan and MRI scans.

Treatment: is by *surgical removal* of the adenoma (usually solitary, occasionally two) or hyperplastic glands, leaving a small amount (100 mg) of functioning tissue either in situ or implanted into the soft tissues of the arm. An alternative is total parathyroidectomy with replacement treatment (calcium/calcidiol). Recurrence >6 months after surgery can be due to inadequate neck exploration with removal of insufficient parathyroid tissue, or ectopic glands. Better preoperative localisation now allows minimally invasive procedures targeted at the single abnormal gland in primary hyperparathyroidism. However, limitations in scan sensitivity can lead to missed multifocal disease and higher recurrence rates. There is histological overlap in the features of adenoma and nodular hyperplasia and designation is more appropriately decided by the number of enlarged glands (adenoma is usually solitary) and the clinical context. What is of crucial importance is that *the pathologist confirms, often by frozen section, to the surgeon that parathyroid tissue has been excised at neck exploration and not lymph node, thymic remnant or thyroid nodule.* To this end each submitted specimen is carefully weighed and its nature confirmed; indicating whether there is any need for further surgical exploration.

Parathyroid carcinoma: is *rare (<1% of cases of primary hyperparathyroidism)* and occurs in elderly patients with high levels of parathyroid hormone. It may infiltrate adjacent soft tissues resulting in *difficulty in surgical excision.* Suspicion is raised by an irregular, large and adherent gland. Histologically the tumour has a solid or trabecular pattern with thick fibrous bands traversing it. Cytological atypia and mitoses may be present but these can also be seen in an adenoma. More reliable indicators are soft tissue, perineural and lymphovascular invasion. It may be resected in continuity with the ipsilateral lobe of thyroid gland, and neck dissection is considered for palpable metastases. Cervical and

mediastinal lymph nodes, lungs, bone and liver are the most common sites for metastatic spread. Parathyroid carcinoma is *slow growing* with a tendency for *multiple local recurrences* after a long disease-free interval and *late metastases*. Ten year survival rates are 49–70%, *death is due to the complications of metastases and hypercalcaemia. Chemotherapy may be used if surgery is contraindicated and radiotherapy adjuvantly or in recurrent cases.*

Adrenal Gland

Metastatic carcinoma in the adrenal gland, particularly from lung, breast and kidney, is *more common than a primary malignancy* and usually detected by CT scan during post surgical follow up. Adrenal gland is the fourth commonest site for metastatic carcinoma after lungs, liver and bone. Sometimes CT guided needle core biopsy or FNAC is used to make this distinction and to avoid progressing to surgical excision.

Otherwise, primary adrenal neoplasms variably present either as *an asymptomatic or incidental radiological mass ("incidentaloma"), an abdominal mass,* or are characterised by their *endocrinological symptoms and signs* and resultant *biochemical profiles*. Appropriate investigations include

- Serum cortisols, sodium and aldosterone: for an adrenocortical lesion
- Plasma free metanephrines or urinary fractioned metanephrines: for adrenal medullary lesions

Nodular lesions within the adrenal cortex are a relatively common finding, affecting up to 10% of the population (incidence increases with age). *Some 80% are non-functioning adenomas* and they are usually <5 cm in size. The incidence of adenomas is rising, and this is likely due to increased use of CT imaging with a resultant rise in adrenal *'incidentalomas'*. CT/MRI scans can assess the size, characteristics and bilaterality of adrenal lesions helping to distinguish *hyperplasia* (secondary to hyperpituitarism and usually bilateral) from *adenoma* (usually solitary). Adrenal imaging can also help to distinguish benign from malignant lesions in many cases but degenerate alterations within an adenoma, including haemorrhage, can mimic carcinomas on scans.

Adrenal carcinoma cells may be dysfunctional or produce mixed hormonal effects, and the tumour often reaches a significant size *(most are > 10 cm/50–100 g)* before presentation. The best histological indicators of malignancy are *increased mitoses* (>5/50 HPFs), *atypical mitotic figures, confluent necrosis, clear cells comprising equal to or less than 25% of the tumour* and *capsular invasion;* criteria that are used in the *modified Weiss scoring system*. An alternative scoring system (the Lin-Weiss-Bisceglia criteria) is used for categorisation of oncocytic adrenocortical neoplasms. However, it can be difficult to distinguish an adenoma from a well differentiated adenocarcinoma and the term *adrenal cortical neoplasm of uncertain malignant potential* may be used, qualified by a morphological assessment of its likelihood to recur.

Spread of adrenal carcinoma involves local organs including retroperitoneal invasion into the kidney and inferior vena cava. In advanced disease, metastases are to regional lymph nodes, liver, bone, lungs and brain. Treatment is aimed at *complete local excision* which may be laparoscopic for small lesions or an open thoracoabdominal approach for a larger tumour. Overall, *adrenal carcinoma* has a *35–65% 5 year survival*.

Characteristic hormonal effects of adrenal cortical tumours are hypercortisolism (Cushing's syndrome), hyperaldosteronism (Conn's syndrome) and virilisation. CT chest may also be done to exclude the possibility of a primary lung small cell carcinoma as a source of *ectopic ACTH* (adrenocorticotrophic hormone) causing bilateral adrenal cortical hyperplasia and a *paraneoplastic syndrome.*

Phaeochromocytoma is medullary in location, commonly 3–5 cm in diameter and 75–150 g in weight, or larger, with a pale to tan coloured cut surface. *Phaeochromocytoma is a contraindication to biopsy* due to the risk of a

catecholamine induced hypertensive crisis. It is usually unilateral and solitary, but if bilateral and multicentric can be associated with MEN2 A/2B, von Hippel Lindau disease and neurofibromatosis. Mutations in the SDH family of genes (which encode subunits of succinate dehydrogenase) have emerged in recent years as the most common cause of hereditary phaeochromocytoma. Similar extra-adrenal paragangliomas are found elsewhere along sympathetic/parasympathetic nervous system sites in the retroperitoneum, mediastinum, carotid body, middle ear and urinary bladder. They have variable secretory capacity and functionality e.g. chemodectomatous head and neck paragangliomas. Radioisotope (MIBG) scan has a role to play in their detection.

Adrenal phaeochromocytoma usually has a characteristic nested "Zellballen" pattern, but may show a variety of morphologies. Phaeochromocytoma cells secrete catecholamines. This may induce paroxysmal or sustained symptoms of flushing, tachycardia, tremor and hypertension, but the classical triad of headache, palpitations and sweating is seen in a minority of patients. *Surgical excision requires careful control of blood pressure to avoid a hypertensive crisis. The prognosis is dependent on complete resection and on the genetic profile of the tumour. The risk of progressive disease and metastases is higher in tumours associated with hereditary SDHB mutations.*

Malignant behaviour is difficult to predict on the basis of histology alone, but no phaeochromocytoma should be considered to be entirely benign. Spread is usually to lymph nodes, then the axial skeleton, liver and lung. Some morphological indicators used for risk stratification are: large nests of cells or diffuse growth, confluent necrosis, spindle cells, mitoses >3/10HPFs, profound nuclear pleomorphism, capsular and vascular invasion. These parameters and others are used in the PASS (phaeochromocytoma of the adrenal gland scoring scale) system.

Neuroblastoma and ganglioneuroblastoma are characteristically seen in infants and children and are not further considered.

Immunophenotype of Adrenal Neoplasms

- *Adrenal carcinoma:* variable positivity for inhibin, synaptophysin, calretinin and melan-A. CEA negative. Variable Ki-67 (MIB 1) proliferation index—usually 5–20%. Strong positivity for cytokeratin, EMA, PAX8 and CD10 would favour metastatic renal clear cell carcinoma, one of its main differential diagnoses.
- *Phaeochromocytoma:* positive for chromogranin, synaptophysin, neurofilament and intervening S100 positive sustentacular cells. Immunohistochemistry for SDHB can help to identify tumours associated with SDH mutations.

TMN8 Stage of Adrenal Cortical Carcinoma

pT1	Tumour confined to adrenal gland and ≤5 cm in greatest dimension
pT2	tumour confined to adrenal gland and >5 cm in greatest dimension
pT3	Tumour of any size, locally invasive but not involving adjacent organs
pT4	Tumour of any size with invasion of adjacent organs: kidney, diaphragm, great vessels, pancreas and liver.
pN0	No regional lymph node metastasis
pN1	Metastasis in regional lymph node (s)

Other adrenal malignancy includes malignant lymphoma and malignant melanoma; usually secondary to disseminated disease. Occasional benign lesions are ganglioneuroma, schwannoma, adrenal cysts, adenomatoid tumour and myelolipoma.

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D. P. Boyle, D. C. Allen (eds.), *Histopathology Reporting*,
https://doi.org/10.1007/978-3-030-27828-1

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