SURGICAL PATHOLOGY

STRUCTURED ORGAN-WISE APPROACH

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Preface

Surgical pathology continues to evolve in both scope and expectation. The introduction of updated WHO classifications and the increasing importance of standardized, synoptic reporting have made it essential to adopt a more structured and integrated approach in the subject. In current practice, accurate diagnosis depends not only on microscopy, but on a seamless progression from anatomical understanding to specimen handling, gross examination, and clear reporting.

In response to these developments, the present book has been comprehensively compiled. Each organ system is now presented in a systematic format that includes relevant anatomy, grossing techniques, gross pearls, microscopy, and synoptic reporting. The aim is to provide a cohesive, practical framework that guides the reader step by step—from specimen receipt to final diagnosis.

Significant changes keeping in mind practical routine have been made; the core strengths of the fundamental principles in surgical pathology have been retained. The sections on grossing and microscopy have been carefully outlined with current techniques and classifications and immense experience of the author. Every effort has been made to ensure that the text remains concise, accessible, and directly relevant to day-to-day reporting.

This book is intended primarily for postgraduate residents, who stand at the interface of learning and independent practice. It is hoped that the book will serve not only as a guide during training, but also as a dependable reference in routine diagnostic work.

I remain grateful to my students and colleagues, whose questions, observations, and shared experiences over the years have quietly shaped this work.

The book is dedicated to my residents; past and present; who continue to inspire careful observation, disciplined practice, and a commitment to clarity in diagnosis.

Dr Shameem Shariff, MD, PhD (Path)

Dedicated to

My Post Graduates

&

Residents In Pathology

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Chapter 1
Female Genital Tract

Chapter outline – A – Cervix ; B- Uterus ; C- hysterectomy specimen (cervix and uterus); D- Ovaries and Fallopian tubes ; E- external genitalia; F- Placenta; G – Foetus .

A I. Cervix Anatomy; A II. - Cervix Grossing Techniques; A I & BI Cervix and Uterus Anatomy ; A II & B II – Hysterectomy specimen Grossing Techniques; A III- Cervix Gross Pearls; A IV – Cervix Microscopy; A V .- Cervix Synoptic Reporting. B II . Endometrial Curettage Grossing; B III – Endometrium and Myometrium Gross Pearls ; B IV Endometrium and Myometrium Microscopy ; B V. Endometrium Synoptic Reporting; D 1 Ovary and Fallopian tube Anatomy ; D II Ovary and Fallopian tube Grossing Techniques ; D III Ovary and Fallopian tube Gross Pearls ; D IV Ovary and Fallopian tube Microscopy ; D V Ovary and Fallopian tube Synoptic Reporting; E I External Genitalia Anatomy; E II -External Genitalia Grossing Techniques; E III External Genitalia Gross Pearls ; E IV External Genitalia Microscopy ; E V External Genitalia (Vulva) Synoptic Reporting ; F I Placenta Anatomy ; F II Placenta Grossing Techniques; F III Placenta Microscopy; G I Foetus Anatomy;. G II Foetus. Grossing Techniques.

A I CERVIX ANATOMY

The female genital tract comprises of the external and internal genitalia. Functionally it interacts closely with the hypothalamic-pituitary axis and other endocrine organs to regulate reproduction.

Cervix

Region	Epithelium
Ectocervix	Stratified squamous non-keratinized
Endocervix	Simple columnar mucin-secreting

A II CERVIX GROSSING TECHNIQUES

The types of biopsies done on the cervix are : Punch, Wedge , Cone and Large loop excision biopsy.

A. Cervix - Punch Biopsy

- This is normally done when CIN is suspected. It is performed as a colposcopically directed biopsy.
  - Measure along the three dimensions (Fig 1.1)
  - The description should include colour, presence or absence of 'mucus nodules' and cut surface appearance. Ideally the stroma should measure 3-5 mm in thickness.

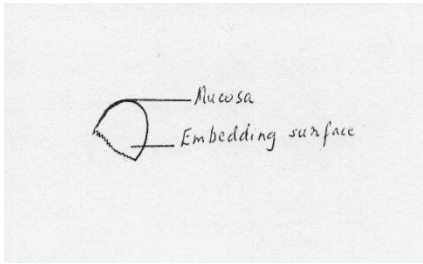


FIG 1.1

- Identify mucosal surface and mark the bedding surface straight away if the biopsy is 4mm or less in thickness. Embed in such a way that the mucosal surface is at right angles to the cutting surface so that the mucosa would be on one aspect of the section as shown in the figure above
- If more than 4mm thick, bisect and again mark the embedding surface in such a way that the mucosa covers the tissue on one aspect (Fig 1.2)

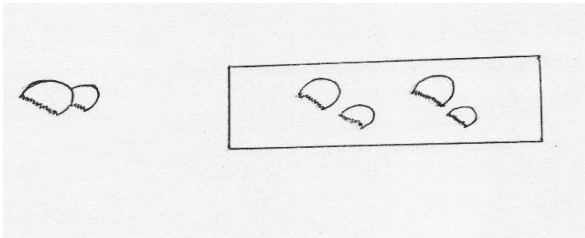


Fig 1.2

- If Bouin's fluid is used as a fixative then the mucosa automatically curls up over
- the tissue thus enabling easy identification as shown in the figure Fig 1.3 .

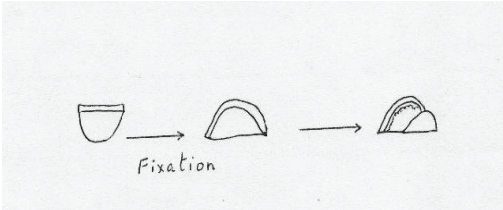


Fig 1.3

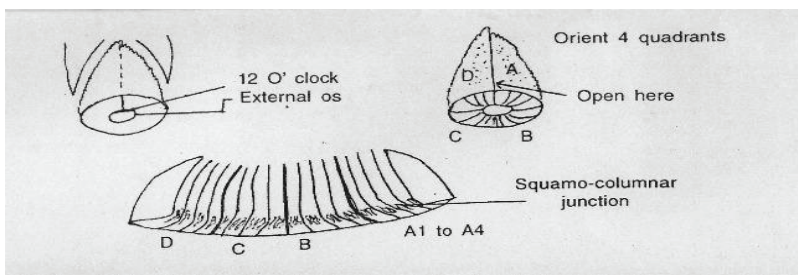
B. Cervix - Wedge Biopsy

- It is performed when an invasive carcinoma is suspected and when facilities for colposcopy are not available

- Measure along three dimensions
- Description should be as for punch biopsy
- Identify mucosal surface and treat as above

C. Cervix - Cone biopsy

- Indications: a) when a glandular lesion is suspected b) colposcope is not available and c) in cases of a high transformation zone.
- Formerly a cone biopsy was performed using just a scalpel but now it is done using a laser or low voltage large loop diathermy: LEEP (loop electro-surgical excision) or LLETZ (large loop excision of the transformation zone) or DLE (diathermy loop excision): (A wire loop is used through which an electrical current is passed at variable power settings. Various shapes and sizes of loop can be used depending on the size and orientation of the lesion. The transformation zone and lesion are excised to an adequate depth which in most cases is at least 8mm, extending 4-5 mm beyond the lesion. A second pass with a more narrow loop can also be done to obtain an endocervical specimen for further histologic evaluation.)
- Description – complete cone or fragmented; colour, presence of irregularities, erosions, polyps (size, shape, location), cysts, previous biopsy sites etc.
- Diameter and depth of the cone to be taken. Ink the surgical margin/surface
- Take the length of the endocervical canal
- Ideally the cone when taken should be marked by the clinician at the 12 o'clock position (centre of anterior lip) to enable identification of the various quadrants
- If the 12 o'clock position has been marked give bits representing each quadrant (Fig 1.4)
- Cut the anterior surface and open it and fix for 24 hours unless it is urgent when sectioning may be done immediately



(Fig 1.4)

- Take serial bits, maximum of 3mm thickness (see figure above), in a clockwise direction, each in a separate block. The squamo-columnar junction (SCJ) should be present in every bit. Trim the periphery if necessary and embed separately for involvement of surgical margin. Mark the embedding surfaces serially
- Do not compromise on the number of blocks
- If tumour is seen grossly then one bit from each quadrant is adequate

- If the cone is not marked then serial sectioning and blocking is to be done without labeling the quadrants
- Bread slice in small loop excision
- The other method of cutting a cone is shown below (Fig 1.5). Either way the entire cone is embedded.

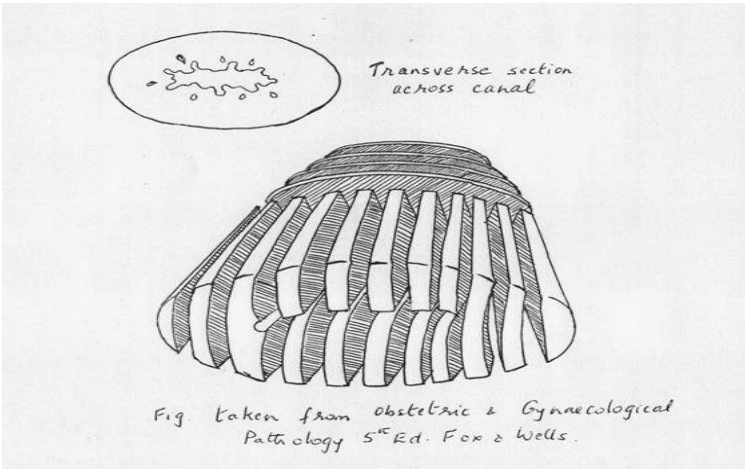


Fig 1.5

- "Critical Tips for all Cervical Biopsies"- punch, wedge, cone, LEEP, etc
- Always include SCJ in sections.
- Mark mucosal surface for embedding.
- Submit entire tissue if tumour not seen grossly and if small.
- Label serial sections clearly,1,2,3, etc

A I & B I CERVIX AND UTERUS -ANATOMY

Reproductive Organs

- Uterus (anteverted and anteflexed position)
- Fundus
- Body
- Isthmus
- Cervix (internal os and external os clearly demarcated)
- Endometrial cavity (thin slit-like)
- Vaginal canal (with rugae)
- Posterior fornix (deepest part)
- Broad ligament (as thin peritoneal fold)

- The Uterus is a pear-shaped organ: fundus, body, isthmus, cervix. Layers: endometrium (functional and basal layers), myometrium (smooth muscle), perimetrium (serosa) (Fig 1.5)
- Cervix — ectocervix with stratified squamous epithelium; endocervical canal lined by mucin-secreting columnar epithelium; transformation zone is clinically important for neoplasia screening.

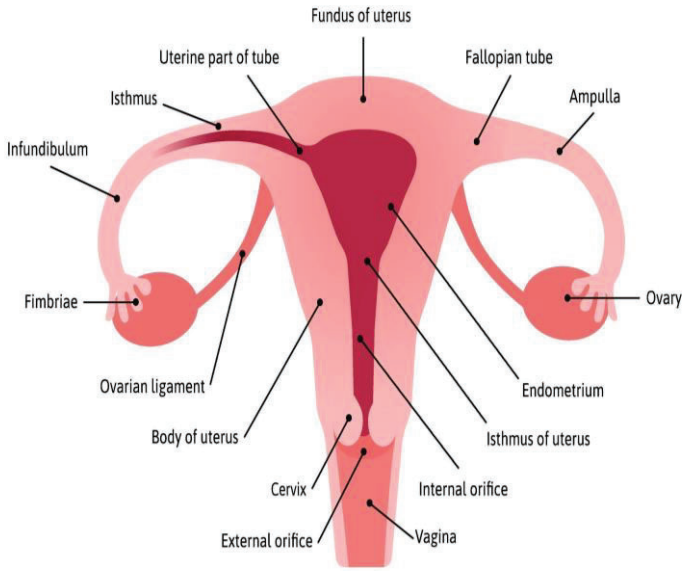


Fig 1.6

A II &B II HYSYERECTOMY SPECIMEN -GROSSING TECHNIQUES

1. Uterus and Cervix: Types of Surgery

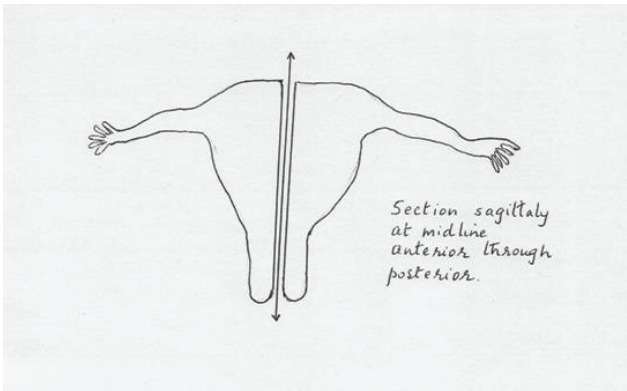
- Simple hysterectomy: Removal of uterus and cervix.
- Hysterectomy with bilateral salpingo-oophorectomy: Removal of uterus, cervix, both tubes, and ovaries.
- Subtotal hysterectomy**:** Cervix not removed.
- Radical (total abdominal) hysterectomy: Removal of uterus, cervix, both tubes and ovaries, upper one-third of vagina, and pelvic lymph nodes.

Note: When describing the specimen grossly, mention the type of surgery if known (e.g. total or radical hysterectomy). Otherwise, describe the organs received, such as "uterus with cervix, both tubes, and ovaries."

Grossing Technique

1. Weigh the specimen (uterus ± adnexae).
   - Uteri are often received opened along the anterior wall by the surgeon.

2. If unopened, identify anterior and posterior surfaces — note that the posterior peritoneal reflection is lower.
3. Section longitudinally through the uterine cavity, endocervical canal, and squamo-columnar junction (SCJ) (Fig 1.7)
    - This allows half the specimen to be preserved for mounting or photography.



(Fig 1.7)

Measurements

Record:

- Length of uterine cavity
- Length of endocervical canal
- Average myometrial thickness (give range if asymmetric)

Description

- Endometrial cavity: Distortion, polyps, or cysts — describe and measure.
- Fibroids (leiomyomata): Number, size (smallest/largest), location (submucosal, intramural, subserosal), and changes (hyalinisation, red degeneration, calcification, cystic change, necrosis).
- Serosa: Adhesions or nodules.
- Cervix: Examine SCJ for erosions, nabothian cysts, or polyps — describe size, surface ulceration, etc.

Tip: After initial bisection, make additional parallel cuts through each half to reveal deeper pathology.

Alternatively, the uterus may be sliced coronally into parallel sections (as shown in the diagram below FIG 1.8).

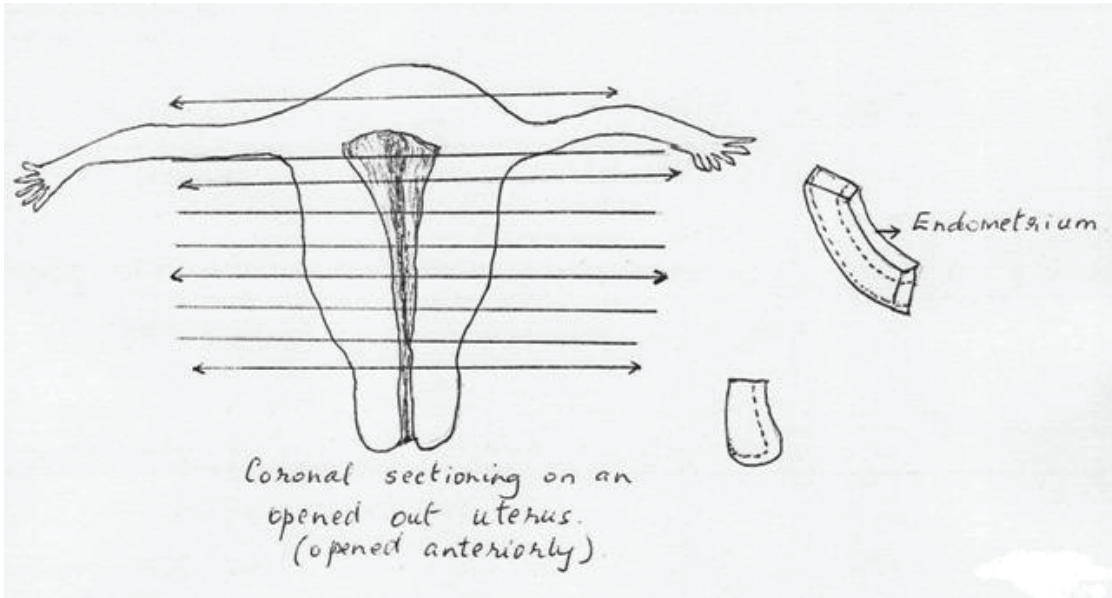


Fig 1.8: Sectioning of uterus coronally.

Bits to be Taken (1.9)

Label	Site	Details
A	Cervix – anterior lip	Include SCJ and any abnormal area
B	Cervix – posterior lip	Include SCJ and any abnormal area
C & D	Endo-myometrium	Two bits from opposite walls; ensure full thickness if needed
E, F, G	Leiomyomata	One from largest (through centre); maximum of 2–3 routinely
+	Endometrial polyps	Include with endomyometrium if small; separate if large (see fig 1.9 below)

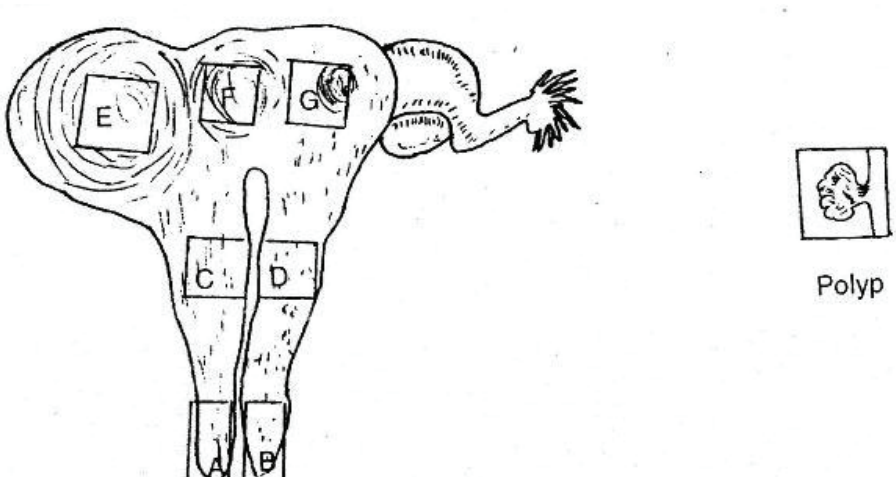


Fig 1.9

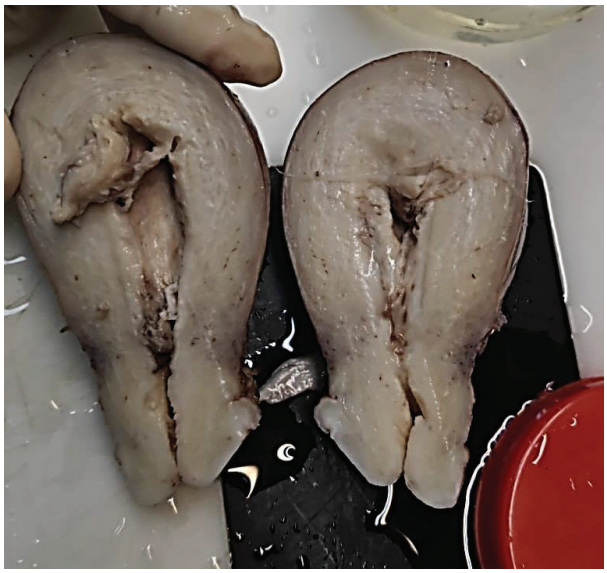


Fig 1. 10 Shows endometrial cavity with a polyp (obtained in a surgical Pathology lab) which can be given in 2 bits.

Carcinoma of the Cervix

Gross Description

- Note whether the growth is polypoid or ulcerative.
- Record size, extent of involvement (entire or part of cervix), and site (specify which portion).
- Comment on infiltration into the isthmus, myometrium, and surrounding structures.

Bits (Fig 1.11)

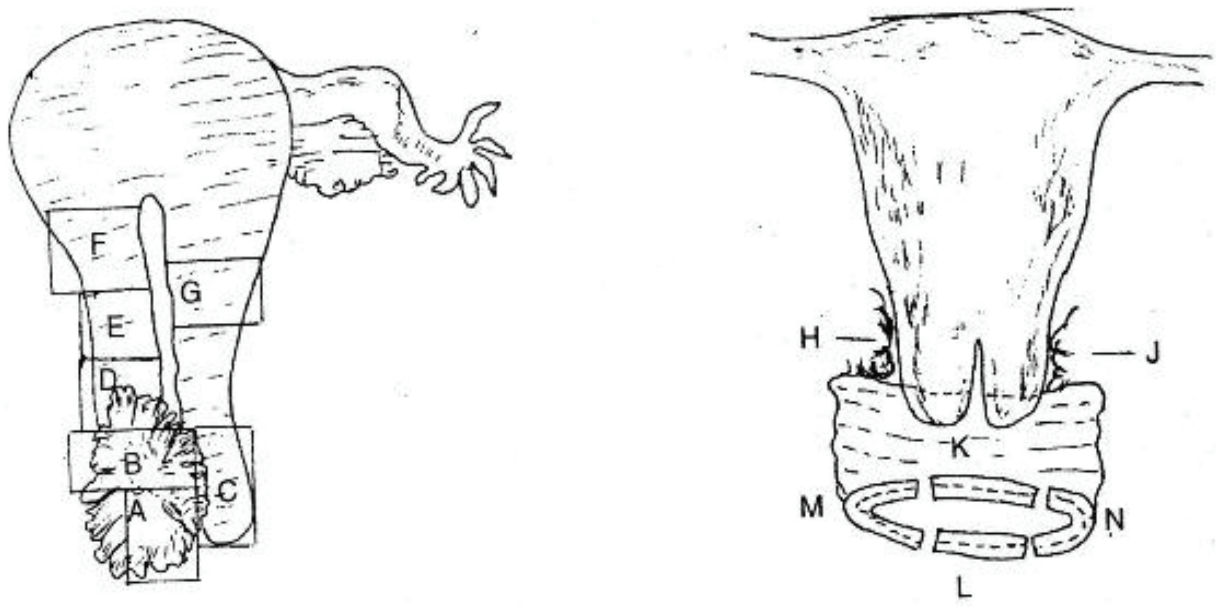


Fig: 1 .11: Carcinoma Cervix **Label**	**Description**
A & B	Two bits from the cervix with tumour – routinely adequate.
C	Uninvolved cervix (opposite lip).
D, E, F	Isthmus, endometrium, and myometrium — to be taken in continuity (F may be avoided if the tumour is very small).
G	Opposite endometrial wall.
H & J	Parametrium (right and left).
K, L, M & N	Vaginal cuff — to be bitted entirely and marked as anterior, posterior, right, and left lateral.
P & Q	Fallopian tubes and ovaries as usual; if involved, take more bits from suspected areas.

Lymph Nodes

Groups should be separately marked and sent by the clinician:

Node Group	Side	Remarks
External iliac	Right / Left	…….
Internal iliac	Right / Left	…….
Obturator	Right / Left	……
Common iliac	Right / Left	…….
Others	Specify …	

- Record size of smallest and largest nodes, number in each group, and number showing gross involvement.
- Grossing: Bisect each node. If >5 mm, give only half; if ≤5 mm, submit entire node.
- Mark the half not given with **eosin** for reference, to avoid duplicate grossing.

Carcinoma of the Endometrium

Gross Description

- Note size and appearance of tumour: solid, cystic, papillary, colour, ulceration, necrosis, etc.
- Record depth of myometrial invasion (<50% or >50%).
- Describe involvement of cervix, isthmus, parametrium, or other adjacent structures.

Bits (Fig 1.12)

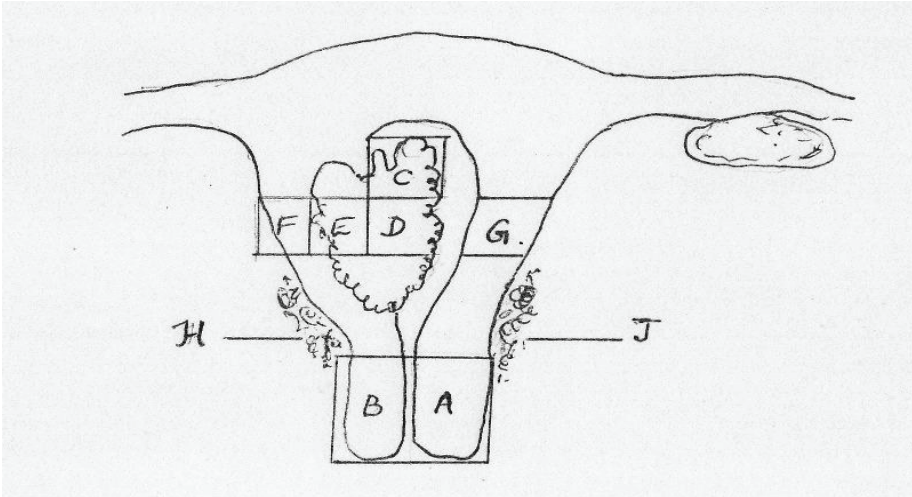


Fig 1.12: Shows bits in carcinoma of endometrium

Label	Description
A, B	Cervix as routine; more bits if involved.
C, D, E, F	Tumour – four bits to include neoplasm and, in continuity, the full thickness of endo-myometrium from mucosa to serosa.
G	One bit from uninvolved endometrium (opposite wall).
H & J	One bit each from right and left parametrium.
Fallopian tubes and ovaries	Routine unless involved — then take more bits.
Lymph nodes	As for carcinoma cervix.

Note:

- Record the maximum thickness of myometrial invasion.
- Invasion < half → Stage IB; ≥ half → Stage IC.
- Ideally, measure total myometrial width and depth of invasion together.
- CAP (College of American Pathologists, 2023) recommends measuring *exact depth of invasion (in mm)* and *total myometrial thickness* in the same block.

Fig 1.13 shows the gross appearance of a carcinosarcoma (infiltrating uterine cavity & myometrium)

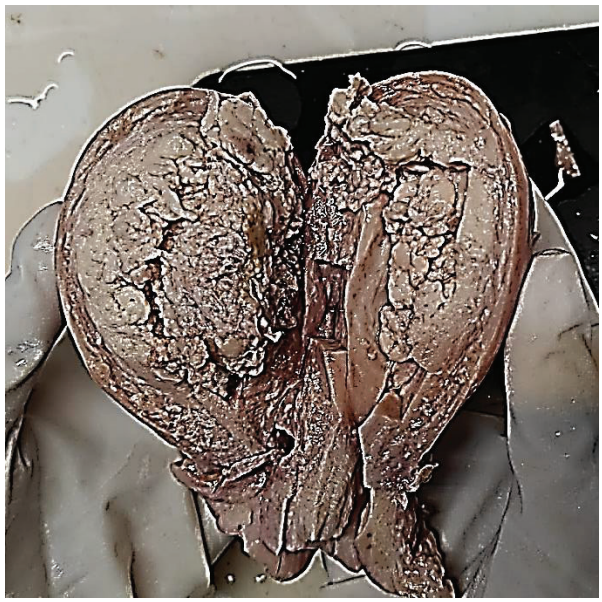


Fig 1.13

In Cases with No Gross Tumour

(e.g. superficial carcinoma, endometrial hyperplasia, atypical hyperplasia)

- Preferably sample the entire endometrium or at least six blocks — three from the anterior and three from the posterior walls.
- Each section should include endometrium–myometrium–serosa in continuity.
- Lymph nodes: as for carcinoma cervix.

Pelvic Exenteration

Performed in advanced carcinomas of the female genital tract (especially cervix).

Type	Structures Removed
Anterior Pelvic Exenteration	Uterus, cervix, tubes & ovaries, upper vagina, pelvic lymph nodes, urinary bladder, segments of ureters and urethra
Posterior Pelvic Exenteration	Uterus, cervix, tubes & ovaries, vagina, rectum, pouch of Douglas, pelvic lymph nodes

Grossing Guidelines

- Gross uterus, cervix, and appendages as described for individual organs.
- Take representative bits from adjacent structures (rectum, bladder wall, ureter) to assess invasion.
- Include surgical margins of rectum, bladder, ureter, and urethra.
- Sample pelvirectal lymph nodes separately.

A III CERVIX GROSS PEARLS

- **Well-defined cysts:** Nabothian cysts; may be multiple, sometimes arranged along the ectocervix, TZ (transformation zone), and extending parallel to the endocervical canal. Cysts contain mucus.
- **Myxoid tumour-like appearance:**
  1. Extensive myxoid change & stromal reaction
  2. Aggressive angiomyxoma
  3. Angio -myofibroblastoma
  4. Sarcoma botryoides (in children & infants)
- **Small superficial ulcer:** Erosion or benign ulcer; both associated with chronic cervicitis microscopically.
- **Slender & small polypoid mass:** Benign endocervical polyp; smooth grey-white cut surface, generally pedunculated.
- **Small to large polypoid mass:**
  1. Globular with whorled cut surface → leiomyomatous polyp
  2. Fibro-epithelial polyp – stromal polypoid mass covered by squamous epithelium.
  3. Firm, grey-white, friable, sometimes with surface ulceration → carcinoma (polypoid type)
- **Cauliflower-like mass with grey-white cut surface:** Carcinoma; generally extends into cervical stroma and upwards into uterine isthmus.
- **Elongated endocervical canal with thickened ectocervical mucosa:** Uterine prolapse; mucosa thickened due to parakeratosis & hyperkeratosis. Small decubitus ulcers may be present.
- **Irregular small to large ulcer, firm base, poorly defined margins, everted:** Carcinoma cervix (ulcerating type).
- **Tumour with brown-black pigment:** Haemorrhage in carcinoma; malignant melanoma (rare in cervix).
- **Polyp extending from uterine cavity:**
  1. Endometrial polyp (benign)
  2. Leiomyomatous submucosal polyp; round, globular, whorled cut surface

A IV CERVIX MICROSCOPY

1) Metaplasias :

The Mullerian epithelium which lines most of the female genital system is well known for its capacity to differentiate into epithelium of ciliated, mucinous, endometriod, transitional and squamous types.

- Squamous metaplasia : occurs commonly in the reproductive age group due to chronic inflammation as a result of proliferation and metaplasia of reserve cells. The stages are i) reserve cell hyperplasia ii) immature squamous metaplasia wherein the epithelium shows multilayering with mucus cells in the upper reaches iii) mature squamous metaplasia where the full thickness is replaced by cells showing intercellular bridges.
- Transitional metaplasia : seen in the exocervix of older women and often associated with atrophy. Resembles the transitional epithelium of urinary bladder and involves the entire thickness of the mucosa. IHC shows +ve CK 13, 17, & 18 as in normal urothelium.
- Tubal metaplasia : cervical epithelium contains 3 cell types as in normal Fallopian tubes ciliated, secretary and intercalated cells. Deep seated glands may be mistaken for carcinoma. Usually found following conisation so represents an aberrant differentiation following injury.
- Intestinal and oncocytic metaplasia occur but are extremely rare.

2) Inflammatory conditions :

- *Herpes Simplex* infection is quite common. Microscopy shows nuclear homogenization, subepithelial cleavage leading to bullae formation but rarely multinucleation.
- *Chlamydia trachomatis* : common venereal infection. Histology shows non specific chronic inflammation with lymphoid follicles. Organisms are not seen at histology but can be demonstrated by IHC.
- *Amoebiasis* occurring in the cervix produces a mass which can be mistaken for carcinoma. Diagnosis lies in the demonstration of Entamoeba histolytica on either the H &E stain or by special stains like the per iodic acid Schiff and Iron Haematoxylin stains.
- *Actinomycosis* is seen in IUCD users : organisms are seen as cotton wool colonies and stain +ve for silver stains(Gomori's methenamine silver stain).
- *Parasitic infections* like Bilharziasis may be observed, diagnosed by demonstration of the ova.- *Condyloma acuminatum* : -Grossly polypoid lesion characterized by undulating epithelium with papillomatosis, acanthosis, koilycytosis and variable inflammation of the stroma.
- - Presence of *severe atypia* should be reported as CIN II or CIN III. Mild atypia is part of the diagnostic feature of the lesion.
- - HPV 6 & 11 are the etiologic factors in 70-90% of the cases, occasionally HPV 16 may be encountered which is associated with high grade dysplasia. Other morphologic variants of HPV infection are more common and include "flat", "spiked" and "inverted" condyloma and warty atypia.
- Histology : normal basal cells, expanded parabasal cell layer, orderly maturation & mitotic activity with no abnormal mitoses. Koilocytotic atypia seen. Proliferative rate is higher than in metaplastic squamous epithelium.

3) Non –neoplastic glandular lesions :

- -*Endocervical polyp* : exaggerated versions of polypoid cervicitis, composed of a fibrovascular stalk and covering endocervical epithelium with squamous metaplasia. Stroma shows varying degrees of inflammation.
- *Tunnel Clusters :* localized proliferations of endocervical glands with side channels growing out from them. Cystic change (type B) may occur with inspissated secretions. In Type A lesions the lobular configuration is retained with absence of cystic change.
- *Microglandular hyperplasia* : originally described in women on oral contraceptives and in pregnancy, can also be seen in the absence of these. Microscopy shows complex proliferations of small glands lined by flat cells with little or no atypia. Accompanying squamous metaplasia may simulate a carcinoma. The proliferation is negative for CEA which differentiates it from an adenocarcinoma.
- *Diffuse laminar endocervical glandular hyperplasia* : is a non –neoplastic condition characterized by proliferation of medium sized, evenly spaced well differentiated glands within the inner third of the endocervical wall, sharply separated from the underlying stroma and often accompanied by stromal inflammation. Differential diagnosis is adenoma malignum - Stromal infiltration, desmoplastic stromal response and cytologic atypia are absent in the glandular hyperplasia.
- *Lobular endocervical glandular hyperplasia* : distinct lobular proliferation of small to medium sized glands often centered around a larger central gland. Differential is again adenoma malignum and the same features as stated above differentiate the two.
- *Mesonephric duct rests*: can show atypical hyperplastic change. They are superficially located.
- *Endometriosis* : occur grossly as blue red nodules. Both glands and stroma should be present, if only stroma prevails it is known as “stromatosis”

4) Non neoplastic stromal reactions :

- *Multinucleated stromal giant cell response* :may appear polypoid. An oedematous cervical stroma shows reactive fibroblastic/myofibroblastic cells which may be mistaken for malignancy.
- *Decidual reaction :* occurs during pregnancy as multiple yellow elevations of cervical mucosa, soft & friable, bleeds easily on trauma. Microscopically decidual cells with abundant pale pink cytoplasm and bland nuclei. IHC –ve for keratin.
- *Placental site nodule* : well defined hyalinised lesion beneath the mucosa. Microscopy ; intermediate trophoblastic cells show cytoplasmic vacuolation. Some nuclear atypia may be seen. DD is carcinoma and cartilage. Cells are reactive for keratin and human placental lactogen.
- *Fibroepithelioma.*- Has a fibrovascular stalk covered by squamous epithelium but with no changes of koilocytosis

5) Updated WHO Classification of Cervical tumours :

The 2020 WHO classification of female genital tumors (5th edition) includes a detailed classification of tumors of the uterine cervix (sections on squamous , adenocarcinoma and other epithelial tumours. Upto germ cell tumours were updated, the rest remain the same)

- **Classification**

  Squamous epithelial tumours

  - mimics of squamous precursor lesions
    - squamous metaplasia
    - atrophy of the uterine cervix
  - squamous cell tumours and precursors
    - condyloma acuminatum
    - squamous intraepithelial lesions
    - squamous cell carcinoma, HPV-associated
    - squamous cell carcinoma, HPV-independent
    - squamous cell carcinoma NOS

  Glandular tumors and precursors

  - Benign glandular lesions
    - endocervical polyp
    - Mullerian papilloma
    - Nabothian cyst
    - tunnel clusters
    - microglandular hyperplasia
    - lobular endocervical glandular hyperplasia
    - diffuse laminar endocervical hyperplasia
    - mesonephric remnants and hyperplasia
    - Arias-Stella reaction
    - endocervicosis
    - tuboendometrioid metaplasia
    - ectopic prostate tissue
  - Adenocarcinomas
    - adenocarcinoma in situ, HPV-associated
    - adenocarcinoma, HPV-associated
    - adenocarcinoma in situ, HPV-independent
    - adenocarcinoma, HPV-independent, gastric type
    - adenocarcinoma, HPV-independent, clear cell type
    - adenocarcinoma, HPV-independent, mesonephric type
    - other adenocarcinomas

- Other epithelial tumours
  - carcinosarcoma
  - Adeno-squamous and mucoepidermoid carcinomas
  - adenoid basal carcinoma
  - carcinoma of the uterine cervix, unclassifiable
- Mixed epithelial and mesenchymal tumours
  - adenomyoma
  - adenosarcoma
- Germ cell tumours

MESENCHYMAL TUMOURS AND TUMOUR LIKE CONDITIONS

Leio- myosarcoma

Endometroid stromal sarcoma, low grade

Undifferentiated endocervical sarcoma

Sarcoma Botryoides

Alveolar Soft part sarcoma

Angiosarcoma

Malignant peripheral nerve sheath tumour

Leiomyoma

Genital rhabdomyoma

Post operative spindle cell nodule

MELANOCYTIC TUMOURS

Malignant melanoma

Blue Naevus

MISCELLANEOUS TUMOURS

Tumours of germ cell type

- yolk sac tumour
- dermoid cyst
- mature cystic teratoma

LYMPHOID AND HAEMOPIETIC TUMOURS

Malignant lymphoma (specify type)

Leukaemia (specify type)

SECONDARY TUMOURS

Highlights:

- All carcinomas of the cervix are broadly divided into squamous cell types and others. Some of the morphologic variants of **squamous cell carcinomas (SCC)** are- papillary squamous cell carcinoma, lymphoepithelioma like carcinoma, verrucous carcinoma (see fig 1. 16.), condylomatous carcinoma, spindle cell carcinoma (carcinosarcoma).
- The WHO 2020, emphasises cervical squamous cell carcinomas as *HPV-associated* vs *HPV-independent* types of carcinoma. HPV associated high risk types are primarily 16,18; also 31,33,35,39,45,51,52,56,58,59,68,73, & 82.

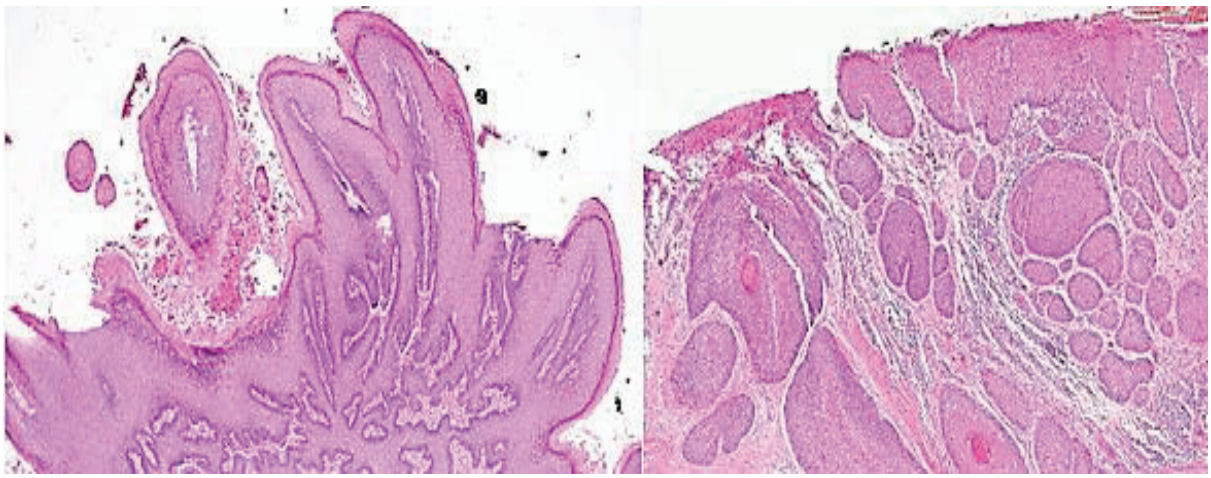


Fig 1.14: shows squamous cervical carcinoma, verrucous in morphology with infiltration into stroma on the right.

Cervical Tumours WHO 2020 : Key updates

- Dual categorisation: HPV-associated (HPVA) and HPV-independent (HPVI) carcinomas.
- Squamous cell carcinoma remains the most frequent type, now subclassified by HPV status.
- **Adenocarcinomas** include:
  - Usual type (HPV-associated)
  - Mucinous types – intestinal, gastric (HPV-independent), signet-ring cell

- Clear cell adenocarcinoma
- Mesonephric and mesonephric-like adenocarcinoma

- **Precursor lesions:** Intraepithelial neoplasias are defined by HPV status (p16 IHC as a surrogate marker).
- **Grading of squamous cell carcinomas :**
  - Grading of squamous cell carcinomas is usually done on a 3 tier system- well, moderately and poorly differentiated. Alternatively the classification and grading of Reagen and co-workers can be used : large cell keratinizing, large cell non-keratinizing and small cell carcinoma. This system originated on the basis that large cell keratinizing carcinomas are radio-resistant relative to non-keratinising carcinomas. The small cell carcinoma has the worse prognosis. The main disadvantage of this system is that the small cell variant of this classification may be misinterpreted by the clinicians for the oat cell type of carcinoma which it is not, the latter having an even worse prognosis and a different line of therapy as compared to the squamous cell carcinoma.
- **Cervical intraepithelial neoplasia (CIN) :** usually develops in the metaplastic squamous epithelium of the transformation zone of the cervix and thus lies on the ectocervix in the younger women and within the endocervical canal in the older patients.
  - Differentiation of CIN is rarely uniform throughout the affected area- it tends to be better differentiated towards the ectocervical region and lesser differentiated towards the endocervical region. the progression to squamous cell carcinoma occurs in about 22% of cases with CIN 3 within 20 years of diagnosis
  - CIN I : cytoplasmic maturation occurs in superficial 2/3 rds of the epithelium. Loss of polarity, nuclear enlargement and hyperchromasia as well as mitotic activity are seen in the lower one third. (see illustration below).
  - CIN 2 : the above mentioned changes are seen in the lower 2/3rds and maturation seen in the upper third.
  - CIN 3 : the changes are seen in the entire thickness of the metaplastic epithelium. HPV types 16 & 18 are most commonly encountered in CIN 3 though they may also be associated with CIN 1 & 2. (see illustration (Fig 1.15) below)

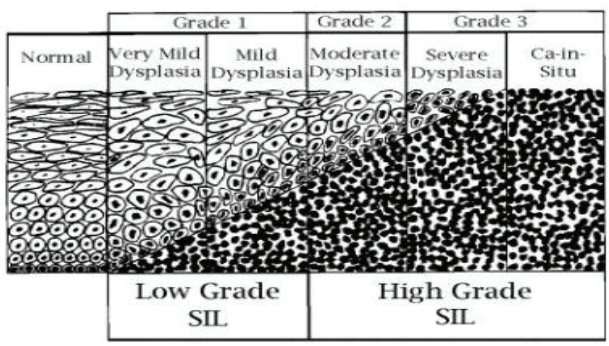


Fig 1.15

- **IHC of carcinomas cervix** : all are +ve for keratins (wide range). CEA +ve in 90% of cases. p63+ve, cathepsin B +ve (not as frequent as in adenocarcinoma), β hCG +ve. ER decreased expression as compared to the normal mucosa.

There is increasing evidence that **p16/Ki-67 dual-staining cytology** can be used as an alternative biomarker, showing overall high sensitivity and specificity for identifying high-grade CIN and cervical cancer. In cells with HPV infections, E7 protein competes with other cell cycle regulatory proteins like pRb, resulting in the release of E2F1 from pRb and the activation of the cell cycle. The disturbance of pRb-E2F1 pathway by E7 induces the overexpression and accumulation of p16 in the cells through a negative feedback loop causing cell cycle activation. Strong and diffuse cytoplasmic and nuclear expression of p16 in cervical squamous cell carcinomas is predominantly associated with HR-HPV infection. Therefore, p16 is considered a surrogate marker for persistent HR-HPV infection and p16 overexpression has been observed in the majority of cervical precancers and cancers. p16 is a tumor suppressor and on the other hand Ki-67 is a cellular marker for proliferation. The over-expression of p16 and expression of Ki-67 under physiological situations are mutually exclusive and are not seen in the same cervical epithelial cell. Therefore, the p16/Ki-67 co-expression implies deregulation of the cell cycle induced by HR-HPV and detection of p16/Ki-67 co-expression can serve as a marker to predict the cell transformation by HR-HPV and the presence of high-grade CIN lesions .

- Some squamous cell carcinomas show intense infiltration by eosinophils with or without accompyning blood eosinophilia, products of eosinotactic substances are produced by tumour cells.
- A minority of SCCs show mucin in cytoplasm. Should be termed as squamous cell carcinoma with mucin secretion and not adenosquamous carcinoma.
- Basaloid squamous cell carcinoma : prominent peripheral palisading, squamous differentiation within islands, infiltrative growth, minimal stromal reaction and aggressive behaviour.
- Spread of SCC : sequential spread to paracervical, hypogastric, obturator, external iliac groups etc. Distant spread to lungs and bone.
- Microinvasive carcinoma : 4 -7% of CIN are associated with superficially invasive ie.not more than 5mm from the basement membrane carcinoma. Criteria for invasion include - desmoplastic response in the adjacent stroma, blurring of the epithelial-stromal interface, focal conspicuous maturation of the neoplastic epithelium with prominent nucleoli, scalloping of margins at the epithelial stromal interface and loss of polarity of nuclei and absence of palisading.
- Adenocarcinomas **:** Constitute 5-15% of all carcinomas of the cervix. There is an association between endocervical adenocarcinoma, ovarian mucinous adenocarcinoma and tubal adenocarcinoma.Can be of the conventional type (*endocervical type*)(70% of cases), or special types as given below. The conventional type is composed of neoplastic glands of the endocervical mucinous type ranging from well to poorly differentiated. Simple complex glands, solid sheets or cords of cells are seen.

- Histochemistry : Alcian Blue and Mucicarmine +ve in all conventional adenocarcinoma, similar to insitu carcinoma but different from the normal cervical mucus.
- IHC : CK, CEA, EMA +ve ; vimentin –ve. p16 ie ink 4a +ve (demonstrates link with HPV virus); HPV 16 & 18 +ve; ER, PR +ve in 1/4th cases; peptide hormones & amylase +ve.
- Prognosis : overall prognosis is less favourable as compared to SCC. The endometriod variety behaves in a slightly better manner. CA 125 may be elevated and is a poor prognostic marker.
- DD : from an endometrial adenocarcinoma extending into cervix is difficult. Some of the differentiating features are :

  The 5th edition of the WHO Classification of the cervix has incorporated the new International Endocervical Adenocarcinoma Criteria and Classification (IECC) system for endocervical adenocarcinomas, based on morphological features linked to aetiology (Human Papillomavirus -HPV infection). It predicts patients at risk of lymph node metastases, recurrences , prognosis and death. The incidence of invasive endocervical adenocarcinomas (ECAs) and precursors has increased, representing about 25% of all cervical cancers.

  The major change in WHO 2020 classification is that ECAs are divided into HPVAs and HPVIs. The only direct precursor glandular lesion for invasive adenocarcinomas is in situ adenocarcinoma, similarly divided into HPVA in situ adenocarcinoma (most frequently associated with HPV 18 and 16 types) and HPVI in situ adenocarcinoma. HPVA in situ adenocarcinoma is represented by usual type and less frequently by stratified mucin producing intraepithelial lesion (SMILE) while HPVI in situ adenocarcinoma is represented by atypical lobular endocervical glandular hyperplasia (LEGH) and gastric type. Both atypical lobular endocervical glandular hyperplasia (LEGH) and gastric type in situ adenocarcinoma are precursors of invasive adenocarcinoma of gastric type, the most frequent HPVI type of ECA. Other precursor lesions for HPV-independent ECAs (such mesonephric, clear cell, endometrioid type) have not been described yet. *Usual type invasive adenocarcinoma*: represents the vast majority (80%) of ECAs and has a characteristic morphology, architecturally represented by glands of various sizes and shapes, lined by columnar tumour cells with intracytoplasmic mucin depletion (although up to 50% of tumour cells may contain mucin) and nuclear pseudostratification.[7] The nuclei are elongated, hyperchromatic and cigar-shaped, while mitotic figures and apoptotic bodies are easily identifiable at scanning magnification.
- **Insitu adenocarcinoma :** is now accepted as a well recognized entity. The features for diagnosis are i) nuclear hyperchromasia & pleomorphism

  ii) ↑ mitoses

  iii) ↑ apoptotic bodies

  iv) +ve for CEA

DD : tubal metaplasia & tubal endometrial hyperplasia

IHC **:** carcinoma in situ is vimentin –ve, diffusely +ve for ink 4, bcl 2 –ve, ↑index for MIB -1

- **Morphologic variants of adenocarcinoma :**
  - *endometriod adenocarcinoma* ; well differentiated tumour, can be associated with metachronous / synchronous endometriod carcinoma of the ovary. DD : primary from endometrium with endocervical spread.
  - *papillary serous carcinoma* :similar to endometrial & ovarian tumours.
  - *adenoma malignum* : minimum deviation adenocarcinoma constitutes 1-2% of all cervical carcinomas. This is so well differentiated structurally and cytologically that it could easily be missed if the deeply situated irregularly branching abnormal glands are not carefully examined. Called malignant due to presence of distorted glands and irregular outlines deeply positioned in the cervix and with stromal response as well as vascular and perineural invasion. Mucin produced is of the neutral type. CEA +tivity differentiates it from benign lesions like microglandular hyperplasia etc.
  - *DD*: 1) endocervical type of cervical adenomyoma-Deeply located endocervical glands (in which stromal response & architectural disarray are absent, CEA is -ve.) 3) Conventional adenocarcinoma cervix (has to be differentiated for prognosis and treatment purposes : see the table below)

	Conventional Adenocarcinoma	**Adenoma malignum**
Differentiation	Well differentiated But admixed with less differentiated Foci	Well differentiated throughout
Stromal response	+ve	+ve
CEA	+ve	+ve
HPV association	+ve (16, 18)	-ve
Ink 4 (HPV associated)	+ve	-ve
Mucin	Alcian blue +ve (acid mucins) Mucicarmine +ve	Neutral type PAS +ve
P53	Over expressed frequently	Lacks p53 expression
Association with P-J syndrome	-ve	+ve

  - *Villoglandular papillary adenocarcinoma :* exophytic polypoid lesion with papillae lined by endocervical, endometrial or intestinal type epithelium with mild atypia. The

superficial part looks like colorectal villous adenoma. Evidence of HPV infection observed. Prognosis is excellent.

- *Adenosquamous carcinoma :* Mixture of adnocarcinoma and SCC ; common during pregnancy.
- *Glassy cell carcinoma :* Distinct type of poorly differentiated adenosquamous carcinoma, occurs in the younger age group and associated with pregnancy. Cytoplasm is moderate with a ground glass or finely granular appearance; PAS +ve cell wall; large nuclei with prominent nucleoli. Increased mitoses. Stroma shows eosinophils. Foci of squamous and glandular differentiated elements may be seen.
- *Clear cell carcinoma :* (mesonephroid carcinoma of mullerian origin). Exophytic tumour ; glands are lined by large cells with abundant clear cytoplasm. Hobnail cells are common as also intraglandular papillary projections. Common in young patients, a smaller peak at 70 years of age. A relationship with DES exposure during prenatal period is known *DD:* Microglandular hyperplasia Aria Stella reaction IHC : CK, CAM 5.2, 34 βE12, CEA, CD15,vimentin bcl 2 and CA125 +ve . Variable +vity for ER. Negative for PR and CK 20. Prognosis is good in this case.
- *Other types of adenocarcinomas :* are adenoid cystic, adenoid basal carcinoma and mesonephric adenocarcinoma.
- *Extra pulmonary small cell carcinoma / atypical carcinoid and neuro endocrine carcinoma are the other carcinomas.*

Differences between small cell carcinoma & small cell variant of SCC

	Small cell carcinoma	Small cell variant of SCC
Precursor lesion	CIN changes in adjacent epithelium is rare. Endocrine cell hyperplasia	CIN +ve
Cell of origin	Argyrophil/ neuro endocrine	Basal reserve cell
HPV assoc	+ve	+ve
Histology	Organoid arrangement in better differentiated tumours, else, insular, trabecular & rosettes. ↑ mitoses, areas of necrosis ++	Diffuse sheets and large nests Necrosis & mitoses may be seen
Combinations	May be combined with adenocarcinoma Squamous cell carcinoma (amphicrine car)	Nil
Argyrophilic	+++; NSE +ve, chromogranin, synaptophysin, 5HT, etc +ve	Argyrophilia very rare; others -ve
CK, CEA	+ve	+ve
Behaviour	Clinically aggressive	Less aggressive than small cell carcinoma

Metastatic carcinomas : common sites of metastasis are from the ovary, large bowel,stomach breast and kidney (clear cell carcinoma- RCC). Mesonephroid adenocarcinoma and RCC are both +ve for vimentin , but RCC is also +ve for CD10 and the former is not.

A V AND B V SYNOPTIC REPORTING – CERVIX & UTERUS

A. Cervical Biopsy (tumour & non tumour)

Patient data :

Name ..... Age .....Date of birth ....……..... Sex....….

Hospital .....……..……..... Hospital No .....……...…… ...

Date of request ……..... Date of reporting……..….....

Biopsy No ……..….

Referring Clinician …….....…….....

Site of biopsy …….

Gross :

Wedge ☐ Cone ☐ Loop ☐ Punch ☐ Other ☐

Measuring ….....mm **x** …….. mm x …… mm

The cervix was divided into …….. blocks, labelled ……..

Separate fragment(s) labelled ……., was (were) also received.

No …….. block, labelled……

Microscopy:

Non tumour :

Non- specific chronic cervicitis ☐

Chronic papillary cervicitis ☐

Chronic herpetic cervicitis ☐

Nabothian cysts ☐

Squamous metaplasia ☐

Ulceration ☐

Regenerative change ☐

Wart virus (HPV) infection Absent ☐ Koilocytosis ☐

Tumour :

CIN (cervical intra-epithelial neoplasia) Absent ☐

Epithelial changes of uncertain significance☐

CIN 1☐ CIN 2 ☐ CIN 3 ☐

Endocervical edge Clear ☐ Involved ☐

Ectocervical edge Clear ☐ Involved ☐

Deep lateral edge Clear ☐ Involved ☐

Endocervical epithelium Normal ☐ Low grade CGIN ☐

High Grade CGIN☐

Endocervical epithelium at end of canal Yes ☐ No ☐

Invasive malignancy Absent ☐

Microinvasive ☐

Squamous cell carcinoma ☐

Adenocarcinoma ☐

Adenosquamous carcinoma ☐ Others ☐

Microinvasive carcinoma, early stromal invasion ☐

Microinvasive carcinoma, small confluent tumour ☐

Maximum horizontal dimension …….. mm Single focus ☐ multiple foci ☐

Maximum depth of invasion ……mm

Is there lymphovascular space involvement? Yes ☐ No ☐

Distance from closest resection margin (minimum tumour free margin)……..mm*

Comments…….

Signature .....…… Date……..

Useful hints :

1. Margins need not be commented on in punch biopsies
2. Microinvasive carcinoma : upto 5 mm in depth. Early stromal invasion : invades to a depth of 1 mm or less. Depth of 3 mm is taken by most authors as the upper limit for conservative management.
3. Microinvasive adenocarcinoma is a possible but difficult diagnosis. Criteria include:
   a) obvious invasion to 5mm or less
   b) usually complete obliteration of the normal

c) endocervical crypts
d) extension beyond the normal glandular field
e) a stromal response characteristic of invasive carcinoma

7. Microinvasive carcinoma can only be diagnosed on a cone or large loop excision biopsy specimen in which the lesion is seen in its entirety. **N.B.** If invasive foci are seen in three or more blocks of tissue, the third dimension of the lesion (which is not routinely measured) may exceed 7 mm (i.e. more than Stage IA2).

8.The following detail should be recorded for all cases of invasive disease : tumour type, tumour grade, size of the lesion-max horizontal dimension, max depth of invasion, the presence or absence of lymphovascular invasion and adequacy of excision.

9. * when all margins are free

Hysterectomy specimen for Cervical Cancer

Gross description

Dimensions of uterus: Length .....mm Transverse .....mm Antero-posterior.....mm

Vaginal cuff: Present ☐ Absent ☐ Length .....mm

Maximum dimension of tumour: ….....mm

Histology

Type: squamous carcinoma ☐

adenocarcinoma ☐

adenosquamos ☐

other (specify)…….

Histological differentiation: Well ☐ Moderate ☐ Poor ☐

Tumour size: maximum horizontal dimension .....mm

depth of invasion .....mm

Distance from closest resection margin (minimum tumour-free rim) .....mm position of this
Parametrical involvement: Yes ☐ Right ☐ Left ☐ Both ☐ No ☐

Vaginal involvement: Yes ☐ Distance from vaginal margin .....mm No ☐

Lymphovascular invasion: Present ☐ Absent ☐

CIN: Present ☐

Grade 1 ☐ 2 ☐ 3 ☐ Absent ☐

CGIN: Present ☐ Grade : High ☐ Low☐ Absent ☐

Pelvic nodes including obturator, internal and external iliac)	right ☐ left☐	**Common iliac nodes**	right ☐ left☐
total number of nodes retrieved	…….	total number of nodes retrieved	…….. ….. .
lymph nodes with tumour deposits	…… ……	lymph nodes with tumour deposits	…….. ……
Extranodal spread	Yes ☐ No ☐		Yes ☐ No ☐

Para-aortic nodes: not sampled ☐ positive ☐ negative ☐

Extranodal spread: Yes ☐ No ☐

Endometrium: Normal ☐ Abnormal (please state)

Myometrium: Normal ☐ Abnormal (please state)

Right ovary/tube: Normal ☐ Abnormal (please state)

Left ovary/tube: Normal ☐ Abnormal (please state)

Comments ……

Signature…… Date ……..

FIGO (The Federation of Gynaecology and Obstetrics) Staging for carcinoma of the cervix uteri

Stage 0 Carcinoma in situ , intraepithelial carcinoma

Stage I The carcinoma is strictly confined to the cervix (extension to the corpus should be disregarded.

Stage I A Invasive carcinoma of the cervix, diagnosed only by microscopy. All macroscopically visible lesions even with superficial invasion are Stage IB

Stage IA-1 Microscopically evident stromal invasion no greater than 3mm in depth and 7mm or less in horizontal apread.

Stage IA-2 Stromal invasion >3mm but should not show a depth of invasion of > than 5mm taken from the base of the epithelium, either surface or glandular from which it originates, and a second dimension, the horizontal spread must not exceed 7mm. Larger lesions should be classified as stage IB. Vascular space involvement either venous or lymphatic does not affect classification.

Stage IB Lesions of greater dimension than stage IA-2, whether seen clinically or not.

Stage IB1 Clinically visible lesion less than or equal to 4cm in greatest dimension.

Stage IB2 Clinically visible lesion more than 4cm in greatest dimension.

Stage II Tumour invades beyond uterus but not to pelvic wall or to lower third of vagina

Stage II A without parametrial invasion Stage II B with parametrial invasion

Stage III tumour extends to pelvic wall and or involves the lower third of vagina and or causes hydronephrosis or non functioning kidney

Stage III A involves lower third of vagina but does not extend to pelvic wall

Stage IIIB tumour extends to pelvic wall and or causes hydronephrosis or non functioning kidney

Stage IV A tumour invades mucosa of bladder or rectum and or extends beyond true pelvis. Note: the presence of bullous oedema is not sufficient to classify the tumour as stage IV A.

Stage IV B Distant metastasis.

B II. ENDOMETRIUM CURETTAGE – GROSSING TECHNIQUE

Endometrium – Curettage / Products of Conception / Foetus

- Preferably use a metal strainer or filter paper on funnel to collect the material.
- Weight to be taken in gms .
- Description –colour and consistency; proportion of blood clot; whether the tissue is suggestive of products; decidual cast identified; shape of villi; presence or absence of gestational sac (under dissecting microscope if available.
- Measurement of villi if distended.
- Embryo –CH (crown heel), CR (crown rump) lengths , appearance- malformed, macerated.
- Foetus – may sometimes be sent along with curettings. Look for the cord, if it is seen measure the length.

Bits (blocks)

- If curettage, all for section (1g per cassette)
- If products – upto 2g for processing (choose the representative bits)
- If small vesicles are present, all can be blocked (–upto 3 blocks can be taken)
- Embryo –block either entire or half
- Foetus – no bits need be taken unless specified. If cord is seen then take a bit from it (transverse section)

B III ENDOMETRIUM AND MYOMETRIUM- GROSS PEARLS

- **Thickened endometrium (>0.5–1 mm):**
  1. Secretory/hypersecretory endometrium
  2. Superficial spreading endometrial carcinoma
- **Membrane-like white covering of endometrial cavity:** Ichthyosis uteri (rare squamous metaplasia; rule out squamous cell carcinoma with multiple bits)
- **Polypoid mass in endometrial cavity:**
  1. Benign endometrial polyp
  2. Endometrial stromal polyp
- **Leiomyomatous polyp** (whorled cut surface) (also called Fibroid Uterus. Leiomyoma is the commonest tumour of the female genital tract, occurring in approximately 20–25% of women of reproductive age. Usually multiple, round, well-circumscribed nodules varying in size from a few millimetres to over 20 cm. Cut surface: firm, white, and shows the characteristic whorled appearance. May show hyaline, cystic, or myxoid degeneration, focal necrosis, or calcification.
- **Pedunculated leiomyomas** may derive part of their blood supply from adjacent structures and become independent of the uterus ("parasitic leiomyoma").
- **Small well-circumscribed solid whorled masses:** Leiomyomata; single/multiple, intramural, submucosal, or subserosal (Fig 1.16); may show cystic change, hyalinisation, myxoid change
  1. "Red degeneration" → infarction in pregnancy; modern term: (apoplectic/haemorrhagic leiomyoma)

Fig 1.16 Show gross appearance of multiple leiomyomata in the uterus

- **Polypoid endometrial carcinoma** (firm grey-white cut surface)
- **Trabeculated myometrium with haemorrhagic cysts:** Adenomyosis

- **White worm-like tumour masses merging with endometrium:** Endolymphatic stromal myosis / low-grade endometrial sarcoma
- **Markedly enlarged uterus, variegated tumour:** Malignant mixed Müllerian tumour (carcinosarcoma)
- **Translucent grape-like structures (few mm–1–2 cm):** Hydatidiform mole
  1. Complete mole → extensive villous formation
  2. Partial mole → only some villi affected
- **Tumour with extensive haemorrhage, occupying uterine cavity & myometrium:** Choriocarcinoma
- **Circumscribed nodule, less haemorrhage than choriocarcinoma:** Placental site trophoblastic tumour

B IV ENDOMETRIUM AND MYOMETRIUM – MICROSCOPY

1) Stages of Normal Menstrual Cycle

Proliferative Phase (Fig 1.17)

Early Proliferative Phase (Day 4–7):

- Surface epithelium: thin
- Glands: straight, short
- Stroma: compact, cells show some mitotic activity and large nuclei

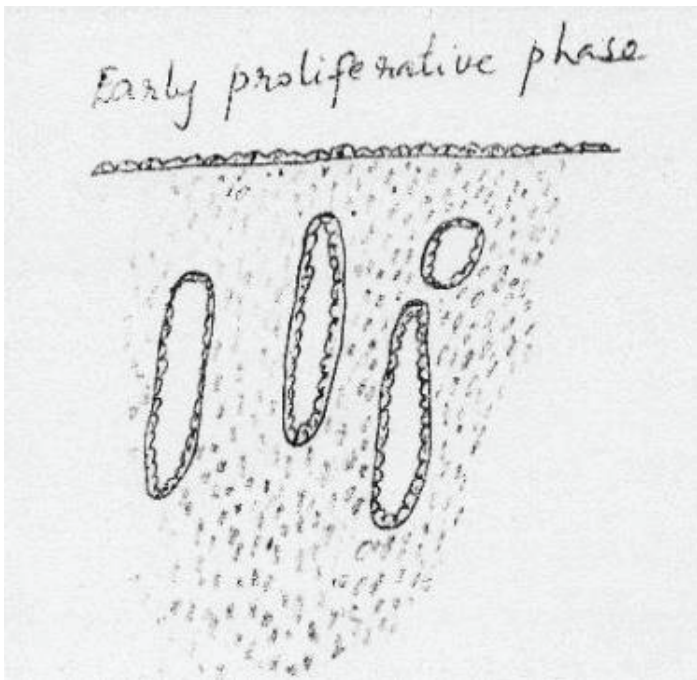


Fig 1.17

Mid Proliferative Phase (Day 8–10) (Fig 1.18):

- Surface epithelium: columnar
- Glands: long, curving
- Stroma: variable edema, numerous mitoses in stromal cells

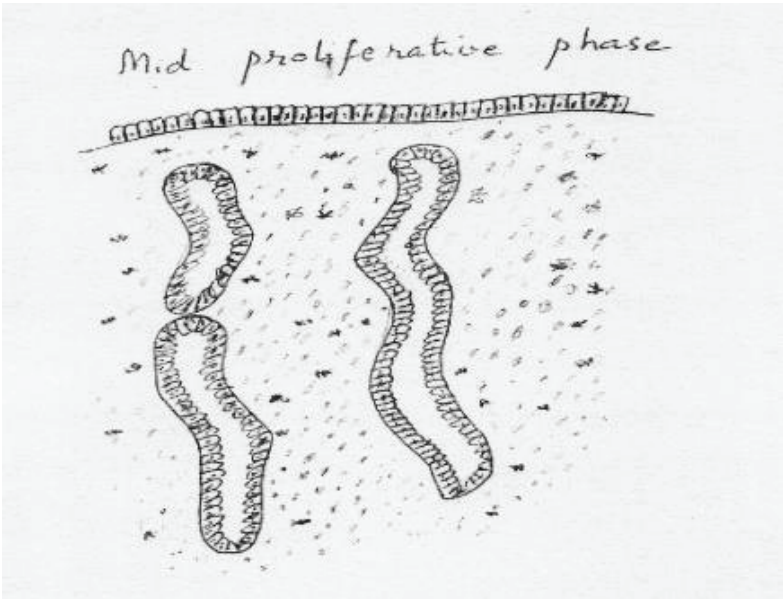


Fig 1.18

Late Proliferative Phase (Day 11–14) (Fig 1.19):

- Surface: undulant
- Glands: tortuous, showing active growth and pseudostratification
- Stroma: moderately dense

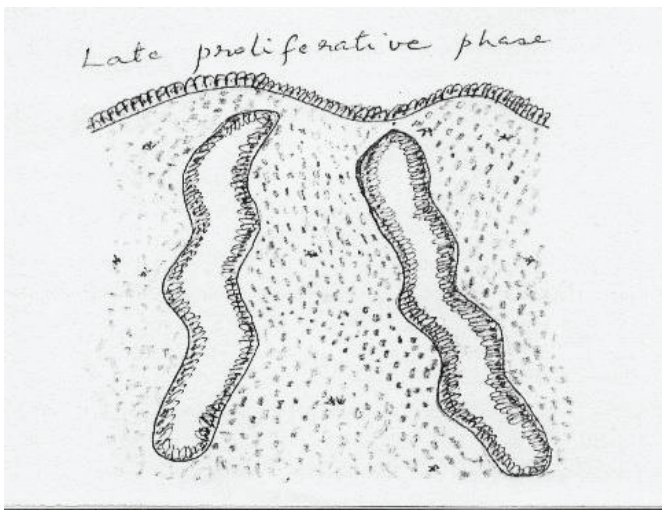


Fig 1.19

Secretory Phase

Early Secretory Phase (Fig 1.20):

- Up to 48 hours after ovulation: no microscopic change
- Day 16–17: subnuclear vacuolation; vacuoles increase in size
- Day 18–19: vacuoles decrease in size; nuclei at base of cells; luminal secretion starts

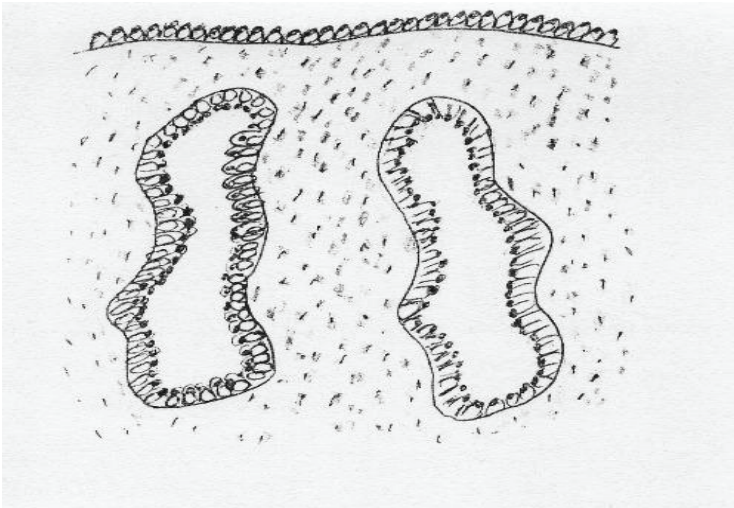


Fig 1.20

Mid Secretory Phase (Fig 1.21):

- Day 20: peak of acidophilic intraluminal secretion
- Day 21: stromal edema begins
- Day 22: stromal edema reaches peak

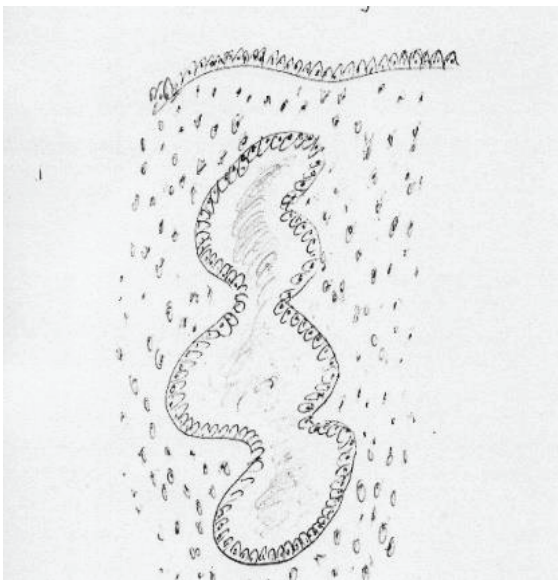


Fig 1.21

Late Secretory Phase (Fig 1.22)

- Day 23–24: spiral arterioles become prominent; predecidual cells appear around spiral arterioles
- Day 25: predecidual cells appear beneath surface epithelium
- Day 26: predecidua form solid sheets
- Day 27: neutrophils appear
- Day 28: necrosis and hemorrhage are prominent

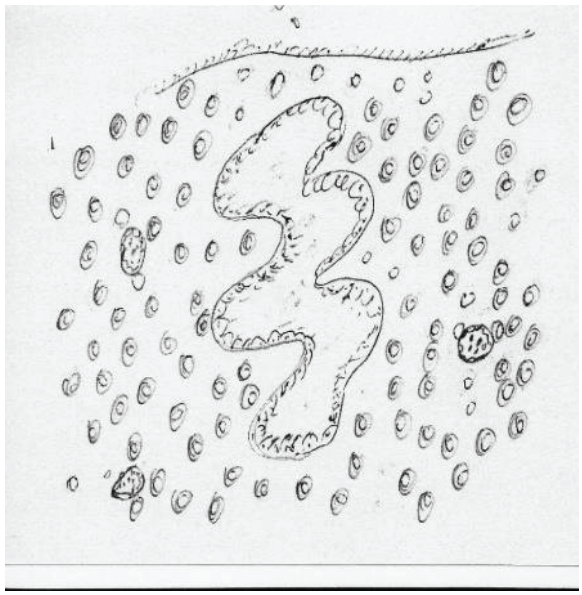


Fig 1.22

2) Metaplasias

The normal endometrial epithelium is composed of cuboidal to columnar cells with faintly eosinophilic cytoplasm and oval to elongated nuclei. **Ciliated cells** are common, especially in the proliferative phase; their presence should **not** be taken as indicative of tubal/ciliated metaplasia.

Endometrial metaplasias can be **focal or diffuse**. They are especially common in:

- Tamoxifen-associated endometrial polyps
- Non-polypoid endometrium

Associations:

- Exogenous hormone therapy (especially unopposed estrogen)
- IUCD
- Chronic endometritis
- Pyometra

Classification (International Society of Gynecological Pathologists):

1. Squamous metaplasia
2. Mucinous metaplasia (including intestinal)
3. Ciliary change
4. Hobnail cell change
5. Clear cell change
6. Eosinophilic/oxyphil cell change (including oncocytic)
7. Surface syncytial change
8. Papillary proliferation
9. Aria Stella change

Details of Common Metaplasias

Squamous Metaplasia:

- Common in endometrial adenocarcinoma and hyperplasia
- Squamous component in carcinoma may be benign, indeterminate, or malignant
- WHO classifies adenocarcinoma with squamous differentiation as a variant of endometrioid adenocarcinoma
- **Morules:** Lack intercellular bridges and keratinization; often coexist with mature squamous metaplasia
- May also occur in pyometra, chronic endometritis, cervical stenosis, and IUCD presence

Mucinous Metaplasia:

- Rare; endometrial cells replaced by abundant mucin-containing cytoplasm
- Intestinal metaplasia (presence of goblet cells) is very rare
- Differential diagnosis: mucinous carcinoma, microglandular hyperplasia

Ciliated / Tubal Metaplasia:

- Diagnosis: one or more glands predominantly lined by ciliated epithelium with eosinophilic cytoplasm
- Commonly occurs in endometrial hyperplasia

Clear Cell Metaplasia:

- Abundant clear cytoplasm, often in pregnancy
- Important to **not misdiagnose** as clear cell carcinoma
- Features favoring metaplasia: focal lesion, no hysteroscopic lesion, absence of stromal invasion, ER positivity

Hobnail Cell Metaplasia:

- Reparative phenomenon, often after endometrial biopsy

Oxyphil / Eosinophilic Metaplasia:

- Epithelial cells with abundant eosinophilic cytoplasm, seen in hyperplasia

Aria Stella Reaction:

- Exaggerated gestational hyperplasia
- Secretary and proliferative changes with prominent nuclear changes (hyperchromasia, enlargement)
- Seen post-abortion, normal/ectopic pregnancy, rarely after exogenous hormones
- Differential diagnosis: clear cell adenocarcinoma

Table summarizing normal, metaplastic, and secretory endometrium:

Phase / Feature	Days	Glandular Epithelium	Stroma	Special Features / Notes
Early Proliferative	4–7	Thin surface epithelium; short straight glands	Compact stroma with mitotic activity	Large nuclei; no secretions
Mid Proliferative	8–10	Columnar surface epithelium; long curving glands	Variable stromal edema	Numerous stromal mitoses
Late Proliferative	11–14	Undulant surface; tortuous glands; pseudostratification	Moderately dense stroma	Active glandular growth
Early Secretory	15–19	Subnuclear vacuolation begins; luminal secretion starts	Slight stromal edema	Up to 48h post-ovulation: no change
Mid Secretory	20–22	Peak acidophilic intraluminal secretion	Stromal edema reaches peak	Stromal changes prominent
Late Secretory	23–28	Predecidual cells appear; necrosis towards day 28	Spiral arterioles prominent	Neutrophils (day 27); hemorrhage (day 28)
Squamous Metaplasia	–	Replacement of endometrial cells by squamous cells	–	Often in tamoxifen, hyperplasia, carcinoma

Phase / Feature	Days	Glandular Epithelium	Stroma	Special Features / Notes
Mucinous / Intestinal Metaplasia	–	Glands with abundant mucin; goblet cells in intestinal type	–	Rare; DD: mucinous carcinoma
Ciliated / Tubal Metaplasia	–	Predominantly ciliated epithelium	–	Seen in hyperplasia; distinguish from tubal epithelium
Clear Cell Metaplasia	–	Abundant clear cytoplasm; gland architecture maintained	–	Can mimic clear cell carcinoma; usually focal, ER+
Hobnail / Oxyphil Metaplasia	–	Reparative or eosinophilic cells	–	Seen post-biopsy or in hyperplasia
Aria Stella Reaction	–	Hyperchromatic, enlarged nuclei; focal	–	Post-abortion or gestation; mimic carcinoma

3) Inflammations

Acute Endometritis

- Seen in: abortions, post-partum states, and instrumentation
- Histology: infiltration by neutrophils
- Note: neutrophils are normally present in endometrium on days 26–28 of the cycle

Chronic Endometritis

- Characterized by infiltration of lymphocytes and plasma cells
- Causes: chronic cervicitis, pelvic inflammatory disease (PID), IUCD
- Diagnostic tip**:** lymphoid follicles (with or without germinal centers) in the functional layer are normal; plasma cells must be identified for diagnosis
- Immunohistochemistry: CD38, VS38, Syndecan-1 can enhance plasma cell detection

IUCD-related Changes

- Causes focal or extensive chronic endometritis, necrosis, and squamous metaplasia
- May lead to salpingitis and PID with tubo-ovarian abscess

- Actinomycosis: colonies of radiating filamentous organisms; stain positive with Gomori's methenamine silver and periodic acid-Schiff

Pyometra

- Accumulation of pus in the endometrial cavity
- Causes: obstruction and infection

Hematometra

- Accumulation of blood in the endometrial cavity due to cervical occlusion
- May result in diffuse xanthogranulomatous endometritis or nodular cystic hyperplasia (focal response)

Endometrial Tuberculosis

- Presents as infertility/sterility
- **Histology:** granulomatous inflammation with epithelioid cells and caseous necrosis

Viral Endometritis

- CMV: occurs in immunocompromised patients; shows large nuclear and cytoplasmic inclusions
- Other viral**:** condyloma

Chlamydial Infections & Malakoplakia

- Rarely reported in the endometrium

4) Table 1 and Table 2 :outline the features of hyperplasia as occurs in the endometrial lining and polyps of the endometrium respectively.

Table 1: Endometrial Hyperplasia (Kurman & Norris Classification, 1986; WHO 2002)

Category	Subtype	Gland Architecture	Cytologic Features	Notes
Simple Hyperplasia	Without atypia	Straight, non-branching glands; variably dilated; occasional luminal protrusions acceptable	No cytologic atypia	Often shows altered differentiation patterns (papillary, eosinophilic, squamous)
Simple Hyperplasia	With atypia	Same as above	Cytologic atypia present	May resemble adenocarcinoma in small/poorly oriented samples

Category	Subtype	Gland Architecture	Cytologic Features	Notes
Complex Hyperplasia	Without atypia	Glands arranged close together ("back-to-back"); prominent branching/in-folding	No cytologic atypia	Architecture is the main distinguishing feature
Complex Hyperplasia	With atypia	Same as above	Cytologic atypia present	High risk for progression to carcinoma

Table 2: Endometrial Polyps

Feature	Description
Gross	Protrude into endometrial cavity; some are pedunculated
Epithelium	Active pseudostratified epithelium in pre-menopausal; flat inactive epithelium post-menopause
Stroma	Fibroblast-like cells; abundant connective tissue; thick-walled blood vessels
Secondary Changes	May show simple or complex hyperplasia with papillary endometrial proliferations
Functional Polyps	Rare
Tamoxifen Effect	Frequency increases after exposure
Polypoid Adenomyoma	Polyps containing smooth muscle not connected to blood vessels

5) Adenomyosis and Endometriosis

Adenomyosis

- Definition: Presence of endometrial glands and stroma deep within the myometrium
- Microscopy:
  - Endometrial glands and stroma should be present at least one low-power field (10x) below the endomyometrial junction
  - Haemosiderin-laden macrophages are usually present in long-standing cases, resulting from cyclical hemorrhage of ectopic endometrial tissue
  - Diagnostic criteria: at least two of the three components—endometrial glands, stroma, and haemosiderin-laden macrophages—should be identified

Endometriosis

- Definition: Presence of endometrial glands and stroma outside the uterus
- Common sites**:** ovary, cervix, vagina, vulva, fallopian tubes, rectovaginal septum, broad ligament, pelvic peritoneum, abdominal wall, small and large bowel
- Microscopy: similar to eutopic endometrium; may see hemorrhage, hemosiderin-laden macrophages, and stromal reaction
- Pathogenesis: multiple theories exist; the metaplastic theory is the most widely accepted

6) Adenomyoma and Variants

Definition

A benign, mixed epithelial and mesenchymal tumour of the uterus composed of endometrial glands and stroma intermingled with smooth muscle. It represents a localized form of adenomyosis.

Epidemiology

- Occurs mainly in women of reproductive age (30–50 years).
- Commonly involves the uterine corpus, sometimes the **cervix**.

Gross Features

- Well-circumscribed, firm nodules within the myometrium.
- Cut surface: gray-white, trabeculated; may show small cystic spaces containing old blood or endometrial debris.
- Usually <5 cm in size, but may be larger and polypoid when cervical.

Microscopy

- Endometrial glands (usually proliferative type) embedded in endometrial-type stroma within bundles of smooth muscle.
- The smooth muscle component resembles leiomyoma.
- No significant atypia or mitotic activity.
- May show cyclic changes (proliferative, secretory, or menstrual pattern).
- Occasionally contains areas of hemorrhage or cystic dilatation of glands.

Variants:

1. Atypical Polypoid Adenomyoma (APA):
    - Benign biphasic tumour with architecturally complex glands and fibromuscular stroma.
    - May show cytologic atypia but lacks invasive features.

- Microscopy: irregular glands with squamous morules; smooth muscle-rich stroma.
- May recur if incompletely excised.
- Occasionally associated with or precursor to endometrioid carcinoma.

2. Cervical Adenomyoma:
   - Well-circumscribed polypoid lesion in the endocervix.
   - Glands lined by mucinous columnar epithelium resembling endocervical glands.
   - Smooth muscle stroma, no atypia.

Immunohistochemistry (IHC)

Component	Positive markers	Negative markers
Epithelial (glandular)	**CK7, EMA, ER, PR**	**CK20**
Stromal / smooth muscle	**Desmin, SMA, h-caldesmon, vimentin**	**CD10 (variable)**

- Atypical polypoid adenomyoma: usually ER/PR positive; Ki-67 shows low proliferative index (<10%).

Prognosis

- Excellent — completely benign if excised.
- Recurrence may occur with incomplete removal (especially APA).
- Rarely progresses to carcinoma in long-standing cases with atypia.

5) WHO CLASSIFICATION OF THE TUMOURS OF THE UTERUS-OLD CLASSIFICATION :

1. EPITHELIAL TUMOURS AND RELATED LESIONS

Endometrial carcinoma

Endometriod adenocarcinoma

- Variant with squamous differentiation
- villoglandular variant
- secretary variant
- ciliated cell variant

Mucinous adenocarcinoma

Serous adenocarcinoma

Clear cell adenocarcinoma

Mixed cell adenocarcinoma

Squamous cell carcinoma

Transitional cell carcinoma

Small cell carcinoma

Undifferentiated carcinoma

Others

Endometrial hyperplasia

Non atypical hyperplasia

- Simple
- Complex (adenomatous)

Atypical hyperplasia

- Simple
- Complex (adenomatous)

Endometrial polyp

Tomaxifen related lesion

II. MESENCHYMAL TUMOURS

Endometrial stromal and related tumours

Endometrial stromal sarcoma, low grade

Endometrial stromal nodule

Undifferentiated endometrial sarcoma

Smooth muscle tumours

Leiomyosarcoma

- -epitheliod variant
- myxoid variant

Smooth muscle tumours of uncertain malignant potential

Leiomyoma, NOS

Histological variants

- Mitotically active variant
- Cellular variant
- Haemorrhagic cellular variant
- Epitheliod variant
- Myxoid
- Atypical variant
- lipoleiomyoma variant

Growth pattern variants

- diffuse leiomyomatosis
- dissecting leiomyoma
- intravenous leiomyomatosis
- metastasizing leiomyoma

III. MIXED EPITHELIAL AND MESENCHYMAL TUMOURS

Carcinosarcoma

Adenosarcoma

Carcinofibroma

Adenofibroma

Adenomyoma

- atypical polypoid variant

IV. GESTATIONAL TROPHOBLASTIC DISEASE

Trophoblastic neoplasms

Choriocarcinoma

Placental site trophoblastic tumour

Epithelioid trophoblastic tumour

Molar pregnancies

Hydatidiform mole

- complete
- -partial
- -invasive
- metastatic

Non Neoplastic non molar trophoblastic lesions

Placental site nodule & plaque

Exaggerated placental site reaction

V. MISCELLANEOUS TUMOURS

Sex cord like tumours

Neuroectodermal tumours

Melanotic paraganglioma

Tumours of germ cell type

Others

VI. LYMPHOID & HAEMATOPOIETIC TUMOURS

Malignant lymphoma (specify)

Leukaemia (specify)

THE WHO Classification of Tumours of the Uterus (5th Edition, 2020) : (UPDATED). The updated 2020 Classification places a greater emphasis on key molecular events and on the integration of morphological and molecular features and represents a major step-forward from the prior Classification.

Category	Entities / Subtypes
1. Endometrial epithelial tumours and precursors	• Endometrial hyperplasia (without atypia / with atypia) • Endometrioid carcinoma of the uterine corpus (including variants with squamous, mucinous, secretory and ciliated differentiation) • Serous carcinoma of the uterine corpus • Clear cell carcinoma of the uterine corpus • Undifferentiated carcinoma / Dedifferentiated carcinoma of the uterine corpus • Mixed endometrial carcinoma (≥2 histologic types, each >5%) • Carcinosarcoma (malignant mixed Müllerian tumour)
2. Tumour-like lesions of the endometrium	• Endometrial polyp • Endometrial metaplasia and reactive changes • Arias–Stella reaction
3. Mesenchymal tumours of the uterus	**(a) Smooth muscle tumours** • Leiomyoma (conventional and variants) • Smooth muscle tumour of uncertain malignant potential (STUMP) • Leiomyosarcoma **(b) Endometrial stromal and related tumours** • Endometrial stromal nodule • Low-grade endometrial stromal sarcoma • High-grade endometrial stromal sarcoma • Undifferentiated uterine sarcoma **(c) Miscellaneous mesenchymal tumours** • Perivascular epithelioid cell tumour (PEComa) • Uterine tumour resembling ovarian sex cord tumour (UTROSCT)

Category	Entities / Subtypes
4. Mixed epithelial and mesenchymal tumours	• Adenomyoma and variants • Atypical polypoid adenomyoma • Adenosarcoma
5. Gestational trophoblastic disease	• Hydatidiform mole (complete / partial) • Invasive mole • Choriocarcinoma • Placental site trophoblastic tumour (PSTT) • Epithelioid trophoblastic tumour (ETT)
6. Miscellaneous tumours	• Sex cord-like tumours • Neuroectodermal tumours • Germ-cell tumours of the uterus
7. Haematolymphoid tumours involving the uterus	• Lymphomas and leukaemic infiltrates (secondary or rarely primary)

WHO Classification (5th Edition, 2020) – Key Updates

Endometrial Tumours

- The WHO now endorses an **integrated histomolecular classification**, comprising four molecular subgroups as per the molecular subtyping based on "The Cancer Genomic Atlas (TCGA) classification and other molecular classifications :
    1. POLE-ultramutated
    2. MMR-deficient (MMRd)
    3. p53-abnormal (p53abn)
    4. No specific molecular profile (NSMP)
- These subgroups refine prognosis and help guide therapy.
- Histological types include:
    1. Endometrioid carcinoma (most frequent)
    2. Serous carcinoma
    3. Clear cell carcinoma
    4. Mixed carcinoma
    5. Undifferentiated / Dedifferentiated carcinoma
    6. Mesonephric-like adenocarcinoma
- **Stromal and mesenchymal tumours:**
    1. Endometrial stromal nodule
    2. Low-grade ESS (with *JAZF1* or *YWHAE-NUTM2* rearrangements)
    3. High-grade ESS (with *BCOR* abnormalities)

4. Undifferentiated uterine sarcoma

(a) Endometrioid adenocarcinoma (conventional type):

Approximately 80% of endometrial carcinomas are of the conventional *endometrioid* type. The tumour is composed of endometrial glands lined by tall, columnar, stratified epithelium showing mitoses, nuclear stratification, and a back-to-back arrangement (Fig 1.23). Most tumours are well differentiated, although some show solid areas composed of malignant cells. Nuclear pleomorphism is usually mild.

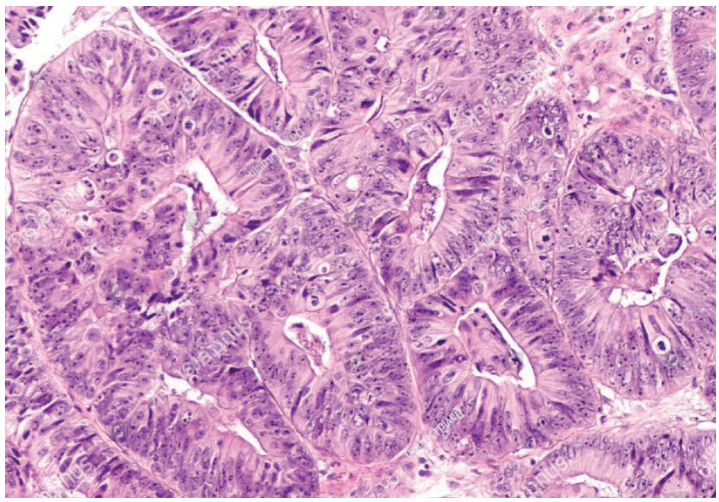


Fig 1.23 : Adenocarcinoma endometrium well differentiated . H& E x 400

Grading (FIGO system):

The *Federation of International Gynecologists and Obstetricians (FIGO)* grading system is applied to conventional endometrioid adenocarcinomas and their variants. Updates to the FIGO staging system incorporate molecular features, allowing for more tailored treatment approaches. Advances in immunotherapy, targeted therapies, and novel therapeutic combinations can improve clinical management :

- **Gx:** Grade cannot be assessed
- **G1:** ≤5% non-squamous solid growth
- **G2:** 6–50% non-squamous solid growth
- **G3:** >50% non-squamous solid growth
  (Grade is increased by one if there is marked nuclear atypia.)

Immunohistochemistry (IHC):

- **ER and PR:** Positive; expression correlates with FIGO grade and stage
- **Cytokeratins (CK7, 8, 18, 19):** Positive
- **Vimentin:** Positive in 65–80% of cases
- **CEA:** Focal positivity, especially in areas with squamous metaplasia
- **CA125, secretory component, Ulex europaeus agglutinin:** Positive in ~12% of cases

- **CD114:** Positive in ~50% of endometrial carcinomas and ~90% of normal proliferative endometrial glands

Differences between endocervical adeno carcinoma of cervix and that of endometrium.

Criteria	Primary endocervical Adenocarcinoma	Primary endometrial Adenocarcinoma
Mucin	Diffuse & abundant	Focal /minimal
In situ carcinoma	Will be seen in adjacent epithelium	Not seen
Vimentin	-ve	+ve
CD 10	+ve in 33% (luminal, limited)	+ve in 77% (cytoplasmic, Membrane or both)
CEA	Strongly +ve	Focal / minimal
ER & PR	Weakly +ve or negative	Significantly +ve
P16	++ 100 % of cases	+ less than 10%

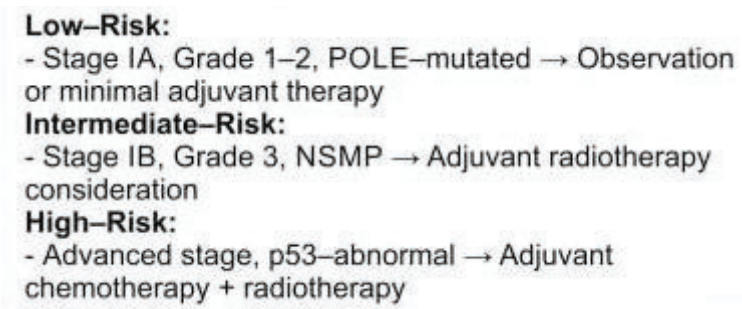
Variants of Endometrioid Adenocarcinoma

- **Villoglandular variant:** Found in about one-fourth of cases; shows superficial papillary foci that should not be mistaken for papillary serous carcinoma (an aggressive variant).
- **Adenoacanthoma:** Well-differentiated endometrioid carcinoma containing benign-appearing squamous elements derived from metaplasia of tumour glands.
- **Adenosquamous carcinoma:** Mixed tumour with malignant glandular and squamous components (up to 30% squamous). Prognosis is poorer than pure adenocarcinoma or adenoacanthoma, largely due to poor differentiation of the glandular component.
- **Glassy cell carcinoma:** Rare type of adenosquamous carcinoma occasionally seen in the endometrium.
- **Secretory carcinoma:** Resembles late secretory (17th day) endometrium with subnuclear vacuolation in neoplastic glands, often under progesterone influence.
- **Ciliated carcinoma:** Rare variant composed of ciliated tumour cells; must be distinguished from benign ciliated cell metaplasia
- **Mucinous adenocarcinoma:**
  Characterized by abundant mucin secretion. Architectural distortion and cytologic atypia distinguish it from mucinous metaplasia. Some mucin-secreting cells may occur in endometrioid carcinoma; hence, this type is technically considered endometrioid and graded (G1–G3) accordingly.
- **Serous (papillary) carcinoma: (common of the type II endometrial carcinoma)**
  A highly aggressive carcinoma resembling its ovarian counterpart. Pure serous

carcinomas are rare (~1% of endometrial carcinomas) and may coexist with endometrioid carcinoma.

- **Microscopy:** Complex papillary architecture, marked cytologic atypia with pleomorphic, hyperchromatic nuclei, macronucleoli, numerous mitoses, necrosis, and psammoma bodies.
- **Myometrial invasion:** Usually present.
- **Differential diagnosis:** Endometrioid carcinoma with villoglandular pattern; both may show psammoma bodies and coexist.
- **IHC:** *WT1* is positive in ovarian serous carcinoma but negative in endometrial serous carcinoma.

- **Clear cell carcinoma:**
  Composed of large clear cells with distinct borders, abundant glycogen, and prominent nucleoli. Shows papillary, tubulocystic, or solid patterns; hobnail cells may be seen. Tumour is of Müllerian origin (Grade 3 by definition) and is not associated with DES exposure (as in cervix).
- **Small cell carcinoma (neuroendocrine type):**
  Aggressive tumour, often presenting as a bulky polypoid mass. May coexist with adenocarcinoma or form part of a malignant mixed Müllerian tumour.
  IHC: Positive for NSE, synaptophysin, and chromogranin.
- **Squamous cell carcinoma:**
  Pure endometrial squamous carcinoma is extremely rare; cervical extension must be excluded. Typically arises in elderly women with pyometra and pre-existing squamous metaplasia.
- **Other rare variants:**
  Include giant cell carcinoma, endometrial carcinoma with trophoblastic differentiation, oxyphilic cell carcinoma, and true mesonephric carcinoma.
- **Treatment decisions are based on Risk Stratification :**



Microscopy of leiomyoma

- Composed of **interlacing fascicles** of uniform **spindle-shaped smooth muscle cells** with elongated “cigar-shaped” nuclei.

- **Mitotic activity:** little or absent; this remains the most important predictor of malignant potential.
- **Mitoses:**
  - ≤ 4 mitoses/10 HPFs → benign
  - ≥ 10 mitoses/10 HPFs → malignant
  - 5–9 mitoses/10 HPFs → **STUMP** (*Smooth Muscle Tumour of Uncertain Malignant Potential*)
- Assessment of mitotic figures must be done in the most active areas. **Coagulative necrosis** and **cytologic atypia** are significant predictors of aggressive behaviour.

Variants

1. Cellular leiomyoma: hypercellular but otherwise typical; behaves as benign.
2. Haemorrhagic (Apoplectic) cellular leiomyoma: seen in pregnancy or oral contraceptive use; shows circumscribed cellular proliferation with central haemorrhage and oedema.
3. Lipo-leiomyoma: contains adipose tissue component.
4. Bizarre (Symplastic) leiomyoma: contains multilobated, hyperchromatic, and pleomorphic nuclei without mitotic activity or necrosis; of no clinical significance.
5. Epithelioid leiomyoma: composed of round or polygonal cells (leiomyoblastoma) or "signet-ring" type cells with clear/vacuolated cytoplasm; may recur rarely.
6. Schwannoma-like leiomyoma: exhibits palisaded nuclei and Antoni A/B-like pattern resembling schwannoma.
7. Mitotically active leiomyoma: increased mitotic figures (up to 15/10 HPFs), but no atypia or necrosis.
8. Benign metastasizing leiomyoma**:** histologically benign leiomyoma associated with nodules in the lungs or lymph nodes years after hysterectomy; indolent course.

Diagnostic Pearls

- Mitoses alone do not define malignancy—atypia and necrosis must be assessed.
- STUMP should always be mentioned specifically, as clinical follow-up is required.
- Immunohistochemistry: positive for desmin, SMA, and h-caldesmon; negative for CD10 (helpful in differentiating from stromal sarcomas).

Leiomyosarcoma

Leiomyosarcoma accounts for approximately 0.7% of all hysterectomy specimens and is a malignant counterpart of leiomyoma. It most often arises *de novo* rather than from a pre-existing leiomyoma.

Clinical Features

- Typically occurs in peri- or postmenopausal women.
- Presents with uterine enlargement, abnormal bleeding, or pelvic pain.

Gross Features

- Usually solitary, though may occasionally be multiple.
- Size: generally large (>5 cm).
- Cut surface: shows poor circumscription, with areas of haemorrhage, necrosis, and soft fleshy consistency.
- Can closely mimic leiomyoma grossly.

Microscopy

- Composed of interlacing fascicles of spindle-shaped smooth muscle cells with blunt-ended ("cigar-shaped") nuclei, but with marked pleomorphism and atypia.
- Mitotic count: high (typically 15–30 mitoses/10 HPFs) with abnormal mitotic figures.
- Coagulative tumour necrosis is characteristic.

When the malignant features are not overt, diagnosis rests on a combination of the following **criteria** (any one sufficient):

Criterion	Description
i.	>10 mitoses/10 HPFs with significant atypia (focal or diffuse) and necrosis
ii.	>10 mitoses/10 HPFs with diffuse atypia, with or without necrosis
iii.	≤10 mitoses/10 HPFs with significant atypia and necrosis

STUMP (Smooth Muscle Tumour of Uncertain Malignant Potential)

Diagnosis of **STUMP** is made when a tumour shows equivocal malignant features that do not fulfil criteria for leiomyosarcoma, e.g.:

Criterion	Description
i.	>10 mitoses/10 HPFs, focal atypia, no necrosis
ii.	≤10 mitoses/10 HPFs, diffuse atypia, no necrosis
iii.	≤10 mitoses/10 HPFs, no/mild atypia, necrosis present

All mitotic counts should be done in consecutive HPFs in the most mitotically active areas.

Immunohistochemistry

- Positive: Desmin, Smooth Muscle Actin (SMA), h-Caldesmon.

- Negative: CD10 (distinguishes from endometrial stromal sarcoma).

Prognosis

- Poor, with a tendency for recurrence and distant metastasis (especially to lungs, liver, and peritoneum).
- Tumour stage and mitotic index are key prognostic factors.

Malignant Mixed Müllerian Tumour (Carcinosarcoma)

General Features

- Occurs most often in postmenopausal women.
- Presents with uterine bleeding and uterine enlargement.
- Represents a biphasic malignant neoplasm containing both carcinomatous (epithelial) and sarcomatous (mesenchymal) elements.
- Now classified by WHO as a metaplastic carcinoma rather than a true mixed tumour, since both components share a common clonal origin.

Gross Features

- Usually located in the fundus or posterior wall of the uterus.
- The tumour is fleshy, polypoid, and friable, projecting into the uterine cavity.
- Areas of necrosis and haemorrhage are common.

Microscopy

- Biphasic pattern with an intimate admixture of:
  - Epithelial component: most often endometrioid adenocarcinoma, but may also show serous, clear cell, squamous, or undifferentiated types.
  - Mesenchymal (sarcomatous) component: may resemble endometrial stroma or smooth muscle (leiomyosarcoma), or show heterologous differentiation such as rhabdomyosarcoma, chondrosarcoma, liposarcoma, or osteosarcoma.
- IHC: Both epithelial and sarcomatous elements express cytokeratins and EMA, confirming their common epithelial (carcinomatous) origin.

Types

- Homologous type: sarcomatous element intrinsic to the uterus (e.g., stromal or smooth muscle).
- Heterologous type: contains non-uterine tissue elements such as rhabdomyoblastic, chondroblastic, or osteoblastic differentiation.
- *Note:* Prognosis is similar for both types.

Pathogenesis

- Evidence supports a monoclonal origin, with sarcomatous differentiation arising via metaplastic transformation from the epithelial component.
- The carcinomatous component is often dominant and determines overall tumour behaviour.

Prognosis

- Highly aggressive with poor outcome.
- Early myometrial invasion, lymphovascular spread, and distant metastases (lung, peritoneum) are common.
- Median survival is <2 years in advanced cases.
- Prognostic factors include stage, depth of invasion, and percentage of epithelial component.

Key Diagnostic Points of Malignant Mixed Müllerian Tumour (Carcinosarcoma)

Feature	Description / Diagnostic Clues
Nature of tumour	Biphasic malignant neoplasm with both epithelial (carcinomatous) and mesenchymal (sarcomatous) components.
Typical site	Body/fundus of uterus, often polypoid and projecting into uterine cavity.
Epithelial component	Usually endometrioid, serous, or clear cell carcinoma; predominates and dictates behaviour.
Mesenchymal component	May be homologous (endometrial stromal or smooth muscle) or heterologous (rhabdomyoblastic, chondroblastic, osteoblastic, lipoblastic).
IHC profile	Both elements express cytokeratin and EMA, supporting common clonal (epithelial) origin.
Spread	Early myometrial, lymphovascular, and peritoneal dissemination; distant metastases common.
Pathogenesis	Monoclonal tumour with sarcomatous metaplasia of carcinoma.
Prognosis	Aggressive; determined mainly by the epithelial component and stage.

ADENOSARCOMA OF THE UTERUS

Definition:

Adenosarcoma is a biphasic mixed epithelial and mesenchymal tumour composed of benign epithelial glands and a malignant stromal component. It represents the low-grade end of the spectrum of mixed Müllerian tumours, distinct from carcinosarcoma.

Gross Features:

- Usually polypoid and intracavitary, projecting into the endometrial cavity.
- Size: often 3–7 cm, may fill the uterine cavity.
- Cut surface: soft, fleshy, grey-white, with cystic spaces due to entrapped glands.
- May involve the endocervical canal.
- Deep myometrial invasion may be seen in aggressive tumours.

Microscopy:

- Biphasic tumour: benign endometrial-type glands and malignant stroma.
- Glands: lined by benign endometrioid epithelium; may show mild hyperplasia or squamous metaplasia.
- Stroma:
  - Hypercellular, usually low-grade sarcomatous type.
  - Cells are spindle to oval, with mild-to-moderate atypia and low mitotic rate (2–5/10 HPF).
  - Periglandular cuffing: characteristic condensation of stromal cells around glands ("cambium layer").
  - Papillary or phyllodes-like architecture may be seen.
  - Myometrial invasion and heterologous elements (cartilage, skeletal muscle) may occur in higher-grade or recurrent cases.

Immunohistochemistry (IHC):

Marker	Expression / Significance
CD10, ER, PR	Usually positive (endometrial stromal differentiation)
WT1	Often positive
Desmin, SMA	Focally positive
p53	Wild-type (mutant pattern indicates dedifferentiation)
Cyclin D1	May be positive in high-grade areas
β-catenin (nuclear)	Seen in some cases
Ki-67	Usually low to moderate proliferative index

Molecular findings:

- JAZF1, PHF1, or MED12 rearrangements have been described in some cases.
- Lack of TP53 and MMR abnormalities distinguishes it from high-grade sarcomas or carcinosarcomas.

Differential Diagnosis:

Lesion	Key Distinguishing Features
Atypical polypoid adenomyoma	Benign stromal component, absence of periglandular cuffing, minimal atypia
Carcinosarcoma (MMMT)	Both epithelial and stromal components malignant; high-grade atypia
Low-grade endometrial stromal sarcoma with glandular differentiation	Lacks well-developed biphasic architecture; glands not benign

Prognosis and Behaviour:

- Generally low-grade malignancy.
- Recurrence in 20–30%, especially if deep myometrial invasion or sarcomatous overgrowth (>25% pure sarcoma).
- Metastases are uncommon but may occur late.
- Prognostic factors:
  - Depth of invasion
  - Sarcomatous overgrowth
  - Mitotic activity
  - Necrosis and vascular invasion

Recent WHO Notes (2020, 5th Edition):

- Adenosarcoma is categorised under Mixed epithelial and mesenchymal tumours of the uterus.
- The presence of sarcomatous overgrowth (≥25% pure sarcoma) must be specifically reported, as it confers a worse prognosis.
- Molecular correlations (JAZF1, PHF1) strengthen its distinction from other stromal or mixed tumours.

High lights - Uterine Adenosarcoma

- **Definition:** Biphasic tumour with benign epithelial (glandular) and malignant stromal components.
- **Age group:** Usually postmenopausal women (average 50–60 years).
- **Common site:** Endometrium — especially the **fundus** or **posterior wall**.
- **Gross:** Polypoid, often projecting into the endometrial cavity; may fill it completely.
- **Microscopy:**
  - Benign endometrial-type glands lined by columnar epithelium.

- Malignant **sarcomatous stroma** showing cellular atypia, mitoses, and periglandular cuffing ("**cambium layer**").
- Occasionally shows heterologous elements (rhabdomyoblastic, cartilaginous, or osteoid).

- **IHC profile:**
  - Stroma: CD10+, vimentin+, ER/PR+, WT1+.
  - Epithelial component: CK7+, EMA+.
  - Loss of CD10 or diffuse p53 positivity → may indicate higher-grade transformation.
- **Prognosis:**
  - Low-grade tumours — good prognosis; recurrences in ~25%.
  - High-grade (sarcomatous overgrowth or heterologous elements) — poorer prognosis, higher risk of recurrence and metastasis.
  - Stage and grade are the main prognostic factors.

ENDOMETRIAL STROMAL TUMOURS

- Occur in middle-aged women, presenting with vaginal bleeding.
- Composed of stromal cells resembling normal endometrial stroma; reticulin fibres surround individual cells and spiral arterioles.
- Tumours often show abundant thin-walled vessels, foci of hyalinisation, and scattered eosinophilic cells.
- Stromal cells are **vimentin** and **CD10 positive**, and **h-caldesmon** and **inhibin negative** (helping distinguish from smooth muscle and granulosa cell tumours).

Classification:

- **Benign:** Endometrial stromal nodule (pushing margins) or stromatosis (mildly infiltrative).
- **Malignant (low grade):** Infiltrative margins, lymphatic permeation (*endolymphatic stromal myosis*), increased mitoses, and invasion into adnexal structures. Recurrent but slow-growing.
- **High-grade endometrial stromal sarcoma:** High mitotic index and mortality. Evans classified these as either morphologically stromal or undifferentiated (pleomorphic) tumours, the latter overlapping with carcinosarcomas.

Variants:

- *Combined smooth muscle–stromal tumour:* If >1/3 of the tumour shows smooth muscle differentiation, it is classified as such. Collagen shows a "star-burst" pattern within smooth muscle areas.

Differential diagnosis:

Feature	Endometrial Stromal Sarcoma	Leiomyosarcoma
Gross	Multinodular, worm-like	Localised, irregular infiltrating margins
Reticulin stain	Spiral arterioles highlighted	Absent
Large vessels	Absent	Present
CD10	Positive	Negative
Endometrial granulocytes	Positive	Negative
h-caldesmon	Negative	Positive
Desmin	Negative	Positive

B and C V ENDOMETRIUM (& CERVIX) SYNOPTIC REPORTING

Endometrial Biopsy Histopathology Report (tumour & non tumour)

Gross :

Type of specimen : Dilatation & curettage ☐

Other ……

Weight ……g

Microscopy :

Endometrium

Proliferative ☐

Secretary ☐ Early ☐ Late ☐

Hypersecretary ☐

Aria – stella reaction ☐

Anovulatory ☐

Acute endometritis ☐

Chronic endometritis ☐

Granulomatous inflammation ☐ Tuberculosis ☐ Other ☐

Specify…….

Hyperplasia : Yes ☐ Simple ☐ Complex ☐ No ☐

With atypia ☐ Without atypia ☐

Benign polyp Yes ☐ Type ……. No ☐

Placental tissue (products) Yes ☐ No ☐

Embryo : Yes ☐ CH ……mm CR……mm No ☐

Hydropic villi : Yes ☐ No ☐

Vesicles (hydatidiform Mole) : Yes ☐ Complete☐ Partial ☐ No☐

Invasive mole : Yes ☐ No ☐

Presence of other tumour : Yes ☐ Type …… No ☐

Other findings; comments : …….

Signature .....……..
Date……...../……...../……..

D. Hysterectomy Specimen – Endometrium (non-tumour)

Gross:

Simple hysterectomy (uterus & cervix) ☐

Hysterectomy with bilateral adnexae ☐

Subtotal hysterectomy (without cervix) ☐

Other ☐ Specify ……..

Weight of uterus with adexae (if present)……..g

Dimensions :Length :…....mm, transverse…….. mm, antero-postero …… mm

Length of uterine cavity…….mm

Average myometrial thickness ……mm

Endometrial thickness …….mm

Length of endocervical canal …….mm

Right ovary ……..mm x……mm

Left ovary……mm x……mm

Right Fallopian tube : Normal ☐ Distended ☐ Congested ☐

Left Fallopian tube : Normal ☐ Distended ☐ Congested ☐

Right tubo-ovarian mass : Yes ☐ No ☐

Left tubo-ovarian mass : Yes ☐ No☐

Microscopy :

Cervix

Chronic cervicitis ☐

Chronic papillary cervicitis ☐

Follicular cervicitis ☐

Other ☐ Specify ……..

Squamous metaplasia ☐

Nabothian cysts ☐

Changes consistent with prolapse ☐

Polyp ☐ specify type ……

Other findings…….

Endometrium

Proliferative ☐

Secretary ☐ Early ☐ Late ☐

Hypersecretary ☐

Aria – stella reaction ☐

Anovulatory ☐

Chronic endometritis ☐ Granulomatous inflammation ☐

Tuberculosis ☐ Other ☐ Specify…….

Hyperplasia : Yes ☐ Simple ☐ Complex ☐ No ☐

With atypia ☐ Without atypia ☐

Stromatosis ☐

Pyometra Yes ☐ No ☐

Benign polyp Yes ☐ Type …… No ☐

Placental tissue : Yes ☐ No ☐

Hydropic villi Yes ☐ No ☐

Vesicles (hydatidiform Mole) yes ☐ Complete ☐ Partial ☐ No☐

Other findings…….

Myometrium

Unremarkable

Trabeculated with adenomyotic Yes ☐ No ☐

Leiomyoma Yes ☐ No ☐

Single ☐

Multiple ☐ State number ……

Secondary change Yes ☐ Type of change ……No ☐

Submucosal ☐

Intramural ☐

Subserous ☐

Other findings ……..

Right ovary : unremarkable

cystic follicle/follicles ☐

cystic corpus luteum ☐

Follicular cyst ☐

single ☐ multiple ☐ No……

Corpus luteum cyst ☐

Right Fallopian tube : unremarkable

Hydrpsalpinx single ☐ multiple ☐ No……

Tubo-ovarian mass Yes ☐ No ☐ Etiology……..

Other finding ……..

Left ovary : unremarkable ☐

cystic follicle ☐

cystic corpus luteum ☐

Follicular cyst ☐

single ☐ multiple ☐ No……

corpus luteum cyst ☐

Left Fallopian tube : unremarkable ☐

Hydrosalpinx ☐

Tubo-ovarian mass Yes ☐ No ☐ Etiology……..

Other finding …

Comments ……..

Signature…….. Date…….

E. Hysterectomy Specimen - Endometrial Cancer

Simple hysterectomy (uterus & cervix) ☐

Hysterectomy with bilateral adnexae ☐

Subtotal hysterectomy (without cervix) ☐

Other ☐ Specify…….

Weight of uterus with adexae (if present)……..g

Dimensions : …...mm **x** …….. mm and …… mm

Other tissues sent ☐ Specify……..

Peritoneal washings ☐

Gross description of tumour :

Size :…….mm x ……mm x ……mm

Colour :…….

location : ……

Polypoid ☐

Ulcerating ☐

Variegated appearance ☐

Diffusely infiltrating ☐

Cystic change ☐

Necrosis ☐

Haemorrage ☐

Myometrial involvement <50% ☐

>50% ☐

Gross involvement of cervix ☐

Gross involvement of right adnexa ☐

Gross involvement of left adnexa ☐

Microscopy :

Histological subtype :

Adenocarcinoma of endometrium ☐

Endometriod adenocarcinoma ☐

Endometriod adenocarcinoma with squamous metaplasia (adenoacanthoma) ☐

Endometriod adenocarcinoma with villoglandular differentiation☐

Adenocarcinoma –clear cell type☐

Papillary serous carcinoma☐

Mixed type composed of :…….

Leiomyosarcoma ☐

Low grade ☐

High grade ☐

Endometrial strmal sarcoma ☐

Low grade ☐

High grade ☐

High grade undifferentiated sarcoma ☐

Malignant mixed mullerian tumour

homologous type ☐

with heterologous elements ☐

Histological grade (for endometriod type only)

FIGO I ☐

FIGO II ☐

FIGO III ☐

Depth of invasion

limited to endometrium ☐

invades <50% of myometrial thickness☐

invades >50% of myometrial thickness ☐

Endocervical invasion

no invasion ☐

present in endocervical mucosa only☐

present in endocervical stroma only ☐

present in mucosa and stroma ☐

Lymphovascular invasion

Yes ☐ No☐

Tumour invades serosa : Yes ☐ No ☐

Endometrium

Unremarkable☐

Atrophy☐

Simple hyperplasia☐

Complex hyperplasia☐

Atypia seen ☐ Not seen ☐

Polyps ☐

Radiation change☐

Myometrium

Unremarkable ☐

Adenomyosis ☐

Leimyomata/leiomyoma Yes ☐ No☐

Secondary change Yes ☐ No☐

Adnexae

Both adnexae unremarkable ☐

Right Fallopian tube shows…….

Right ovary shows……

Left Fallopian tube shows……..

Left ovary shows……..

Other adnexae unremarkable ☐

Vaginal involvement : Yes ☐ distance from vaginal margin…….mm

No ☐

Pelvic nodes (including obturator, internal and external iliac)	right left	Common iliac nodes	right left
total number of nodes retrieved	…….	total number of nodes retrieved	…….. ….. .
lymph nodes with tumour deposits	……. ……..	lymph nodes with tumour deposits	…….. ……
Extranodal spread Para-aortic nodes : Not sampled ☐ Positive ☐ Negative ☐	Yes ☐ No ☐		Yes ☐ No ☐

Other tissues involved Yes ☐ Specify……..

No ☐ Specify…….

Peritoneal washings Positive ☐ Negative ☐

Comments ……..

Signature…….. Date…….

FIGO staging for endometrial carcinoma (Chicago, 1994, Society of Gynaecologic Oncologists)

Stage O – insitu carcinoma

Stage 1 – Confined to corpus

1A – Grade 1,2, 3) : Tumour limited to endometrium

1B – Grade 1,2,3 : Invasion to < one half of myometrium

1C – Grade 1,2,3 :Invasion to > one half of myometrium

Stage II – Extension to cervix

II A – Grade 1,2,3 : Endocervical glandular involvement only

IIB – Grade 1,2,3: Cervical stromal invasion

Stage III – Local and /or regional as specified below

IIIA – Grade 1,2,3 :Tumour invades serosa and /or adnexa, and /or +ve peritoneal cytology

IIIB – Grade 1,2,3 : Vaginal metastasis

IIIC – Grade 1,2,3 : Metastasis to pelvic and /or paraaortic lymph nodes

Stage IV A – Grade 1,2,3 : Tumour invasion of bladder and or bowel mucosa

IV B – Distant metastasis including intra abdominal and or inguinal lymph nodes

Histological grading of endometriod type of adenocarcinoma

FIGO grade 1 (5% or less of non-squamous or non morular solid growth pattern)

FIGO grade 2 (6% to 50% of non squamous type or non morular solid growth pattern)

FIGO grade 3 (> 50% of non squamous or non morular solid growth pattern)

Notes on pathological grading

1. Notable nuclear atypia, inappropriate for the architectural grade raises the grade of a grade 1 or grade 2 tumour by 1.
2. In Clear cell adenocarcinoma ; serous adenocarcinoma and squamous cell carcinomas nuclear grading takes precedence. The former 2 are considered high grade tumours.
3. Adenocarcinomas with squamous differentiation are graded according to the nuclear grade of the glandular component, squamous element is ignored.

Rules related to staging

1) Because corpus cancer is now staged surgically, procedures previously used for determination of stages are no longer applicable, such as findings from fractional D& C to differentiate between stage 1 & 2
2) Ideally width of the myometrium should be measured along with the width of tumour invasion.
3) The staging system for uterine corpus carcinoma is used for uterine sarcomas

D I - OVARIES AND FALLOPIAN TUBES -ANATOMY

Anatomy of the Ovaries and Fallopian Tubes (Fig 1.24)

Ovaries

The ovaries are paired, almond-shaped gonads situated in the ovarian fossa on either side of the uterus. They are attached to the uterus by the ovarian ligament and to the lateral pelvic wall by the suspensory ligament (infundibulopelvic ligament), which contains the ovarian vessels.

Each ovary has:

- **Surface (germinal) epithelium** – a single layer of modified mesothelium.
- **Cortex** – contains ovarian follicles in various stages of development (primordial, primary, secondary, and Graafian follicles) embedded in cellular stroma.
- **Medulla** – composed of loose connective tissue containing blood vessels, lymphatics, and nerves.

Functionally, the ovary is responsible for:

- Oogenesis
- Production of steroid hormones (estrogen and progesterone)

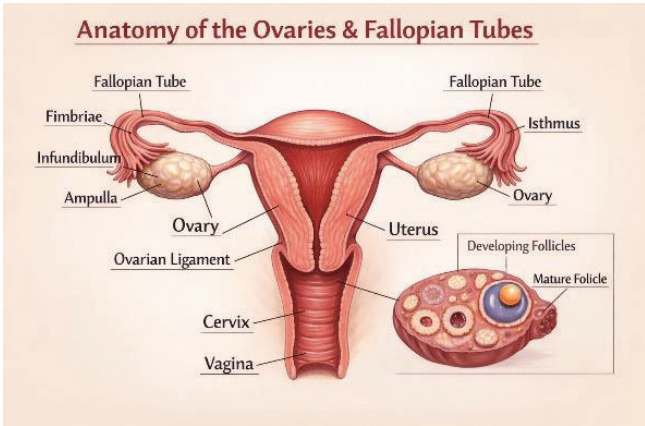


Fig 1.24

Fallopian Tubes (Uterine Tubes)

The fallopian tubes extend from the uterine cornua to the ovaries and measure approximately 10–12 cm in length. They are intraperitoneal structures supported by the mesosalpinx.

Each tube is divided into four anatomical segments:

- **Infundibulum** – the funnel-shaped distal end with finger-like fimbriae that capture the ovum.
- **Ampulla** – the widest and longest segment; the usual site of fertilization.
- **Isthmus** – narrow, thick-walled medial segment.
- **Interstitial (intramural) portion** – traverses the uterine wall.

Histologically, the tube consists of:

- **Mucosa** with complex plicae lined by ciliated columnar epithelium and secretory cells
- **Muscularis** (inner circular and outer longitudinal layers)
- **Serosa**

The coordinated ciliary activity and muscular contractions facilitate transport of the ovum or zygote toward the uterine cavity.

D II - OVARIES AND FALLOPIAN TUBES -GROSSING TECHNIQUES

Ovary

Measurements
Record in three dimensions and note **weight** (especially if sent alone).

External surface:
Smooth or irregular; presence of adhesions or rupture.

Cut surface:

- **Capsule:** Normal or thickened; adhesions, haemorrhage, rupture, etc.
- **Cortex and medulla:** Describe appearance.
- **Cysts:** Location, number, size.
- **Corpus luteum:** Describe if present.
- **Additional findings:** Calcification or other notable changes.

Sectioning:
Cut along the maximum diameter of the ovary.

Bits (see fig 1.25)

Label	Site / Description
H	One bit routinely to include cortex and medulla; if a single cyst < 2.5 cm, include adjacent cyst wall. May be combined with the Fallopian tube of the same side.
J	For larger cysts (> 2.5 cm): 3 bits including solid areas.

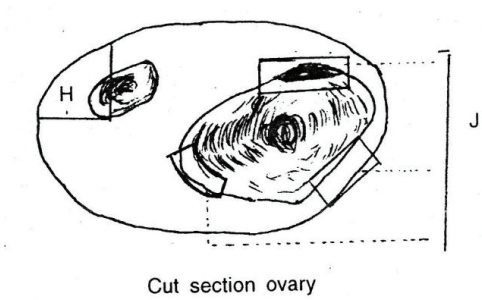


Fig 1.25

Fallopian Tube

Measurements:
Length and greatest diameter (without opening).

Description:

- External surface: smooth or irregular; presence of **tubectomy scar**, fibrin, haemorrhage, rupture, placental tissue, blood clot, or embryo.
- Section tube perpendicular to its length, taking multiple parallel cuts to include proximal, middle, and fimbrial portions.
- Lumen: Patent or dilated; if dilated, record maximum diameter.
- Masses**:** Size, appearance, invasion into wall.
- **Cysts:** Location (paraovarian or fimbrial), size, and appearance.
- In ectopic gestation, open along the entire length of the tube.

Bits (Fig 1.26)

Label	Site / Description
H	One bit routinely from the ampulla; may be combined with the ovary of the same side in routine hysterectomy specimens.
Tubectomy specimen	Two bits — one proximal and one distal to the tubectomy scar. Keep blocks separate from ovary.
Ectopic pregnancy	Two or more bits , as necessary

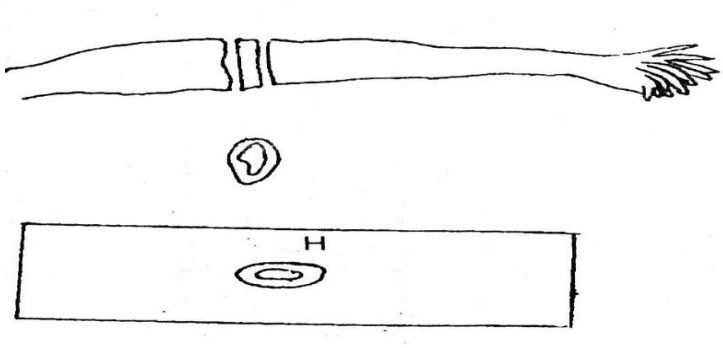


Fig 1.26 Routine bit- Fallopian tube

F. Ovarian Tumours

Gross Description

- **Capsule:** Intact or ruptured
- **Surface:** Smooth or nodular; note adhesions or areas of rupture
- **Cut surface:** Comment on the following –
  - Consistency (solid or cystic)

- Nature (papillary, uniloculated or multiloculated cyst)
- Contents (serous, mucinous, haemorrhagic etc.)
- Areas of haemorrhage, necrosis, calcification
- Presence of normal ovarian tissue (if seen)

After initial bisection along the **greatest dimension**, take **parallel sections** 0.5–1 cm thick across the tumour.

Bits (Fig 1.27)

Solid Tumours

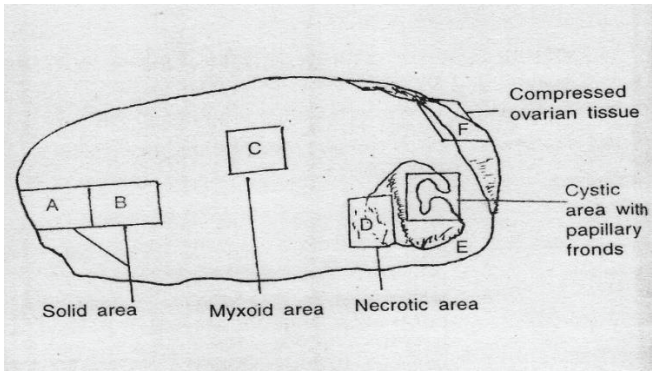


Fig 1.27.

- 3–4 bits if tumour < 5 cm.
- If > 5 cm: one block per centimetre along the greatest dimension, especially through variegated areas.

Cystic Neoplasms (Fig 1.28)

- Gross as shown (serially slice the cyst through its maximum diameter).
- Submit representative bits from:
  - Cyst wall (3 -4 bits including any solid or papillary areas)

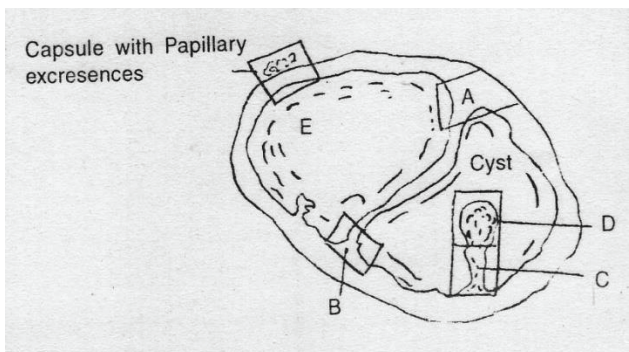


Fig 1.28

  - Thickened or necrotic zones
  - Capsule and adjacent normal ovarian tissue

Tumours of the Fallopian Tube

Gross Description

- Note distension, nodularity, or mass formation on outer surface.
- Make multiple cross sections perpendicular to the long axis.
- Describe:
  - Tumour nodules
  - Papillary processes
  - Haemorrhage and necrosis
- SEE-FIM protocol (Sectioning and Extensively Examining the FIM briated end) is now advised for risk-reduction surgery and for identifying STIC lesions (serous tubal intra-epithelial carcinoma -this lesion is limited to the fallopian tube epithelium and a precursor to extrauterine (pelvic) high grade serous carcinoma)

Bits (Fig 1.29)

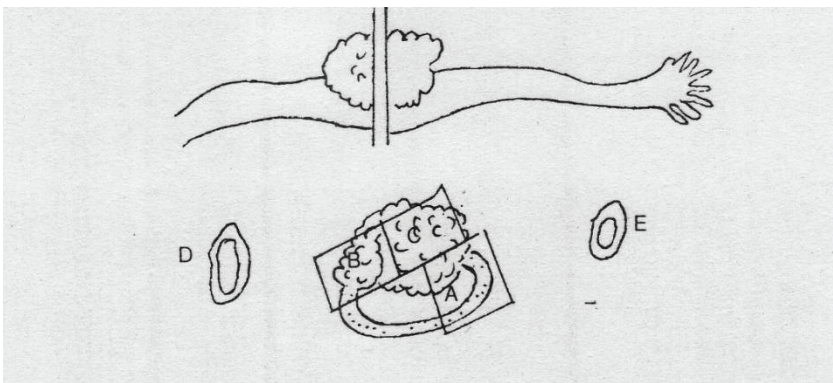


Fig 1.29

Label	Description
A, B, C	Tumour with adjacent wall (≥3 bits).
D & E	Proximal and fimbrial ends uninvolved by tumour.
"In prophylactic salpingo-oophorectomy and high-grade serous carcinoma work-ups, the SEE-FIM protocol should be used to examine the fimbrial end completely."	

D III - OVARIES AND FALLOPIAN TUBES -GROSS PEARLS

- **Ovarian cyst:** any type of cyst like Bartholin, epidermoid etc. >2.5 cm qualifies as "cyst"; smaller cysts may be graafian follicle or corpus luteum
- **Single cyst:** Solitary follicular cyst (5–10 cm)
- **Multiple small cysts (<0.5 cm):** Polycystic ovarian disease (PCOD / Stein–Leventhal syndrome), sclerotic cortex
- **Cyst with yellow rim:** Corpus luteum cyst (<6 cm)
- **Haemorrhagic cyst with thin/thick wall:**
    - Chocolate cyst / endometriotic cyst
    - Torsion in follicular cyst
    - Torsion in corpus luteal cyst
- **Cystic ovarian tumour:**
    - Serous (serous fluid/mildly viscous)
    - Mucinous (mucoid contents, may be multilocular)
    - Yolk sac tumour (with necrosis & haemorrhage)
    - Benign cystic teratoma (hair, pultaceous material, tooth, Rokitansky protuberance)
- Solid ovarian tumour:
    - Fibroma-thecoma group
    - Granulosa-theca cell tumours
    - Sertoli-Leydig cell tumours
    - Brenner tumour
    - Dysgerminoma
    - Clear cell carcinoma
    - Undifferentiated carcinoma
- **Solid tumour with yellow/tan cut surface:** Thecoma or fibroma-thecoma
- **Cystic tumour with papillary/polypoid excrescences:** Serous papillary cystadenoma/carcinoma
- **Papillae or polypoid fronds on outer surface:** Surface serous papilloma
- **Bilateral ovarian tumours:** Serous, mucinous, germ cell (dysgerminomas), Krukenberg tumour
- **Tubo-ovarian mass:**
    - Chronic salpingo-oophoritis
    - Endometriotic cyst with secondary adhesions
    - Malignant tumour of fallopian tube/ovary infiltrating the other
- **Ovarian tumours:**

- WHO 2020 classification emphasises **tubo-ovarian origin** of high-grade serous carcinoma.
- **Krukenberg tumour:** Always bilateral; may originate from gastrointestinal primary — note mucin-producing signet-ring cells.

- **Distended Fallopian tube:**
  - Soft & cystic → Hydrosalpinx
  - Haemorrhagic with rupture → Ectopic tubal gestation
  - Firm to hard → Carcinoma
- **Fallopian tube carcinoma:** Recognised as primary site for many serous carcinomas; histological evaluation should include fimbrial end extensively. SEE-FIM protocol recommended for risk-reducing salpingo-oophorectomy and to detect STIC lesions.

D IV OVARIES AND FALLOPIAN TUBES -MICROSCOPY

WHO Classification of Ovarian Tumours (2020, 5th Edition)

Ovarian tumours are classified according to the cell of origin and their histopathological characteristics. The 2020 WHO classification incorporates molecular features and refines diagnostic categories.

I. Epithelial tumours

1. **Serous tumours** – benign, borderline, and malignant (high-grade and low-grade serous carcinoma).
2. **Mucinous tumours** – benign, borderline, malignant.
3. **Endometrioid tumours** – including borderline and carcinoma.
4. **Clear cell tumours** – clear cell adenofibroma, clear cell carcinoma.
5. **Brenner tumours** – benign, borderline, malignant.
6. **Seromucinous tumours** – benign and borderline (malignant category discontinued in 2020).
7. **Mixed epithelial tumours.**

II. Sex cord–stromal tumours

1. Granulosa cell tumour (adult and juvenile types).
2. Thecoma and fibroma group.
3. Sertoli–Leydig cell tumour.
4. Steroid cell tumour, NOS.
5. Microcystic stromal tumour (newly recognized).
6. Sclerosing stromal tumour.

III. Germ cell tumours

1. Dysgerminoma.
2. Yolk sac tumour.
3. Embryonal carcinoma.
4. Choriocarcinoma (nongestational).
5. Teratomas – mature, immature, monodermal.
6. Mixed germ cell tumours.

IV. Miscellaneous and secondary tumours

- Small cell carcinoma (hypercalcaemic and pulmonary type).
- Metastatic carcinoma (commonly Krukenberg tumour).

Epithelial Tumours

Serous Tumours

- **Benign:** Lined by a single layer of ciliated columnar epithelium resembling fallopian tube mucosa. Papillary structures may be seen.
- **Borderline:** Shows hierarchical papillary architecture, mild nuclear atypia, and epithelial stratification without stromal invasion.
- **Malignant (High-grade Serous Carcinoma):** Complex papillae with slit-like spaces, marked nuclear atypia, and mitoses. Necrosis and psammoma bodies may be present.
    - **IHC:** WT1+, PAX8+, p53 (mutant pattern), p16 diffuse, ER+.
    - **Prognosis:** High-grade tumours are aggressive; low-grade serous carcinoma (BRAF/KRAS mutated, WT1+, p53 wild-type) has a more indolent course.
    - SET (solid, endometrial-like, transitional) patterns harbor p53 mutations and are now under the category of high grade serous carcinoma, They may also be associated with BRCA gene mutations (somatic or germline).

Mucinous Tumours

- Show mucin-filled glands and cysts lined by tall columnar epithelium resembling endocervical or intestinal mucosa.
- **Borderline:** Epithelial stratification and mild atypia without stromal invasion.
- **Carcinoma:** Stromal invasion with complex glandular pattern or solid areas.
    - **IHC:** CK7+, variable CK20+, PAX8+, negative WT1.
    - **Prognosis:** Most are unilateral and have better outcome than serous carcinoma.

Endometrioid Carcinoma

- Resembles endometrial adenocarcinoma; may arise from endometriosis.

- Cribriform or confluent glandular pattern with squamous differentiation.
    - **IHC:** ER+, PR+, PAX8+, β-catenin nuclear positivity; WT1−.
    - **Prognosis:** Intermediate; associated with synchronous endometrial carcinoma.

Clear Cell Carcinoma

- Large polygonal cells with clear or hobnail cytoplasm, forming tubulocystic or papillary structures.
    - **IHC:** HNF1β+, Napsin A+, PAX8+, WT1−, ER−.
    - **Prognosis:** Often chemoresistant; stage-dependent survival.

Brenner Tumour

- Nests of transitional-type (urothelial) epithelium in dense fibrous stroma.
    - **IHC:** GATA3+, CK7+, uroplakin II+, PAX8−.
    - **Prognosis:** Benign in most cases.

Mixed and Sero-mucinous Tumours

- Show admixture of cell types; the malignant category has been removed from the 2020 classification.

Sex Cord–Stromal Tumours

Granulosa Cell Tumour (Adult Type)

- **Microscopy:** Microfollicular, trabecular or diffuse patterns; Call–Exner bodies; coffee-bean nuclei with nuclear grooves.
    - **IHC:** Inhibin+, Calretinin+, FOXL2 mutation (C134W) in ~95%.
    - **Prognosis:** Indolent; late recurrence may occur even after decades.

Thecoma–Fibroma Group

- Composed of spindle cells producing collagen (fibroma) or lipid-laden cells (thecoma).
    - **IHC:** Inhibin+, Calretinin+.
    - **Prognosis:** Benign; may cause Meigs' syndrome (ascites, pleural effusion).

Sertoli–Leydig Cell Tumour

- Tubules of Sertoli cells (see fig 1.30) with interspersed Leydig cells containing Reinke crystals.
    - **IHC:** Inhibin+, Calretinin+, SF-1+.

- **Prognosis:** Usually low-grade malignancy; recurrence in poorly differentiated forms.

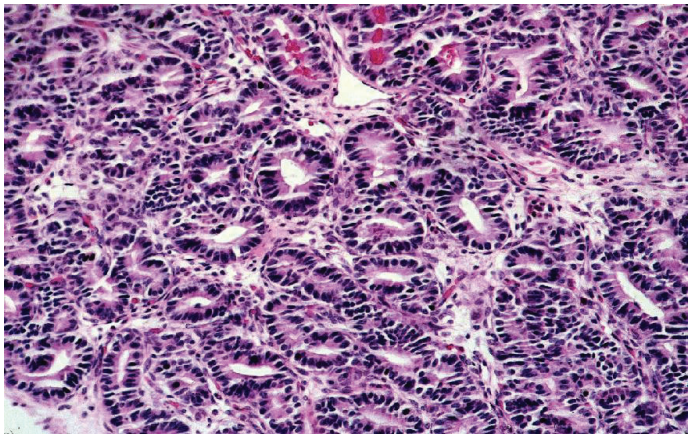


Fig 1.30 : Shows tubules in Sertoli Leydig Cell tumour of the ovary.

Steroid Cell Tumour (NOS)

- Composed of polygonal cells with abundant eosinophilic or vacuolated cytoplasm.
  - **IHC:** Inhibin+, Calretinin+, Melan-A+.
  - **Prognosis:** Variable; 25–40% show malignant behaviour.

Germ Cell Tumours

Dysgerminoma

- Sheets of uniform polygonal cells with clear cytoplasm, central nuclei, separated by fibrous septa infiltrated by lymphocytes.
  - **IHC:** PLAP+, OCT3/4+, c-Kit+, SALL4+.
  - **Prognosis:** Radiosensitive; excellent outcome.

Yolk Sac Tumour

- Reticular, microcystic, or endodermal sinus pattern (Schiller–Duval bodies).
  - **IHC:** AFP+, Glypican-3+, SALL4+.
  - **Prognosis:** Aggressive; early chemotherapy response.

Immature Teratoma

- Contains immature neuroepithelium in addition to mature elements.
  - **Grading** depends on amount of immature tissue.
  - **Prognosis:** Grade and stage dependent; good response to chemotherapy.

Mature Teratoma (Dermoid Cyst)

- Cyst lined by squamous epithelium with skin appendages, sebaceous material, and variable tissues from all germ layers.
    - **IHC:** Not required for diagnosis.
    - **Prognosis:** Benign; rare malignant transformation (~1–2%).

Miscellaneous Tumours

Small Cell Carcinoma of Ovary

- **Types:** (1) Hypercalcaemic type (SCCOHT), (2) Pulmonary type.
    - **IHC:**
        - Hypercalcaemic: EMA+, INI1 loss, inhibin−.
        - Pulmonary: NSE+, Chromogranin+, Synaptophysin+.
    - **Prognosis:** Extremely poor; SCCOHT is associated with SMARCA4 mutation.

WHO -ovarian tumours : key updates :

Ovarian tumours are classified into **epithelial**, **sex-cord stromal**, **germ-cell**, **mixed**, and **mesenchymal** categories.

Epithelial tumours:

- *Serous tumours* – high-grade and low-grade serous carcinoma
- *Mucinous tumours*
- *Endometrioid carcinoma*
- *Clear cell carcinoma*
- *Seromucinous, Brenner, and mixed carcinomas*
- **New or emphasized entities:**
    - Mesonephric-like adenocarcinoma
    - Dedifferentiated and undifferentiated carcinoma
- **Molecular associations:**
    - High-grade serous carcinoma → *TP53*, *BRCA1/2*
    - Low-grade serous carcinoma → *KRAS*, *BRAF*
    - Endometrioid and clear cell → *ARID1A*, *PTEN*
- WHO first classified low and high grade serous carcinoma as different subtypes of the same tumor; now it is evident that these are 2 different types of carcinoma have different molecular characteristics, IHC profile, different morphology as also pathogenesis and prognosis .

- Most of the high grade serous carcinomas (of ovary) arise from the distal fimbria of the fallopian tube from a precursor lesion known as serous tubal intraepithelial carcinoma (STIC)
- Low grade serous carcinomas of the ovary arise from benign or borderline serous neoplasms

Microscopy of Fallopian Tube

The fallopian tube may show a variety of inflammatory, benign and malignant lesions on microscopic examination.

Inflammatory lesions:
Acute salpingitis shows edema and congestion of the mucosa with dense neutrophilic infiltrate, often involving the mucosal folds and lumen. In chronic salpingitis, the mucosa shows lymphocytes and plasma cells with fibrosis and distortion of the plicae. Tuberculous salpingitis demonstrates granulomas composed of epithelioid cells, Langhans giant cells and caseous necrosis.

Endometriosis:
Fallopian tube endometriosis is characterized by the presence of endometrial glands and stroma within the tubal wall, often associated with hemorrhage and hemosiderin-laden macrophages.

Benign epithelial proliferations:
Papillary epithelial hyperplasia and adenofibroma may be seen rarely. The epithelium forms papillary or glandular structures without significant cytologic atypia or stromal invasion.

Serous Tubal Intraepithelial Carcinoma (STIC):
This lesion, usually arising in the fimbrial end, shows marked epithelial atypia with nuclear enlargement, hyperchromasia, loss of polarity and increased mitotic activity, without stromal invasion. It is regarded as a precursor of high-grade serous carcinoma of the ovary and peritoneum.

Primary carcinoma of the fallopian tube:
Most are serous adenocarcinomas and show complex papillary or glandular architecture lined by markedly atypical epithelial cells, with high mitotic activity and stromal invasion. Psammoma bodies may be present.

Metastatic tumours:
The fallopian tube may also be involved secondarily by metastatic carcinoma from ovary, endometrium or gastrointestinal tract, showing infiltration of the tubal wall by malignant cells.

Microscopic evaluation should include careful examination of the fimbrial end, as many early serous carcinomas originate in this region.

D V OVARIES AND FALLOPIAN TUBE-SYNOPTIC REPORTING

Ovary and Fallopian tube

Non – neoplastic / Benign tumours

Macroscopy

Nature of specimen : …….

Wedge resection ☐

Complete removal☐ ☐ Other ☐

Right : ☐ Left ☐ Both ovaries ☐

Dimensions : Right ……x……x……cm Left ……x……x……cm

Fallopian tube : Present ☐ Length ……cm Absent ☐

Hydrosalpinx ☐ Rupture ☐

Tubo-ovarian mass: Yes ☐ No ☐

Surface papillary tumour Yes ☐ No ☐

C/S : cystic ☐ haemorrhage ☐ solid ☐ Solid & cystic ☐

Microscopy : Ovary unremarkable ☐

Fallopian tube unremarkable☐

Cystic follicle ☐

Cystic corpus luleum ☐

Follicular cyst ☐

Corpus luteum cyst ☐

Endometriotic cyst/cysts ☐

Stromal hyperplasia ☐

Stromal hyperthecosis ☐

Neoplasm seen : Yes ☐ No ☐

Histological subtype ……

Chronic salpingitis ☐

Chronic salpingo-oophoritis ☐

Granulomatous inflammation ☐ Tuberculous ☐ Actinomycosis ☐ Foreign body type☐

Ectopic tubal gestation☐

Other ☐ Specify…….

Other findings……..

Signature…… Date…/…/…. Snomed code

OVARY – TUMOUR

(Based on current WHO classification and FIGO staging principles)

1. Specimen

- Type of procedure:
    - Oophorectomy ☐
    - Salpingo-oophorectomy ☐
    - TAH + BSO ☐
    - Staging laparotomy specimen ☐
- Laterality:

o Right ☐
o Left ☐
o Bilateral ☐

2. Tumour Identification

- Histologic type (WHO classification)
    - High-grade serous carcinoma ☐
    - Low-grade serous carcinoma ☐
    - Mucinous carcinoma ☐
    - Endometrioid carcinoma ☐
    - Clear cell carcinoma ☐
    - Brenner tumour ☐
    - Sex cord–stromal tumour ☐ (specify)…….
    - Germ cell tumour ☐ (specify) ……
    - Metastatic tumour ☐ (specify origin)……..

Tumour grade

- For serous carcinoma: Low-grade ☐ / High-grade ☐
- For endometrioid carcinoma: FIGO grade 1 / 2 / 3 ……..

3. Tumour Size

- Greatest dimension (cm) ……..
- Additional dimensions (if relevant)……

4. Tumour Extent

- Limited to ovary ☐
- Ovarian surface involvement (Present ☐/Absent ☐)
- Capsule:
    - Intact ☐
    - Ruptured (intraoperative ☐ / preoperative ☐)
- Involvement of:
    - Fallopian tube ☐
    - Uterus ☐
    - Contralateral ovary ☐
    - Omentum ☐
    - Peritoneum ☐
    - Other pelvic/abdominal structures (specify)…….

5. Lymphovascular Invasion

- Present / Absent……..

6. Peritoneal Cytology

- Positive / Negative / Not submitted …….

7. Lymph Nodes (if sampled)

- Number examined ……..
- Number positive ……..
- Largest metastatic focus (mm) ……
- Extranodal extension (Present/Absent) …….

8. Margins (if applicable)

- Involved / Uninvolved ……
- Specify site ……

9. Pathologic Staging (FIGO)

- pT: ……..
- pN: …….
- pM (if applicable) ……
- Final FIGO Stage: …….

10. Ancillary Studies (if performed)

- p53 (wild-type / mutant pattern) ……
- WT1 ……..
- ER/PR ……..
- Napsin A (clear cell) ……..
- Inhibin / SF1 (sex cord stromal) …….
- AFP, SALL4 (germ cell) ……
- MMR status (if indicated) …….
- BRCA testing (if performed) ……..

FALLOPIAN TUBE

(Particularly important in the era of tubal origin of serous carcinoma)

1. Specimen

- Salpingectomy ☐
- Salpingo-oophorectomy ☐
- Risk-reducing salpingo-oophorectomy ☐

Laterality:

- Right ☐
- Left ☐
- Bilateral ☐

2. Tumour Identification

- Histologic type:
    - High-grade serous carcinoma ☐
    - Low-grade serous carcinoma
    - Endometrioid carcinoma
    - Clear cell carcinoma
    - Transitional/Brenner-type
    - Primary tubal carcinoma
    - Metastatic carcinoma
- Serous Tubal Intraepithelial Carcinoma (STIC):
    - Present / Absent
    - Location (fimbria / other)

3. Tumour Size

- Greatest dimension (cm)

4. Tumour Extent

- Confined to tube
- Serosal involvement
- Extension to:
    - Ovary
    - Uterus
    - Peritoneum
    - Omentum
    - Other pelvic organs

5. Lymphovascular Invasion

- Present / Absent

6. Lymph Nodes

- Number examined
- Number positive

7. Peritoneal Cytology

- Positive / Negative

8. Margins

- Involved / Uninvolved

9. Pathologic Staging (FIGO – shared ovarian/tubal/peritoneal system)

- pT:
- pN:
- pM:
- Final FIGO Stage:

10. Ancillary Studies

- p53 (mutant pattern supports STIC)
- Ki-67 (high in STIC)
- WT1
- BRCA testing (if performed)

Important Concept: High-grade serous carcinomas of ovary, fallopian tube and primary peritoneum are staged using the same FIGO staging system, reflecting the tubal origin theory.

OVARY – GERM CELL TUMOURS

(Applicable to dysgerminoma, yolk sac tumour, immature teratoma, mixed germ cell tumours, etc.)

1. Specimen

- Procedure:
  - Oophorectomy
  - Salpingo-oophorectomy
  - Fertility-sparing staging procedure
  - TAH + BSO
  - Staging laparotomy specimen
- Laterality:
  - Right
  - Left
  - Bilateral

2. Tumour Identification

Histologic Type (WHO Classification)

- Dysgerminoma
- Yolk sac tumour
- Embryonal carcinoma
- Choriocarcinoma (non-gestational)
- Immature teratoma
- Mature teratoma with malignant transformation
- Mixed germ cell tumour (specify components and %)

3. Tumour Size

- Greatest dimension (cm)
- Additional dimensions

4. Tumour Focality

- Unifocal
- Multifocal

5. Tumour Grade (if applicable)

Immature Teratoma (Grade Required)

- Grade 1
- Grade 2
- Grade 3
  (Based on amount of immature neuroepithelium per low-power field)

Note: Most other malignant germ cell tumours are not graded.

6. Tumour Extent

- Confined to ovary
- Capsule:
  - Intact
  - Ruptured (preoperative / intraoperative)
- Ovarian surface involvement:
  - Present / Absent
- Involvement of:
  - Contralateral ovary
  - Fallopian tube
  - Uterus
  - Omentum
  - Peritoneum
  - Other pelvic/abdominal organs (specify)

7. Lymphovascular Invasion

- Present
- Absent

8. Lymph Nodes (if sampled)

- Number examined
- Number positive
- Largest metastatic deposit (mm)
- Extranodal extension (Present/Absent)

9. Peritoneal Cytology

- Positive
- Negative

- Not submitted

10. Margins (if applicable)

- Involved / Uninvolved
- Specify site

11. Pathologic Staging

(Staged using the same FIGO system as epithelial ovarian carcinoma)

- pT:
- pN:
- pM:
- Final FIGO Stage:

12. Serum Tumour Markers (if available)

(Preoperative or postoperative — useful for clinicopathologic correlation)

- AFP
- β-hCG
- LDH
- CA-125 (if performed)

Correlation with histologic subtype recommended.

13. Ancillary Studies (if performed)

- SALL4
- OCT3/4
- PLAP
- AFP (IHC)
- Glypican-3
- CD30
- β-hCG (IHC)

Key Reporting Pearls

- Always document percentage of each component in mixed germ cell tumours.
- Grade is mandatory only for immature teratoma.
- Capsule status and surface involvement significantly impact staging.
- Bilaterality is common in dysgerminoma – check for this.
- Extensive sampling required in mature teratoma in postmenopausal women (to exclude malignant transformation).

OVARY - MALIGNANT SURFACE EPITHELIAL TUMOURS

Macroscopy

Nature of specimen : …….

Ovaries

Right : □ Dimension ……x……x……cm Tumour involvement Yes □ No □

Capsule intact □ Breached by tumour □ Not assessable □

Surface involvement Yes □ No □

Left : □ Dimension ……x……x……cm Tumour involvement Yes □ No □

Capsule intact □ Breached by tumour □ Not assessable □

Surface involvement Yes □ No □

Fallopian tubes

Right □ Normal □ Abnormal □ Comment…….

Left □ Normal □ Abnormal □ Comment…….

Uterus and cervix

Normal □ Abnormal □ Comment…….

Omentum Biopsy □ Omentectomy □ Normal □ Abnormal □
Comment……

Size of largest tumour nodule …….cms

Peritoneal biopsy

Not received □ Normal □ Abnormal □ Comment……

Microscopy

Right

Benign □ None □ Serous □ Mucinous □ Other (specify)……

Borderline epithelial change: None □ Serous □ Mucinous □ Other (specify)……

Microinvasion : Not present □ Present □

Invasive carcinoma : Not present □ Serous □ Mucinous □ Endometroid

Transitional □ Clear cell □ Mixed epithelial type □ (tick all that apply)

Other (specify)……

Differentiation (if appropriate) well(grade1) □ Moderate (grade2) □ Poor (grade3) □

Left

Benign □ None □ Serous □ Mucinous □ Other (specify)……

Borderline epithelial change: None □ Serous □ Mucinous □ Other (specify)……

Microinvasion : Not present □ Present □

Invasive carcinoma :Not present □ Serous □ Mucinous □ Endometroid

Transitional □ Clear cell □ Mixed epithelial type □ (tick all that apply)

Other (specify)……

Differentiation (if appropriate) well(grade1) □ Moderate (grade2) □ Poor (grade3) □

Microscopic features : other structures

Fallopian tubes

Right □ Involved □ Not involved □

Left □ Involved □ Not involved □

Endometrium Normal □ Abnormal □ Comment …….

Myometrium Normal □ Abnormal □ Comment …….

Uterine Serosa : Not involved □

Boderline changes (non-invasive implants) □

Invasive carcinoma □

Omentum Not involved □ Boderline changes (non-invasive implants) □

Invasive carcinoma □

Peritoneal biopsies (if received) Not involved □ Boderline changes (non-invasive implants) □

Epithelial ☐ Desmoplastic ☐ Invasive carcinoma ☐

Peritoneal washings (if received) Not involved □ Involved □

Appendix (if received) Not involved □ Involved □ Comment…….

Lymph nodes Sites……. Total no…… No involved…….

Comments /additional
information……..

…….

…….

Signature…… Date…/…/…. Snomed code

Note : Borderline tumours should only be diagnosed after rigorous sampling has been done to exclude invasion

A distinction should be made between capsular breach due to surgical procedure and breach due to tumour invasion.

Microinvasion in serous, mucinous tumours is< 5mm from the basement; and in endometriod tumours is < 5mm in diameter.

NON-EPITHELIAL TUMOURS OVARY

Macroscopy :

Right : □ Dimension ……x……x……cm Tumour involvement Yes □ No □

Capsule intact □ Breached by tumour □ Not assessable □

Left : □ Dimension ……x……x……cm Tumour involvement Yes □ No □

Capsule intact □ Breached by tumour □ Not assessable □

Fallopian Tubes

Right : Normal □ Abnormal □

Left : Normal □ Abnormal □

Uterus and cervix : Normal □ Abnormal □ Comment……

Omentum: Biopsy □ Omentectomy □

Normal □ Abnormal □ Comment……

Peritonial biopsies Not received □ Normal □ Abnormal □
Comment……

Microscopy :

Tumour involvement : Right □ Left □ Bilateral □

Granulosa cell tumour : Adult □ Juvenile □

Fibroma □ Thecoma □ Fibrosarcoma □

Sclerosing stromal tumour □

Sertoli □ Leydig cell □ Sertoli Leydig cell □

Heterologous elements: present □ Absent □

Differentiation (sertoli/Leydig)

well □ intermediate □ poor □ retiform □ not assessable □

Gynandroblastoma □ Steroid cell tumour □ SCTAT □ Gonadoblastoma □

Mitoses / 10 hpfs ……..

Mature teratoma □ Mature teratoma with malignant change □

Immature teratoma □ low grade □ high grade□

Dysgerminoma □ Yolk sac tumour □

Embryonal carcinoma □ Polyembryoma □ Choriocarcinoma □

Mixed germ cell □ Germ cell tumour in gonadoblastoma □

Mixed germ cell tumour NOS □

Soft tissue tumour not specific to ovary ……. Specify …….

Metastasis primary site if known …….

Vascular permeation present Not seen

Microscopy : Other findings

Right Fallopian tube : Involved □ Not involved □

Left Fallopian tube : Involved □ Not involved □

Endometrium Normal □ Abnormal □ Comment …….

Myometrium Normal □ Abnormal □ Comment …….

Uterine Serosa Involved □ Not involved □

Peritoneum (site/s) Involved □ Not involved □

Omentum Involved □ Not involved □

Lymph nodes Site/s : Number examined □ Number involved □

Comments ……..

Signature …….. Date…….

Note : Mixed tumours are reported if the minor component is > 10% of the tumour.

FIGO Staging for Ovarian malignancy :

FIGO Stage I ; tumour limited to the ovary

IA : tumour limited to one ovary ; capsule intact, no tumour on ovarian surface; no malignant cells in peritoneal washings or ascites

IB : : tumour limited to both ovaries ; capsule intact, no tumour on ovarian surface; no malignant cells in peritoneal washings or ascites.

IC : tumour limited to one or both ovaries with any of the following : capsule ruptured, tumour on ovarian surface; malignant cells in peritoneal washings or ascites.

Stage II : tumour involves one or both ovaries with pelvic extension

IIA : extension and /or implants on the uterus and /or tube(s); no malignant cells in peritoneal washings or ascites.

IIB : extension to other pelvic tissues; no malignant cells in peritoneal washings or ascites.

IIC : pelvic extension (2A or 2B) with malignant cells in peritoneal washings or ascites.

Stage III : tumour involves one or both ovaries with microscopically confirmed peritoneal metastasis outside the pelvis and /or regional lymph node metastasis.

IIIA : microscopic peritoneal metastasis beyond pelvis

IIIB : macroscopic peritoneal metastasis beyond pelvis 2cm or less in greatest dimension

IIIC : peritoneal metastasis beyond pelvis > 2cm in greatest dimension and /or regional lymph node metastasis.

Stage IV : distant metastasis (excludes peritoneal metastasis)

FALLOPIAN TUBE CARCINOMA

Macroscopy :

Nature of specimen :

Fallopian Tube

Right : Normal □ Abnormal □

Size of tumour …x….x….cm Serosal involvement Yes □ No□

Left : : Normal □ Abnormal □

Size of tumour …x….x….cm Serosal involvement Yes No

Ovaries:

Right Dimensions …x…..x….cm Tumour involvemrnt Yes No

Left : Dimensions …x…..x….cm Tumour involvemrnt Yes No

Uterus and cervix : Normal □ Abnormal □ Comment……

Omentum: Biopsy □ Omentectomy □

Normal □ Abnormal □ Comment……

Peritoneum Not received □ Normal □ Abnormal □ Comment…….

Microscopy :

Fallopian tubes

Right

Borderline epithelial change: None □ Serous □ Mucinous □ Other

Invasive carcinoma:

Invasive carcinoma :Not present □ Serous □ Mucinous □ Endometroid

Transitional □ Clear cell □ Mixed epithelial type □ (tick all that apply)

Other (specify)……

Differentiation (if appropriate) well (grade1) □ Moderate (grade2) □ Poor (grade3) □

Left

Borderline epithelial change: None □ Serous □ Mucinous □ Other

Invasive carcinoma:

Invasive carcinoma :Not present □ Serous □ Mucinous □ Endometroid

Transitional □ Clear cell □ Mixed epithelial type □ (tick all that apply)

Other (specify)……

Differentiation (if appropriate) well (grade1) □ Moderate (grade2) □ Poor (grade3) □

Ovaries

Right Not involved □ Involved □

Left Not involved □ Involved □

Endometrium Normal □ Abnormal □ Comment …….

Myometrium Normal □ Abnormal □ Comment …….

Uterine Serosa Not involved □

Boderline changes (non-invasive implants) □ Invasive carcinoma □

Omentum Not involved Boderline changes (non-invasive implants) □

Invasive carcinoma □

Peritoneal biopsies (if received) Not involved □ Boderline changes (non-invasive implants) □

Invasive carcinoma □

Peritoneal washings (if received) Not involved □ Involved □

Appendix (if received) Not involved □ Involved □ Comment…….

Lymph nodes Sites……. Total no…… No involved…….

Comments /additional information……..

…….

…….

Signature…… Date…/…/….

E I EXTERNAL GENITALIA -VULVA -ANATOMY

- External genitalia (vulva) (Fig 1.31)
- Mons pubis — fatty pad over pubic symphysis.
- Labia majora and labia minora — protective folds; labia minora enclose the vestibule.
- Clitoris — erectile organ formed from paired corpora cavernosa, highly innervated.
- Vestibule — contains urethral meatus and vaginal introitus; includes Bartholin (greater vestibular) glands and Skene's paraurethral tissue.

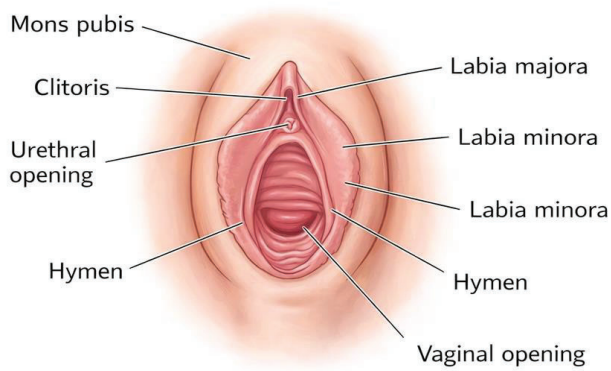


Fig 1.31

E II EXTERNAL GENITALIA -VULVA -GROSSING TECHNIQUES

Specimens Received

- Small biopsies (for non-tumoural lesions)
- Simple vulvectomy
- Radical vulvectomy

Biopsies for Non-tumoural Conditions

- Measure in three dimensions.
- Describe any lesion present.
- If < 4 mm → block entirely.
- If > 4 mm → section, describe cut surface, and block all tissue.

Vulvectomy Specimens

State type (simple / radical).

Gross Description

- Overall size (in 3 dimensions) — first measurement horizontally including inguinal region if present.
- **Tumour:** location, type of growth, ulceration, surface features, size.
- **Non-neoplastic skin:** atrophy, keratosis, ulceration.
- **Lymph nodes:** separate into groups and fix in individual containers.

After removing lymph nodes, pin the specimen on a cork board with pins at regular intervals and fix overnight.
Ideally photograph twice—one with sites of sections marked.

Sectioning

- Tumours < 2 cm → **radial sections** from tumour to closest surgical margin.
- Tumours > 2 cm → **vertical sections** 1 cm apart as shown.
- Record size of largest lymph node and number showing gross involvement.

Bits (Fig 1.32)

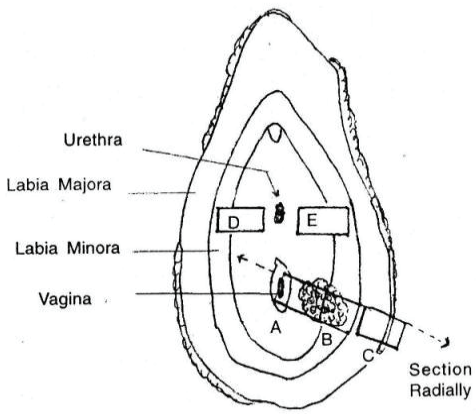


Fig 1.32

Simple Vulvectomy

Label	Description
A, B	Two bits tumour (< 2 cm lesions).
C	Closest surgical margin.
D	Labia majora & minora (right).
E	Labia majora & minora (left).
	For tumours > 2 cm — take additional bits proportionate to size.

Radical Vulvectomy (Fig 1.33)

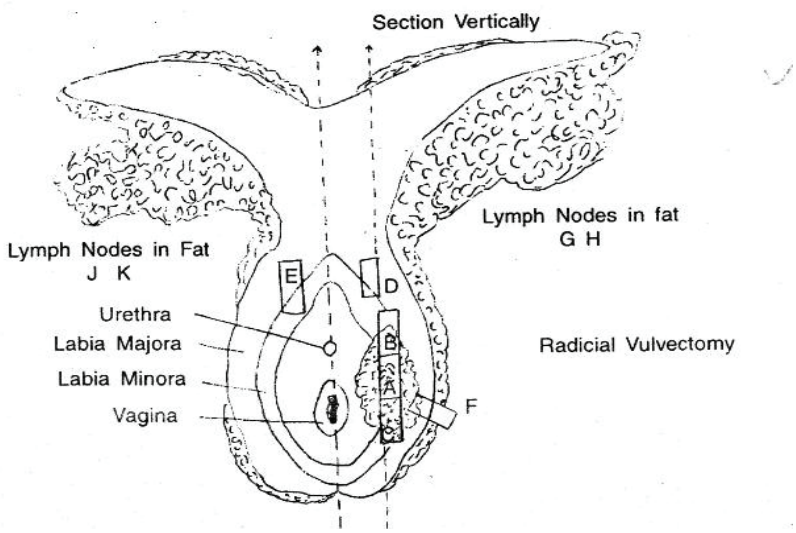


Fig 1.33

Label	Description
A, B, C	Tumour (3 sections including edge with normal skin and deepest invasion).
D, E	Non-neoplastic skin from labia majora & minora (right & left).
F	Closest soft tissue surgical margin(s).
G, H, J, K	Lymph nodes (superficial and deep inguinal), grouped separately.

E II EXTERNAL GENITALIA -VULVA -GROSS PEARLS

- Raised ulcerating lesion : non specific ulcer, Granuloma inguinale, LGV
- Warty Lesion : viral lesion, papilloma
- Raised papule : Papilloma
- Multiple raised lesions: Bowenoid papillomatosis, Wart
- Hypopigmented patches of varying sizes : Leukoplakia.
- Polypoid lesion : Squamous papilloma. Fibroepithelial polyp.

E II EXTERNAL GENITALIA -VULVA -MICROSCOPY

A. Vulva

The mons pubis, clitoris, labia majora and minora constitute the vulva.

1) Cysts : The Bartholin gland is the major vestibular gland with a tubulo-alveolar structure, made up of acini composed of mucus secreting cells and a duct lined by transitional epithelium. **A Bartholin gland cyst** is a distension of the duct lined by trasitional or stratified squamous epithelium, It is filled with mucin which is non sulphated sialomucin.

Other cysts : are the epidermoid cyst, cyst of the canal of Nuck, Gardner duct cyst, the latter being more common in the vagina.

2) Inflammations*: Granuloma inguinale (Donovanosis)* is a chronic inflammation caused by Calymmatobacterium granulomatis ie a Gram –ve, non motile encapsulated bacillus. Presents in the vulvar region as a soft nodule which ulcerates. The diagnosis rests on the demonstration of Donovan bodies which are small encapsulated round to oval bodies in the cytoplasm of histiocytes, well identified by the Giemsa and Warthin Starry stain which gives them a "safety pin appearance".

- *Lymphogranuloma Venereum* a veneral disease produced by Chlamydia group of organisms, serotypes L1, L2, L3. A small ulcer is seen at the site of contact which often goes unnoticed. Subsequently inguinal lymph nodes enlarge and give rise in their parenchyma, microscopically to stellate abscesses surrounded by pale epitheliod cells. Organisms are Gram negative and stain black with Warthin- Starry silver impregnationtechnique.This later leads to fistula formation, strictures of the urethra,

vagina and rectum. Diagnosis is confirmed by the Frie's test which is an intra dermal skin test; complement fixation and /or by immunoflurescence.

Viruses : Herpes simplex causes vulvar ulcers-mononuclear and multinucleate giant cells show ground glass nuclei or eosinophilic intranuclear inclusions. Human papilloma viruses are among the most common vulvar infections and cause squamous proliferations of the skin and mucous membranes (see below).

3) Leukoplakia – Refers to a white coloured skin patch accompanied by pruritus. It is a clinical term. At times the lesions appear as red patches with excoriation and atrophy, called "Kraurosis vulva". Histologically they prove to be either psoriasis, lichen planus, lichen simplex chronicus, lichen sclerosis, or squamous intra-epithelial lesion.

- Lichen sclerosis et atrophicus (chronic vulvar dystrophy.) : Occurs at any age. Histology shows i) atrophic epidermis ii) hypocellular upper dermis which is oedematous in the early stages and shows sclerosis later iii) inflammation beneath this composed of lymphocytes. Rarely goes onto squamous cell carcinoma.
- Keratosis (squamous hyperplasia): acanthosis, prominent stratum granulosum, hyperkeratosis and a mild chronic inflammatory cell infiltrate, absence of atypia.
- Mixed vulvar dystrophy is used when keratosis and lichen sclerosis et atrophicus co-exist.

4) Condyloma acuminatum **:** venereal disease caused by HPV type 6 and characterized by one or more soft elevated masses of variable size.

- microscopically : papillary processes of well differentiated squamous epithelium are seen with delicate , well vascularised connective tissue stalks. Low power shows the epithelium to be undulating.
- a flat condyloma is more common than a papillary one. Koilocytosis of the surface epithelium is seen in both, ie perinuclear vacuoles, minimal nuclear atypia and wrinkling of nuclear membranes "nuclear raisins". Normal mitoses are seen in contrast to abnormal mitoses. The latter together with nuclear pleomorphism and nuclear enlargement are features of VIN ie vulval intraepithelial neoplasia.

5) Squamous papilloma **:** papillary lesion without the cytological features characteristic of HPV infection.

6) ***Epidermolytic hyperkeratosis*** **:** related to Hailey- Hailey disease and shows similar histology with suprabasal clefts and dyskeratotic cells.

7) ***Bowenoid papulosis*** **:** -multiple often pigmented papules in or near the vulva of young patients. Clinically they resemble verrucae (HPV 16 associated), small condylomas or nevi.

- Characterised microscopically by a degree of atypia approaching Bowen's disease. The intra-epidermal portion of the hair follicle (acrotrichium) is spared. Acrosyringium is involved.

8) VIN (Bowen's disease) : -elevated plaque-like lesion with a red velvety appearance, occurs in the labia majora but may extend down to the perineum and anus. Several cases are associated with HPV infection. The lesion may be single or multiple, scaly or eczymatoid

- microscopically : hyperkeratosis, parakeratosis, acanthosis and a variable number of multinucleated dyskeratotic cells with abnormal mitoses *involving the entire thickness of the epidermis.* It corresponds to CIN III type of lesion in the cervix. Intraepidermal part of hair follicle is involved.

9) Carcinomas **: a) Squamous cell carcinoma :** constitutes 95% of malignant tumours of the vulva

- - frequently associated with malignant tumours elsewhere in the lower genital tract.
- - Two patterns are seen in the vulva : i) the common one occurs in the elderly and is not related to HPV. Microscopically characterized by a typical keratinizing squamous cell carcinoma and associated with keratosis.

  ii) younger women frequently HPV +ve , often with basaloid or warty histology and associated with VIN. Common on the labia majora and is generally well differentiated.

- WHO 2020 divides squamous lesions of the vulva into:
  - *HPV-associated* (vulvar HSIL / usual-type VIN)
  - *HPV-independent* (differentiated VIN, often with p53 abnormality)

b)- Microinvasive carcinoma : Depth of invasion in squamous cell carcinoma should

be < 5mm.

c) - Verrucous carcinoma is distinguished from condyloma acuminatum by its larger size and the presence of club shaped fingers of neoplastic epidermis invading the underlying stroma in a pushing fashion. DD from a squamous cell carcinoma is by the absence of cytologic atypia and a pushing infiltrative margin of growth as compared to an irregular infiltrative margin in squamous cell carcinoma.

d) Table to show differences between Condyloma acuminatum, Verrucous carcinoma and Squamous cell carcinoma :

Feature	Condyloma acuminatum	Verrucous carcinoma	Squamous cell carcinoma
Gross	Exophytic growth Variable size	Exophytic growth Huge	-Exophytic growth -Infiltrative
Micros	Papillomatosis Acanthosis Koilocytosis Variable inflammation in Stroma	Papillomatous Acanthosis Club shaped prolife-ration into stroma	Papillary pattern Diffuse infiltration

Feature	Condyloma acuminatum	Verrucous carcinoma	Squamous cell carcinoma
Atypia	Minimal	Nil, Well differentiated	Pleomorphism Nuclear atypia Marked
Margins	Nil, exophytic growth	Pushing	Infiltrative
HPV	+ve (subtype 6 & 11)	Rare / absent	+ve (16 & 18)
Cytokeratin	Homogenous +ve	+ve	Less uniform
Metastasis	Nil	Local invasion	Local & distant

e) Melanomas : rare cases of these occur in the vulvar, in the labia minora , clitoris or majora. They may be of the superficial spreading, nodular or acral lentiginous type.

f) Paget's disease : Carcinoma of multipotential cells located along the epidermal basal layer that differentiates along glandular (sweat gland) lines.

- it presents as crusty, elevated, scaly erythematous lesions
- Microscopy : Large pale tumour cells that form solid nests, glandular spaces with a cleft between them and the upper keratinocytes of the epidermis. Cella contain acidic mucin
- presence of melanin pigment does not rule out Paget's disease.

IHC : +ve for MUC 1, MUC 5AC, CK, EMA, CEA, B72.3, GCFD-15, and androgen receptors. ER and PR –ve

CK 7+vity and CK20-vity rules out any extension from urothelial malignancy the incidence of underlying invasive carcinoma in vulvar Paget's disease ranges from 0-30%

g) Differences between the various myxoid lesions of the vulva

Criteria	Aggressive angiomyxoma (AA)	Angiomyofibroblastoma (AMFB)	Superficial angiomyxoma (SAM)(cutaneous myxoma)	Cellular angiofibroma (CA)
Age at presentation	Reproductive years	Reproductive years May extend to older age	Reproductive years	
Sex	Mostly women	Mostly women, has been reported in males	Either sex	Either sex
Clinical presentation	Simulates Bartholin gland cyst	Polypoid submucosal	Small dermal lobulated, sharply circumscribed	Well circumscribed; Dermal

Size & site	Often >5cm, around 10cm; vulva, vagina, perineum	<5cm; commonly vulva, less common vagina	Small can occur anywhere – dermal or subcutis	1.5 to 2cm; vulva, pelviperineal region may be larger
Margins	Infiltrative	Compressive/ circumscribed borders	Sharply circumscribed/ almost encapsulated	Well circumscribed
Cut surface	Oedematous / gelatinous	Tan, pink, yellow	myxoid	White
Cellularity & cells	Paucicellular bland stellate cells	More cellular than AA; hyper & hypocellular areas; perivascular concentration of plump; stromal or epithelioid cells	Stellate or spindle shaped fibroblasts in a vascular myxoid matrix. Epithelial component may be present as squamous keratinous cysts	Cellular Monotonous spindle cells, indistinct cytoplasm, wavy nuclei, wispy collagen, with small foci of mature fat
Vessels	Medium caliber, thick walled, pin wheel collagen	Smaller than AA, perivascular concentration of plump cells	Thin walled, inconspicuous	Small to medium thick walled with mural hyalinisation
Other findings	Extravasated RBCs	-	Stromal neutrophils; Carney Complex	Mitoses readily identified, no atypia
Clinical course	Local recurrence; never metastasises	Does not recur	Local recurrence	Local recurrence is very infrequent
ER & PR	+	+	-ve	+
Desmin	+(focal)/ -ve	+	-ve	Mostly –ve, focal +
SMA	+ (focal)/ -ve	-ve	+	Mostly –ve focal+
MSA	+(focal)	-ve	-	-

CD34	+ (variable)	+/-	+(variable)	+ (variable)
HMGA2	+	+	-	-
Keratin	-ve	-ve	+ve in squamous elements	-ve

E V EXTERNAL GENITALIA VULVA SYNOPTIC REPORTING

(Primarily for invasive squamous cell carcinoma; applicable with minor modification to other vulvar malignancies)

1. Specimen

- Type of procedure:
  - Punch/incisional biopsy ☐
  - Wide local excision ☐
  - Partial vulvectomy ☐
  - Radical vulvectomy☐
  - Vulvectomy with sentinel lymph node biopsy ☐
  - Vulvectomy with inguinofemoral lymphadenectomy ☐
- Specimen laterality:
  - Right ☐
  - Left ☐
  - Midline ☐
  - Bilateral ☐

2. Tumour Identification

Histologic Type

(As per current WHO classification)

- Squamous cell carcinoma, HPV-associated ☐
- Squamous cell carcinoma, HPV-independent ☐
- Verrucous carcinoma ☐
- Basal cell carcinoma ☐
- Melanoma ☐
- Extramammary Paget disease (invasive / in situ) ☐
- Bartholin gland carcinoma ☐
- Other (specify)……..

3. Tumour Size

- Greatest dimension (cm) ……
- Additional dimensions …..

4. Tumour Focality

- Unifocal ☐
- Multifocal ☐

5. Depth of Invasion (Mandatory)

- Measured in mm …..
- Method of measurement:
  - From epithelial–stromal junction of adjacent most superficial dermal papilla to deepest point of invasion …….

Depth of invasion is critical for staging (≤1 mm vs >1 mm).

6. Tumour Extension

- Confined to vulva ☐
- Involvement of:
  - Lower urethra ☐
  - Lower vagina ☐
  - Anus ☐
  - Upper urethra ☐
  - Upper vagina ☐
  - Bladder mucosa ☐
  - Rectal mucosa ☐

7. Lympho-vascular Invasion

- Present ☐
- Absent ☐

8. Margins

- Peripheral (lateral) margins:
  - Involved / Uninvolved ……..right ☐ left ☐
  - Closest margin distance (mm) …….
- Deep margin:
  - Involved / Uninvolved ☐
  - Closest margin distance (mm)……

- HSIL / differentiated VIN at margin: ☐
  - Present / Absent ☐

9. Associated Precursor Lesion

- High-grade squamous intraepithelial lesion (HSIL) ☐
- Differentiated vulvar intraepithelial neoplasia (dVIN) ☐
- Lichen sclerosus ☐
- None identified ☐

10. Lymph Nodes (if submitted)

- Sentinel lymph nodes:
  - Number examined ……..
  - Number positive…….
  - Size of largest metastasis (mm)……
  - Micrometastasis (<2 mm) / Macrometastasis ☐
- Non-sentinel (inguinofemoral) lymph nodes:
  - Number examined……..
  - Number positive ……..
- Extranodal extension:
  - Present / Absent ☐

11. Pathologic Staging

(AJCC/TNM or FIGO — specify system used)

- pT:……
- pN:……
- pM (if applicable):……
- .Final Stage: ……

12. Ancillary Studies (if performed)

- p16 (block-positive supports HPV-associated pathway) ……
- p53 (aberrant pattern supports HPV-independent pathway)……..
- HPV testing (if done)…….
- Melanoma markers (if applicable: S100, SOX10, HMB45) ……

Key Reporting Points in Vulvar Carcinoma

- Depth of invasion must be measured in millimetres
- Margin distance should be documented precisely.

- Sentinel lymph node status significantly impacts staging.
- HPV-associated and HPV-independent carcinomas are biologically distinct

F I PLACENTA – ANATOMY

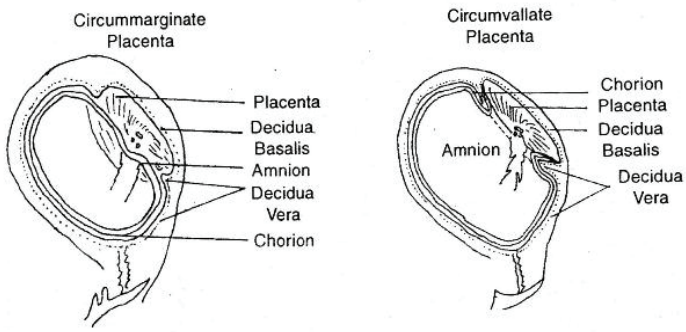


Fig 1.34

Fig 1.34. Diagrammatic representation of the uterus of a pregnant woman showing normal placenta in situ.

TWIN PLACENTAS (Fig 1.35)

Types of twin placentas

Type	*Incidence*	*Gross*	*Twin type*
Dichorionic-diamniotic (separate)	35%		Monozygotic or dizygotic
Dichorionic-diamniotic (fused)	34%		Monozygotic or dizygotic
Monochorionic-diamniotic	30%		Monozygotic
Monochorionic-monoamniotic	1%		Monozygotic

(From Ackerman's Surgical Pathology, 1996)

Fig 1.35

F II and III PLACENTA -GROSSING TECHNIQUES AND GROSS PEARLS

Conditions for which the placental examination may be performed are a)any maternal complication or disease during pregnancy such as diabetes, toxaemia and infection b) any complication of labour or delivery c) any abnormality of the infant including stillbirth, abortion or abnormality of the placenta itself.

Singleton type of placenta

1. Examine the placents as soon as possible as possible in the fresh state after delivery. Handle with care avoiding any laceration . Samples for smearing and microbial culture may be taken. Also take a small sample each for viral studies, immunoflurescence or for special fixation for electrn microscopy. For chromosal studies chorionic villi are more appropriate (as foetal and umbicical cord tend to autolyse rapidly after intra-uterine death). Gross photographs should be taken for record.
2. Weigh the placenta – ideally this should be done after trimming of the membranes, cutting the cord and removal of any extraneous material such as blood clot etc
3. Note the amount of blood clot in the container or separate pieces of membranes, cord or placenta and record the findings.
4. Fixation : 10% neutral buffered formalin is ideal as a fixative. Formalin fixed tissue can be used for light microscopy and for immunopathologic techniques. The placenta should be allowed to lie flat in an adequate sized, round bottomed container with atleast 3-4 times its own volume of fixative. Ideal time for fixation is 48-72 hours.
5. Exa mination of the placenta should be done in the following order : examination of the membranes, cord, foetal surface and maternal surface
6. membranes : i) measure the distance from the placental margin to the nearest point of rupture, in cases of placenta previa this is zero. Examine for completeness, meconium staining, colour and transparency, insertion into placental margin, oedema, necrosis and retromembranous haemorrhage, etc

   ii) Strips of membrane about 4-5 mm wide are cut starting from the site of rupture upto the placental margin (inclusive of it). These bits can be kept aside for blocking which may be done by :

   a. orienting the cut edge on the embedding surface, or
   b. making a swiss roll with the ruptured site as the starting point and the placental margin as the end point, the amniotic surface being the inner aspect of the role. (Fig 1.36)

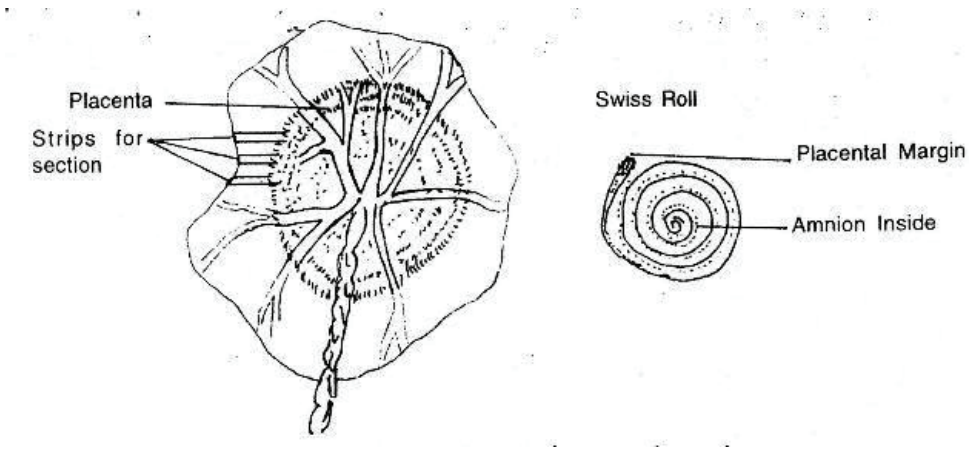


Fig 1.36 : Grossing of singleton Placenta

iii) trim the rest of the membranes from the placental margins.

At this stage some of the some of the abnormal types of placenta may be described :

a. Placenta membranacea : a rare condition in which most of the foetal membranes are covered by placental tissue thereby reducing the area of free membranes. This results in an extremely thin placenta with a large surface area of functional villous tissue . Complications of this are placenta accrete and placenta previa with accompanying premature separation.

b. Placenta extrachorialis (see figures below): In this type of placenta the chorionic plate from which the villi arise is smaller than the basal plate, the transition from villous to non-villous chorion takinf place not at the placental margin but at some distance within the circumference of the foetal surface of the placenta. There are 2 types : circummarginate in which the membranes lift directly from the chorionic plate and circumvallate in which the membranes fold back on the villi. The shortened chorionic plate thus results in a smaller than normal cavity limiting foetal growth. Also denuded placental tissue not covered by chorion results in marginal and retroplacental haematomas and frequently in antepartum bleeding. Chorioamnionitis may also ensue. These complications are associated more with the circumvallate type of extrachorial placenta.

Cord : i) Measure the length of the cord. Those over 70 cm in length and under 30 cm are considered to be abnormal.

ii) The shortest distance between the cord insertion and placental margin is to be measured thereby showing a central, paracentral or eccentric (1-2 cm from the margin eg. Battledore placenta) insertion.

iii) Insertion of the cord (membranous or non-membranous): A velamentous insertion is one in which the cord is implanted on the membranes away from the margin of the placenta. The vessels divide and course within the membranes before reaching the chorionic plate. In a velamentous insertion look whether the vessels are intact or ruptured. Trauma may result in bleeding due to unprotected umbilical vessels running through the membranes.

iv) Examine the colour and look for true knots, tortion, stricture, haematomas and thrombosis.

v) Make transverse sections of the cord at two or more points and assess the number of umbilical vessels. (normally the umbilical cord shows 2 arteries and one vein). Occasionally one artery may be absent.

vi) Remove the cord from the placenta by sectioning at 3 cm proximal to the insertion and take a 3mm thick bit at its midpoint for blocking.

1. Foetal surface : examine this for colour, meconium staining, appearance of fetal vessels, bleeding around these vessels or beneath the membranes, thrombosis of fetal vessels , subchorionic fibrin cysts (size & number), chorangioma (an increase in the number of vascular channels per villus), and presence of nodules on the amnion (amnion nodosum). Microscopically these nodules are composed of deposits of amorphous cellular debris (vernix) which may be periodic acid Schiff (PAS) positive and contain fragments of hair. The nodules should be distinguished from squamous metaplasia of the amnion – this shows stratified squamous epithelium with keratohyaline granules. Squamous metaplasia is of no clinical significance whereas amnion nodosum reflects oligohydramnios of any cause. Frequently associated with oligohydramnios are congenital abnormalities of the fetal genitourinary system (as fetal urine takes part in the formation of amniotic fluid).
2. Maternal surface : i)examine the maternal surface for completeness, normal fissures, laceration (and its extent), retroplacental haemorrhage (size and distance from margin), gross haematoma and pale oval zones (areas of infarction – a fresh placental infarct is moderately firm and dark red; as it ages it becomes progressively harder and its colour changes to brown, yellow and white. An old infarct therefore appears as an amorphous, hard and white plaque).

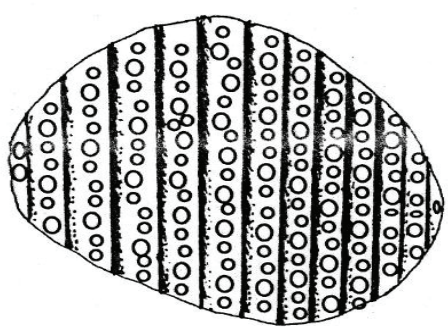


Fig 1.37 : Gross specimen sectioned serially

ii) Measure the maximum diameter and now the placenta may be weighed without the cord & membranes. Make a note of its shape.

iii) Place the placenta maternal surface upwards on a flat surface and make parallel sections with a large sharp knife at an interval of 1 cm firmly holding the placenta onto

the table with the left hand. The foetal surface will not cut through and will hold the slices together (bread loafing the specimen).(Fig 1.37)

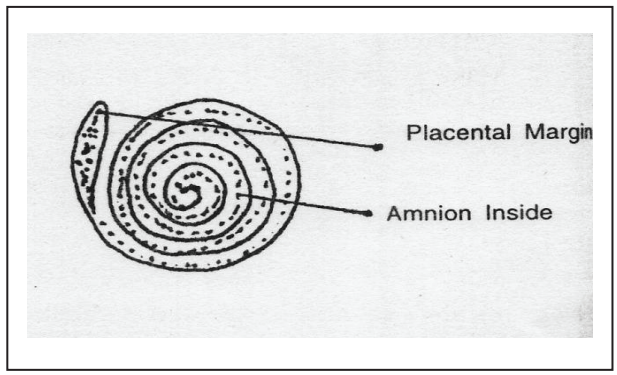
iv) examine all the cut surfaces for presence of infarcts (location, size, fibrin deposition, pallor, consistency , calcification, consistency & age), cysts and tumours.

v) describe any other lesion , location (central, lateral or marginal and depth (basal, intermediate, subchorionic).

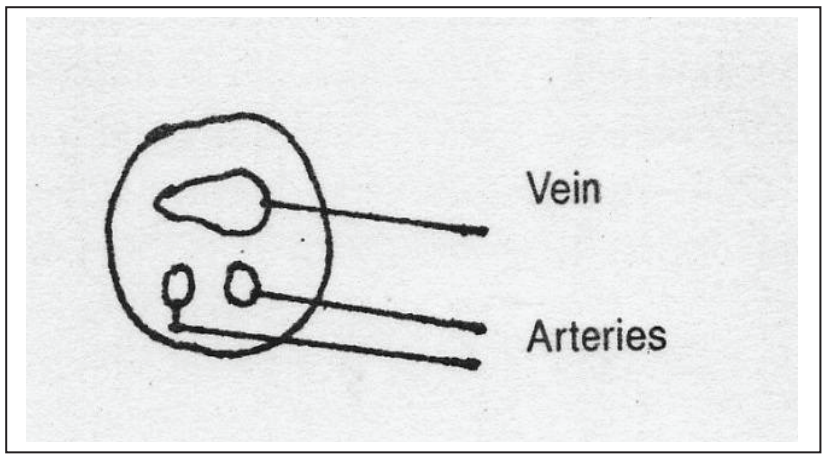
vi) representative tissue blocks can be selected from appropriate marginal and central areas to include normal and abnormal tissue. One section should include the chorionic plate in an area with minimal subchorionic tissue.

Bits

A & B –membranes, three or more bits in 2 blocks or swiss rolls including placental margins (Fig 1.38, seen alongside).



c- one bit cord, more if necessary (see Fig 1.39 adjacent)



D, E, F – placenta, to include normal & abnormal areas (see fig 1.40 below)

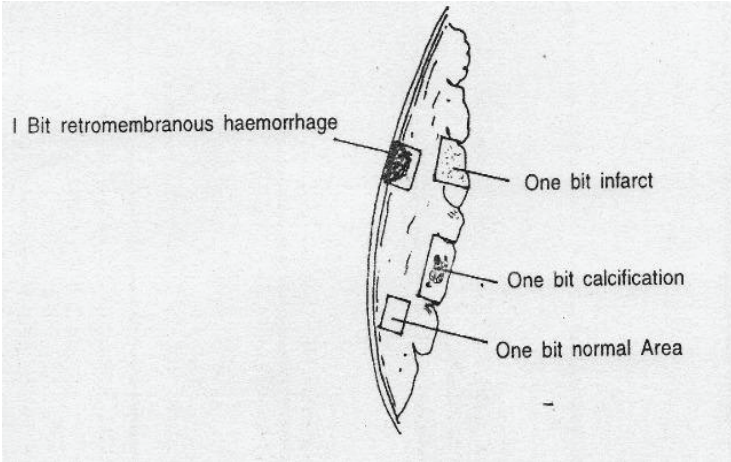


Fig 1.40

Twin type Placentas

1. If the placentas are separate (non-fused) then examine each placenta as a singleton.
2. If the placentas are fused
    a) note whether the two cords are labelled Twin A & Twin B, if not label them arbitrarily and make a statement to that effect.
    b) determine the presence and type of dividing membranes and state it :
        i) if absent, monochorionic- monoamniotic
        ii) if present check whether the dividing membrane is thick & opaque and is difficult to separate by stripping.
        iii) also not presence of vascular anastomosis between the membranes, whether numerous or not

(if the dividing membrane is thick and opaque, difficult to separate by stripping and the vascular anastomosis few, then the placenta is dichorionic diamniotic. If the dividing membrane is thin & transparent and can be separated easily by stripping, and if the vascular anastomosis are numerous then the placenta is monochorionic diamniotic in type. The point of attachment of the membranes to the foetal surface is smooth in the case of monochrionic diamniotic placenta).

1. Remove a rectangle 3mm width of the dividing membrane and make a swiss roll and embed it .

2.Record the kind and number of vascular anastomosis in monochorionic diamniotic placenta; artery to artery, vein to vein and artery to vein (A-V) shunts if possible. The veins are larger and deeper than the arteries. For this 30-50 ml of saline solution containing a dye may be injected into the artery of one twin along the plane of fusion of the placenta and noting whether the fluid emerges from the vein of the other twin. The placenta must be intact to perform this test.

a) Divide the fused twin placenta along the vascular equator rather than through the base of the dividing membranes.
b) Examine each half as a singleton placenta.

Bits

Twin A

A & B - membranes three or more bits in 2 blocks or Swiss rolls including placental margins.

C - one bit cord (more if necessary)

D,E,F – placenta, to include normal and abnormal areas.

Twin B

Same as above.

F IV PLACENTA – MICROSCOPY

I. Non-neoplastic Conditions

1. Inflammatory Lesions

Terminology

- **Villitis:** Inflammation of placental villi.
- **Chorioamnionitis:** Inflammation of fetal membranes.
- **Funisitis:** Inflammation of the umbilical cord.

Etiopathogenesis:

- **Ascending infection:** The most common route, via the birth canal, due to bacteria from urinary tract infection, sexual intercourse, or instrumentation.
  - *Gross:* Cloudy amniotic fluid; membranes thickened and dull.
  - *Microscopy:* Polymorphonuclear infiltrate in chorion and amnion, extending toward the amniotic cavity.
- **Hematogenous infection:** Less common, caused by transplacental spread of organisms such as *Toxoplasma gondii*, *Treponema pallidum*, *Listeria monocytogenes*, or viruses (TORCH group).
  - *Microscopy:* Affects villi predominantly with villitis, necrosis, and calcification.

IHC (if needed): Organism-specific stains or immunomarkers — *Toxoplasma*, CMV, or HSV antigens.

2. Toxaemia of Pregnancy (Preeclampsia / Eclampsia)

Due to defective trophoblastic invasion of maternal spiral arteries leading to placental ischemia.

Gross:

- Placenta smaller than expected for gestational age.
- Numerous infarcts and retroplacental hematomas.

Microscopy:

- Villous ischemia, increased syncytial knots.
- Thickened trophoblastic basement membranes and hypovascular villi.
- Fibrinoid necrosis and intramural lipid deposition in small decidual arteries (acute atherosis).
- Occasional chronic villitis suggestive of an immunologic component.

3. Diabetes Mellitus (Maternal)

Changes:

- Villous immaturity with enlarged, edematous villi and thickened basement membranes.
- Fibrinoid necrosis and atherosclerosis in utero-placental vessels.
- Increased placental weight; sometimes chorangiosis.

II. Gestational Trophoblastic Disease (GTD)

1. Hydatidiform Mole

A trophoblastic lesion with cystic swelling of chorionic villi and trophoblastic proliferation. Occurs in the 4th–5th month of pregnancy with uterine enlargement and elevated β-hCG levels.

2.Complete Mole : hydropic change**;** absence of foetus

Gross:

- "Bunch of grapes" appearance with hydropic villi filling and distending the uterus.
- No identifiable embryo, cord, or membranes.

Microscopy:

- Diffuse villous swelling with central cistern formation.
- Circumferential trophoblastic hyperplasia (cyto- and syncytiotrophoblast).
- Absence of villous blood vessels.
- Atypia may be present in trophoblasts.

IHC / Molecular:

- **hCG:** Diffuse positivity in syncytiotrophoblast.
- **p57:** Negative (absence of maternal genome).
- **Ploidy:** Diploid, androgenetic (46,XX or XY, paternal origin).

Prognosis:

- 10% develop into invasive mole, 2% progress to choriocarcinoma.

3.Partial Mole

Gross:

- Presence of an embryo or fetal parts (often blighted).
- Uterine size appropriate or slightly small for gestation.
- Mixture of normal and hydropic villi.

Microscopy:

- Focal villous enlargement with irregular scalloped outlines.
- Vessels with nucleated fetal RBCs.
- Trophoblastic hyperplasia mild to moderate, focal.
- Stromal inclusions of trophoblastic cells.

IHC / Molecular:

- **p57:** Positive.
- **Ploidy:** Triploid (69,XXY or 69,XXX — both maternal and paternal genomes).

Prognosis:

- Rarely develops into persistent trophoblastic disease.

Diagnostic Table: Hydropic Abortus vs. Complete vs. Partial Mole

Feature	Hydropic Abortus	Complete Mole	Partial Mole
Karyotype	Diploid (46XX/XY)	Diploid (paternal only)	Triploid (69XXY/XXX)
Fetus	Present	Absent	Often present/blighted
Villous change	Minimal	Diffuse hydropic	Mixed, scalloped villi
Trophoblastic hyperplasia	Minimal, polar	Marked, circumferential	Mild, focal
Vascularity	Present	Absent	Decreased
Cistern formation	Absent	Prominent	Variable
p57 expression	Positive	Negative	Positive
hCG	+	++++	+
Progression risk	Rare	10–15% invasive mole, 2% choriocarcinoma	Rare

2. Invasive Mole (Chorioadenoma destruens)

A hydatidiform mole (usually complete) with villi invading the myometrium and blood vessels.

Microscopy:

- Hydropic villi infiltrating the myometrium, often associated with hemorrhage and necrosis.
- Villous structures help differentiate it from choriocarcinoma.

Prognosis:

- Locally destructive; may embolize but rarely metastasizes.
- Excellent outcome with evacuation and chemotherapy.

3. Choriocarcinoma (Gestational Type)

- Highly malignant trophoblastic neoplasm without villous formation.

Gross:

- Soft, hemorrhagic, necrotic mass; often distends uterus or arises at extra-uterine sites.

Microscopy:

- Biphasic pattern: sheets of cytotrophoblast and syncytiotrophoblast without villi.
- Marked anaplasia and numerous mitoses (in cytotrophoblast).
- Hemorrhage and necrosis are extensive.
- Myometrial and vascular invasion prominent.

IHC:

- **hCG:** Strong diffuse positivity.
- **hPL:** Focal or negative.
- **Ki-67:** Very high proliferative index.

Prognosis:

- Highly malignant but **chemosensitive**.
- Metastases: lungs (50%), vagina (30–40%), brain, liver, kidney.
- Nearly all curable with chemotherapy.

4. Placental Site Trophoblastic Tumour (PSTT)

Derived from intermediate trophoblast at implantation site.

Gross:

- Nodular or ill-defined mass infiltrating myometrium; tan-yellow with necrosis.

Microscopy:

- Sheets of polygonal intermediate trophoblasts infiltrating myometrium and vessel walls.
- Absence of chorionic villi.
- Venous sinuses often preserved.
- Mitotic activity: 1–10/10 HPF.

IHC:

- **hPL:** Diffuse strong positivity.
- **hCG:** Focal.
- **Mel-CAM / HSD3B1:** Positive.
- **Ki-67:** Moderate (10–30%), lower than choriocarcinoma.

Prognosis:

- Usually localized; 30% mortality in advanced (FIGO III–IV) stages.

5. Epithelioid Trophoblastic Tumour (ETT)

Variant composed mainly of chorionic-type intermediate trophoblast.

Microscopy:

- Nests and cords of monomorphic cells with eosinophilic or clear cytoplasm; necrosis and hyaline-like material.
- Resembles squamous carcinoma histologically.

IHC:

- **hPL:** Focal.
- **hCG:** Scanty.
- **p63:** Positive.
- **Ki-67:** Moderate (20–25%).

Prognosis:

- Intermediate between PSTT and choriocarcinoma; metastasis possible.

G I FOETUS – ANATOMY

Gross Anatomy on Foetus and Embryo :

I. Standard Weights of a Foetus

Weight of foetus in relation to age in grams

Lunar month	Streeter	Scammon & Calkins
II	1.1	3.5
III	14.1	14.3
IV	108.0	86.8
V	316.0	260.9
VI	630.0	551.6
VII	1045.0	971.4
VIII	1680.0	1519.0
IX	2378.0	2196.1
X	3405.0	2998.8

(From Edith L. Potter: Pathology of the Foetus and Infant, ed. 2nd, Chicago, Year Book of Medical Publication, page11, 1961)

II. Standard Foetal Measurements

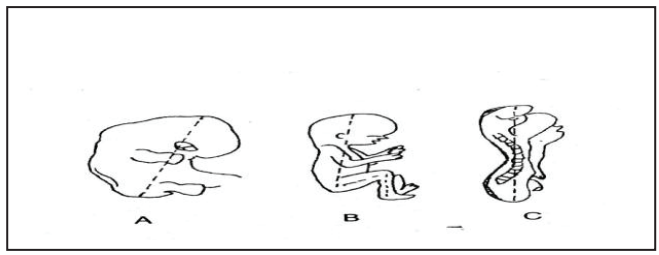
Diagrammatic representation of the measurement of embryos. (Fig 1.41)

A : Crown heel length

B : Crown rump length

C; Greatest length (Fig 1.41)

(from jeft to right)



Length of foetus in relation to age (in cm)

Lunar	Grown rump length		Crown heel length	
Month	Streeter	Scammon &Calkins	Dietrich	Scammon & Calkins
II	2.3	-	3.00	-
III	7.4	5.1	9.8	7
IV	11.6	10.7	18.00	15.5
V	16.4	15.5	25.0	22.7
VI	20.8	19.7	31.5	29.2
VII	24.7	23.6	37.1	35.0
VIII	28.3	27.1	42.5	40.4
IX	32.1	30.5	47.0	45.4
X	36.2	33.6	50.0	50.2

(From Edith L. Potter: Pathology of the Foetus and Infant, ed. 2nd, Chicago, Year Book of Medical Publication, page11, 1961)

III. Developmental changes of an Embryo

Schematic drawing of the dorsal aspect of an 18-day presomite embryo (Fig 1. 42). The embryo has a pear shaped appearance and shows at its caudal end the primitive streak and node of Henson.

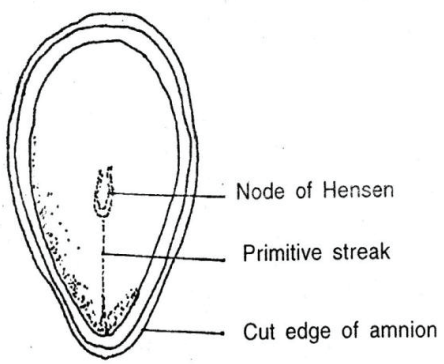


Fig 1. 42

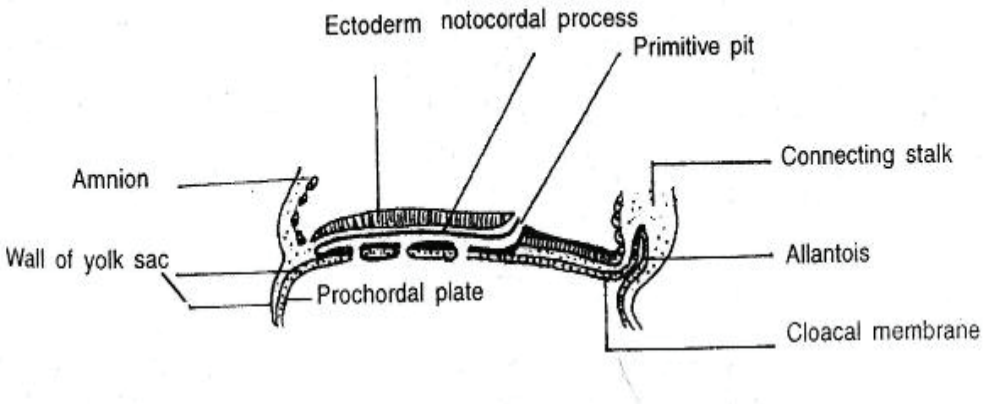


Fig 1.43

Drawing of a cephalo-caudal section of an 18-day embryo (Fig 1.43) showing the notochordal process extending from the primitive pit to the procordal plate and fused with the endoderm. The notocordal -endoderm plate gradually disappears and the remaining part of the notochord is then intercalated in the endodermal germ layer.

The left side of a 25-somits embryo approximately 28days old (Fig 1.44) . The first 3 pharyngeal arches and the optic & the otic placodes are clearly visible

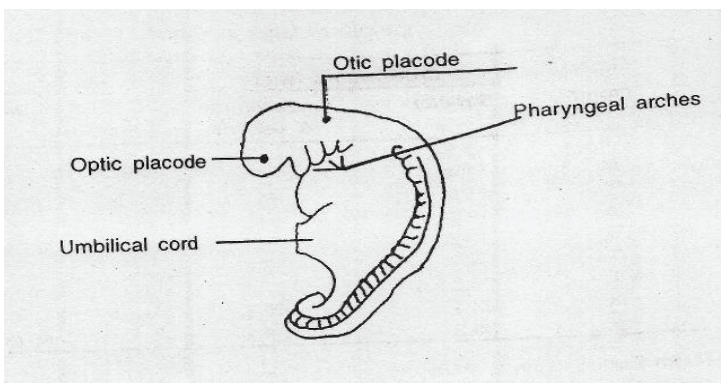


Fig 1.44

A 5 week human embryo seen from the left. Crown rump length is approximately 7 mm. Note the paddle shaped limb buds. The otic & optic vesicles are seen. (Fig 1.45)

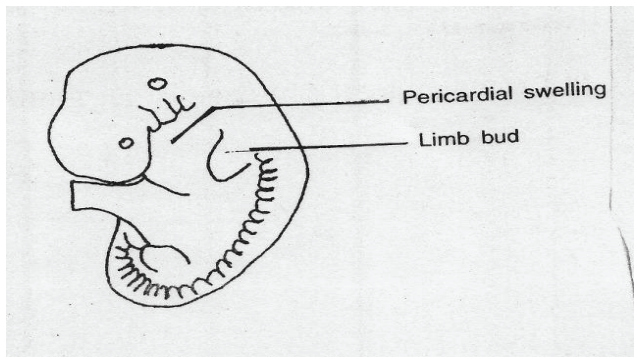


Fig 1.45

A 6 week human embryo seen from the left . Crown rump length is approximately 133 mm. The upper limb buds show a flattened terminal portion with four radial grooves. Note the formation of the eye and external auditory meatus flanked on each side by 3 hillocks derived from the mandibular and hyoid arches(Fig 1.46).

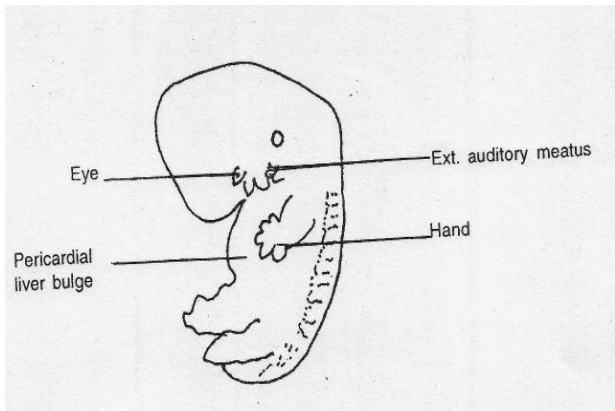


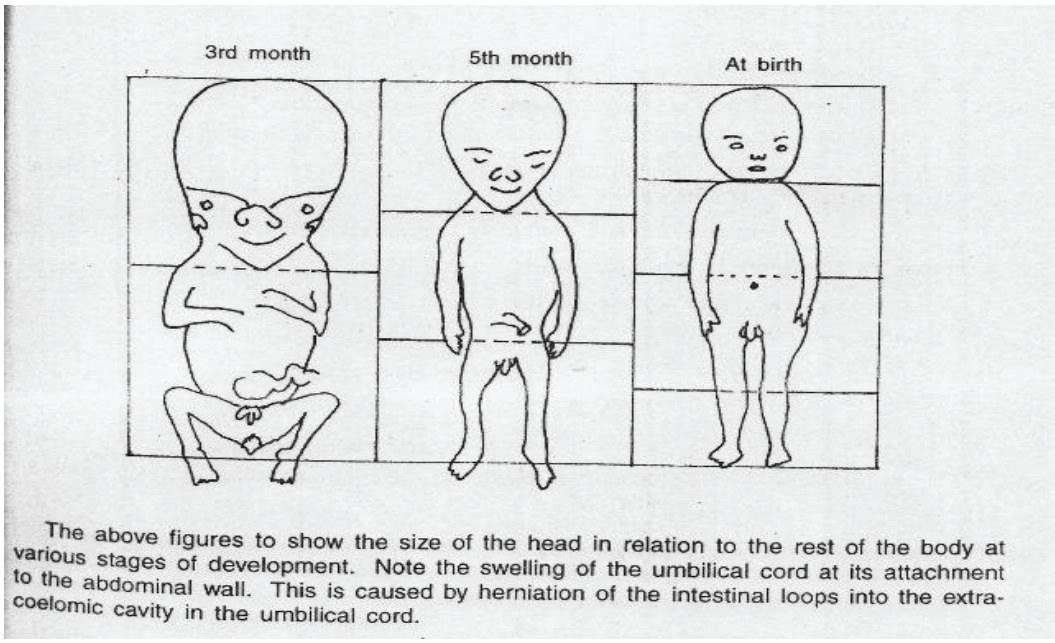
Fig 1.46

A 7 week human embryo seen from the left. Crown rump length is approximately 18 mm. The upper extremities are more advanced in development than the lower ones. (Fig 1.47)

Fig 1.47

A 8 week human embryo seen from the left. Crown rump length is approximately 30 mm. Note the size of the head in comparison with the remaining parts of the body. The eyelids are not present and the eyes are wide open (Fig 1.48)

Fig 1.48



The above figures to show the size of the head in relation to the rest of the body at various stages of development. Note the swelling of the umbilical cord at its attachment to the abdominal wall. This is caused by herniation of the intestinal loops into the extra-coelomic cavity in the umbilical cord.

Fig1.49

G II FOETUS – GROSSING TECHNIQUE

Elective termination of pregnancy is often preferred in cases where foetal or chromosomal abnormalities would result in non-survival of the foetus. At times a medical termination of pregnancy may also be done for maternal causes. Though biochemical screening tests such as hCG and α feto protein on the maternal serum, ultra sonography, chromosomal karyotyping and chorionic villi sampling can detect foetal abnormalities, these procedures sometimes may not yield conclusive results particularly in the first trimester of pregnancy. Therefore there is a need to do a histopathological examination.

Types of specimens

1. Material from Dilatation and Evacuation (D&C) procedures : This often results in foetal parts and placenta getting fragmented and admixed, rendering gross diagnosis and tissue sampling for histopathology difficult.

2. Abortion contents

   The foetal and placental parts after D&C usually come in bits and pieces. They should be placed without any fixative in 2 or more sterile containers labeled with the mother's name and medical record number.

   If cytogenetic or biochemical testing is to be performed :

   a. remove foetal and placental parts from the containers and place them on a sterile field.
   b. Using sterile instruments obtain tissues from atleast 2 separate bits (ie. foetal parts like lung, cartilage, skin, chest wall etc. these tissues grow well in cell cultures) from these two separate containers. Approximately 0.1 to 0.5g of each sample is taken.
   c. These specimens should be washed in 50 to 100ml of sterile saline and placed in pre-thawed cell culture media (like Rosewell Park Memorial Institute (RPMI) media) and left at room temperature for transport to the cytogenetic laboratory for chromosomal studies.
   d. A separate sample may be taken for biochemical studies in the appropriate media. Biochemical studies may be performed on blood samples for estimation of bilirubin levels in cases of erythroblastosis foetalis and α feto-protein levels in neural tube defects.
      1. After the above samples have been taken then the specimen may be put in fixative.
      2. Sort placental fragments from foetal parts. Weigh placental fragments. Assemble segments of unbilical cord and note the length, diameter and number of vessels.
      3. Sort foetal parts into body regions usuing skeletal fragments as a guide. Weigh the foetal parts and take any external measurements from recognizable body parts. Eg, crown rump, crown heel lengths, hand and foot lengths etc. Compare with standard tables of foetal measurements for the estimation of gestational age.
      4. Examine the head. Note the morphologic appearance of the cranium and that of any residual brain tissue. Identify the vertebral column and spinal cord to determine the presence of any neural tube defect. Intracranial abnormalities such as Arnold- Chiari malformation, Dandy-Walker malformation etc can also be determined.
      5. Examine skeletal fragments if present, orient these and take a radiograph to detect any skeletal abnormality.
      6. Examine the viscera. If intact then examine their relationship to each other before separating them. Weigh them. Start with the most recognizable

organs. Examine the gross appearance and take representative bits of both recognizable as well as from non-recognisable tissue. (Note : do not put more than one unknown into a tissue cassette)

7. If all or part of the heart and lungs are recognizable, leave them together and perform as much of a gross dissection of the heart and attached vessels as possible; along the line of blood flow.
8. Examine gastric contents for meconium stain, blood or otherwise.
9. If one or both kidneys are identified intact in the retroperitoneum, note their size and position & trace the ureters to the bladder if possible. Section any tissue formed at the sites suspected for kidney.
10. Carefully examine the foetal fragments for presence of external & internal genitalia. Place into tissue cassettes any material in the pelvis to determine the histologic appearance for presence of gonads.
11. Placenta tissue : see section on placenta.

Bits

- For small embryos : submit whole or one half depending on the size.
- Large fetuses: submit representative portions from lung, stomach, kidneys, liver, spleen and genitalia.
- For placenta : see section on placenta.

Some of the diagnoses that can be made by submitting tissue for histopathologic examination are: 1. Osteogenesis imperfecta

2. Polycystic kidney/ Dysplastic kidney
3. Bilateral renal agenesis / Urethral atresia
4. Conjoined twinning
5. Tetrology of Fallot.
6. Atrial/Ventricular septal defect
7. Meningomyelocoele

ASSESS YOURSELF – FEMALE GENITAL TRACT

Essay Questions (10 marks each)

1. Describe the grossing technique of a hysterectomy specimen for carcinoma cervix. Add the bits to be taken and their significance.
2. Discuss the gross and microscopic features of carcinoma cervix, including the recent WHO classification.

3. Describe the grossing and microscopic features of endometrial carcinoma, including assessment of myometrial invasion.
4. Discuss the classification, morphological features, and clinical significance of endometrial epithelial metaplasias.
5. Explain the differences between adenomyosis and endometriosis, including histopathological diagnostic criteria and associated microscopic findings.

Short Answer Questions (5 marks each)

1. Cone biopsy of cervix – indications and grossing technique.
2. Cervical intraepithelial neoplasia (CIN) – grading and morphology.
3. Endometrial hyperplasia – classification and significance.
4. Leiomyoma – gross features and degenerative changes.
5. Adenomyosis – definition and diagnostic criteria.

Short Notes (3 marks each)

1. List the Germ Cell of the ovaries
2. Endocervical polyp
3. Products of conception – gross examination
4. Hydatidiform mole – gross appearance
5. Placental site nodule

MCQs

1. **The most common site for development of CIN is:**
   A. Endometrium
   B. Transformation zone
   C. Myometrium
   D. Vaginal fornix
2. **Depth of invasion in microinvasive carcinoma cervix is:**
   A. <1 mm
   B. <3 mm
   C. <5 mm
   D. <10 mm
3. **Whorled cut surface is characteristic of:**
   A. Adenomyosis
   B. Leiomyoma
   C. Endometrial carcinoma
   D. Choriocarcinoma

Answer Key for MCQs

1. 1 B
2. 2 C
3. 3 B

Chapter 2
Male Genital System

Chapter outline -A Penis; B Prostate; C Testis

A 1 Penis Anatomy; A II Penis Grossing Techniques; A III Penis Gross Pearls; A IV Penis Microscopy; A V Penis Synoptic Reporting.

B I. Prostate Anatomy; B II Prostate Grossing Techniques; B III Prostate Gross Pearls; B IV Prostate Microscopy; B V Prostate Synoptic Reporting

C I Testis Anatomy; C II . Testis Grossing Techniques ; C III Testis Gross Pearls; C IV Testis Microscopy; C V Testis Synoptic Reporting

AI, BI AND CI PENIS, PROSTATE & TESTIS ANATOMY

The male genital tract (see figs 2.1 and 2.2 below) consists of the penis, prostate, testes and associated ductal structures, which function together in the production, maturation and transport of sperm. The penis is the external copulatory organ composed of erectile tissues and the penile urethra. The prostate is a fibromuscular gland situated below the urinary bladder and surrounding the proximal urethra, contributing secretions to the seminal fluid. The testes, located within the scrotum, are the primary male gonads responsible for spermatogenesis and testosterone production. Sperm formed within the seminiferous tubules of the testis pass through the epididymis and vas deferens before entering the prostatic urethra during ejaculation.

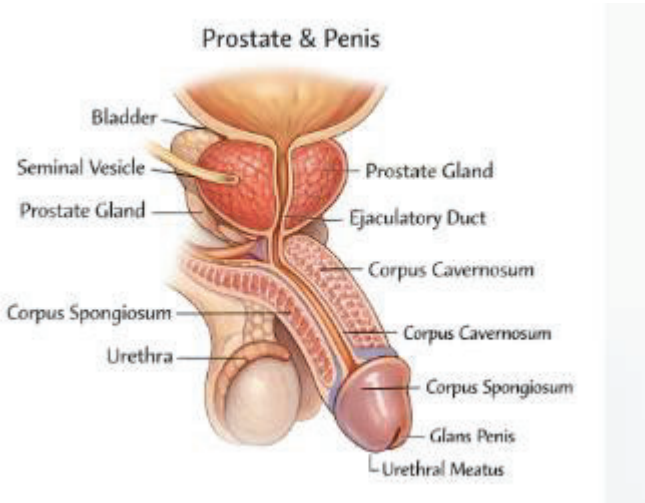


Fig 2.1

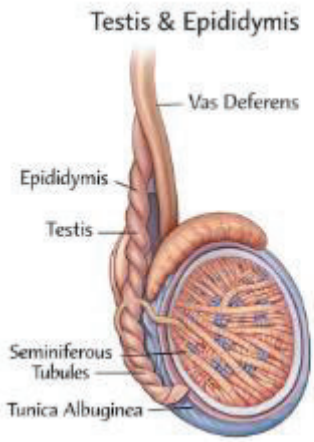


Fig 2.2Penis

The **penis** is composed of two corpora cavernosa and one corpus spongiosum containing the urethra, all surrounded by the tunica albuginea and covered by skin and prepuce. The penis is the external male copulatory organ and functions in urination and sexual intercourse. It consists of three cylindrical erectile bodies:

- Two corpora cavernosa, located dorsally.
- One corpus spongiosum, located ventrally and enclosing the urethra.
- The distal expansion of the corpus spongiosum forms the glans penis, which is covered by the prepuce (foreskin) in uncircumcised individuals.
- Each corpus cavernosum is surrounded by a dense fibrous sheath called the tunica albuginea, which plays an important role in penile erection by trapping blood within vascular sinusoids. The erectile tissue is composed of vascular spaces lined by endothelium separated by trabeculae of smooth muscle and connective tissue.
- The penile urethra runs through the corpus spongiosum and opens at the external urethral meatus at the tip of the glans.

Prostate

The prostate is a zonal gland with most carcinomas arising in the peripheral zone, while **benign prostatic hyperplasia** originates in the **transition zone**; the testis contains seminiferous tubules producing germ cells with testosterone-secreting Leydig cells in the interstitium. The prostate is a fibromuscular gland located below the urinary bladder and surrounding the proximal urethra. In adults it weighs approximately 20 g and measures about 3 × 4 × 2 cm.

Anatomically, the prostate is divided into several zones:

- Peripheral zone – forms the posterior and lateral portions of the gland and is the most common site for prostatic carcinoma.
- Transition zone – surrounds the proximal urethra and is the principal site for benign prostatic hyperplasia (BPH).

- Central zone – surrounds the ejaculatory ducts.
- Anterior fibromuscular stroma – contains mainly smooth muscle with few glands.

Histologically, the prostate is composed of branching tubuloalveolar glands embedded in fibromuscular stroma. The glandular epithelium consists of luminal secretory cells and a basal cell layer. Prostatic secretions contribute to seminal fluid.

Testis

The testes are paired ovoid organs located within the scrotum and suspended by the spermatic cord. Each testis measures approximately 4–5 cm in length, 2–3 cm in width and weighs 15–25 g.

- The testis is divided by fibrous septa into lobules containing seminiferous tubules for spermatogenesis, while the interstitium contains Leydig cells responsible for testosterone production.
- The testis is covered by a dense fibrous capsule, the tunica albuginea, which thickens posteriorly to form the mediastinum testis. Fibrous septa extend from the capsule and divide the testis into lobules, each containing 1–4 seminiferous tubules where spermatogenesis occurs.
- The seminiferous tubules drain into the rete testis within the mediastinum, which then connects to the efferent ductules leading to the epididymis. The epididymis consists of head, body and tail and serves as the site of sperm maturation and storage.
- Two principal cell types are present in the testis:
  - Germ cells within seminiferous tubules responsible for spermatogenesis.
  - Leydig cells located in the interstitium, which produce testosterone.
- The testis has a rich vascular supply from the testicular artery, and venous drainage occurs via the pampiniform plexus.

A II PENIS: – GROSSING TECHNIQUES

Small biopsies :

1. These are usually sent as :
   a. specimens of prepuce from patients having circumcision for phimosis
   b. as small biopsies from glans penis or any other lesion eg. leukoplakia, Bowen's Disease, carcinoma etc.
2. Take measurements along the 3 dimensions. If more than one piece of tissue is sent , measure the largest and smallest.
3. Description : Mucosa / skin , any gross lesion.

Bits

a) Sample the entire specimen for epithelial changes in case of suspected malignancy.
b) A maximum of 3 blocks including muco-cutaneous junction, if it is only for inflammatory conditions.

Penectomy Specimens

Total amputation specimens

Partial amputation specimens

Total amputation with inguinal lymph node block dissection

a) If sent with inguinal block dissection, separate them and handle differently
b) If fresh, put a catheter through the urethra, leave in formalin and fix overnight. Pass a probe to check the patency of the urethra.
c) Paint surgical margins, including urethral with India ink.
d) Measure the length of the specimen
e) Section longitudinally to expose urethra as well as prepucial sac.
f) Description
    a) Type of specimen – partial / total amputation
        - with / without - scrotal skin
        - testis
        - inguinal lymph nodes
    b) Tumour
        - location in relation to glans penis, prepuce, skin, urethra
        - size
        - colour, cut surface
        - borders
        - depth of invasion into the shaft
        - distance from surgical margins
        - involvement of corpora cavernosa/ spongiosa
    c) glans penis – balanitis
        - leukoplakia
    d) urethra –whether involved by tumour or not
    e) any other tissue sent – describe whether normal or otherwise.

Bits (Fig 2.3)

A, B - two bits tumour

C - one bit tumour with urethra

D - one or more bits to include adjacent skin and prepucial mucosa with changes if any.

E, F, G – complete surgical margin including skin and urethra, (one block urethral surgical margin and two blocks skin and rest of soft tissue surgical margins)

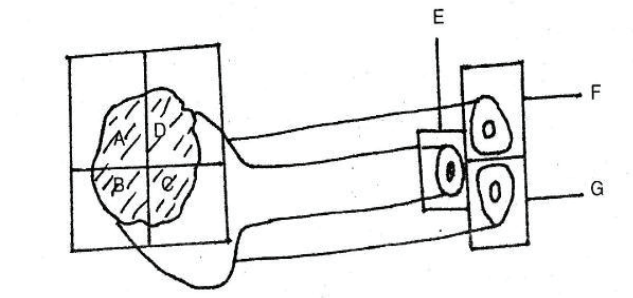


Fig 2.3

Inguinal Lymph Node Dissection

These nodes may be sent as separate groups marked superficial and deep, to the right and left side respectively.

1. Section the fibrofatty tissue containing lymph nodes along the greatest dimension.
2. Serial sections should be made parallel to this at a distance of 5mm.
3. Description – number of nodes
   - size of largest lymph node
   - cut surface
   - presence or absence of internal saphenous vein

Bits

1. At least 12 lymph nodes should be found normally in a nodal resection of one side
2. All lymph nodes may be submitted as a single group unless the surgeon has given the superficial and deep groups separately
3. If there is a large node give only half but sample the entire cut surface, if not grossly involved. If grossly involved give the bit from that area.
4. For small lymph nodes 5mm or less give the entire node.
5. A cross section of the internal saphenous vein should also be taken.

A III . PENIS -GROSS PEARLS

1. Developmental abnormalities : -

   Urethral opening *:* due to improper closure of the urethral groove, The Urethral meatus may open abnormally .

1. *Hypospadias* is an abnormal opening of the urethra on the ventral surface of the penis.

- *Epispadias* is when the opening is on the dorsal surface of the penis.

- **Phimoses :** the orifice of the penis is too small to permit its normal retraction over the glans penis.

1. **Red to brown papillary warty excrecences** over the prepuce or coronal sulcus of the shaft : condyloma acuminatum./ verrucous carcinoma.
2. **Nodular ulcerating growth over the glans penis**, coronal sulcus or prepucial skin : squamous cell carcinoma.
3. **Locally aggressive exophytic large verrucous growth** - Buschke–Löwenstein tumour.

A IV PENIS- MICROSCOPY PEARLS

1. Balanoposthitis : Inflammatory condition of the glans (balano) and prepuce (posthitis). Can result due to any infective etiology; bacterial or fungal or even non specific inflammation.
2. Phimosis : due to the narrow prepucial orifice leading to non - retraction of the prepucial skin over the glans, it predisposes to infection and squamous cell carcinoma.
3. Premalignant conditions : a) *Erythroplasia of Queyrat* : appears as red soft raised plaques with sharp margins over the glans and coronal sulcus b) *Bowen's Disease* : sharply demarcated scaly erythematous plaques over the skin of the shaft of the penis with ulceration and crusting in middle aged men

   c*) Bowenoid papulosis*: occurs at a younger age and presents as multiple reddish verrucoid papules on the glans penis and shaft. Associated with HPV 16.

All three lesions are microscopically identical and show severe dysplasia and carcinoma in situ changes. They may progress to invasive carcinoma over the years.

4. Peyronie's dieaease : (plastic induration of the penis):
   - is a disease of unknown etiology and may be related to fibromatosis; may also be secondary to an inflammatory or fibrosing reaction to urethritis.
   - Affects middle aged men and is characterized by development of circumscribed fibrous tissue between corpora cavernosa and tunica albuginea.
   - Palpated as firm plaques on the dorsal surface of the erect penile shaft.
   - Histolgically fibrosis is seen which is inelastic and leads to penile curvature and pain.
5. Balanitis Xerotica Obliterans : is a disorder of unknown etiology which may result in phimosis, meatal stenosis and fissue formation.
   - - Clinically white papules or plaques involve the foreskin & glans.
   - Histologically shows epidermal atrophy, interface dermatitis, dermal
   - edema & fibrosis. Features are similar to lichen sclerosis et atrophicus of the vulva.

6. Condyloma Acuminatum : is a benign tumour caused by HPV types 6 & 11 and similar to its vulvar counterpart..
   - Great majority are sexually transmitted.
   - Grossly it is sessile or pedunculated with red papillary excrecences.
   - Histologically long papillae with central fibrovascular cores covered by squamous epithelium exhibiting hyperkeratosis, parakeratosis and koilocytotic atypia (cytologic features of HPV change) are seen .
7. Squamous cell carcinoma (SCC)

Squamous cell carcinoma (SCC) is by far the most common malignant tumour of the penis. It arises from squamous epithelium of the glans, prepuce/foreskin, coronal sulcus and, less commonly, the shaft skin (see Fig 2.4).

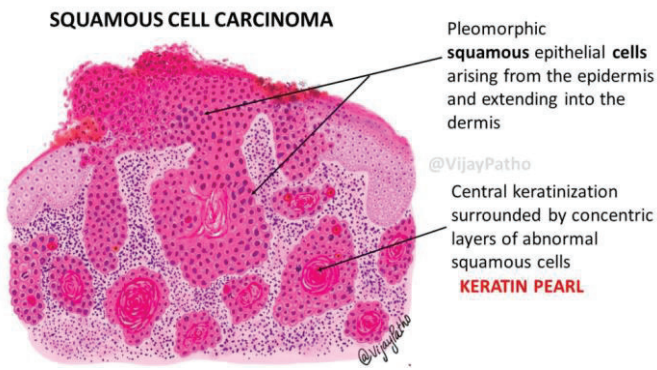


Fig 2.4

Epidemiology & risk factors

- Strongly associated with lack of circumcision, poor genital hygiene, phimosis and chronic inflammatory conditions.
- Human papillomavirus (HPV) — particularly high-risk types 16 and 18 — are detected in a substantial proportion of penile SCCs (variable by geography and subtype); routine p16 immunohistochemistry is recommended as a surrogate marker for HPV-associated tumours. HPV-associated and HPV-independent tumours have distinct precursor lesions (HPV-associated PeIN vs differentiated PeIN) and differing biology.

Clinical patterns of growth

- Superficial spreading — flat lesion spreading horizontally.
- Vertical (infiltrative) — deeper penetration into subepithelial tissues.
- Verruciform — exophytic/warty papillary growth (correlates with verrucous or warty carcinoma).
- Other patterns — mixed or NOS.

Histopathology

- Typical SCC shows pleomorphic squamous cells with keratin production (individual cell keratinisation and/or keratin pearls). Broder's grading (traditional 4-tier) or simplified 3-tier grading is used; report grade and specify which system used.
- Histologic variants: verrucous carcinoma (giant fungating/ulcerated lesion), warty (condylomatous) carcinoma, papillary carcinoma, basaloid carcinoma, sarcomatoid carcinoma, and other specified variants. Reporting the histologic subtype is important because certain variants (e.g., basaloid, sarcomatoid) carry a worse prognosis.

8. Buschke–Löwenstein tumour (giant condyloma acuminatum)
    - Giant condyloma (Buschke–Löwenstein tumour) is a locally aggressive exophytic lesion caused by low-risk HPV but with locally destructive growth. Many authors consider its behaviour and morphology overlapping with verrucous carcinoma; management is surgical and follows the same practical considerations as verrucous penile lesions.

Practical implications for reporting and management

- Because the T1a vs T1b subdivision incorporates LVI/PNI/grade, pathology must specifically comment on those features to allow correct staging and treatment planning.
- p16 IHC / HPV testing is recommended as part of routine pathology workup (helps classify tumours as HPV-associated vs HPV-independent).
- Report the exact anatomic structures invaded (spongiosum vs cavernosum) — these map directly to T2/T3 and have prognostic significance.
- Note presence or absence of extranodal extension (ENE) in nodes (pN3) — ENE changes nodal stage and strongly affects prognosis and adjuvant treatment decisions.

A V SYNOPTIC REPORTING -PENIS

1) Small biopsies

Preliminary data : should be given as usual

Site of biopsy : Glans □

Prepuce□

Skin of shaft□

Other □ Specify……..

Gross : Measurements : …… x…….cm

Ulcer□

Erythema□

Mucosa (of urethra)present □

Warty growth□

Other □ Specify……

Microscopy : Non specific chronic inflammation□

Balanitis□

Posthitis□

Balanoposthitis□

Condyloma□

Balanitis Xerotica Obliterans□

Peyronie's Disease□

Squamous cell carcinoma □

Any other □ Specify……..

Sigature……..Date ……..

2) Resection specimens

Specimen type :

Partial amputation□

Total amputation□

Total amputation with inguinal lymph node block dissection□

Gross

Measurements :…… x ……...cm

Measurement of tumour (if present) …….. x ……..cm

Ulcer present□ Warty growth □

Polypoid growth□

Location of tumour : Glans □ Prepuce □ Shaft □

Urethral meatus □ Other □ Specify ……..

Cut surface : Grey white □ Necrotic □ Haemorrahgic □

Margins : Soft tissue margin involved □

Not involved □ Distance from tumour…….cm

Urethral margin involved□

Urethral margin free □ Distance from tumour……cm

Lymph node resection : Yes □ No□ Site……..

Other tissues sent : Yes □ Specify ……. No □

Microscopy :

Histologic subtype :

Carcinoma in situ □

Squamous cell carcinoma□

Verrucous carcinoma□

Basaloid carcinoma□

Sarcomatoid carcinoma□

Condyloma Acuminatum □

Buschke Lowenstein tumour□

Other □ Specify type ……

Ta : non invasive verrucous carcinoma □

T1 : tumour invades subepithelial connective tissue □

T2 : tumour invades corpus spongiosum □

T3 : tumour invades corpus cavernosum □

T4 : tumour invades adjacent structures like prostate, bone etc □

Margins: Soft tissue margin involved □

Not involved □ Distance from tumour…….cm

Urethral margin involved □

Urethral margin free □ Distance from tumour……cm

Lymphovascular invasion : Yes □ No □

Lymph nodes : No examined ……

No involved……

Site of involved nodes…….

Other tissues involved : Yes□ Specify tissue……

Other tissues free □ Specify tissue ……

Sigature……..Date ……..

A Pathology reports should include: anatomical site, tumour size, histologic type and variant, grade, depth of invasion (including whether corpus spongiosum or corpus cavernosum invaded), lympho-vascular invasion (LVI), perineural invasion (PNI), front/pattern of invasion, surgical margin status (distance to margin), and p16/HPV status. These elements are critical for staging and management decisions.

TNM classification (UICC / AJCC 8th edition

Clinical and pathological categories (condensed):

Primary tumour (T)

- TX — Primary tumour cannot be assessed
- T0 — No evidence of primary tumour
- Tis — Carcinoma in situ (Penile intraepithelial neoplasia — PeIN)
- Ta — Non-invasive verrucous carcinoma
- T1 — Tumour invades subepithelial connective tissue
  - T1a — invades subepithelial connective tissue without lymphovascular invasion or perineural invasion and not poorly differentiated
  - T1b — invades subepithelial connective tissue with LVI or PNI or is poorly differentiated
- T2 — Tumour invades corpus spongiosum (with or without urethral invasion)
- T3 — Tumour invades corpus cavernosum (with or without urethral invasion)
- T4 — Tumour invades adjacent structures (scrotum, prostate, pubic bone, etc.).

Regional lymph nodes (N)

- cNX / pNX — cannot be assessed
- cN0 / pN0 — No palpable or pathologic regional nodes
- pN1 — Metastasis in one or two inguinal lymph nodes (unilateral)
- pN2 — Metastasis in more than two unilateral inguinal nodes or any bilateral inguinal nodes
- pN3 — Metastasis in pelvic lymph node(s) or extranodal extension (ENE) of inguinal or pelvic nodes.

Distant metastasis (M)

- cM0 / pM0 — no distant metastasis
- cM1 / pM1 — distant metastasis.

Stage grouping (summary)

- Stage 0: Tis / Ta N0 M0

- Stage I: T1a N0 M0
- Stage IIA: T1b–T2 N0 M0
- Stage IIB: T3 N0 M0
- Stage IIIA: T1–T3 N1 M0
- Stage IIIB: T1–T3 N2 M0
- Stage IV: T4 any N M0, any T N3 M0, any T any N M1.

The pT, pN, pM categories correspond to the T,N,M categories.

Prognostic features

Regional nodal status (inguinal/pelvic nodes) is the strongest prognostic factor.

- Ulcerating/infiltrative tumours and adverse histologic variants (basaloid, sarcomatoid) are associated with higher rates of nodal metastasis and worse outcome.
- Emerging literature suggests HPV-associated tumours may have different (often more favourable) outcomes compared with HPV-independent tumours, but data are evolving and routine HPV/p16 reporting is recommended

B II PROSTATE : GROSSING TECHNIQUE

Specimens received

Needle biopsy : Popularly obtained by an 18-guage spring driven biopsy gun introduced transrectally with or without radiologic guidance.

Two types of needle biopsies may be received in the department –

- A single needle biopsy targeted at a firm nodule felt on digital rectal examination or of a hypo-echoic area on transrectal ultrasound.
- Multiple quandrantic biopsies from patients with an elevated PSA to detect carcinoma, its histological grade and the extent of the tumour. Six cores are taken three on either side of the gland.

TURP specimen :

Suprapubic prostatectomy

Radical prostatectomy – removal of prostate, seminal vesicles and

portion of vas deferens

Needle Biopsy

Length of longest and smallest bit to be measured

In **TURP** – 1. Weigh accurately

2. Size -3 dimensions

3. Consistency -carcinoma may be hard
4. Colour – carcinoma may be yellow
5. Sometimes nodules of hyperplasia can be made out grossly

Bits

- All for section I needle biopsies
- With regard to TURP, if the tissue weighs less than 10gms, and is sent in one container , take all for section; if more, then for each 10 gms take one block eg. for 30gms- 4 + 2 = 6 blocks are to be taken. for 40 gms – 4+3 = 7 blocks
- If the tissue is sent in separate containers from various lobes and labelled separately, the take more blocks and label separately
- If carcinoma is identified microscopically in a specimen which has not been fully blocked then the remaining entire tissue irrespective of amount should be blocked – in order to identify PIN and to pick up the highest grade.
- Try and pick up fat for staging.
- If PIN is identified block the entire tissue.

Suprapubic prostatectomy for nodular hyperplasia

- Weigh specimen
- Cut first section at the greatest dimension after weighing
- Cut sections at 5mm, parallel to the first
- Examine each slice for various areas – carcinomas are hard, yellow and firm in consistency
- Examine for cysts, nodules, areas suspicious for carcinoma
- **Bits** (as shown in the fig 2.5)

  A & B – one lobe – 2bits

  C & D -other lobe -2 bits

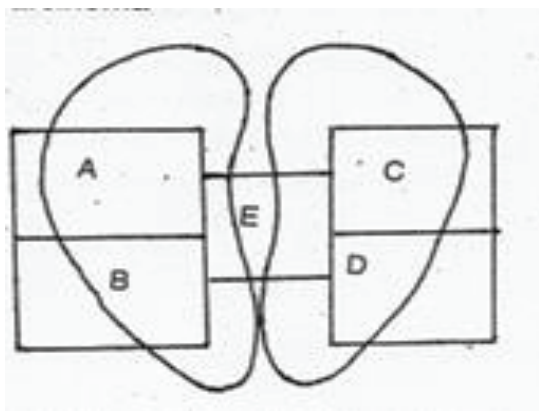


Fig 2.5

E – middle lobe -1 bit, may include urethra

- Suprapubic prostatectomy may at times be done for neoplasms. Then bits are then taken as for a specimen of radical prostatectomy.

Radical Prostatectomy for carcinoma

- Weigh the specimen and measure it in 3 dimensions.
- Measure the seminal vesicles
- Ink the outer surface.
- Take bladder shave area for proximal surgical margin
- Identify the organs sent – ? whole prostate
  seminal vesicles- ? involved by tumour
  Urethra , take the length, Check for patency
  Vas deferens, involved
  Lymph nodes
- Tumour description- size
  -colour , consistency
  - borders
  -Extent & Infiltration into surrounding organs, prostatic and periprostatic
- Remaining prostate – nodular , etc

Bits (Fig 2.6)

Tumour 3 bits A to C

Non -neoplastic prostate one bit – D

Urethral apical surgical margins , 2 bits E & F

Urethral surgical margin at bladder base 1 bit G

I bit right sided uninvolved or involved seminal vesicle + I bit right sided vas deferens at margin H 1 bit left seminal vesicle (involved or uninvolved) and + one bit left vas deferens at margin J

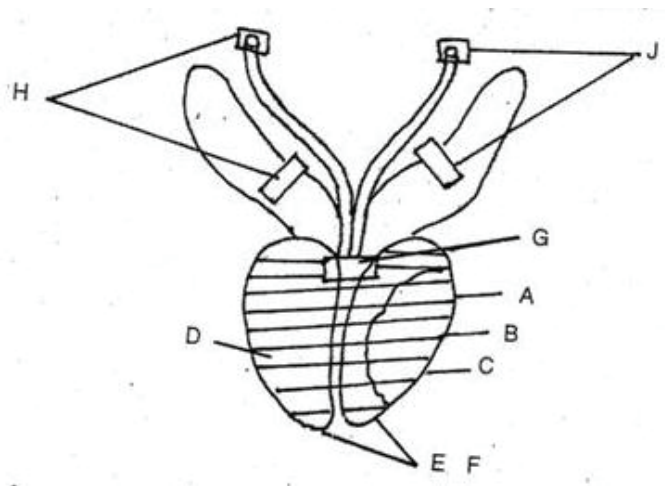


Fig 2.6

B III PROSTATE GROSS PEARLS

- The **normal prostate weighs about 20 g** and surrounds the proximal urethra just below the urinary bladder.
- **Benign prostatic hyperplasia predominantly involves the transition (periurethral) zone**, producing nodular enlargement that may compress the urethra.
- **Most prostatic carcinomas arise in the peripheral (posterior) zone,** which may appear firm or ill-defined on sectioning but are often **not grossly obvious.**
- In **radical prostatectomy specimens**, careful orientation, inking and evaluation of **apical, basal and posterolateral margins** are essential.

B IV PROSTATE - MICROSCOPY

- **Normal :** -prostate is retroperitoneal and normally weighs 20g and encircles the neck of the bladder & urethra can be divided into an inner periurethral and outer cortical zone. The inner zone is the primary site for nodular hyperplasia (and the rare carcinomas arising from the large ducts) whereas the outer zone is the site of predilection for the ordinary adenocarcinomas arising from the ducts and acini.
  - the prostrate is enveloped by a fibromuscular layer referred to as the capsule which is not a well defined anatomic structure; it is more evident on the base and less so on the anterior and apical surfaces.
  - Ducts and acini contain secretary cells, basal cells, and scattered neuroendocrine cells. The ducts can be major (excretory) or peripheral (secondary or minor) normal prostatic secretions are neutral mucins . Most carcinomas secrete a mixture of neutral and acid mucins.
  - Prostate lymph vessels drain into the pelvic lymph nodes and from there into the retroperitoneal chain.

1. **Benign prostatic hyperplasia** : Is the most common urologic disease to affect men. It is related to androgenic effect. Testosterone is converted to dihydrotestosterone by 5α-reductase. Dihydrotestosterone is the mediator of prostatic growth.
   - The gland in hyperplasia also called as nodular hyperplasia weighs about 60-100 g. The periurethral region is the classic site affected. Cut surface is yellow pink to pale grey.
   - Microscopically hyperplasia of both glandular and stromal elements are seen in varying proportions. (Fig 2.7)

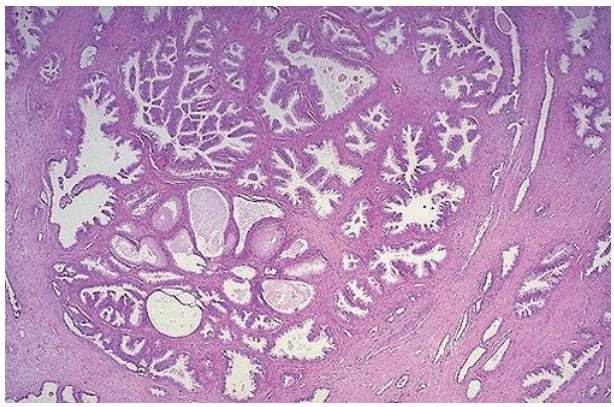


Fig 2.7: Nodular hyperplasia prostate H & E x 100 (Pathology outlines)

   - TURP and retropubic prostateectomies remove only the newly formed nodules.

2. **Inflammatory conditions**: i) Acute bacterial prostatitis : consists of a focal or diffuse suppurative inflammation caused by bacteria similar to those causing a urinary tract infection.. Neutrophilic infiltration, hyperemia and cellular debris with odema are seen.

   ii) Chronic bacterial prostatitis : This is seen in patients with recurrent urinary tract infection. Microscopy shows an infiltration by leukocytes, lymphocytes and plasma cells as well as macrophages.

   iii) Granulomatous prostatitis

   a. Granulomatous prostatitis : (non specific in nature) : A local foreign body giant cell reaction to ruptured prostatic secretions > It is similar in nature to granulomatous mastitis.
   b. Tuberculous granulomatous inflammation : This includes chronic tuberculous prostatitis which may occur as a complication of BCG immunotherapy for bladder cancers or spread of tuberculosis from elsewhere.
   c. Allergic granulomatous prostatitis : A rare manifestation of systemic allergic reaction characterized by multiple granulomata with a diffuse eosinophilic infiltrate.
   d. Post TURP granulomas : occur due to surgical trauma and are composed of epitheliod histiocytes and giant cells around a central area of fibrinoid necrosis.

4. Prostate-Specific Antigen (PSA)

- Definition:
  PSA is a glycoprotein produced primarily by the epithelial cells lining the acini and ducts of the prostate gland.
- Genetics:
  The gene encoding PSA is localized to chromosome 19. It was first discovered in seminal plasma in Japan.
- Physiology and Clinical Significance:
  PSA is normally concentrated in the prostatic tissue; hence, serum PSA levels are very low.
  Disruption of the normal prostatic architecture (as in benign prostatic hyperplasia, prostatitis, or carcinoma) leads to increased PSA release into circulation.
  Prostatic intraepithelial neoplasia (PIN) alone does not raise PSA levels.
- Interpretation of PSA Values:
  - Normal: <4 ng/mL
  - >4 ng/mL – requires further evaluation
  - >10 ng/mL – strongly suggestive of carcinoma
  - Age-adjusted PSA:
    - <40 years: ~2.5 ng/mL
    - Gradual increase with advancing age
  - Correlation with tumour mass:
    Tumour tissue produces nearly 10× more PSA than normal tissue.
    Levels >25 ng/mL indicate extraprostatic extension to seminal vesicles or lymph nodes.
- Derived Indices and Their Use:
  - **PSA Density:**
    $$\text{PSA Density} = \frac{\text{PSA level}}{\text{Prostate gland volume}}$$
    Adjusts PSA values for gland size to improve specificity and reduce unnecessary biopsies.
  - **PSA Doubling Time (PSADT):**
    - Normal: >15 years
    - Prostatic carcinoma: 4–10 years
    - **<6 months** – indicates aggressive tumour with systemic spread.
  - Free vs. Total PSA:
    - Carcinoma: lower free PSA fraction (complexed to proteins)
    - Benign disease: higher free PSA fraction

        - Diagnostic utility:
          In cases with borderline PSA (4–10 ng/mL), a free-to-total PSA ratio <14–28% indicates need for biopsy.
    - PSA Velocity:
        - Measures rate of change of PSA over time
        - A rise of >0.75 ng/mL per year suggests malignancy.
- Clinical Utility:
  PSA, in combination with digital rectal examination (DRE) and transrectal ultrasonography (TRUS), forms a diagnostic triad for early detection of prostatic carcinoma.

5. Indications for Prostatic Biopsy

A biopsy should be performed when:

1. PSA >4 ng/mL
2. Significant rise in PSA between successive tests
3. Abnormal findings on digital rectal examination

6.Prostatic Intraepithelial Neoplasia (PIN)

- Definition:
  A premalignant intraductal process involving prostatic ducts and acini, described as intraductal or duct–acinar epithelial proliferation.
- Location and Distribution:
    - Found predominantly in the peripheral zone of the prostate.
    - Usually multifocal.
    - Seen in 70–80% of prostates harbouring carcinoma (often >10 foci).
- Classification:
    - Low-grade PIN: Grade 1
    - High-grade PIN: Grades 2 and 3
      Only high-grade PIN (HGPIN) is clinically significant and should always be reported in needle biopsies (except in radical prostatectomy specimens).
- Clinical Importance:
  High-grade PIN is regarded as a marker and precursor lesion of prostatic adenocarcinoma.
  If HGPIN is found without carcinoma:
    - Follow-up with ultrasound,
    - Repeat guided biopsies, and
    - Serum PSA monitoring are advised.
      About 50% of such cases show carcinoma on re-biopsy.

Microscopy of PIN

Feature	Low-grade PIN	High-grade PIN
Architecture	2–3 layers of epithelium	3–4 layers with nuclear stratification
Nuclei	Mild enlargement	Prominent nucleomegaly, hyperchromasia
Nucleoli	Small, inconspicuous	Large, prominent nucleoli
Other features	Minimal atypia	Cribriform, tufting or micropapillary patterns may be seen

- Site: Mostly in peripheral ducts and acini.
- Basal cell layer: Present but often attenuated.
- Cytoplasmic features: May show amphophilic or clear cell change.

Special stains and IHC markers for PIN:

- Ulex europaeus lectin (UEA), AgNORs, and DNA ploidy pattern – similar in PIN and carcinoma, supporting its premalignant nature.
- Basal cell markers: High-molecular-weight cytokeratin (34βE12) and p63 highlight the retained basal layer, differentiating PIN from invasive carcinoma.
- AMACR (α-methylacyl-CoA racemase): Positive in both PIN and carcinoma, but absent in benign glands.

7.Adenocarcinoma of the Prostate

- Definition:
  The commonest malignant tumour of the prostate, accounting for >90% of epithelial malignancies, usually of the acinar (conventional) type.
- Anatomic Distribution:
  - Peripheral zone: 68%
  - Transition zone: 24%
  - Central zone: 8%

Gross

- Ill-defined, firm, grey-white or yellowish areas within the prostate.
- May show grittiness due to calcification.
- Advanced lesions may distort the capsule or infiltrate surrounding tissues.

Microscopy (Fig 2.8)

- **Architectural patterns:**
  - Acinar

- Cribriform
- Papillary
- Trabecular
- Solid
  (Often mixed patterns are present.)

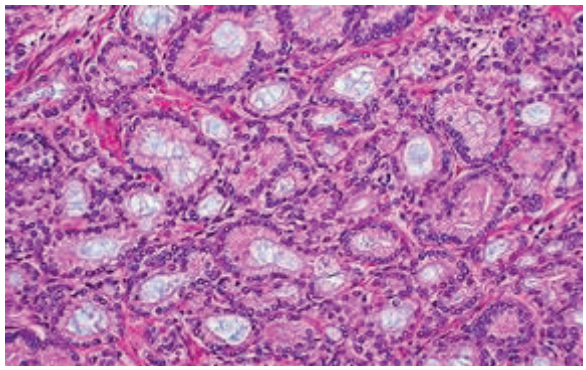


Fig 2.8 Acinar pattern in carcinoma prostate H&E x400

- **Cytology:**
  - Cells have finely granular cytoplasm; sometimes clear or foamy.
  - Tall cell and atrophic variants occur.
  - Infiltrative pattern of growth, nuclear enlargement, and macronucleoli (≥1 μm) are diagnostic hallmarks.

Criteria for Diagnosing Adenocarcinoma (especially well-differentiated lesions)

1. Small gland proliferation with focal infiltration.
2. Back-to-back glandular arrangement.
3. Single cell lining—absence of basal layer (confirmed by negative 34βE12 and p63).
4. Nucleomegaly and macronucleoli with reddish eosinophilic hue.
5. Supportive features (not diagnostic alone):
   - Intraluminal crystalloids (elongated, eosinophilic).
   - Intraluminal acid mucin (alcian blue positive).
   - Collagenous micronodules.
   - Perineural invasion (a common finding).
   - Adjacent high-grade PIN

8.Variants of Prostatic Adenocarcinoma

(Comprise about **10%** of all prostatic carcinomas)

a. Large Duct (Ductal) Adenocarcinoma

- Origin: From large periurethral ducts, typically around the verumontanum.

- Gross:
  - May show a villous or papillary mass protruding into the urethra or an infiltrative lesion.
- Microscopy:
  - Malignant transformation of dilated large ducts with papillary and cribriform growth.
  - Fibrovascular stalks lined by pseudostratified columnar epithelium.
  - Mitoses frequent; necrosis may be present.
- Immunohistochemistry:
  - PSA and PAP positive → confirms prostatic origin.
  - CK7 negative (helps exclude urothelial carcinoma).
- Differential diagnosis:
  - Urethral papilloma, prostatic urethral polyp, high-grade PIN, and urothelial carcinoma (latter is PSA/PAP negative).
- Clinical notes:
  - May present with hematuria or urethral obstruction.
  - Often more advanced stage (C or D) at presentation but may have better short-term survival.

b. Endometrioid (Large Duct, Müllerian Type) Adenocarcinoma

- Location: Periurethral region, often near verumontanum.
- Origin: Earlier thought to arise from prostatic utricle (Müllerian remnant); now regarded as a ductal variant of adenocarcinoma.
- Microscopy:
  - Papillary or glandular pattern with fibrovascular cores.
  - Pseudostratified tall columnar cells, sometimes with secretory or cribriform areas.
  - Frequent mitoses.
- IHC profile:
  - PSA and PAP positive → confirms prostatic origin.
  - Estrogen and progesterone receptor negative → distinguishes from true Müllerian lesions.
- Clinical:
  - Often presents with urethral bleeding.
  - Usually locally advanced at diagnosis.

c. Mucinous (Colloid) Adenocarcinoma

- Incidence: <0.5% of all prostatic adenocarcinomas.
- Diagnostic criteria:

    - At least 25% of the tumour shows extracellular mucin lakes containing tumour cell clusters.
    - Intracytoplasmic mucin alone is not diagnostic.
- Microscopy:
    - Extracellular mucin pools containing floating clusters, acini, or cribriform glands.
    - Non-mucinous component often shows microglandular or solid pattern.
- IHC profile:
    - PSA and PAP positive, CEA negative (distinguishes from colonic adenocarcinoma).
- Clinical:
    - Presentation and prognosis similar to usual acinar adenocarcinoma, though bone metastasis is rare and hormone dependence less evident.

d. Signet-Ring Cell Carcinoma (SRCC)

- Extremely rare, often presenting at advanced stage.
- Microscopy:
    - Infiltrative pattern with signet-ring cells containing intracytoplasmic mucin pushing nuclei to the periphery.
    - Usually associated with high-grade carcinoma (solid or cribriform pattern).
- IHC profile:
    - PSA and PAP positive (confirms prostatic origin).
    - CEA, CK20, and CDX2 negative → rules out metastatic colonic carcinoma.
- Differential diagnosis:
    - Metastatic carcinoma from stomach, bladder, or colon (must be excluded).
- Clinical:
    - Aggressive behaviour, poor prognosis.

e. Primary Transitional Cell Carcinoma (TCC) and Mixed Adeno–TCC

- Site: Arises from prostatic urethra or ducts.
- Histology:
    - Transitional-type epithelium with variable glandular differentiation.
- IHC:
    - PSA/PAP negative, CK7 and CK20 positive, GATA3 positive → consistent with urothelial origin.

- Clinical:
    - Frequently associated with bladder TCC; may present with urethral symptoms or hematuria.

8 Other Rare Variants of Carcinoma prostate

- Small Cell (Neuroendocrine) Carcinoma:
  - PSA may be negative; expresses synaptophysin, chromogranin, CD56.
  - Very aggressive, often associated with paraneoplastic syndromes.
- Squamous or Adenosquamous Carcinoma: Usually post-therapy related (radiation or hormonal).
- Basaloid / Basal Cell Carcinoma: Resembles basal cell neoplasms of skin; rare.
- Sarcomatoid Carcinoma: Biphasic epithelial–mesenchymal tumour; highly aggressive.

B V PROSTATE SYNOPTIC REPORTING

1) Prostatic needle biopsies/TURP chips

Type of specimen:

Needle biopsy □ No of cores sent……..

TURP □ Weight……g

Histology:

Nodular hyperplasia □

Acute prostatitis □

Chronic prostatitis □

Microabscesses □

Granulomatous prostatitis □ Tuberculous □ Non tuberculous □

Infarction present □ Squamous metaplasia □

Predominantly stromal hyperplasia □

Adenocarcinoma present : Yes □ No □

High grade PIN present : Yes □ No □

Gleason's grade & score : largest % first (primary) pattern …..

Second largest % (secondary) pattern…..

Total score ……

Percentage of TURP chips containing tumour*…….

For needle biopsy (single core): Size of core …. and size of tumour….mm

For multiple needle biopsies :

Right 1 -tumour present Yes □ No □

Right 2 -tumour present Yes □ No □

Right 3 -tumour present Yes □ No □

Left 1 -tumour present Yes □ No □

Left 2 -tumour present Yes □ No □

Left 3 - tumour present Yes □ No □

Invasion into seminal vesicle Yes □ No □

Other tumour □ Specify……..

* For non-palpable tumours :a) 5% or less of carcinoma (Gleason's score 2 to 6 ; pT1a)

b) More than 5% carcinoma (Gleason's score 2 to 6 or any amount of carcinoma with a Gleason's score of 7 or above pT1b)

c) tumour detected by needle biopsy (as a result of elevated PSA levels) -T1c ; in one or both lobes.

2) Resected specimens for prostatic carcinoma

Specimen type : Prostate □

Prostate & seminal vesicle □

Bladder □ prostate □

Other Specify ……..

Type of procedure : Radical prostatectomy □

Transurethral resection of prostate (TURP) □

Suprapubic prostatectomy □

Retropubic prostatectomy □

Other □

PSA (if known) ……

Weight : ……g

Measurements : …..x……x…..cm

Carcinoma evident grossly : Yes □ No □

If yes, measurements : …..x……x…..cm

Nodular hyperplasia □

Necrosis □

Bladder measurements : …..x….x…cm

Involved by neoplasm : Yes No

Seminal vesicle measurements ….x…x….cm

Involved by neoplasm : Yes No

Vas deferens : measurements …..x…..cm

Paraffin block key ……

…….

Microscopic features :

1. Tumour type : Adenocarcinoma

Other □ Specify ……

2. High grade PIN (grades 2 and 3) Yes □ No □

3. Gleason's grade & score* : largest % first (primary pattern) …..

Second largest % (secondary pattern)…..

Total score ……

Amount of tumour :

Percentage of tumour in relation to amount of gland removed……..

Number of microscopic foci involved by carcinoma…….(optional)

For radical prostatectomy : Percentage of tumour in relation to weight of gland…….

5. Multicentricity : Present Absent

6. Surgical margins : Posterior : Free □ Involved □

Posterior-lateral : Free □ Involved □

Anterior : Free □ Involved □

Neurovascular bundle : Free □ Involved □

Right : Free □ Involved □

Left : Free □ Involved □

Circumferential margin : Free □ Involved □

Apical margin : Free □ Involved □

Base margin (proximal bladder neck margin) involved Free □ Involved □

Perineural extension : Present □

Within prostate □ Outside □

Absent □

Staging** : Confined to prostate pT2 □

Extraprostatic extension (soft tissue) pT3a □

Invasion of seminal vesical/vesicals pT3b □

Invasion of other structures □ Specify ……..

Lymph nodes sent yes □ no □

No of nodes examined …….

No of nodes involved …….(Record the anatomic site of node involvement)

Other pathology……..

Signature …….Date…….

Gleason's microscopic grading of prostatic adenocarcinoma : (see fig 2.9 and description below)

Fig 2.9: shows grades of prostatic carcinoma (1 to 5)

Grade Description

1. Single separate uniform glands in closely packed masses with a definite usually rounded edge limiting the area of tumour

2. Single separate slightly less uniform glands, loosely packed (separated by small amounts of stroma) with less sharp edge

3a Single separate much more variable glands, may be closely packed but usually irregularly separated; ragged poorly defined edge

3b Like 3a but very small glands or tiny cell clusters

3c Sharply and smoothly circumscribed rounded masses of papillary or loose cribriform tumour ("papillary intraduct tumour")

4a Raggedly outlined, raggedly infiltrating and fused glandular tumour

4b Like 4a , with large pale cells ("hypernephroid")

5a Sharply circumscribed , rounded masses of almost solid cribriform tumour, usually with central necrosis("comedocarcinoma")

5b ragged masses of anaplastic carcinoma with only enough gland formation or vacuoles to identify it as adenocarcinoma

- Basis of grading – Grading is architectural, not nuclear.
- Only invasive carcinoma is graded (not intraductal carcinoma — IDC-P).
- The Gleason's system is used for adenocarcinoma alone. A tumour may show one or several of the histologic patterns outlined above ranging from lowest grade (grade 1) to the highest grade (grade5).
- A tumour is assigned to a particular category according to the grade in which it fits (indicated by the number) irrespective of its subgrade (as indicated by the letter). Combined grade is obtained by adding predominant grade (primary grade) and the next predominant grade(secondary grade) when present. If a tertiary (minor) higher-grade pattern is present (>5% but <secondary component), it is recorded in parentheses and may influence risk stratification.
- If a tumour has the same grade throughout, its combined grade is obtained by doubling the value of its grade.
- The primary and secondary patterns (most and second most prevalent) are added to form the Gleason score (sum). In a needle biopsy when there are more than 2 patterns and the worst grade is neither the predominant nor the secondary pattern, choose the predominat pattern and the highest grade to arrive at the correct score (for instance : the patterns are : grade 3 is 60%, grade 1 is 30% and grade 4 is 10%; the score should be 3+4 = 7.A Gleason score of 7 is an indicator of clinically significant disease even if the disease is restricted to a single microscopic focus.

Problems with the Current Gleason System

- Scores 2-5 are currently no longer assigned and certain patterns that Gleason defined as a score of 6 are now graded as 7, thus leading to contemporary Gleason score 6 cancers having a better prognosis than historic score 6 cancers.
- The combination of Gleason scores into a 3-tier grouping (6,7,8-10) is used most frequently for prognostic and therapeutic purposes, despite 3+4=7 vs. 4+3=7 and 8 vs. 9-10 having very different prognoses.
- In practice the lowest score is now assigned a 6, although it is on a scale of 2-10. This leads to a logical yet incorrect assumption on the part of patients that their cancer is in the middle of the scale, compounding the fear of their cancer diagnosis with the belief that the cancer is serious, thus leading to an expectation that treatment is necessary.
- **Proposal for a new Grading System (John Hopkin's University)**

  To address the above deficiencies, a new 5 Grade Group system has been developed based on a study of >20,000 prostate cancer cases treated with radical prostatectomy and >5,000 cases treated by radiation therapy (see composite photograph for different patterns).
- Grade Group 1 (Gleason score ≤6) - Only individual discrete well-formed glands
- Grade Group 2 (Gleason score 3+4=7) - Predominantly well-formed glands with a lesser component of poorly-formed/fused/cribriform glands
- Grade Group 3 (Gleason score 4+3=7) - Predominantly poorly-formed/fused/cribriform glands with a lesser component of well-formed glands†
- Grade Group 4 (Gleason score 8)
  - Only poorly-formed/fused/cribriform glands or
  - Predominantly well-formed glands with a lesser component lacking glands †† or
  - Predominantly lacking glands with a lesser component of well-formed glands ††
- Grade Group 5 (Gleason scores 9-10) - Lacks gland formation (or with necrosis) with or w/o poorly-formed/fused/cribriform glands (John Hopkin's University)†
- The new grading system, using the above terminology, has been accepted by the 2016 World Health Organization (WHO).
- The ISUP Grade Group system (1–5) has now **replaced numerical Gleason sums** in most modern reports for clarity in clinical communication.
- To avoid confusion, it will be advisable to report the new grading system, if using it , in conjunction with the Gleason system, until it is regularised and practiced [ie. *Gleason score 3+3=6 (Grade Group 1)*].

Recent Gleason / ISUP Grading System (current standard) (2019)

ISUP Grade Groups (2014, reaffirmed 2022)

Grade Group	Gleason Score	Description
1	3 + 3 = 6	Well-formed glands only; best prognosis
2	3 + 4 = 7	Predominant pattern 3 with <50% pattern 4
3	4 + 3 = 7	Predominant pattern 4 (>50% pattern 4)
4	8 (4 + 4, 3 + 5, 5 + 3)	Poorly differentiated, fused/cribriform glands
5	9–10 (4 + 5, 5 + 4, 5 + 5)	Undifferentiated, solid/necrotic carcinoma

Key refinements in the recent system

- Cribriform glands (even if small) are always pattern 4, not 3.
- Poorly formed glands qualify as pattern 4; tiny tangentially cut glands are not.
- IDC-P is not graded, but its presence must be reported, as it implies high-grade disease.
- Percent pattern 4 (in 3 + 4 and 4 + 3 tumours) and tertiary pattern 5 should be recorded for prognostic accuracy.

Current Pattern Definitions (ISUP / WHO 2022)

Gleason Pattern	Architectural features
Pattern 3	Discrete, well-formed individual glands, no fusion or cribriforming.
Pattern 4	Fused, poorly formed, or cribriform glands; glomeruloid structures included. *Large cribriform pattern must always be called pattern 4 (adverse prognostic impact).*
Pattern 5	No gland formation (solid sheets, cords, or single cells); comedonecrosis.

TNM Staging of Prostatic Carcinoma (AJCC / UICC 8th Edition — current)

Primary Tumour (T)

Category	Definition
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
T1	Clinically inapparent tumour, not palpable or visible by imaging

Category	Definition
• T1a	Incidental tumour in ≤5% of resected tissue (TURP)
• T1b	Incidental tumour in >5% of resected tissue
• T1c	Tumour identified by needle biopsy (elevated PSA)
T2	Tumour confined within the prostate
• T2a	Involves ≤ half of one lobe
• T2b	Involves > half of one lobe, but not both lobes
• T2c	Involves both lobes
T3	Extraprostatic extension (EPE) or seminal vesicle invasion
• T3a	Extraprostatic extension (unilateral or bilateral) or microscopic bladder neck invasion
• T3b	Invasion of seminal vesicle(s)
T4	Tumour is fixed or invades adjacent structures other than seminal vesicles (e.g., external sphincter, rectum, bladder, levator muscles, or pelvic wall)

Regional Lymph Nodes (N)

Category	Definition
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in regional lymph node(s) (pelvic, below bifurcation of common iliac arteries)

Distant Metastasis (M)

Category	Definition
M0	No distant metastasis
M1	Distant metastasis present
• M1a	Non-regional lymph node(s)
• M1b	Bone(s)
• M1c	Other sites (with or without bone disease)

Stage Grouping (simplified)

Stage	T	N	M	Grade Group
I	T1–T2a	N0	M0	1
IIA	T1–T2a	N0	M0	2
IIB	T2b–T2c	N0	M0	2
IIC	T1–T2	N0	M0	3–4
III	T3–T4	N0	M0	Any
IVA	Any T	N1	M0	Any
IVB	Any T	Any N	M1	Any

Extra-prostatic extension (EPE) defines T3a — even if microscopic.

- **Seminal vesicle invasion** is **T3b**, regardless of continuity.
- **Bladder neck invasion** is staged **T3a**, not T4 (per 8th edition).
- **Regional nodes** are pelvic (below common iliac bifurcation); para-aortic nodes are **M1a**.
- **Note:** Pathologic staging follows **AJCC/UICC 8th edition (2017)** TNM classification, with T3a defined by extra-prostatic extension, T3b by seminal vesicle invasion, and T4 by invasion of adjacent structures beyond seminal vesicles. No changes have yet been introduced in a 9th edition (as of 2025).

The **Whitmore-Jewett staging system** (also known as the Jewett staging system or ABCD system) is another system of grading for prostate cancer. It was developed by the American Urological Association (AUA). Most societies (including AUA) and clinicians now advocate and use the TNM staging system.

- A: subclinical tumour: found incidentally (T1 in TNM)
  - A1: tissue resembles normal cells; found in a few chips from one lobe
  - A2: more extensive involvement
- B: tumour palpable on digital rectal exam (DRE): no extraprostatic extension (T2 in TNM)
  - B1: less than 50% one lobe
  - B2: more than 50% one lobe
  - B3: both lobes
- C: extracapsular extension (T3/T4 in TNM)
  - C1: unilateral, not seminal vesicles (T3a)
  - C2
    - bilateral, not seminal vesicles (T3b)
    - seminal vesicles (T3c)

- bladder neck, external anal sphincter, and/or rectum (T4a)
- levator muscles, and/or fixed to pelvic sidewall (T4b)

- D: metastasis
  - D1: regional adenopathy (N1 in TNM)
  - D2: distant metastases (M staging in TNM)

C II. TESTIS GROSSING TECHNIQUE-

TESTIS

Specimens received :

Wedge or needle biopsy

Orchidectomy

Wedge or Needle biopsy

1. To be fixed in Bouin's fluid and should go directly into alcohol and not formalin at processing.
2. Give the accession number and measurements (three dimensions) of the largest and smallest fragments. The colour will no doubt be yellow due to the fixative and need not be described.

Bits

All for section

Orchidectomy specimen

1. Open the tunica vaginalis, measure the testis from pole to pole and take the length of the spermatic cord.
2. Weigh the testis with the spermatic cord and tunica vaginalis. The weight is taken after opening the sac in order to drain out any possible hydrocoele fluid
3. Cut the testis and epididymis sagitally such that the spermatic cord is retained in one half. The cut should pass through the epididymitis and rete testis such that invasion of these structures can be recognized in tumours.
4. Cut serial sections parallel to the original section at a thickness of 5 mm
5. Make cross sections of the spermatic cord at different levels to exclude any pathology.
6. In case of neoplasms describe

-size in 2 dimensions

-colour

-consistency

-cut surface : homogenous/variegated

-cysts : present / absent

-necrosis

-haemorrhage

-bony tissue, cartilage

-tumour extension to tunica albuginea, epididymis,

rete testis, spermatic cord and others structures

1. In case of non tumour :

-'plucking test' to be done if the origin of the tissue is in question

-describe atrophy, fibrosis

-cut section : nodules, necrosis, tubercles etc.

Bits - For tumours (Fig 2.10)

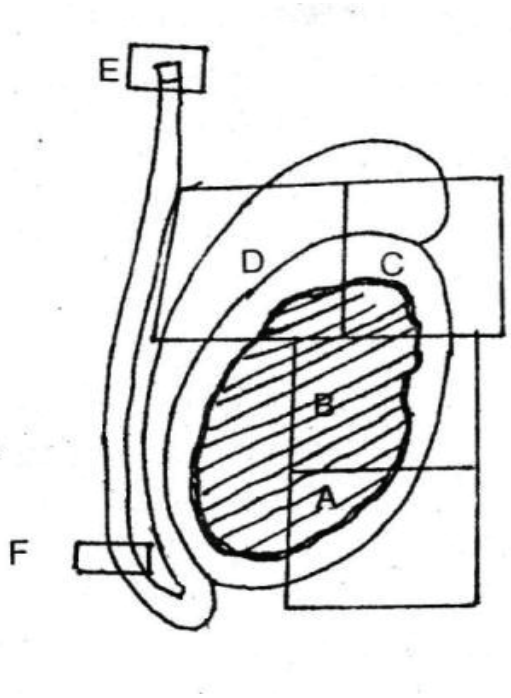


Fig 2 10

A,B, C - tumour – three bits or one bit per centimeter of the tumour measured along the greatest tumour dimension.

They should include tumour with tunica albuginea in most bits, areas of haemorrhage and necrosis, solid areas and tumour with adjacent normal testis in one or two bits in order to look for vascular invasion.

D – uninvolved testis, epididymis and rete testis

E- One bit cross section of spermatic cord at the surgical margin. This is taken preferably before sectioning of the testis in order to avoid contamination

F- Cross section of spermatic cord at the base – one bit

Importance of sampling

Testicular tumours may have different components with varied significances and therefore the tumour should be sampled extensively.

eg. Choriocarcinoma shows early metastasis to the brain.

Seminoma shows bone metastasis.

Embryonal carcinoma shows early widespread metastasis.

For non-neoplastic lesions (Fig 2.11)

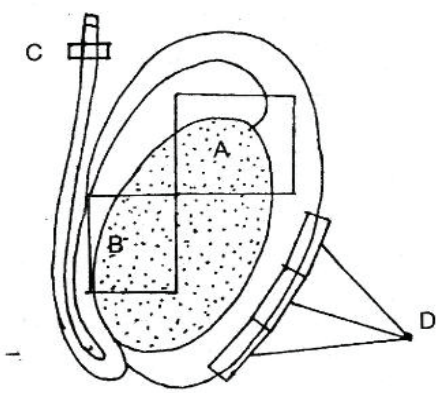


Fig 2.11

A & B – one or two bits testis with epididymis and tunica albuginea

C- spermatic cord to be bitted if any pathology is suspected

D- fibrotic sac in case of hydrocoele.

C III TESTIS GROSS PEARLS

1. **Irregular testicular enlargement** with involvement of epididymis ,showing focal areas of necrosis and fibrosis : **tuberculous epididymo-orchitis**
2. **Small testis, dilated tunical sac**, thickened tunica parietalis, sometimes haemorrahge into the cavity : hydrocoele with secondary changes (calcification may also be observed)- **atrophic testis with hydrocoele** .

3. **Uniformly enlarged testis**. C/S well demarcated neoplasm, homogenous, firm mass, frequently grey white, coarsely lobulated and bulging : **Seminoma** (spermatocytic seminoma is yellow, softer and gelatinous)
4. **Tumour with variegated cut surface**, with haemorrahge & necrosis, grey white and granular, poorly demarcated : **Embryonal carcinoma /Endodermal sinus tumour/** mixed germ cell tumour. Embryonal carcinomas in the pure form are small in size upto 2.5 cm in size.
5. **Well demarcated tumour** from surrounding un-involved testis, solid or multicystic, cysts filled with clear, keratinous or gelatinous material. Cartilage, spicules of bone, patches of pigmented tissue or brain tissue may be seen : Mature teratoma.
6. **Yellow tan firm lobulated mass** : granulosa cell tumour / malakoplakia

C IV TESTIS MICROSCOPY

1. Inflammatory Lesions

Tuberculous Epididymo-orchitis:
A common granulomatous infection in endemic regions such as India. Fine-needle aspiration (FNA) and histology show epithelioid granulomas with caseous necrosis, Langhans giant cells, and a rim of lymphocytes. Acid-fast bacilli may be demonstrated by Ziehl–Neelsen staining.
Pearl: Always exclude tuberculous infection in chronic granulomatous orchitis, especially when bilateral.

Malakoplakia:
May involve testis, epididymis, or both. The parenchyma is replaced by sheets of von Hansemann histiocytes with granular eosinophilic cytoplasm. Within them are **Michaelis–Gutmann bodies**, intracytoplasmic targetoid inclusions that are PAS and von Kossa positive. Background shows mixed inflammatory infiltrate, granulation tissue, and fibrosis.

Hydrocoele Sac:
Microscopy reveals a thickened fibrous wall, granulation tissue, and vascular proliferation. Foreign body giant cells and cholesterol clefts (from old hemorrhage) may be present.

2. Testicular Biopsy in Infertility

Assessment Criteria:

- **Spermatogenesis:** Graded by **Johnson's score (1980)** — each tubule is scored from 1–10 (see below).
  A normal adult testis averages ≥8.9 with ≥60% of tubules scoring 10.
- **Germ cell–Sertoli cell ratio:** Normally constant at **13:1**. About 12 Sertoli cells per tubule cross-section are expected.
- **Syndromic associations:** Note any pattern such as *Sertoli Cell Only Syndrome*; recommend cytogenetic evaluation if suspected.

- **Associated pathology:** Look for granulomatous or other specific inflammation.

Johnson's Score for Spermatogenesis (1980)

Score	Description
10	Complete spermatogenesis and perfect tubules
9	Many spermatozoa, but disorganized spermatogenesis
8	Only a few spermatozoa present
7	No spermatozoa, but many spermatids present
6	Only a few spermatids present
5	No spermatozoa/spermatids but many spermatocytes present
4	Only a few spermatocytes present
3	Only spermatogonia present
2	No germ cells present
1	No germ or Sertoli cells present

Common Microscopic Patterns in Azoospermia

Category	Features	Comments
a) Germ Cell Aplasia (Sertoli Cell Only Syndrome)	Tubules contain only Sertoli cells (100–150 µm diameter), thickened basement membrane, absent germ cells. Leydig cells may be normal or atrophic.	29% of cases
b) Maturation Arrest	Arrest of spermatogenesis at primary spermatocyte (early) or spermatid (late) stage.	26%
c) Generalised Fibrosis	Tubular atrophy and peritubular fibrosis of any etiology.	18%
d) Normal Spermatogenesis	Seen in obstructive azoospermia (e.g., Young's syndrome).	27%
e) Hypospermatogenesis	Reduced but complete maturation sequence in scattered tubules.	—
f) Klinefelter's Syndrome (47,XXY)	Tubular sclerosis, thickened basement membrane, Leydig cell hyperplasia.	Cytogenetic confirmation advised

3. Cryptorchidism

Undescended testes have a **30–50× increased risk** of malignant transformation, most commonly **seminoma**.
Histologically, tubules are hypoplastic with thickened basement membranes and interstitial fibrosis.

4. Classification of Testicular Tumours

Historically, classifications included Dixon & Moore, Collins & Pugh, and the British Testicular Tumour Panel.
The WHO 2022 (5th Edition) now provides the international standard, integrating morphology, immunophenotype, and molecular profile.

WHO 2022 Classification of Germ Cell Tumours

Germ Cell Tumours Derived from GCNIS	Non–GCNIS Derived Germ Cell Tumours
Seminoma	Prepubertal-type teratoma
Embryonal carcinoma	Prepubertal-type yolk sac tumour
Postpubertal-type yolk sac tumour	Spermatocytic tumour (previously *spermatocytic seminoma*)
Choriocarcinoma	—
Teratoma (mature, immature, or with somatic-type malignancy)	—
Mixed germ cell tumour	—

5. Detail on Seminoma

Classic (Typical) Seminoma: (see Fig 2. 12)
Uniform tumour cells with abundant clear (glycogen-rich) cytoplasm, distinct membranes, central nuclei, and prominent nucleoli.
Nests of tumour cells are separated by fibrous septa with lymphocytic infiltration; granulomatous reaction may occur.

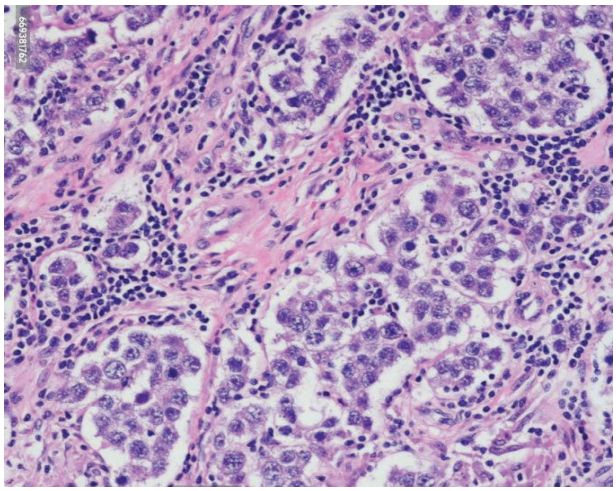


Fig 2.12: Uniform tumour cells with abundant clear (glycogen-rich) cytoplasm, distinct membranes, central nuclei in classic seminoma of testis. H & E x 400

Variants:

- **Anaplastic Seminoma:** >3 mitoses/HPF, marked nuclear pleomorphism and necrosis; prognosis similar to classic type.
- **Seminoma with Syncytiotrophoblastic Giant Cells:** 10–20% of cases; elevated serum β-hCG.
- **Seminoma with Yolk Sac Differentiation:** Rare; shows hyaline globules and AFP positivity.

Immunoprofile:
PLAP+, OCT3/4+, CD117 (c-KIT)+, D2-40+, SALL4+, negative for CD30 and cytokeratin (high MW).
Recent marker: SOX17 positive (helps distinguish from embryonal carcinoma).

6. Spermatocytic Tumour (Previously Spermatocytic Seminoma)

Occurs in older men (>50 years).
Shows three cell types—small lymphocyte-like, medium-sized predominant cells, and scattered giant cells.
No fibrous septa or lymphoid infiltrate.

Immunostains: Negative for PLAP, OCT3/4, and CD30; positive for SALL4 and VASA.
Molecular hallmark: *FGFR3* or *HRAS* mutation.

7. Embryonal Carcinoma

Highly malignant tumour of large pleomorphic cells with indistinct borders, prominent nucleoli, and frequent mitoses.
IHC: Positive for OCT3/4, CD30, cytokeratin (high MW), SOX2, and EMA; negative for

CD117.
Note: Loss of *p53* and *RB1* expression correlates with aggressive behaviour.

8. Teratoma

Comprises mature or immature somatic tissues.
Mature teratomas are benign in prepubertal males but potentially malignant postpuberty.
Search for yolk sac areas as these alter prognosis.
May undergo **somatic-type malignancy** (e.g., sarcomatous or carcinomatous transformation).

9. Yolk Sac Tumour (Endodermal Sinus Tumour)

- **Children (<2 years):** Pure form; excellent prognosis.
- **Adults:** Usually mixed; aggressive course.

Microscopy: Reticular or microcystic pattern; *Schiller–Duval bodies* (central vessel surrounded by tumour cells in a cystic space).
Immunostains: AFP+, glypican-3+, cytokeratin+, SALL4+.
Emerging marker: GATA-4 (positive in paediatric cases).

10. Choriocarcinoma

Highly malignant tumour composed of **cyto- and syncytiotrophoblasts**, often small, hemorrhagic, and necrotic.
Serum β-hCG markedly elevated.
Presence of syncytiotrophoblastic cells alone (as in seminoma) is **not diagnostic**.

11. Germ Cell Neoplasia in Situ (GCNIS)

Previously called *intratubular germ cell neoplasia*.
Seen adjacent to germ cell tumours or in high-risk settings (cryptorchidism, infertility, intersex disorders).
IHC: OCT3/4+, PLAP+, c-KIT+.
Significance: Precursor lesion to all GCNIS-derived tumours; recognition is vital for management and contralateral surveillance.

12. Patterns of Metastasis

- **Initial spread:** Para-aortic and iliac nodes.
- **Later:** Mediastinal and left supraclavicular nodes.
- **Organ tropism:**
    - Choriocarcinoma → brain and lungs
    - Seminoma → bone
    - Embryonal carcinoma → early hematogenous spread
    - Teratocarcinoma → metastasizes as embryonal carcinoma component.

13. Recent Diagnostic Advances

- **Multiplex IHC panels (OCT3/4, SOX17, SOX2, SALL4, CD117, CD30)** refine subtyping.
- **Molecular signatures:**
  - GCNIS-derived tumours show *12p gain (i[12p])* in 80–90%.
  - *FGFR3/HRAS* mutations in spermatocytic tumour.
- **Digital pathology** enables automated seminiferous tubule scoring in infertility biopsies.
- **Liquid biopsy marker:** miR-371a-3p is emerging as a non-invasive marker for active germ cell tumour disease.

Summary and Key Points

- **Spectrum of lesions:** Testicular pathology includes inflammatory, developmental, and neoplastic conditions; among these, germ cell tumours constitute over 90% of neoplasms.
- **Infertility biopsy:**
  - Evaluate spermatogenesis using the **Johnson score** and note the germ cell–Sertoli cell ratio (normal ≈13:1).
  - The most frequent patterns in azoospermia are **Sertoli Cell Only Syndrome**, **maturation arrest**, and **tubular fibrosis**.
- **GCNIS:** The precursor lesion for most germ cell tumours. Always search for it in the parenchyma adjacent to tumours and in high-risk testes (cryptorchid, atrophic, dysgenetic).
- **WHO 2022 Classification:** Divides germ cell tumours into **GCNIS-derived** and **non–GCNIS-derived** groups, reflecting pathogenesis and prognosis.
- **Seminoma:** Most common testicular tumour. Shows clear cells, fibrous septa, lymphoid infiltrate, and PLAP/OCT3/4 positivity.
  SOX17 is a reliable new marker for seminoma.
- **Spermatocytic tumour:** Distinct from seminoma; occurs in older men, has a benign course, and lacks GCNIS association.
- **Yolk sac tumour:** Shows Schiller–Duval bodies and AFP positivity. **GATA-4** expression supports diagnosis in paediatric cases.
- **Choriocarcinoma:** Marked hemorrhage and necrosis, very high β-hCG, early hematogenous spread.
- **Mixed tumours:** Common; staging and treatment are based on the most aggressive component.
- **Molecular advances:**
  - *i(12p)* is characteristic of GCNIS-derived tumours.
  - *FGFR3* or *HRAS* mutations define spermatocytic tumours.

- **miR-371a-3p** serves as a promising serum biomarker.

- **TNM staging (AJCC 8th, 2025):**
  - pT category is based on local invasion (testis, tunica, spermatic cord, scrotum).
  - pN and M reflect nodal and distant spread;
  - S category incorporates serum tumour markers (AFP, β-hCG, LDH).
- **Diagnostic emphasis:**
  - Always document lymphovascular invasion and rete testis involvement.
  - Use a comprehensive IHC panel (OCT3/4, SOX17, SOX2, CD117, CD30, SALL4).
  - Integrate morphology, immunoprofile, and molecular data for accurate classification.

C V TESTIS SYNOPTIC REPORTING

1) Testicular biopsy

Specimen type : True cut needle □ Wedge □

Measurements : ….. mm x ….. mm x …..mm

Spermatogenesis : Adequate □ Inadequate □

Johnson's Score :……

Germ cell : Sertoli cell ratio (normal 13: 1) : Maintained □ Altered □

Average no of Sertoli cells / tubular cross section (normal -12) : ……

Basement membrane thickening : Yes □ No □

Interstitium : Leydig cells : Present □ Hyperplastic : Yes □ No □ Absent □

Inflammation : Present □ Acute □ Chronic □ Absent □

Granulomas : Present □ Type : Tuberculous □

Spermatocytic □

Other □ Specify ..…..

Absent □

Any other comment …….

Signature …….. Date……

2) Resection specimen for Testicular Cancer

Gross :

Size of testis : ……x……x……cm

Size of tumour ……x……..x…….cm

Cut surface :

Homogenous □

Haemorrhage □

Necrosis □

Cystic change □

No of blocks taken from tumour ……

Length of spermatic cord : …….cm

Epididymus : Present □ Absent□

Measurement …..x…..cm

Histology :

Histologic subtype :

Seminomatous □

With syncitiotrophoblasts □

With yolk sac features □

With anaplasia □

Spermatocytic □

Non –seminomatous □

Yolk sac tumour □

Embryonal carcinoma □

Choriocarcinoma □

Teratoma mature □

Teratoma immature □

Teratoma with embryonal carcinoma □ and or

with yolk sac tumour □

Mixed seminoma and NSGCT (……) □

Non- germ cell tumour □

Sex cord tumours □ specify :…….

Mesenchymal tumour □specify……

Haemopoietic tumour □ specify…….

Metastatic tumour □ specify ……

Miscellaneous □ specify …….

Other features :

Tumour : limited to testis □

invades tunica albuginea □

present in cord □

invades rete testis □

invades epididymus □

invades scrotum □

Vascular invasion Yes □ No □

Areas of scarring only □

Excision margin of cord : involved □ not involved □

Biopsy of contralateral testis Yes □ No □

Intratubular germ cell neoplasm present Yes □ No □

Lymph nodes : Specify group :

No examined ……. No positive……..

TNM stage : …….

Summary and comments : …….

Signature : Date :

Pathologic (pTNM) Staging of Testicular Tumours (AJCC 8th Edition, (2025)
(AJCC / UICC 8th Edition)

Category	Criteria
Primary Tumour (T)	
Tis	Germ cell neoplasia in situ (GCNIS)
T1	Tumour limited to testis ± rete testis invasion; no lymphovascular invasion
T2	Tumour limited to testis with lymphovascular invasion OR tunica vaginalis involvement
T3	Tumour invades spermatic cord
T4	Tumour invades scrotum
Regional Lymph Nodes (N)	
N0	No regional lymph node metastasis
N1	Node mass ≤2 cm; ≤5 nodes involved
N2	Node mass >2–5 cm OR >5 nodes involved OR extranodal extension
N3	Node mass >5 cm
Distant Metastasis (M)	
M0	No distant metastasis
M1a	Non-regional lymph nodes or lung metastasis
M1b	Other distant metastases (e.g., liver, bone, brain)
Serum Tumour Markers (S)	
S0	Normal markers
S1	LDH <1.5× normal; hCG <5000 IU/L; AFP <1000 ng/mL
S2	LDH 1.5–10× normal; hCG 5000–50,000; AFP 1000–10,000
S3	LDH >10× normal; hCG >50,000; AFP >10,000

Staging Note:
Serum tumour markers (AFP, β-hCG and LDH) are an integral component of staging in testicular germ cell tumours and are assessed after orchiectomy. Prognostic grouping is determined by the combination of TNM category and post-orchiectomy serum marker levels (S stage).

ASSESS YOURSELF MALE GENITAL SYSTEM

Essay Questions (10 marks each)

1. Describe the grossing of a penectomy specimen. Add the important points to be included in reporting of carcinoma penis.
2. Discuss the grossing technique of prostate specimens (TURP chips and radical prostatectomy). Add the salient features to be noted in carcinoma prostate.
3. Describe the grossing of orchidectomy specimens for testicular tumours. Add the important prognostic factors to be assessed.

Short Answer Questions (5 marks each)

1. Write a note on inguinal lymph node dissection in carcinoma penis.
2. Enumerate the zones of prostate and their pathological significance.
3. Describe the gross features of seminoma and non-seminomatous germ cell tumours.

Short Notes / Point-wise Questions (3 marks each)

1. Enumerate the premalignant lesions of penis.
2. List the risk factors for carcinoma prostate.

MCQs

1. **The most common malignant tumour of the penis is:**
   a) Melanoma
   b) Basal cell carcinoma
   c) Squamous cell carcinoma
   d) Sarcoma
2. **Benign prostatic hyperplasia most commonly arises in the:**
   a) Peripheral zone
   b) Central zone
   c) Transition zone
   d) Anterior fibromuscular stroma

3. **The most common testicular tumour in young adults is:**
   a) Seminoma
   b) Teratoma
   c) Yolk sac tumour
   d) Leydig cell tumour

Answer Key for MCQs

1. c
2. c
3. a

Chapter 3
Gastro-Intestinal Tract

Chapter outline A Oesophagus ;B Stomach; C Small and Large Intestine

AI Oesophagus Anatomy; A II Oesophagus Grossing Techniques ; A III Oesophagus Gross Pearls; A IV Oesophagus Microscopy; A V Oesophagus Synoptic Reporting.

BI Stomach Anatomy; BII Stomach Grossing Techniques ; BIII Stomach Gross Pearls; BIV Stomach Microscopy; B V Stomach Synoptic Reporting.

C I Small and Large Intestine Anatomy; C II Small and Large Intestine Grossing Techniques; C III Small and Large Intestine Gross Pearls; C IV Small and Large Intestine Microscopy; C V Small and Large Intestine Synoptic Reporting.

A I OESOPHAGUS ANATOMY

The esophagus begins in the hypopharynx at the crico-pharyngeus muscle and ends at the gastroesophageal junction (GEJ) to the left of midline opposite the 10th or 11th thoracic vertebrae. It measures 25 to 35 cm in adults .

It follows the course of the vertebral column maintaining close proximity with the trachea, left mainstem bronchus, aortic arch, descending aorta, and left atrium.

- It is customarily divided into 3 parts —upper, middle and lower.
- The normal esophagus has constrictions :
    1. at its cricoid origin,
    2. along its left side at the aortic arch,
    3. at the crossing of the left mainstem bronchus,
    4. at the fifth thoracic vertebra and left atrium.
- The right side of the GEJ appears smooth, whereas the left side forms a sharp angle known as the incisura or angle of His.
- Lymphatic drainage of oesophagus.

    Upper 1/3: Cervical lymph nodes.

    Middle 1/3: paratracheal and paraesophageal lymph nodes.

    Lower 1/3: Nodes around aorta and celiac axis.

Normal histology :

The wall of the esophagus from the lumen outwards consists of mucosa, submucosa (connective tissue), layers of muscle fibers and an outer layer of connective tissue. The mucosa is a stratified squamous epithelium of around three layers of squamous cells.

The transition between these two types of epithelium is visible as a zig-zag line. Normally, the cardia of the stomach is immediately distal to the z-line.

- Non keratinised squamous epithelium: Basal cells (1to 4 layers) constitute < 15% of the thickness of the normal epithelium. Melanocytes and neuroendocrine cells are seen in basal layer
- Sub epithelial connective tissue is composed of loosely arranged stromal cells and a few inflammatory cells
  - Muscularis mucosa is thicker than other portions of the GIT.
  - Papillae of the lamina propria do not exceed > 50% of the total thickness of the epithelium
- Sub mucosa contains mucus secreting glands and the ducts open onto the lumen, in the distal oesophagus the mucosa also contains mucous glands.
- Foci of pancreatic acini may be seen**.**
- Oesophagus has no serosa, therefore lack of serosa permits ready and widespread dissemination of various infections and tumours to the posterior mediastinum, the spread is further facilitated by the rich network of longitudinally placed lymphatics in the mucosa and submucosa[2]**.** When these lymphatics traverse the muscularis to reach the adventia, lymph node involvement at sites away from the level of the tumour can occur.

A II OESOPHAGUS GROSSING TECHNIQUES

Types of specimens received are:

1. Endoscopic biopsy – to diagnose viral, fungal infections, Barrett's oesophagus,etc
2. Oesophagectomy specimens for
   a) Neoplastic lesions – depending on the extent of neoplasm a partial, subtotal; or total oesophagectomy with portion of stomach may be performed.
   b) Non-neoplastic lesions- strictures due to corrosives, etc

Endoscopic Biopsy

- Description: Note the number and colour of the tissue fragments. They are separated from the underlying filter paper/cardboard on which they are sent or fixed. The mucosal surface should face upwards on the filter paper. Orientation of the fragments is done preferably using hand lens/dissection microscope.

- Measurement: Note the size of the largest and the smallest of the fragments in the greatest dimension.

Bits Process all the tissue.

Oesophagectomy specimens : Surgeries performed may be :

- Transhiatal oesophagectomy is done for tumours anywhere in the esophagus or gastric cardia. No thoracotomy is performed.

Right thoracotomy (Ivor-Lewis Procedure): good for exposure of upper to mid esophageal lesions.

- Left thoracotomy : appropriate for lower third of esophagus and gastric cardia.
- Radical (enbloc resection): for tumour anywhere in the esophagus or gastric cardia. Benefit of more extensive lymphadenectomy and potentially better survival, but increased operative risk.

Specimen handling :

- Describe the parts included in the specimen, i.e the oesophagus with or without proximal part of the stomach. Measure the length and circumference of the oesophagus as also the length of the greater and the lesser curvatures of the stomach when present.
- Open the oesophagus longitudinally from one end to the other (in presence of tumour, the oesophagus is opened along the opposite longitudinal side of the tumour). Extend the cut along the lesser or greater curvature of the stomach if it is included in the specimen.
- Describe the tumour by noting the following points:
    - Size (three dimensions)
    - Configuration – whether endophytic (ulcerative), exophytic (fungating) or diffusely infiltrative
    - Location – its distance from the gastro- oesophageal junction and proximal resection margin..
    - Extent circumferentially - Whether focal or annular involving the entire circumference of the organ.
    - Estimated depth of invasion (measurement to be noted at the maximum
    - depth) and note the layers involved by the tumour
    - Whether involving the stomach or adjacent organs
    - Note the proportion of tumour located in the oesphagus and in the stomach
    - for those located at gastro-oesophageal junction.
    - Evidence of perforation if any
    - Cut surface - colour and consistency
    - Margins - Note the distance of tumour from the margins:
    - Proximal; distal and radial (distance of the soft tissue margin closest to
    - deepest tumour penetration).

- Ink (India ink/Eosin) the outer surface of the oesophagus at the level of the deepest tumour penetration and mop dry before taking the radial margin bit (this enables to identify the outer radial margin limit on the slide). Also ink the proximal and distal surgical margins.
- Describe the uninvolved mucosa with regard to presence of erosions, haemorrhages, ulcers, any thickened areas, varices (identified as bluish cordlike easily movable structures), diverticula (commonly seen in the anterior wall) and areas of Barrett's mucosa (seen as reddish velvety areas).
- Lymph nodes: Regional lymph nodes: Para and peri oesophageal lymph nodes in fat (the oesophagus has no serosa) are dissected out. Gastric lymph nodes, from the lesser and greater curvatures are dissected and grouped separately. Other groups eg. coeliac etc if sent should be grossed separately.
- Note the following features in these lymph nodes: The number, colour, consistency, any involvement of the adjacent soft tissue and the greatest dimension of the largest and the smallest involved lymph nodes.
- Application of Lugol's iodine: Painting oesophageal mucosa with Logol's iodine will normally stain the normal mucosa mahogony brown while the pathological mucosa fails to show the colour change due to lack of glycogen. Hence, by this staining procedure one can localise the abnormal sites for tissue sampling.

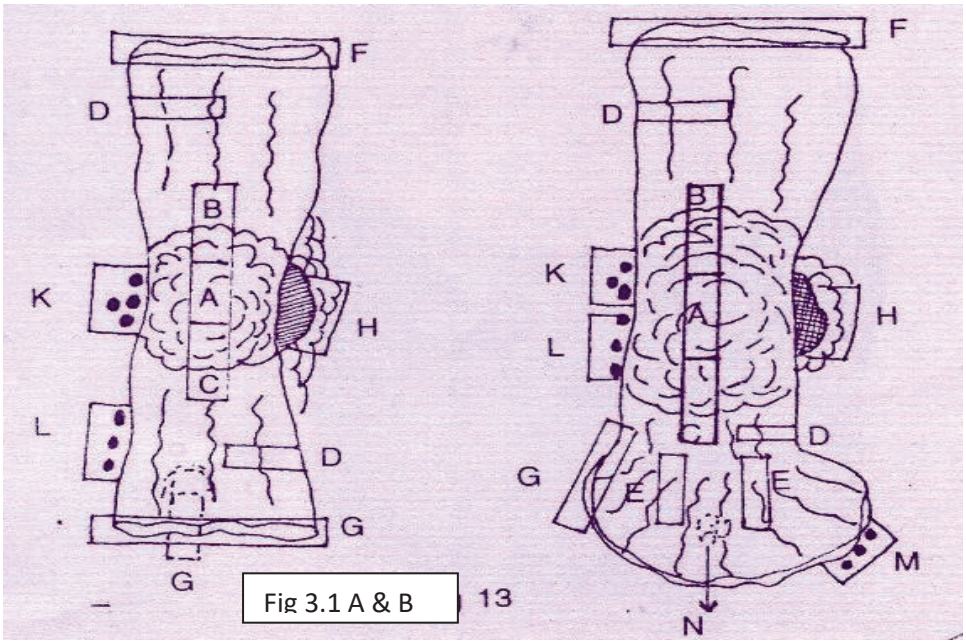


Figure 3.1 *A & B : Shows opened up oesoghagectomy specimens and the sites of bits taken*

Bits

A,B, and C : 3 bits tumour (may be 4 depending on size of bits)

A: To include maximum depth *of* tumour infiltration

B: To include interface with adjacent proximal oesophagus

C: To include interface with. adjacent distal oesophagus

D: Two transverse bits from proximal and distal oesophagus when there is no gross pathology. In case *of* any obvious pathology, separate blocks may be given.

E: Two bits from stomach with one to include gastro-oesophageal junction

F: One bit - proximal cut margin

G: One bit - distal cut margin (horizontal-if the rim is small and grossly free *of* tumour, and vertically if the tumour is close to the margin - see dotted line)

H: One bit - radial margin

If the specimen includes a wider portion *of* the stomach, bit additionally as follows: (Fig 3.1B)

J: Two bits - random from stomach one from anterior wall; one from posterior wall

K: One half each of all lymph nodes submitted. (Whole lymph nodes are submitted if less than 5mm)

M: Lymph nodes along greater curvature

N: Lymph nodes along lesser curvature.

If the lymph nodes are few, then one block for oesophageal lymph nodes; one block for gastric lymph nodes is adequate.

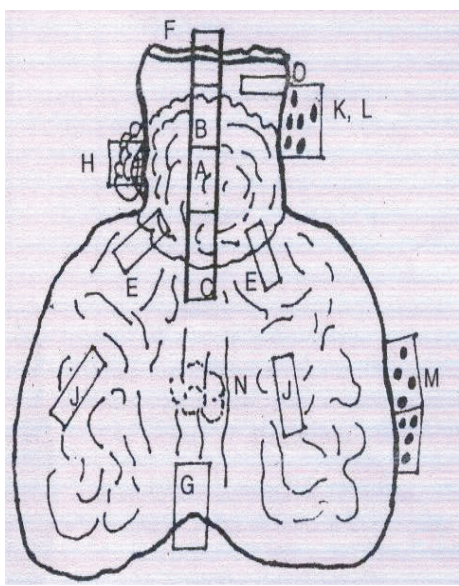


Fig 3. 2: Fig Shows lower part of oesophagus with rim of stomach mucosa with it and bits taken perpendicular to the mucosal folds .

Note:

1. Sections should always preferably be taken perpendicular to the direction *of* mucosal folds (see fig 3.2).

2. Sections to evaluate the proximal and distal cut margins can be oriented in two ways:

Enface, i.e. sections parallel to the resected edge / margin; or longitudinal i.e sections perpendicular to the margin.

- Grossing in non neoplastic lesions : Bits should be taken from the concerned areas of the mucosal lesion to include full thickness of the wall as well as adjacent normal appearing mucosa. One representative bit from the mucosa away from the lesion is adequate.

A III GROSS PEARLS : ABNORMAL FINDINGS AT ENDOSCOPY :

- *Gastro-Esophageal Reflux Disease (GERD)*: -linear or punctuate erosions / ulceration with accompanying inflammatory exudates; mucosal erythema
- In 50% of cases the endoscopic findings are normal or minimal.
- Infections : - *Candidiasis* : Discrete or confluent white plaques issuing from erythematous ulcerated or friable mucosa mainly in the mid or distal esophagus. Plaques are the ones which contain spores at microscopy.

  -*Herpes* : Small vesicles that become discrete shallow ulcers known as volcano ulcers. These coalesce to from larger ulcers.

  - *CMV* : findings seen in immunocompromised patients, rarely those with normal immunological status. Similar findings to herpes.
- *Barrett's esophagus* : Red tongue like irregular processes extending into the pale esophageal mucosa. May be long segment, 3cm or more in length or short segment <3cm in length.
- *Squamous cell papilloma* : seen as a single well demarcated sessile intra-luminal tumour usually less than 1.5 cm in size most common in the lower third of the esophagus. Inflammatory fibroid polyp may also appear like this but is less commonly pedunculated and more often ulcerated; occupying the mid or lower third of the esophagus.
- *Fibrovascular polyp* : occurs at all levels of esophagus, but commonly reported in the upper third : typically solitary pedunculated lesions often with long stalks which achieve a considerable size and cause dysphagia.

Gross : Non endoscopy :

- *Circumferential ulcerated growth* with sharply demarcated margins ; cut surface is grey white infiltrating part or all of the muscular wall. – Carcinoma ; malignant melanoma; lymphoma . Melanomas of the esophagus may be amelanotic and not produce pigment for gross identification.

- *Polypoid forms of large dimension :* Carcinosarcoma, carcinoma , melanoma.
- *Cysts in the wall :* diverticulae; acquired retension cysts, congenital esophageal cysts, gut duplication, bronchogenic and neurenteric cysts. Diverticulae communicate with the lumen; and gut duplication, bronchogenic and neurenteric cysts may or may not communicate with the esophageal lumen.

A IV OESOPHAGUS - MICROSCOPY-PEARLS.

Reflux oesophagitis :

- Hyperplasia of basal layer >15% of thickness of the normal epithelium.
- Elongation of papillae >50% of the height of the epithelium
- Widened and congested papillae
- Exudation by leukocytes and lymphocytes .
- Eosinophils in the epithelium. Intra epithelial eosinophils are a frequent and important indicator

Candidal oesophagitis :

- Neutrophils infiltrate the epithelium
- Pseudohyphae/spores which can be highlighted by special stains-PAS /Grimelius stain
- Desquamation of superficial epithelial cells.
- Diagnosis can be confirmed by Brush Cytology

Viral oesophagitis ***:***

Herpes

- Volcano ulcers at endoscopy
- Subepithelial congestion
- Homogenisation of nuclei
- Subepithelial cleavage and bullae
- Very rarely multinucleate giant cells and well formed nuclear inclusions (Cowdry typeA)
- Ulceration of epithelium
- CD68+ Mononuclear cells with convoluted nuclei adjacent to the inflamed epithelium constitute an important diagnostic clue

CMV

- same endoscopic findings as herpes simplex
- large cells with basophilic cytoplasm
- Intranuclear inclusions

Barrett's oesophagus (BE) **:**Characterised by endoscopic appearance of abnormal mucosa (intestinal metaplastic type or foveolar type) proximal to the GE junction. However, the definitions of BE have not been standardized worldwide; columnar-lined epithelium ie foveolar

type without intestinal metaplasia (IM) and located < 1 cm of GE junction is not diagnosed as BE in most countries. Eg. As per the American Society of Gastro-enterology BE should be diagnosed when there is extension of salmon-colored mucosa into the tubular oesophagus *extending ≥1 cm proximal* to the gastroesophageal junction (GEJ) with biopsy confirmation of intestinal metaplasia.

- BE has been traditionally defined as the presence of at least 1 cm of metaplastic columnar epithelium that replaces the stratified squamous epithelium normally lining the distal oesophagus. Segments <1 cm have been classified as "specialized IM of the esophagogastric junction" (SIM-EGJ) and not BE is because of the low risk for oesophageal adenocarcinoma (EAC). Patients with SIM-EGJ have not demonstrated an increase in the development of dysplasia or EAC in large cohort studies after long-term follow-up, in contrast with patients with segments of IM >1 cm. The American Society of GE requires the *presence of intestinal epithelium as mandatory* for a diagnosis of Barrett's oesophagus.
- Recent trends by the British Society of Gastro-enterology state that presence of either specialised columnar *or* foveolar epithelium with or without intestinal epithelium are said to be indicative of Barretts. The specialised columnar epithelium should be seen lining a segment of distal oesophagus and extending 1cm or more above the level of the lower oesophageal sphincter (GE junction).
- The German Society of Gastroenterology too requires oesophageal biopsies *showing intestinal metaplasia with goblet cells* as mandatory to establish the diagnosis; they claim intestinal metaplasia alonen(see Fig 3.4) predisposes to malignancy as studies suggest that cancer most commonly occurs in columnar metaplasia with goblet cells compared with columnar metaplasia without goblet cells.

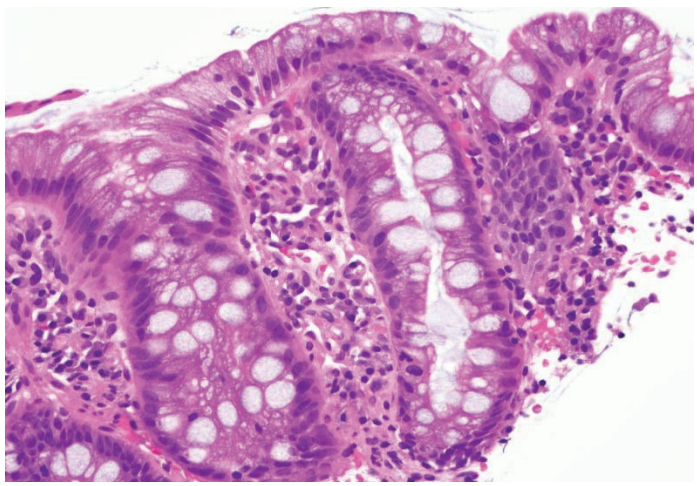


Fig 3.4: Barrett's oesophagus with intestinal metaplasia H&E x 400

- BE is Subdivided into long segment (the classic form in which the changes involve 3cm or more of the oesophagus above the GE junction and a short segment (less than 3 cm). Some refer to an ultra-short segment defined as total involvement of 1 cm in length.

- Long standing GERD induced tissue damage plays a vital role in the pathogenesis of Barrett's Oesophagus (10 % risk of developing BE in GERD patients.
- At the histochemical level the mucin present in the specialised columnar epithelium is of the neutral type . In over 70% of cases there are also sialomucins and sulpho-mucins (ie small intestinal type and large intestinal type of mucins respectively). Regarding MUC type of mucin classification, the intestinal type of differentiation is evidenced by the presence of intestinal type mucin ie MUC 2 in addition to gastrin type mucins (MUC 5AC; MUC 6 and TFF peptides).
- Intestinal metaplasia can also occur in the gastric cardia in the absence of Barrett's oesophagus . Distinction of BE (due to GERD) is made by knowing the level of biopsy and the immunohistochemical keratin profile ie Barrett's CK7/20 phenotype (superficial CK 20 staining of superficial epithelium and strong CK 7 staining of both the superficial and deep glands in Barrett's). In gastric epithelium with intestinal metaplasia CK 20 is positive in both superficial epithelium and deep glands but there is absent staining of CK 7.[8B]
- Muscularis mucosae duplication is a distinct feature of BE [10], and occurs in upto 92% of cases (in one study) and involves from 5% to >90% of the Barrett segment.
- *Complications* **:** peptic ulcer, stricture, bleeding , dysplasia / adenocarcinoma.

Dysplasia in Barrett's Esophagus

-Occurs more commonly in intestinal metaplastic epithelium with goblet cells and less commonly in metaplastic columnar epithelium without goblet cells (foveolar or gastric type dysplasia).

-Dysplasia is divided into two grades; low grade and high grade.

i) Oesophageal glandular dysplasia (intra-epithelial neoplasia) low grade

ii) Oesophageal glandular dysplasia (intra-epithelial neoplasia) high grade)

Dysplasia is diagnosed on - *architectural and cytological abnormality* (architectural abnormality: budded, branched, crowded irregular glands, papillary infoldings and villiform configuration of the mucosal surface). *Nuclear features:* enlargement with ↑ N/C ratio, hyperchromatism and ↑ abnormal mitoses. *Cytoplasmic* basophilia with loss of mucus and excessive nuclear stratification extending from the epithelial basement membrane to the luminal surface.

- *Carcinoma in-situ of oesophagus* and high grade dysplasia are synonymous as per WHO.
- The Vienna Classification of Dysplasia for Barrett's Oesophagus[11] :

Negative for neoplasia/dysplasia

Indefinite for neoplasia : Changes are not adequate enough for dysplasia

Non-invasive low grade neoplasia (low grade adenoma dysplasia)

Non invasive high grade dysplasia

- High grade dysplasia

- Non Invasive carcinoma (carcinoma in situ)
- Suspicious for invasive carcinoma

Invasive neoplasia

-Intra mucosal adenocarcinoma : is carcinoma through the basement membrane of the glands into the lamina propria but not beyond the muscularis mucosae.

-Submucosal carcinoma or beyond.

- The risk of developing carcinoma in Barrett's with high grade dysplasia is 30-40 fold higher than in the general population. The morphological type of carcinoma is adenocarcinoma**.** Cancer in Barrett's occurs as a sequential change from inflammation to metaplasia to low grade and finally high grade metaplasia leading to carcinoma with multiple genetic and epigenetic alterations- mutations in PCNA, CCND1, TP53, SMAD$, AMACR etc
- *Treatment of Barrett's E*: Confirmed BE should be monitored by annual or once in 3 years endoscopy, depending on the severity. Endoscopic resection, radiofrequency ablation or cryotherapy are some of the treatment regimes.

WHO CLASSIFICATION OF ESOPHAGEAL TUMOURS (5th edition, 2019)

Benign epithelial tumors and precursors

- Squamous cell papilloma, NOS
  - Squamous papillomatosis
- Esophageal glandular dysplasia (intraepithelial neoplasia), low grade
- Esophageal glandular dysplasia (intraepithelial neoplasia), high grade
- Esophageal squamous intraepithelial neoplasia (dysplasia), low grade
- Esophageal squamous intraepithelial neoplasia (dysplasia), high grade

Malignant epithelial tumors

- Adenocarcinoma, NOS
- Adenoid cystic carcinoma
- Mucoepidermoid carcinoma
- Adeno-squamous carcinoma
- Squamous cell carcinoma, NOS
  - Verrucous squamous cell carcinoma
  - Squamous cell carcinoma, spindle cell
  - Basaloid squamous cell carcinoma

- Carcinoma, undifferentiated, NOS
  - Lymphoepithelioma-like carcinoma
- Neuroendocrine tumor, NOS
  - Neuroendocrine tumor, grade 1
  - Neuroendocrine tumor, grade 2
  - Neuroendocrine tumor, grade 3
- Neuroendocrine carcinoma, NOS
  - Large cell neuroendocrine carcinoma
  - Small cell neuroendocrine carcinoma
- Mixed neuroendocrine – non-neuroendocrine neoplasm (MiNEN)
  - Combined small cell - adenocarcinoma
  - Combined small cell - squamous cell carcinoma

The Fifth edition of the World Health Organization classification of digestive system and American Joint Committee on Cancer staging manual contain substantial refinements of information for esophageal tumors. The epithelial tumors of esophagus are classified as benign, dysplasia, and malignant groups. Dysplasia is divided into Barrett dysplasia and squamous dysplasia and graded into either low-grade or high-grade. Malignant esophageal tumors are often adenocarcinoma or squamous cell carcinoma. The main update in cancer staging in esophageal tumors is the subdivision of the prognostic staging into 3 groups; squamous cell carcinoma, adenocarcinoma, and carcinoma after adjuvant therapy. HER-2 amplification is recognized as a molecular target for therapy of esophagogastric adenocarcinoma. The other esophageal tumors are adenoid cystic carcinoma, mucoepidermoid/adenosquamous carcinoma, undifferentiated carcinoma and neuroendocrine neoplasms.

- **Squamous papilloma** : of the oesophagus is a benign papillary growth covered by squamous epithelium. Seen in the middle or lower third of owsophagus and easily visualised on endoscopy. It's incidence is low 0.01 to 0.45%. Etiology is mucosal inflammation due to infections (HPV); irritation and subsequent regeneration and reflux disease. Squamous papillomatosis is associated with genetic disorders such as focal dermal hyperplasia or Golz-Gorlin syndrome and angioma serpiginosum , both conditions affecting the X chromosomes.

Histopathology conforms to a papilloma occurring elsewhere with an exophytic growth pattern. Scattered vacuolated cells are noted in the epithelium with features suggestive of koilocytes. Has to be differentiated from a low grade squamous cell carcinoma and fibrovascular polyp occurring in the upper oesophagus. The latter lacks papillary proliferative growth.

Squamous papilloma or papillomatosis rarely becomes malignant.

- **Squamous dysplasia**: is a unequivocal neoplastic alteration of the oesophageal squamous epithelium without invasion. It occurs any where in the oesophagus and precedes squamous cell carcinoma.

As per WHO; divided into 2 tier grading with low grade (involving lower half of epithelium) or high grade (involving more than half the thickness of epithelium). The latter is also considered as carcinoma in situ.

Some authors use the 3 -tier grading system based on involvement of the lower third, 2/3rds or upto surface involvement ; the last category being synonymous with carcinoma in situ.

- **Malignant Tumours of oesophagus**

i) Carcinoma of the oesophagus : Squamous cell carcinoma incidence varies depending on the countries; being the commonest malignant neoplasm (90% of oesophageal tumours) in Asian countries particularly China. It is more common in the middle and lower third in the areas of normal anatomic constrictions. In western literature the incidence of adenocarcinoma is increasing remarkably and may parallel or exceed this.

Etiopathogenesis : several factors contribute- low socio economic status; alcohol consumption; tobacco smoking; dietary factors such as hot beverages; consumption of pickles due to the nitrosamine content and genetic diseases like tylosis (a keratotic disorder caused by a mutation in the autosomal dominant gene RHBDF2 at 17q25.3) ; and Fanconi syndrome.

A diet rich in fresh fruits and vegetables reduces the risk of developing the carcinoma. HPV has no definite role in the pathogenesis.

Gross : More common in the middle and then lower third of the oesophagus -protruded / polypoid – 60% of cases

- Flat thickening & narrowing within the wall -15% of cases
- Excavated, ulcerated – 25% of cases

The Japanese Society Macroscopic Classification of squamous cell carcinoma of the oesophagus:

Macroscopic classification (Fig 3.5):

- Type 0 Superficial type
- Type 1 Protruding type
- Type 2 Ulcerative and localized type
- Type 3 Ulcerative and infiltrative type
- Type 4 Diffusely infiltrative type
- Type 5 Unclassifiable type

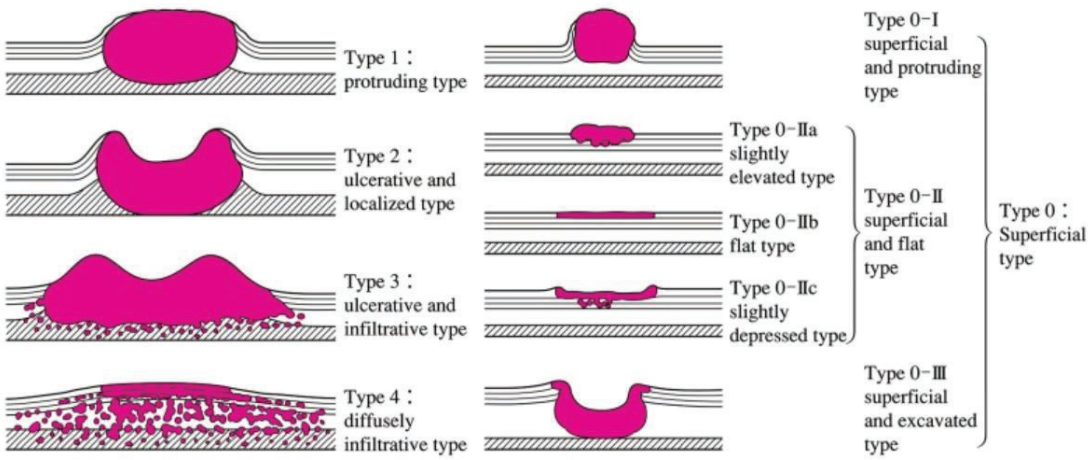


Fig 3.5: Japanese Classification of Esophageal Cancer, (11th Edition: part I)

Microscopy : tumours show a range of differentiation - well to moderately differentiated as well as poorly differentiated tumours. True glandular and mucus secreting components are found focally in 1/5th of cases. Graded histologically as well, moderate and poorly differentiated.

- IHC : CK +ve, some express vimentin and others B72.3, poorly differentiated carcinomas may react for hCG.

The most common genetic abnormality is TP53 mutation, found in about 90% of cases. Mutations are also found in several cell cycle regulatory genes.

Verrucous carcinoma : variant of SCC characterised by broad papillae of well differentiated keratinising squamous epithelium, minimal cytologic atypia and pushing edges into underlying tissues as broad based tongues of epithelium. The invasive islands are characterised by broad, bulbous pushing projections. It is an exceedingly slow growing tumour with uncommon metastasis.

Basaloid squamous cell carcinoma : solid nests of tumour cells with squamous differentiation but peripheral palisading. Affects elderly males and has an aggressive behaviour.

Sarcomatoid (spindle cell) carcinoma. Presents grossly as a large polypoid mass. Microscopy shows spindle cell cytology akin to a sarcoma. Positive for cytokeratin as well as vimentin markers at IHC.

- *Others* : adenosquamous carcinoma; mucoepidermoid carcinoma; adenoid cystic carcinoma , etc
- **ii) Adenocarcinoma :**

  Adenocarcinoma of the oesophagus and oesophago-gastric junction (carcinoma of the junction with it's epicenter upto 2 cm from the G-E junction and extending into the oesophagus) are treated as one entity. Carcinoma whose epicenter is > 2cm from the G_E junction is staged and managed as carcinoma of the stomach.

Incidence of adenocarcinoma varies with geographic region; on the rise in western countries, occurs predominantly in men. The incidence has gradually risen in the western countries and replaced squamous cell carcinoma in being the predominant type of carcinoma of the oesophagus in UK, parts of Europe and USA [13, 14]. It has risen to constitute almost 50% of oesophageal carcinomas in the western countries; arises more commonly from Barrett's oesophagus, can also arise from a focus of heterotopic gastric mucosa, and /or from oesophageal glands. Usually occur in the lower part, rarely in the middle and upper third where it arises from the oesophageal glands.

Etiopathogenesis : occurs in the elderly (7^{th} decade); associated with reflux disease; males. Other factors include H pylori infection and smoking abd Barrett's oesophagus. In the latter dysplasia develops as a result of accumulation of multiple genetic and epigenetic mutations of the cell cycle regulatory (PCNA, CCND1, TP53 and AMACR) genes , losses and gains of chromosome functions etc

Gross : may occur in the early stage as irregular plaques or small nodules. Later develop into structuring, polypoid fungating , ulcerating or diffusely infiltrative lesions.

Microscopy : shows gastric (proximal gastric type), intestinal (with goblet cell differentiation) or mixed differentiation. The mucosa adjacent to the tumour may show Barrett's oesophagus. Various patterns have been described as in carcinoma of the stomach-papillary, tubular, mucinous and signet ring type. The tubular pattern is most common with glands lined by neoplastic cells. Mucinous pattern shows small groups of cells floating in extracellular mucin with or without signet ring cells. Papillary pattern is characterised by papillae covered by neoplastic cells.

Grading is characterised by 3 grades- grade I with >95% of carcinoma showing well formed glands; grade 2, showing 50-95% gland formation and grade 3 showign< 50% of tumour with gland formation.

iii) Neuro-endocrine neoplasms are covered in the section on neoplasms of the intestine.

iv) Malignant melanoma : primary oesophageal malignant melanoma is rare but well documented cases occurring in the mid or lower third have been reported. Grossly may be indistinguisable from primary carcinoma as not all tumours produce sufficient melanin to be identified grossly or even microscopically. In such cases HMB-45 (which detects pre-melanosomes) and DOPA reaction may be used for confirmation.

Junctional activity is common and features are similar to melanomas occurring elsewhere.

Stromal tumours : a wide variety can occur in the oesophagus– smooth muscle tumours, nerve sheath tumours, GIST including gastrointestinal autonomic nerve tumours (GNAT), vasculars tumours, malignant fibrous histiocytomas etc

A V OESOPHAGUS : SYNOPTIC REPORTING

Oesophageal biopsy histopathology report

Type of specimen : endoscopic biopsy ☐

Other ☐

Measurements : ……mm x ……mm x…….mm

Microscopy :

Adequate ☐ Not adequate ☐ Superficial ☐

Inflammation : Acute esophagitis ☐

Chronic esophagitis ☐

Esophageal esophagitis ☐

Herpes Simplex ☐

CMV ☐

Granulomatous ☐ Tuberculosis ☐

Sarcoidosis ☐

Other ☐ Specify……..

Changes of reflux : Yes ☐ No ☐

Barrett's oesophagus : Yes ☐ No ☐

Complication in Barrett's : ulcer ☐

intestinal metaplasia ☐

dysplasia ☐

adenocarcinoma in situ ☐

Neoplasm present : Yes ☐ No☐

Histological type ;……

Benign ☐

Suspicious for malignancy ☐

Malignant ☐

Comments …….

Signature …… Date

Oesophageal resection for carcinoma

Gross description

- Maximum length of specimen: mm

Length of oesophagus: mm

Length of stomach ……mm

Length of tumour ……mm

Width of tumour…….mm

Type of tumour

Polypoid ☐

Ulcerating ☐

Infiltrative ☐

Other ☐

b) Tumour edge to nearest distal margin: ….. mm

Tumour edge to nearest proximal margin: …. mm

Siewert tumour type …… ☐ 1 ☐2 ☐3

(cardiac cancers only : Type 1 -adenocarcinoma arising in Barrett's oesophagus ; Type 2 -true junctional carcinoma; Type 3 -subcardiac carcinoma which may extend into OGJ junction)

Cancers involving the oesophagogastric junction (OGJ) whose epicenter is within the proximal 2 cm of the cardia (these are Siewart type I & II) are to be staged as oesophageal cancers. Cancers whose epicenter is more than 2cm from the OGJ are to staged as stomach cancers even though the OGJ is involved.

Histology :

c) Type of tumour

☐ Squamous cell carcinoma

☐ Adenocarcinoma

☐ Other (specify)

Differentiation by worst area:

☐ Well ☐ Moderately

☐ poorly differentiated

d) Depth of invasion

☐ Tis high-grade dysplasia/Carcinoma in situ

☐ T1 invasion of lamina propria/submucosa

☐ T2 invasion of muscularis propria

☐ T3 invasion beyond muscularis propria

☐ T4 invasion of adjacent structures

e) Proximal margin

☐ Normal ☐ Dysplasia ☐ Carcinoma ☐ Barrett's

f) Distal margin

☐ Normal ☐ Dysplasia ☐ Carcinoma

g) Circumferential margin

Involvement (<1 mm): ☐ Yes ☐ No ☐ N/A

(If no: distance of carcinoma to nearest circumferential margin mm)

h) Other features

Vascular invasion ☐ Yes ☐ No

Barrett's metaplasia Yes No

adjacent to tumour

i) Lymph nodes

Number examined……. Number positive…….

(N0 if no nodes positive, otherwise N1)

j) Distant metastases

Coeliac axis node positive ☐ Yes ☐ No

Cervical node positive ☐ Yes ☐ No

Other distant metastasis (M1b) ☐ Yes ☐ No

PATHOLOGICAL STAGING

Complete resection ☐ Yes (R0) ☐ No (R1 or R2)

TNM

pT…… pN…… pM…….

Comments …….

Signature ……... Date …../……/…..
SNOMED codes T …….. / M ……

pTNM Staging for esophageal carcinomas :

T : Primary tumour

pTX Primary tumour cannot be assessed

pT0 No evidence of primary tumour

pTIS Carcinoma in situ/high grade dysplasia

pT1 Tumour invades lamina propria, muscularis mucosae or submucosa

(T1a – invades lamina propria or muscularis mucosa; T1b- invades submucosa)

pT2 Tumour invades muscularis propria

pT3 Tumour invades adventitia

pT4 Tumour invades adjacent structures

N : Regional Lymph Nodes

pNX : Regional lymph Nodes cannot be assessed

pN0 : No regional lymph node metastasis (Histological examination of a mediastinal lymphadnectomy specimen should ordinarily include atleast 7 lymph nodes).

pN1 :1-2 regional lymph node metastasis

pN2 : 3-6 regional lymph nodes

pN3 : 7 or more nodes +ve

M : Distant metastasis

pM1 : Distant metastasis (microscopically confirmed)

G : Histopathological grading

GX : Grade of differentiation cannot be assessed

G1 : Well differentiated

G2 : Moderately differentiated

G3 : Poorly differentiated

G4 : Undifferentiated

A I STOMACH ANATOMY

Normal anatomy:

- The stomach is an intraperitoneal organ which extends from the lower end of the esophagus, at the level of the 11th thoracic vertebrae, crossing to the right of the midline, and ending in the duodenum.
- The stomach is often divided into four anatomic regions: Cardia, fundus, body, and antropyloric region. . The cardia is a narrow, ill-defined region, is not grossly distinctive and is identified Histologically by the presence of cardiac glands. The fundus is that portion of the gastric body that protrudes over a horizontal line drawn from the esophagogastric junction. It blends into the gastric body, which constitutes most of the stomach. The body is demarcated from the distal portion, called the pyloric antrum, by a notch in the lesser curvature, the incisura angularis.
- Numerous longitudinal, greyish pink mucosal folds (called rugae) lie parallel to the lesser curvature and characterize the mucosa of the gastric body.
- The lesser curvature and greater curvature have folds of peritoneum extending as continuation of their visceral serosa called the lesser and greater omentum. These are important routes of spread of tumour.
- Normal histology of the gastric mucosa in the various areas (see fig 3.6 A too) is :-

Cardiac region :

- Foveolae constitute upper half of the mucosal thickness
- Looser package of glands in the lamina propria as compared to the other parts of the stomach
- Lined by mucin secreting cells with basal flattened nuclei
- Inflammatory cells are maximum in the cardia.

Fundic region and Corpus (body) :

- Foveolae constitute ¼ of the mucosal thickness
- Cells : mucus neck cells, chief (pepsinogen secreting) cells –short columnar cells with basophilic cytoplasm and basal nuclei, parietal (acid secreting) cells –triangular in shape with eosinophilic cytoplasm and round central nuclei; and endocrine cells with a clear pale cytoplasm around the nucleus like a halo (Fig #.6 A & B)
- inflammatory cells are least in the region of the body with a few small aggregates

Antrum and pylorus : similar to the cardia :

- Foveolae occupy upper one half
- cytoplasm is bubbly, glassy and vacuolated with subnuclear vacuoles.
- Ciliated cells seen iv) G cells, EC (entero-chromaffin cells) cells and D (delta) cells are present

Mucus of gastric glands is neutral mucin which is PAS +ve and Alcian blue negative.

Foveolar epithelium mucin is MUC I and MUC 5AC. Glandular mucin is MUC 6.

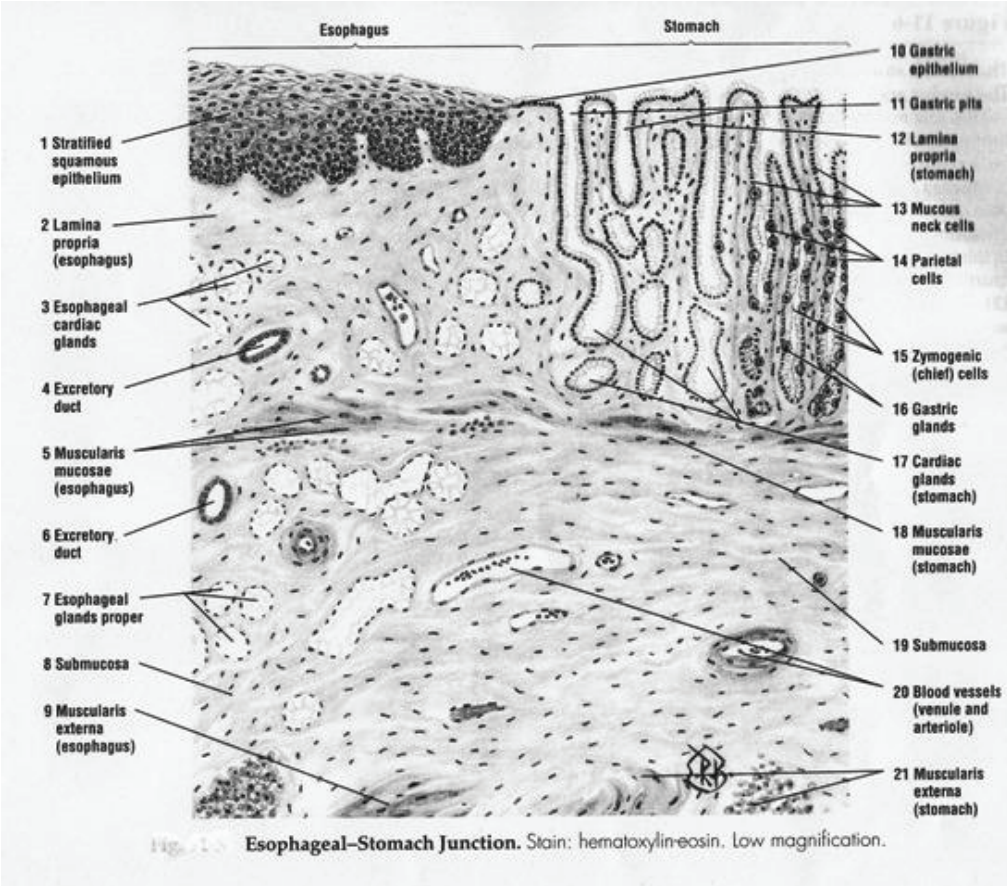


Figure 3.6 A : Lining epithelial cells of gastric mucosa

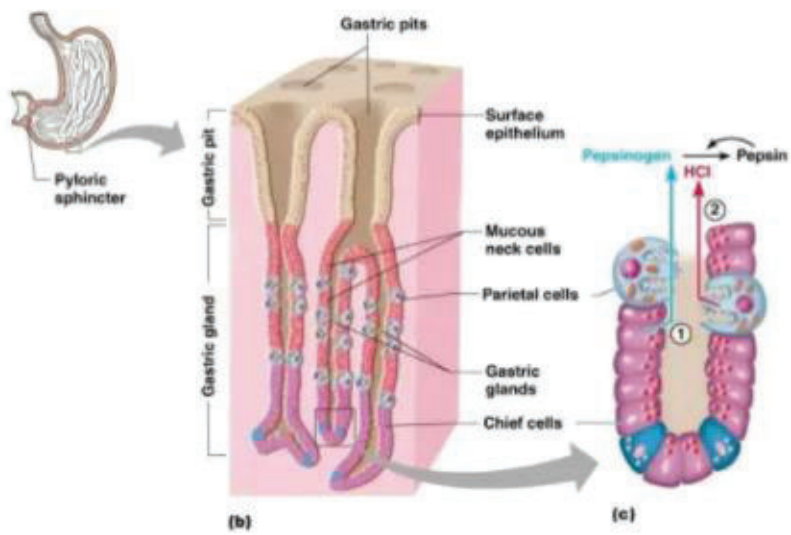


Fig 3.6 B: Cells lining gastric pits

B II STOMACH GROSSING

Types of specimens received are :

1. Endoscopic biopsies

2. Gastrectomy specimens:

- Total (to include cardia & pylorus)
  - Subtotal (to include pylorus)
- Partial (antrum or body): commonly done for antral and pyloric tumours.
- -Endoscopic mucosal resection : For small tumours limited to mucosa (T1a) which generally have no lymph node metastasis.

Endoscopic Biopsies

1. Description: Note the number and colour of the tissue fragments. They need to be separated from underlying filter paper/cardboard on which they were sent or fixed. Orientation of the fragments is done preferably using hand lens/dissection microscope.
2. Measurement: Note and measure the largest and the smallest of the fragments in greatest dimension.

Bits

Process the entire tissue.

Gastrectomy specimen (for peptic ulcer)

Examine the specimen in the fresh state.

The resected portion normally includes the antrum, pylorus and portion of the first part of duodenum. .

- Take the measurements of the stomach along the greater and lesser curvatures.
- Open the specimen along the greater curvature when the suspected lesion is along the lesser curvature (as guided by radiology/clinical information/palpation) and when the lesion is along the greater curvature, open along the lesser curvature. The cut on the greater curvature should pass anterior to the attachment of the omentum.
- Examine the mucosa for ulcer - its location, size, depth of penetration, shape, edges and floor. The surrounding mucosa should also be examined.
- Measure the distance of the ulcer from the closest proximal and distal surgical
- margins.
- Cut through the centre of the ulcer along its long axis to see the base of the ulcer for fibrosis and presence of large vessels, if any.

- If an ulcer is not identifiable (and the clinical diagnosis is that of a peptic ulcer), contact the surgeon to find out whether the ulcer was resected. This information should be recorded as part of the gross description.
- Examine the rest of the mucosa for oedema, haemorrhage, atrophy, erosions, any mucosal irregularities and nodules if any.
- Dissect the lymph nodes and group them separately as per the figure 3.7 shown below. Measure the greatest dimension of the largest and the smallest lymph nodes of each group

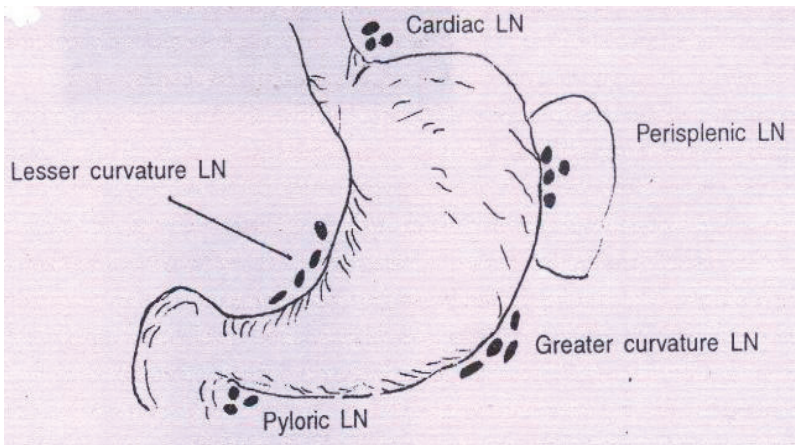


Figure 1.7 : Various groups of lymph nodes in gastrectomy specimen

Bits (see figure 3.8 below)

A, B, C and D - Ulcer- Four bits, also to include the centre of the lesion

E - Lesser curvature - two bits} If the surrounding mucosa of the

F - Greater curvature - two bits ulcer appears apparently normal.

G - Duodenum - one bit, if present.

H - Two bits from the rest of the stomach showing any mucosal lesion if present. When these bits are taken, then E and F need not be taken.

J,K, etc : Lymph nodes, depending on the number.

Bit entire lymph node if less than 5mm. Bisect and take one half if more than 5mm in size. Mark the remaining half in the specimen with eosin.

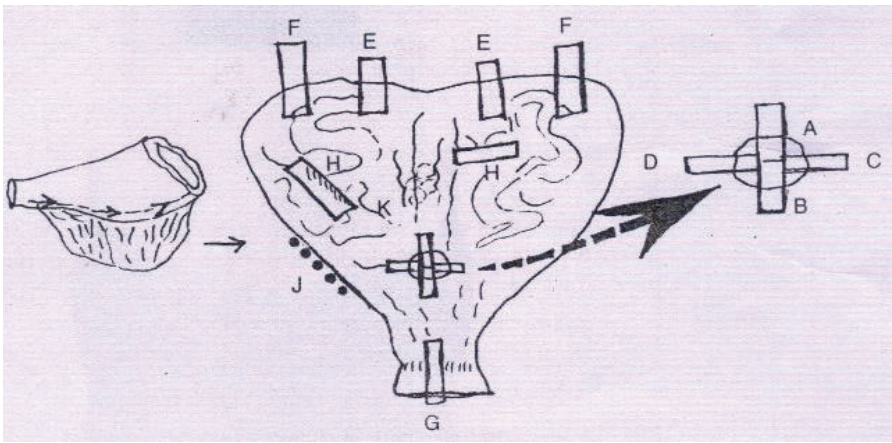


Figure 3.8 : Bits taken in gastric ulcer

Gastrectomy specimen (for malignant lesions)

Lymphadenectomy :

- Gastrectomy specimens with malignancy are accompanied by regional lymph node resections. In 'general surgical departments' most times level 1 (N1) nodes are removed. Metastasis to various regional LN stations depends on the depth of gastric-wall invasion.
- A gastrectomy specimen is labelled – as D1, D2, D3 depending on the groups of lymph nodes removed. D1 is removal of N1 nodes (stations 1 to 6); D2 is removal of N2 nodes (station 7 to 11). Removal of D2 nodes depends on the location of tumour in the stomach; however when needed, recent surgical guidelines clearly state that for non-locally advanced gastric cancers, surgery has a central role among all available therapeutic modalities and D2-lymphadenectomy is the "gold-standard" surgical technique. The Japanese and European guidelines enclose the D2 lymphadenectomy as the gold standard in R0 (complete) resection.
- In the individualized, stage-adapted gastric cancer surgery the Maruyama computer program (MCP), a software programme, estimates lymph node involvement preoperatively with accuracy and in addition; the Maruyama Index less than 5 has a better impact on survival, than D-level guided surgery. For these reasons, the preoperative application of MCP is recommended routinely, with an aim to perform "low Maruyama Index surgery".
- Also the sentinel lymph node biopsy (SNB) may decrease the number of redundant lymphadenectomy intraoperatively with a high detection (93.7%) and accuracy rate. (A dual tracer method which uses radioactive colloids and blue dyes is a reliable method of mapping sentinel lymph node). More accurate stage-adapted surgery could be performed using the MCP and SNB in parallel fashion in gastric cancer .
- The benefit of the role of extending lymphadenectomy in removing D3 (N3) nodes is debatable and is rarely done.

- It becomes mandatory to receive these groups of nodes with proper labelling (particularly for the N2 groups) in separate containers and process them separately to assess the spread at various levels for an accurate TNM staging. The responsibility of sending the resected N2 nodes separately labelled and the assessing of these for involvement rests on the surgeon and pathologist respectively.
- A representation of these lymph node stations (N1, N2 and N3) is given below in the table below -.

Broad categories of Lymph node stations as per the Japanese Cancer Research Society.

1	Right cardiac nodes
2	Left cardiac nodes
3	Nodes along the lesser curvature
4	Nodes along the greater curvature
5	Suprapyloric nodes
6	Infrapyloric nodes
7	Nodes along the left gastric artery
8	Nodes along the common hepatic artery
9	Nodes around the coeliac axis
10	Nodes at the splenic hilus
11	Nodes along the splenic artery
12	Nodes in the hepatoduodenal ligament
13	Nodes at the posterior aspect of the pancreas head
14	Nodes at the root of the mesenterium
15	Nodes in the mesocolon of the transverse colon
16	Para-aortic lymph nodes

N1 : stations 1 to 6; N2 : stations 7 to 11; N3 stations 12 to 16. These were further classified depending on the location of nodes along ramifications of the blood vessels.

Grossing :

- Examine the specimen in the fresh state and take measurements along both the curvatures.(In case of tumours involving the upper portion of the stomach, part of the oesophagus may be resected. Measurements should be taken accordingly and resection margin of the oesophagus should be marked and taken).
- Open the specimen as per instructions given for peptic ulcer.
- Examine the opened out specimen for tumour and observe the following features:
    - Type (ulcerative, ulcero-proliferative or polypoid, or diffusely infiltrating)
    - Location (whether on lesser or greater curvature)
- Size along two dimensions
- Note the distance from the closest proximal and distal surgical margins.
- Cut through the the tumour along its long axis and note the depth and extent of infiltration.
- Measure the maximum depth of infiltration.
- Examine the rest of the mucosa for oedema, haemorrhage, mucosal nodules, mucosal irregularities as well as changes of atrophic gastritis as evidenced by loss of rugosity in areas away from Magenstrasse (that portion of the lesser curvature of normal stomach which exhibits longitudinal furrows and lacks mucosal folds and along which the food gets propelled).
- Mark the proximal and distal surgical margins with India ink after mopping any excess fluid on it.
- If splenectomy is included, dissect the hilar lymph nodes of the spleen. Separate the spleen from the rest of the specimen and dissect out as per the requirements.
- Identify the lymph node groups as cardiac, greater curvature, lesser curvature, pyloric and omental nodes, see diagram above. Note the number in each of the groups and measure the greatest dimension of the largest and the smallest lymph nodes in each group. Describe the cut surface, whether grossly involved or not.

Bits (see figure 3.9 below)

A, B and C - tumour - 3 bits, one to include maximum depth of Infiltration and the other two to include the adjacent non-neoplastic mucosa.

D and E - non-neoplastic mucosa/any other finding in mucosa - 2 bits.

F - Closest proximal cut margin - 2 bits (i.e. at lesser curvature in the figure).

G - 2 bits - surgical margin along greater curvature

H and J - Closest distal cut margin - 1 to 2 bits

K, L, M, N, P & Q - Lymph nodes - bits taken depending on the number and grouped into separate blocks. (P & Q not shown)

Note: If thorough mapping of mucosal abnormalities is desired such as in case of intestinal metaplasia, dysplasia, regenerative atypia, a Swiss roll technique (where mucosa alone is shaved off from the underlying muscle layer and rolled up) should be used to cover a large mucosal surface in a minimum number of blocks.

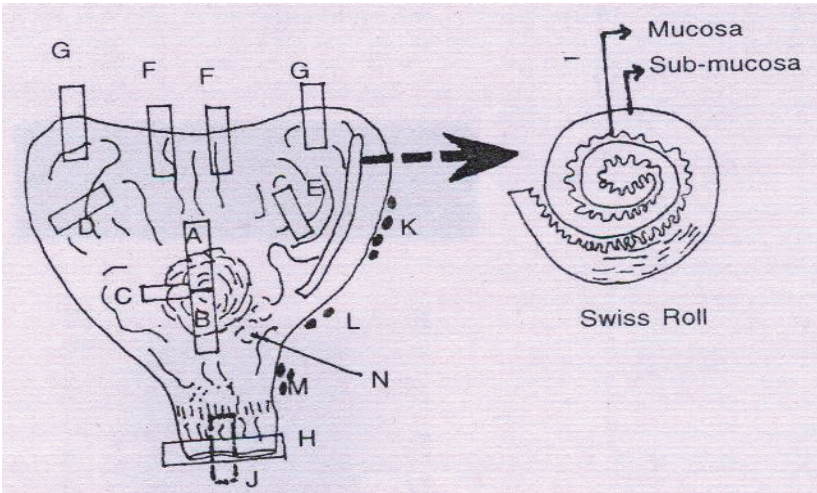


Figure 3.9 : Bits taken in case of gastric tumour

B III STOMACH GROSS PEARLS

- **Hypertrophic pyloric stenosis (Olive tumour) :** 3-12 weeks of age, commonly in males. Greatly thickened pyloric muscle which occludes the pyloric canal and obstructs gastric outflow (ganglionic dysfunction).
    - In adults, hypertrophy of pyloric circular muscle is acquired & is secondary to chronic gastritis.
- **Multiple superficial erosions** : erosive gastritis.
- **Extensive haemorrhage in the mucosa :** Haemorrhagic gastritis
- **Small ulcers** (generally <2cm) with punched margins, clean base, mucosal folds right upto the edge of ulcer : Peptic ulcer. Location -occurs in the stomach (generally in the achorhydric zone of gastric mucosa – ie lined by pyloric type mucosa), duodenum, lower third of oesophagus, margin of gastro-jejunostomy, Meckel's diverticulum and ectopic gastric mucosa.
- 95% of ulcers occur in the lesser curvature (magestrausse).
- **Diverticulae** : located in the juxta cardiac location ie anatomically weak areas
    - Acquired diverticulae are seen due to chronic peptic ulcer disease and occur any where in the stomach.

- **Duplication :** rare anomaly in the stomach . Seen as a unilocular / multilocular cyst lined by gastric mucosa. May or may not communicate with the lumen of the normal stomach. May get distended due to obstruction and lead to a palpable mass.
- **Submucosal cysts** : occur as a result of gastritis cystica profunda.
- **Bezoars (foreign bodies):** are occasionally seen surgical specimens of the stomach. Trichobezoars are foreign bodies composed of hair and phytobezoar composed of vegetable matter. (Factors favouring development of bezoars are lack of teeth, vagotomy and obstructing lesions of the gastric outlet.
- **Single large gastric vessel high on the lesser curvature**, topped with a **small defect** on the overlying mucosa : diagnostic of aneurysm of the gastric vessel due to malformation.
- Endoscopy shows **parallel red stripes at the crest of mucosal folds** : called **water melon stomach** (due to the stripes): gastric antral vascular ectasia (GAVA) : results in blood loss and iron defiency anaemia .
- **Raised small yellow lesions on the mucosa** : Xanthoma, (xanthelasma), due to accumulation of neutral fat in foamy histiocytes in the lamina propria.
- **Loss of rugosity** in portions of the mucosa : chronic atrophic gastritis, may give the appearance of shallow cobblestones, seen in mucosa around carcinomatous ulcer, association with alcoholism & anti-inflammatory drugs.
- **Randomly distributed polyps,** generally small, sessile and multiple with a smooth or slightly lobulated contour, may show central umbilication.: i) Hyperplastic polyps (occur against a background of hypo-chlorhydria, low levels of pepsinogen, hypergastrinemia, chronic gastritis and gastric atrophy). Can co-exist with carcinoma elsewhere in the stomach. Called as type I & II polyps by Japanese authors.
- ii) Polyposis syndrome of the stomach: may also be associated with familial colonic polyposis and Gardner's syndrome.
- **Polyps in the antrum, sessile or pedunculated, single and comparatively large – elevated lesions with a flat surface** : usually adenomas (type III & IV of the Japanese authors); single antral polyps may also be inflammatory fibroid polyp (the latter associated with hypochlorhydria or achlorhydria)
- **Polyps in the fundus 2 mm or slightly small** : i)Fundic gland polyps (associated with Zollinger – Ellison Syndrome; and in those patients on long term treatment with proton pump inhibitor; and associated with colonic polyposis). Fundic gland polyps may also occur in the body of the stomach.

  ii) carcinoid tumours.
- **Giant hypertrophy of gastric rugae ,** resembling cerebral convolutions, centered along the greater curvature : i) Menetrier's Disease ie hyperplastic gastropathy (due to foveolar hyperplasia , accompanied by tortuosity and cystic dilatation of glands at microscopy) . There is localized form with a well circumscribed, cerebroid mass either in the fundus or antrum.

ii) Similar changes of rugae are seen in Zollinger-Ellison Syndrome.

- **Large polypoid mass** with a firm cut surface : Polypoid type of gastric carcinoma
- **Ulcer > 2cm with a firm base, everted edges and dirty floor** : Ulcer cancer ie ulcerating form of gastric carcinoma.
- **Diffusely thickened wall of stomach (leather- bottle stomach)** : i)Linitis plastica - diffusely infiltrating form of carcinoma stomach.
  ii) lymphoma.
    - (Based on Borrmann's classification [18], the gross appearance of advanced gastric carcinomas are divided into type I – polypoid gastric carcinoma, type II – circumscribed excavation, type III – induration with ulceration, and type IV - diffusely infiltrating which is also referred to as linitis plastica)
- **Small nodular lesion with intact mucosa**, single or multiple : carcinoid tumour.

Differences between chronic peptic ulcer and malignant ulcer

	Chronic Peptic Ulcer	Malignant Ulcer
Name	Peptic ulcer ("ulcer cancer" if this turns malignant over years)	Cancer- Ulcer
Location	95% located in lesser curvature (magenstrausse)	Greater curvature , Anterior & posterior walls
Size	Up to 2 cm	More than 2 cm
Edges	Sharp & punched out	Beaded & thickened
Rugae	Seen up to edge of ulcer	Loss of rugosity around ulcer
Floor	Clean due to peptic digestion	Dirty due to necrotic slough
Base	Movable	Fixed & firm
Microscopy	4 zones- from above downwards fibrinous material, inflammatory cells, granulation tissue & fibrosis.	Adenocarcinoma With neoplastic glands

B IV STOMACH MICROSCOPY

- **Endoscopic Gastric mucosal biopsies**

At low power note

a) Overall architecture of the epithelium and connective tissue. An ideal biopsy should include atleast 2 pieces of full thickness mucosa with muscularis mucosae. Muscularis mucosae is usually absent in oesophageal biopsies. Presence of muscularis mucosae is important to assess atrophy.

b) At high power note the detail on the mucosa (Fig 3.10) with regard to the region of biopsy in the stomach-whether antral, cardia or body mucosae. Note whether the region corresponds to the same one indicated in the form.

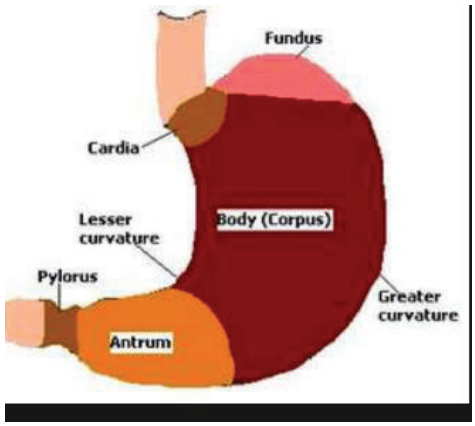


Fig3.10 : shows various anatomical areas of stomach.

Microscopic Reporting of Gastritis: Sydney System (1994)

The Sydney System for the classification of gastritis combines topographical, morphological and etiological information into a scheme that helps generate reproducible and clinically useful diagnoses. It was formulated in 1990 at the World Congress of Gastroenterology at Sydney and updated in 1994 at the Houston Gastroenterology Workshop.

The practical importance of this system lies in the differentiation between H. pylori–associated and non–H. pylori–associated gastritis. H. pylori–positive cases can be further sub-typed based on morphological patterns representing the sequelae of infection, while H. pylori–negative gastritis may represent autoimmune, chemical/reactive, ex–H pylori, *Helicobacter heilmannii*, Crohn's gastritis, eosinophilic, radiation-induced forms etc.

The system includes grading of inflammation severity, activity, glandular atrophy, intestinal metaplasia, and Helicobacter density.

Updated Sydney System – Interpretation Criteria

- Separate biopsies from antrum and corpus, with an additional biopsy from the incisura angularis to be assessed independently. The incisura sample was specifically introduced because maximum atrophy and IM frequently occur here.
- Classify gastritis into H. pylori–induced vs non–H. pylori–induced categories.
- Grade (absent, mild, moderate, severe; visual analogue system):
  - H. pylori density
  - Chronic inflammation (mononuclear infiltrate)
  - Neutrophilic activity
  - Glandular atrophy
  - Intestinal metaplasia
- Concluding summary indicating etiology, topography (antral, corpus, pangastritis), and morphology (activity, atrophy, IM, lymphoid follicles, etc.).

Recent Advances Integrated Into Sydney System Reporting (2020–2024)

1. Integration of OLGA/OLGIM Staging

Modern practice frequently complements the Sydney grading with OLGA (Operative Link on Gastritis Assessment) or OLGIM staging, which translate the extent and severity of atrophy/intestinal metaplasia into gastric cancer–risk stages.

- OLGA/OLGIM I–II: Low-risk, no routine surveillance needed.
- OLGA/OLGIM III–IV: High-risk; endoscopic surveillance recommended. Many pathology labs now include an optional OLGA/OLGIM stage in the final report.

2. Standardised Biopsy Protocols (USSBP) A growing number of centres adopt a **s**tandardised 5-biopsy mapping protocol—two antrum, two corpus, one incisura—to improve detection of multifocal atrophy and IM.
This increases diagnostic yield and allows accurate OLGA/OLGIM staging.

3. Molecular and Phenotypic Advances in H. pylori Gastritis

Recent studies emphasize H. pylori gastritis phenotyping, useful in predicting ulcer vs carcinoma risk:

Duodenal ulcer phenotype: Antral-predominant chronic active gastritis, high acid output; low carcinoma risk. Gastric cancer phenotype / multifocal atrophic gastritis (MAG): Pangastric/ corpus-predominant distribution, marked atrophy + IM; significantly higher cancer risk. Severe corpus gastritis often begins at the incisura and spreads radially.

4. Advances in Autoimmune Gastritis (AIG)

- AIG is increasingly recognised as underdiagnosed; guidelines emphasise serology (anti-parietal cell, anti-IF), serum gastrin, pepsinogen I/II ratio, and endoscopic mapping.
- Histologically, the distinction between "active" autoimmune gastritis (periglandular lymphocytic attack + parietal cell hypertrophy) and "burnt-out" stage (complete oxyntic atrophy, pseudopyloric metaplasia, intestinal metaplasia) is now better defined.
- AIG is associated with ECL cell hyperplasia, hypergastrinemia, and increased risk of Type I gastric NETs; surveillance guidance is increasingly standardized.

5. Additional emerging concepts

- Increasing interest in the gastric microbiome and non–H. pylori organisms (still investigational).
- Enhanced recognition of ex–H. pylori patterns (persistent mild lymphoplasmacytic infiltrates, lymphoid aggregates, and residual IM despite successful eradication).
- IHC for H. pylori is used more frequently in low-density or partially treated cases.

Forms of Gastritis

I. Non–H. pylori Associated Forms of Gastritis

Autoimmune Gastritis / Diffuse Atrophic Corporeal Gastritis (Type A)

- Diffuse involvement with antral sparing.
- Associated with anti-parietal cell antibodies, hypochlorhydria, and marked hypergastrinemia; evolves into pernicious anemia (Vit B12 deficiency).
- Antibodies to intrinsic factor less common than anti-parietal cell antibodies; cell-mediated immunity also implicated.
- Two histologic phases:
  - Active phase: Periglandular lymphocytic attack, parietal cell damage, parietal cell pseudohypertrophy.
  - Burnt-out phase: Total loss of oxyntic glands, intestinal/pseudopyloric metaplasia, mild chronic inflammation.
- G-cell hyperplasia → predisposition to carcinoid (Type I ECL-NETs).
- Associated hyperplastic polyps may occur.
- Strong association with intestinal metaplasia → carcinoma risk.

Other Special Forms

- Eosinophilic gastritis
- Lymphocytic gastritis

- Granulomatous gastritis
- Collagenous gastritis
- Strongyloides, fungal, and other rare infections

Chemical / Reactive Gastritis

Due to endogenous or exogenous mucosal irritants, including bile reflux (post Billroth I/II, GJ, vagotomy) and NSAIDs.
Histology:

- Foveolar hyperplasia
- Lamina propria fibrosis with smooth muscle fibres
- Edema, vascular congestion
- Mild chronic inflammation ± intestinal metaplasia

Ex–H. pylori Gastritis

After eradication therapy; neutrophils disappear, but mild lymphoplasmacytic infiltrate, lymphoid aggregates, and intestinal metaplasia may persist.

Helicobacter heilmannii Gastritis

Rare (~0.1%), often linked to contact with pets.
Organisms are much longer than H. pylori and easily detected by Warthin–Starry stain.
Disease is usually mild, rarely causes atrophy or IM.

Granulomatous Gastritis

About 60% are due to Crohn's disease.
Histology shows granulomas + periglandular lymphocytic infiltrates.
Other causes: foreign body granulomas, sarcoidosis, tuberculosis.

Varioliform Gastritis (Chronic Erosive)

Erosions with central epithelial defects, necrotic base, hyperplastic tortuous pits.
Marked IEL infiltration (≥40/100 epithelial cells).
Muscularis mucosae intact; deep gland cystic dilatation present.

II. H. pylori–Induced Gastritis

Helicobacter pylori (formerly *Campylobacter pylori*) is a spiral bacterium colonizing the antrum and cardia.
Identifiable on H&E; special stains (Giemsa, Warthin-Starry, Steiner, Genta) and IHC improve detection.

Found in ~90% of chronic gastritis, peptic ulcers, and many gastric carcinomas.

Diffuse Antral Gastritis (Type B)

- Strongly associated with H. pylori.
- Chronic active gastritis: lymphocytes + neutrophils.
- Intestinal metaplasia and mild atrophy may be seen.
- Lymphoid aggregates/follicles common.
- Atrophy not as prominent as in autoimmune gastritis.

Phenotypic Variants

Duodenal Ulcer Phenotype

- Antral-predominant, high acid output.
- Very low carcinoma risk.
- Nearly always H. pylori when other causes excluded.

Gastric Cancer Phenotype (Multifocal Atrophic Gastritis – MAG)

- Pangastritis/corpus gastritis beginning at the **incisura**.
- Multifocal atrophy + intestinal metaplasia; may be extensive.
- Serology may be positive even when organisms not seen in biopsies.
- Associated with gastric ulcer and erosive gastritis.
- Increased carcinoma risk; familial clustering possible.

Gastric Atrophy (Fig 3.11)

Loss of native glands with widening of gland–gland spaces, lamina propria fibrosis, thinning of mucosa, and frequent intestinal or pseudopyloric metaplasia.

Glandular atrophy ie loss of glands native to the epithelium of the gastric mucosa is characterized by increased distance between individual glands and condensation of reticulin fibres and fibrosis in the lamina propria. The resultant gastric mucosal atrophy results in thinning of the mucosa and is the end stage of chronic gastritis and increases carcinoma risk. With this is also evident intestinal metaplasia or pseudopyloric metaplasia.

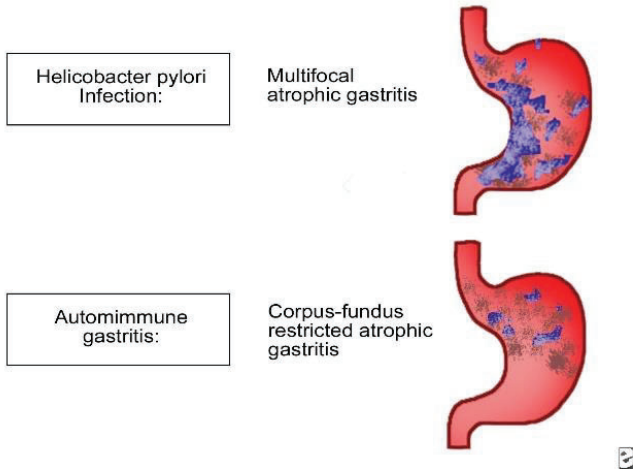


Fig 3.11: Atropic gastritis Nafea & Zayouna 2018.

- **Metaplasias : Pyloric metaplasia:** is replacement of fundic type glands with mucus secreting glands

Intestinal metaplasia *:* is replacement by intestinal epithelium (fig) of either the small or large bowel type- including goblet cells, absorptive (brush border) cells, Paneth cells and a variety of endocrine cells.

Both type of metaplasias may be either complete or incomplete :

Criteria	Type I Complete	Type II Incomplete
Epithelium	Identical to small bowel epithelium with admixture of gastric cells & goblet cells; development of villi & crypts (in advanced cases)	Columnar cells +++ of foveolae, no goblet cells, absorbtive cells are absent. (No change in morphology, therefore rely on mucin stains to diagnose)
Mucin type	Neutral mucin + (of stomach) Sialomucin +++ (small bowel mucin) Sulphomucin + (large bowel mucin)	Type II A : neutral ++ Sialomucin ++ Type IIB : sulphomucin ++
H. pylori	Absent	Present
Association with carcinoma	+ve	Type IIB +ve
IHC	MUC 2; ↓ MUC 1; MUC 5AC; MUC 6	MUC 2 + & mucins normally expressed in the stomach

Neutral mucin is PAS +ve and Alcian Blue negative at pH 2.5 & 0.5.

Sialomucin is PAS +ve , Alcian Blue +ve at pH 2.5 but Alcian Blue –ve at pH 0.5.

Sulphomucin is weakly PAS +ve, Alcian Blue +ve at pH 2.5 as well as 0.5 . It is also High Iron Diamine +ve (brown black colour).

Recent Advances in Chronic Gastritis

- **Refined Sydney System (Updated practice trends)** – Emphasis on topographic mapping with minimum 5 biopsies (2 antrum, 2 corpus, 1 incisura); stronger recommendation for routine *H. pylori* immunostaining when morphology is equivocal.
- **OLGA/OLGIM Risk Staging Integration** – Increasing global adoption for cancer-risk stratification:
    - OLGA (atrophy-based staging)
    - OLGIM (IM-based staging)
      Both systems identify high-risk stages (III–IV) linked with gastric cancer progression.
- **Advanced *H. pylori* Detection** – High-sensitivity tests: multiplex PCR, stool antigen tests with monoclonal antibodies; routine use of IHC in low-density or post-treatment settings.
- **Molecular Insights** – Recognition of autoimmune gastritis as T-cell–mediated injury driven by parietal-cell antibodies with associated ECL cell hyperplasia and MEN-1 mutations in associated NETs.
- **Updated Management Pathways** – Prolonged surveillance recommended for OLGA/OLGIM III–IV; utilisation of endoscopic image-enhancement (NBI, BLI) for targeted biopsies.
- **Gastric Microbiome Data** – Emerging evidence that non-*H. pylori* microbiota contribute to persistent inflammation and possibly carcinogenesis.
- **Serological Markers** – Pepsinogen I/II ratio and gastrin-17 increasingly used to screen for atrophic and autoimmune gastritis in population programs.
- **Autoimmune Gastritis Developments** – Greater emphasis on identifying early hypergastrinemia, iron deficiency in younger patients, and surveillance for Type I gastric NETs.

DIAGNOSTIC ALGORITHM FOR CHRONIC GASTRITIS

Step 1: Clinical & Endoscopic Context

- Dyspepsia, anaemia, B12 deficiency, pernicious anaemia, reflux-like symptoms, family history of gastric cancer.

Step 2: Biopsy Protocol (Updated Sydney)

- Five biopsies minimum:
  - 2 × antrum (lesser + greater curve)
  - 2 × corpus (lesser + greater curve)
  - 1 × incisura angularis

Step 3: Microscopy Evaluation

- Grade: chronicity, activity (neutrophils), atrophy, IM
- Identify *H. pylori*
- Recognise pattern:
  - *H. pylori*-associated
  - Autoimmune gastritis
  - Chemical/reactive (NSAID, bile reflux)
  - Lymphocytic gastritis
  - Eosinophilic gastritis
  - Variants (post-eradication, patchy gastritis)

Step 4: Ancillary Tests

- *H. pylori* IHC if organisms not seen but morphology suggests infection
- Parietal cell & intrinsic factor antibodies if autoimmune suspected
- Serology (PGI, PGII, G17) in severe atrophy or surveillance settings
- Gastrin levels for suspected AIG or NET risk
- PCR if needed (equivocal cases, post-treatment)

Step 5: Apply Staging Systems

- OLGA – based on atrophy (antrum + corpus)
- OLGIM – based on intestinal metaplasia (pattern + location)
- Classify I–IV; III and IV indicate high cancer risk

Step 6: Final Diagnosis

Type of gastritis

- Etiology
- Severity (Sydney grading)
- Stage (OLGA/OLGIM)
- Risk category + recommended surveillance

Polyps of the stomach :

- fundic gland polyp

- Hyperplastic polyp

-Adenomatous polyp -

-Other conditions presenting as polyps -

- *Menetrier's Disease*
- *Giant rugae*
- *Hypertropic hypersecretary polyp*
- *Zollinger-Ellison Syndrome*

- **Fundic gland polyps :** -These are benign gastric epithelial lesions showing hyperplastic expansion of the deep part of the oxyntic mucosa and therefore considered a type of hyperplastic polyp. They constitute 70-90% of all gastric polyps at histopathology.
  - They are located where ever the mucosa has oxyntic glands ie in fundus and body.
  - If more than 20 polyps are noted, a polyposis syndrome should be considered.
  - Microscopy : dilated oxyntic glands with parietal hyperplasia. Foveolar hypoplasia is seen. The dilated glands are lined either with flattened oxyntic epithelium or foveolar type epithelium.
  - Unlike hyperplastic polyps it is inversely related to H pylori gastritis; and therefore less associated with carcinoma.
  - Sporadic cases have mutations of CTNNB1 encoding beta catenin.
  - familial polyposis cases have APC mutations and wild type CTNNB1
- **Hyperplastic polyps** : Occur as a result of foveolar hyperplasia as a response to tissue injury. There is a strong association with chronic gastritis and H pylori associated gastritis. Majority are situated in the antrum, although can occur in other parts of stomach. Constitute about 20% of polyps in the stomach.
  - Gross : generally multiple, dome shaped polyps less than 1 cm in size or large polyps may be lobulated and pedunculated.
  - Microscopy : elongated distorted, branched and dilated hyperplastic foveolae in an inflamed oedematous stroma. About 1-20% harbour dysplasia and in 2% of cases foci of carcinoma may prevail; the latter in polyps >1cm in size.
  - Hyperplastic polyps have to be differentiated from foveolar hyperplasia which is precursor to these polyps but lacks the dilatation of the glands. The other differential is gastric mucosal prolapse polyps in the antrum; these lack the hyperplasia as well as dilatation.
- **Adenomas :** mucosal lesions with changes as described above with raised polypoid nodules.
  - -Intestinal type gastric adenoma-a polypoid lesion consisting of

- dysplastic intestinal epithelium; 60% occur in distal stomach; intestinal
- metaplasia is the precursor lesion.
- -Foveolar type adenoma-occurs where ever there is oxyntic gastric
- epithelium ie body/fundus. No association with gastric carcinoma in
- sporadic cases but may be with the familial type.
- -Gastric pyloric gland adenoma -rare, occurs in the gastric body and
- fundus. MUC6 +ve, Surface coating of MUC5AC representing apical
- mucin.
- -Oxyntic gland adenoma- occurs usually in the upper third of the
- stomach. Composed of columnar cells with differentiation to chief cells
- or oxyntic (parietal) cells.

- **GAVA : gastric antral vascular ectasia(water melon stomach) :** is an acquired vascular disease. Endoscopically parallel red stripes at the crest of the mucosal folds in the antrum resembling stripes of the water melon, sometimes located in the cardia. Microscopically minimal changes with an increase in the number and caliber of the vessels, fibrin thrombi and fibromuscular hyperplasia**[12].**
- **Menetrier's disease :** characterized by hypertrophic folds along the greater curvature of the stomach seen at endoscopy & radiology. Shows marked foveolar epithelial hyperplasia at microscopy. Other conditions with giant gastric folds are- Zollinger Ellison syndrome, hypersecretory hypertrophic gastropathy, malignant infiltration by lymphoma/carcinoma.
- **Zollinger – Ellison Syndrome** : Carcinoid tumours (in fundus) secrete gastrin which leads to hyperplasia of parietal cells leading to increase in acid secretion. This leads to peptic ulcers in the duodenum and jejunum.

Gastric Dysplasia

Unequivocal neoplastic changes of gastric epithelium without evidence of stromal invasion (non polypoid adenomatous change)

Gastric intraepithelial neoplasia -low grade

Gastric intraepithelial neoplasia- high grade

Synonyms : Serrated dysplasia low grade

Serrated dysplasia high grade

Intestinal type dysplasia

Foveolar type

Gastric pit dysplasia

Location of gastric dysplasia is mostly in the antrum, however can occur elsewhere, it mirrors gastric carcinoma in most respects. Endoscopy shows flat depressed or elevated lesions with or without erosions.

- Dysplasia may be graded as per the Vienna dysplasia or WHO system :
  - Vienna System : Category 1: negative for dysplasia
    - Category 2: Indefinite for dysplasia
    - Category 3 : Low grade adenoma/dysplasia
    - Category 4.1 : High grade adenoma/dysplasia
      - 4.2 Non invasive carcinoma
      - 4.3 Suspicious for invasive carcinoma
      - 4.4 Intramucosal carcinoma
  - WHO (2019) grading of dysplasia :
    - Negative for Dysplasia/IEN
    - Indefinite for Dysplasia/IEN
    - Low grade Dysplasia/IEN (low grade adenoma/dysplasia)
    - High grade dysplasia/IEN (high grade adenoma/dysplasia)
    - Intramucosal Invasive neoplasia (intramucosal carcinoma)
  - High grade dysplasia shows mol changes such as MSI, Cp-G island methylation ;
  - loss of TP53 function and overexpression of p53 akin to gastric neoplasia.
- Dysplasia can be divided into 2 morphologic categories : Intestinal; foveolar; mixed type is also seen. Dysplasia occurs denovo or in pre-existing fundic gland or hyperplastic polyps.
  - *Intestinal type dysplasia* consists of tubular, tubule-villous or villous lesions. Glands are lined by columnar cells, nuclei are enlarged and hyperchromatic; elongated and stratified. Cytoplasm is eosinophilic and cells show terminal differentiation to goblet, absorptive cells and Paneth cells. They are +ve for MUC2, CD10, CDX2.
  - *Foveolar type dysplasia* shows tubulo-villous or serrated glands with columnar cells showing round nuclei and prominent nucleoli. Cells are +ve for MUC 5AC (apical neutral mucin) and show MUC 6+ve cells at the bottom.
  - In either type of dysplasia, low grade shows nuclei at the bottom of the cell with minimal architectural disarray. High grade dysplasia (also called carcinoma-in -situ) shows architectural disarray with cribriforming, glandular complexity and *nuclei located at the apical portion of cell.* Sometimes it may be difficult to distinguish this from intramucosal carcinoma which is characterised by invasion of lamina propria. High grade dysplasia invariably leads to adenocarcinoma in about 70% of cases. Morphologically some dysplasia may show a '*serrated surface'* or may be located in the *pits of the crypts*.

WHO CLASSIFICATION OF GASTRIC TUMOURS

I. EPITHELIAL TUMOURS

Benign epithelial tumours and precursors

Glandular Intra-epithelial neoplasia low grade

Glandular Intra-epithelial neoplasia high grade

Serrated dysplasia low grade

Serrated dysplasia high grade

Intestinal type dysplasia

Foveolar type gastric dysplasia

Gastric pit/crypt dysplasia

Intestinal type adenoma low grade

Intestinal type adenoma high grade

Sporadic intestinal type gastric adenoma

Syndromic intestinal- type gastric adenoma

Adenomatous polyp low grade dysplasia

Adenomatous polyp high grade dysplasia.

Malignant epithelial tumours

Adenocarcinoma, NOS

Tubular adenocarcinoma

Parietal cell carcinoma

Adenocarcinoma with mixed subtypes

Papillary adenocarcinoma , NOS

Micropapillary carcinoma, NOS

Muco-epidermoid carcinoma

Mucinous adenocarcinoma

Signet ring cell carcinoma

Poorly cohesive carcinoma

Medullary carcinoma with lymphoid stroma

Hepatoid adenocarcinoma

Paneth cell carcinoma

Squamous cell carcinoma,NOS

Adenosquamous carcinoma

Undifferentiated carcinoma, NOS

Large cell carcinoma with rhabdoid phenotype

Pleomorphic carcinoma

Sarcomatoid carcinoma

Carcinoma with osteoclast like giant cells

Gastroblastoma

Neuroendocrine tumour , NOS

Neuroendocrine tumour grade 1

Neuroendocrine tumour grade 2

Neuroendocrine tumour grade 3

Gastrinoma, NOS

Stomatostatinoma, NOS

Enterochromaffin cell carcinoid

ECL-cell carcinoid, malignant

Neroendocrine carcinoma, NOS

Large cell neuroendocrine carcinoma

Small cell neuroendocrine carcinoma

Mixed neuroendocrine-non neuroendocrine neoplasm (MiNEN)

NON EPITHELIAL TUMOURS

Leiomyoma

Schwannoma

Granular cell tumour

Glomus tumour

Leiomyosarcoma

Gastrointestinal Stromal Tumour (GIST)

- benign
- uncertain malignant potential
- malignant

Kaposi's Sarcoma

Others

Maliganant lymphoma

- Marginal zone B cell lymphoma of MALT type
- Mantle cell lymphoma
- Diffuse large B cell lymphoma
- Others

II. SECONDARY TUMOURS

Carcinoma Stomach : is the most common malignant epithelial tumour of the stomach. H, pylori infection is the commonest etiological factor giving rise to a series of epithelial phenotypic changes (Correa's cascade) – inflammation, intestinal metaplasia and subsequent low grade and high grade dysplasia. This is accompanied by genetic alterations (like TP53) leading to carcinoma. Given above is recent 2019, WHO classification of carcinoma stomach where the various types have been listed.

Eighty percent of cases occur in the distal stomach particularly in Asian countries ie antral location. In USA and UK, about half are situated in the cardia and fundus, in Japan the incidence of proximal carcinoma is on the rise.

About 10% of carcinomas of the stomach develop in a familial or hereditary setting.

The carcinoma is graded as well, moderately and poorly differentiated.

Other Classification systems are : Lauren 1965 : i) Intestinal type of adenocarcinoma

ii) Diffuse type of adenocarcinoma

iii) Mixed type of adenocarcinoma

This classification is easy to use and quite popular.

Intestinal type of adenocarcinoma stomach whether in the Lauren or WHO classification, closely resemble a colon carcinoma. Grossly they are nodular, polypoid or ulcerated masses and microscopically characterized predominantly by a well formed glandular pattern with solid and papillary areas. A moderate quantity of glandular mucin may be present (more in the lumina). Cells with intracytoplasmic mucin are uncommon.

The diffuse type of carcinoma is characterized by a plaque like growth to extensive infiltration (linitis plastica), have sheets of cells with intracytoplasmic mucin separated by a fibrous or myxoid stroma. These tumours as a rule are usually poorly differentiated.

Early gastric carcinoma : (Fig 3.12) is a term used in Japanese literature where a primary invasive growth is confined to the mucosa or submucosa of the stomach, irrespective of tumour size. May or may not have lymph node metastasis. Importance is that they are generally cured by surgery.

(type 1-protruberant; type II-superficial; type III-excavated)

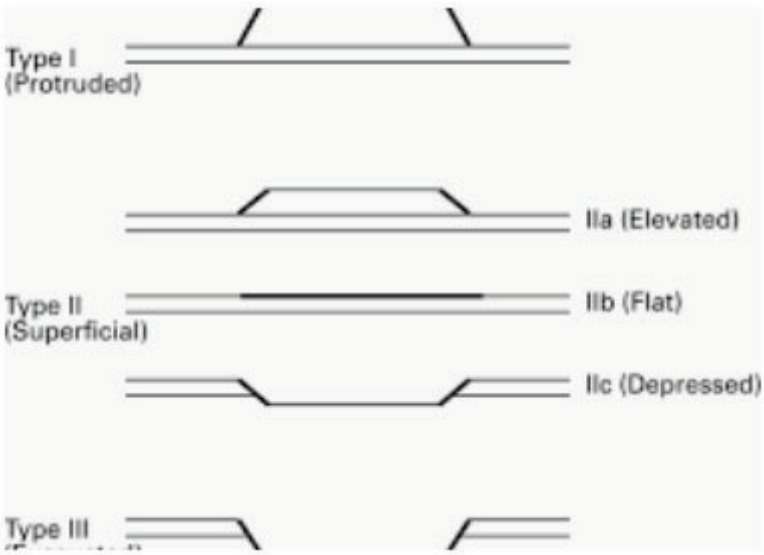


Fig 3.12 Early gastric carcinoma

Molecular classification of gastric carcinoma :

The Cancer Genome Atlas Research Network Group (TCGA) proposed a molecular classification dividing GC into four subtypes: EBV-positive tumors, microsatellite unstable tumors (MSI), genomically stable tumors (GS) and tumors with chromosomal instability (CIN) . Recent studies have shown a correlation between molecular and clinico-pathological characteristics of GCs.

EBV+ve tumours : neoplasm with lymphoid stroma and lympho-epithelial like features. Activation of EBV detected in about 9% of neoplasms. More common in men. Shows PIK3CA and ARID1A mutations and global or genomic wide hypermethylation.

MSI Tumours : show methylation of mismatch repair genes (eg MLH1)& global hypermethylation.

Genomically stable tumours : show diffuse type histology and minimal genetic abberations.

Chromosomal unstable tumours : Intestinal type histology with DNA abnormalities. Amplification of ERBB2, EGFR, MET etc

Gastric Stromal Tumours :

The vast majority of gastric stromal tumours may be divided for simplicity of reporting into i) GISTS which show a distinctive positivity for CD 117 (c-kit) and CD34 (vast majority belong to this group) and ii) of true leiomyomatous origin iii) less commonly tumours of true schwannian origin which are CD34 negative but S-100 +ve and glial acid fibrillary protein +ve.

C-kit and CD 34 is expressed by the interstitial cells of Cajal, the putative pacemakers of gastrointestinal motility and most gastrointestinal stromal tumours are of this lineage and are termed GISTS. The cells have a receptor on their surface called CD117. This CD117 protein is

made by a gene called c-kit. A fault in this gene due to a mutation causes the c-kit gene to make too much CD117 protein expressed by these tumours.

Gastric stromal tumours may affect any part of the stomach and generally occur in adults aged over 30 years. The only recognized predisposing conditions are von Recklinghausen's disease and Carney's triad (syndrome in young women with epitheliod stromal tumour of stomach, extra-adrenal paraganglioma and pulmonary chondromas).

Gross : tumours may be single or multiple and vary in size from tiny intramural lesions to bulky masses. Most project into gastric lumen as endophytic polypoid submucosal growths and are prone to surface ulceration & bleeding. Others may protrude from serosa and still others may grow in both directions as dumbbell shaped lesions. Well circumscribed but lack a capsule. C/S grey pink in color with a rubbery consistency; cystic degeneration, haemorrahge or infarction may be seen.

Tumours > 10cm are known to throw metastasis.

Microscopy : wide range of histologic appearances :

- spindle cells are seen in interlacing bundles or whorls with elongated blunt ended nuclei and eosinophilic cytoplasm that contains a single clear paranuclear vacuole
- Some tumours show a striking neural differentiation including parallel stratification of nuclei, a plexiform growth pattern and merging of tumours with nerve fibres of myenteric or submucosal autonomic plexuses. Occasional cases contain skenoid fibres which are small, globular curvilinear eosinophilic aggregates of filamentous material scattered among tumour cells.
- Epithelioid differentiation may be seen . Such tumours previously termed 'epithelioid leiomyoblastomas ' occur more commonly in the antrum and consist of round vacuolated or clear cells in sheets or nests that impart an epitheliod pattern.
- Stroma may be hyalinised or calcified. May also have hyaluronic acid rich, myxoid connective tissue component.
- Mitotic rates for 50 hpfs should be determined. Upto 50% of tumours with 4 mitoses / 50hpfs will metastasise and 95% of tumours with 5 or more mitoses / 50hpfs will metastasise.
- Clearly malignant spindle cell tumours show foci of liposarcomatous, chondrosarcomatous or even rhabdomyosarcomatous differentiation.
- IHC : CD 117 +ve; negative in <2 % of cases

  CD 34 +ve in 70-80 % of cases

  SMA +ve in 30-40% of cases

  Desmin +ve in < 5% of cases

  S-100 +ve in <5% of cases

  DOG 1 is a new marker that is very sensitive & specific for GIST.

Surgery is the treatment of choice . Chemotherapy and radiotherapy don't work well for GIST, so research has identified other types of treatment. A biological therapy called Glivec (imatinib) is used to treat GIST that is not completely removed with surgery. Glivec is a tyrosine kinase inhibitor. This means it blocks the enzyme tyrosine kinase which transmits impulses for protein synthesis at the level of the interstitial cells of Cajal.

B V STOMACH SYNOPTIC REPORTING

Small biopsy report

Gross

Specimen type

Endoscopic biopsy □

Other (specify)……

Not specified□

Single □ Multiple□

Site of biopsy if known Fundus □ Antrum □ Body □

Microscopy : Non tumour

Gastritis : Nil □ Mild □ Moderate □ Severe □

Activity Nil □ Mild □ Moderate □ Severe □

H. pylori Nil □ Mild □ Moderate □ Severe □

Intestinal metaplasia Nil □ Mild □ Moderate □ Severe □

Type : Complete □ Incomplete□

Atrophy : Present □ Absent□

Type of gastritis ……..

Regenerative atypia : Present □ Absent□

Dysplasia/adenoma : Present □ Absent□

Crohn's Disease □ Ulcer □

Other findings if any …….

Tumour

Polyp : □ Type……

Adenocarcinoma - Intestinal type□

Diffuse type □

Papillary adenocarcinoma□

Tubular adenocarcinoma□

Mucinous adenocarcinoma (>50% mucinous component) □

Signet ring cell carcinoma (>50% signet ring cells) □

Lymphoma □

NET □ NEC □

Other : Specify……..

Carcinoma : type cannot be determined □

Histologic grade – Not applicable□

- grade cannot be ascertained□

- well differentiated□

- moderately differentiated□

- poorly differentiated□

- undifferentiated□

Extent of invasion – (deepest)

Cannot be determined□

Lamina propria□

Muscularis mucosae□

Muscularis propria□

Margins : (for polypectomy only) :

Not applicable□

Cannot be assessed□

Mucosal margin free□

Mucosal margin involved by invasive carcinoma □

Mucosal margin involved by adenoma □

Deep margin for carcinoma :

Uninvolved by invasive carcinoma□

Distance of invasive carcinoma from margin….mm

Involved by invasive carcinoma□

Additional findings……

Signature…….. Date……..

REPORTING TEMPLATE for CHRONIC GASTRITIS (Sydney + OLGA/OLGIM Combined)

Specimens Submitted (Sydney Topographic System):

- Antrum, lesser curvature
- Antrum, greater curvature
- Incisura angularis
- Corpus, lesser curvature
- Corpus, greater curvature

Microscopy (Sydney System Grading)

Feature	Antrum	Corpus
Chronic inflammation (0–3)		
Activity (neutrophils) (0–3)		
Atrophy (0–3)		
Intestinal metaplasia (0–3)		
***H. pylori* (0–3)**		

Other features:

- Lymphoid follicles: Present/Absent
- ECL cell hyperplasia: Present/Absent (AIG)
- Foveolar hyperplasia/chemical injury: Present/Absent
- Eosinophils/infiltrate: Present/Absent
- Dysplasia: Absent/Low-grade/High-grade

Etiologic Impression

- *H. pylori*-associated / Autoimmune / Reactive / Lymphocytic / Eosinophilic / Other

OLGA STAGING (Atrophy)

- Antrum score:
- Corpus score:
- Stage (I–IV):

OLGIM STAGING (Intestinal Metaplasia)

- Antrum score:
- Corpus score:
- Stage (I–IV):

Final Diagnosis

Example format:
"Chronic *H. pylori*-associated gastritis with moderate chronicity, mild activity, mild atrophy, and incomplete intestinal metaplasia. OLGA stage II, OLGIM stage II."

Recommendations

- ***H. pylori*** eradication therapy if positive
- Surveillance for OLGA/OLGIM III–IV: 1–3 yearly endoscopy
- Autoimmune gastritis: monitor B12, iron; consider NET surveillance
- Chemical/reactive gastritis**:** manage reflux/NSAID use

STOMACH CARCINOMA RESECTION HISTOPATHOLOGY REPORT

Gross

-Specimen type

Partial gastrectomy □

Distal gastrectomy □

Proximal gastrectomy □

Other (specify) □

Total gastrectomy □

Other (specify) □

Not specified □

- Tumour site

- Cardia □
- Fundus □
- Body □

Anterior wall □

Posterior wall □

Lesser curvature □

Greater curvature □

- Antrum □
  - Anterior wall □
  - Posterior wall □
  - Lesser curvature□
  - Greater curvature□
- Other□
- Not specified□

-Tumour configuration

- exophytic □
- infiltrative □
- diffuse infiltrative (linitis plastica) □
- expansile (non-infiltrative) □
- ulcerating □
- annular □

-Tumour size

- greatest dimension……..cm
- additional dimensions ……cm x…..cm
- cannot be determined □

-Histological type

Adenocarcinoma, NOS

- Tubular adenocarcinoma □
- Adenocarcinoma with mixed subtypes □
- Papillary adenocarcinoma , NOS □
- Mucinous adenocarcinoma□
- Signet ring cell carcinoma □
- Poorly cohesive carcinoma □

Other …….

- TNM stage ……

Signature……. Date …….

TMN STAGING : STOMACH

Extent of tumour :

T : Primary tumour

pTX : primary tumour cannot be assessed □

pT0 : no evidence of primary tumour □

pTis: carcinoma in situ, intra-epithelial tumour without invasion of the lamina propria, high grade dysplasia □

pT1 : tumour invades lamina propria, muscularis mucosa or submucosa □

pT2 : tumour invades muscularis propria □

pT3 : tumour invades subserosa

pT4 : tumour perforates subserosa (visceral peritoneum) or invades adjacent structures □

Adjacent structures are - like spleen, transverse colon, diaphragm, liver, pancreas, abdominal wall, kidney, retroperitoneum etc) □

N : Regional Lymph Nodes

pNX : Regional lymph Nodes cannot be assessed □

pN0 : No regional lymph node metastasis (regional lymphadenectomy specimen should normally include 16 lymph nodes) □

pN1 : metastasis in 1 to 2 regional lymph nodes □

pN2 : metastasis in 3 to 6 regional lymph nodes □

pN3 : metastasis in 7 or more regional lymph nodes □

M : Distant metastasis

pM1 : Distant metastasis □ (includes peritoneal seeding, +ve peritoneal cytology & omenta tumour not part of continuous extension)

Margins of resection :

Distal margin free of tumour □

Distal margin involved by invasive carcinoma □

Distal margin involved by CIN/adenoma □

Proximal margin free of tumour □

Proximal margin involved by invasive carcinoma □

Proximal margin involved by CIN/ adenoma □

Radial margins : lesser omental radial margin free□

lesser omental radial margin involved by tumour □

Greater omental radial margin free □

Greater omental radial margin involved by tumour □

Distance of invasive carcinoma from closest margin…..mm

Specify margin……..

Lympho-vascular invasion : Present □ Absent □

Perineural invasion : Present □ Absent□

Additional findings None □

Intestinal metaplasia □

Dysplasia □

Gastritis (type) □

Polyp (type) □

Histological grade (same as for esophagus)

Snomed code……

C I SMALL AND LARGE INTESTINE ANATOMY

ANATOMY RELEVANT TO GROSSING

Small Intestine

Extent and Segments

- Extends from pylorus to ileocecal valve
- Length: ~5–6 meters
- Divided into:
  - Duodenum (≈25 cm) – C-shaped, retroperitoneal (except first part)
  - Jejunum (proximal 2/5)
  - Ileum (distal 3/5)

Key Gross Features for Identification

Feature	Jejunum	Ileum
Wall	Thick, firm	Thin
Lumen	Wider	Narrower
Folds (plicae circulares)	Prominent	Sparse/absent
Mesenteric fat	Less	More (encroaches on wall)
Vascularity	Rich (long vasa recta)	Less (short vasa recta)
Peyer patches	Rare	Numerous (antimesenteric side)

Duodenum –

- 4 parts; 2nd part receives bile and pancreatic ducts
- Important landmarks:
  - Ampulla of Vater
  - Periampullary region (common site for tumors)
- Close relation to pancreas → tumor origin may be difficult to define

Important Gross Landmarks

- Ligament of Treitz → marks duodenojejunal junction
- Ileocecal valve → transition to large intestine
- Mesenteric border vs antimesenteric border
  - Ulcers (e.g., typhoid) → typically antimesenteric
  - Vascular supply enters via mesenteric side

Large Intestine Extent and Segments

- From ileocecal valve to anus
- Length: ~1.5 meters
- Segments:
  - Cecum (with appendix)
  - Ascending colon
  - Transverse colon
  - Descending colon
  - Sigmoid colon
  - Rectum and anal canal

Key Gross Identifying Features

- Taenia coli (3 longitudinal bands)
- Haustra (sacculations)
- Appendices epiploicae (fat tags)

Cecum

- Is a Blind pouch
- Ileocecal valve and appendiceal orifice
- Common site for:
  - Tuberculosis
  - Carcinoma

Appendix

- Variable position
- Important to identify:
  - Base
  - Tip
- Lumen may be obliterated in older individuals

Colon

- Ascending & descending → retroperitoneal
- Transverse & sigmoid → intraperitoneal (mobile)

Tumor patterns:

- Right colon → polypoid/exophytic
- Left colon → annular "napkin-ring" lesions

Rectum

- No taenia coli → continuous longitudinal muscle
- No appendices epiploicae
- Important landmark:
  - Peritoneal reflection
- CRM (circumferential resection margin) is critical in cancer specimens

Important Gross Landmarks

- Ileocecal valve
- Hepatic flexure

- Splenic flexure
- Rectosigmoid junction
- Peritoneal reflection (rectum)
- Always orient using mesenteric border

C II SMALL AND LARGE INTESTINE GROSSING TECHNIQUES

Types of specimens

Endoscopic Biopsies

Polypectomy (mostly large intestine)

Resections

Endoscopic Biopsies

These specimens are usually received attached to a piece of filter paper mucosal side up.

- Note number of pieces and take measurements in three dimensions.
- Examine small intestinal biopsies with a dissecting microscope and determine the mucosal pattern and colour without crushing or drying the tissue.

The pattern can be described as finger-like. leaf-like, cerebriform or flat (fig 3.13).

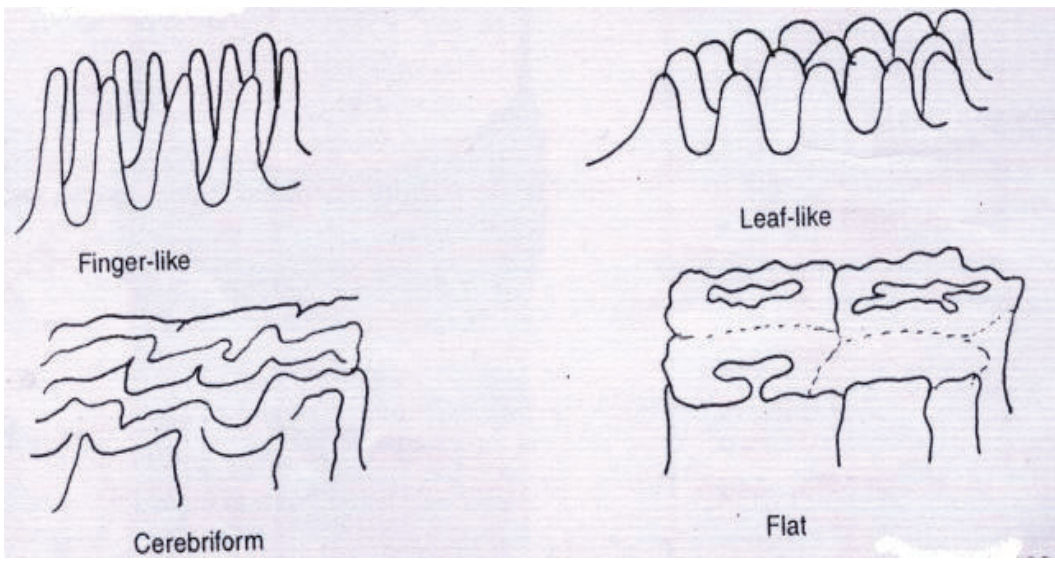


Figure 3.13

- Embed the biopsy so that the mucosal side is at right angles to the cutting surface.

Polypectomy specimens (Large intestine)

- Examine and comment on whether the polyp is sessile or pedunculated.
- Give the measurements of the polyp in three dimensions and the length of the stalk if present.

- Examine the surface and note whether it is ulcerated, smooth or papillary.
- Cut a pedunculated polyp eccentrically as shown in the figure 3.14. If small polyps are bisected through the stalk the latter may get trimmed away, while trimming of the block.

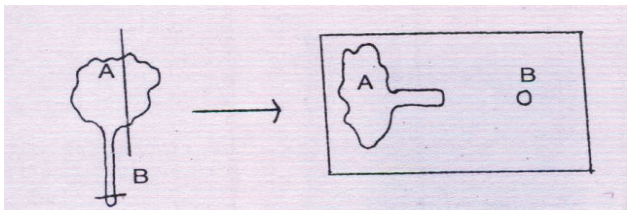


Fig 3. 14

If the polyp (A) is big give as 2 pieces.

- Sessile polyps may be sectioned as shown below .

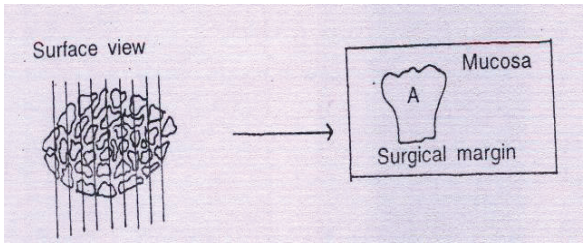


Figure 3.15

- Examine the cut surface for cysts or haemorrhage.

Bits

A - 1 bit polyp including surgical margin.

B - in case of a long stalk one cross section of the stalk at the surgical margin is to be taken in addition.

Resections

These specimens are usually removed in cases of gangrene (fig 3.16A), ischaemic necrosis, perforation, tuberculosis, intussusception (3.16B) or tumour.

- Measure length and diameter
- Identify site of lesion (distance from margins/landmarks)
- Note:
  - Ulcers (site, depth, orientation)
  - Strictures
  - Tumors (polypoid, ulcerative, infiltrative)
- Look for:
  - Perforation (often antimesenteric)
  - Mesenteric lymph nodes

- Open bowel along antimesenteric border (except when tumour dictates otherwise)
- In obstruction:
  - Proximal dilation, distal collapse
- In ischemia:
  - Segmental involvement along vascular territories
- In inflammatory diseases:
  - Distribution pattern (continuous vs skip lesions)

Large intestine resection specimens are identified by the presence of taenia coli and appendices epiploicae. These specimens may be of the following types.

- total colectomy
- right hemicolectomy terminal ileum with ileo-caecal valve, caecum and colon upto hepatic flexure along with attached appendix and mesentery (Fig 3.16B)
- left hemicolecomy includes colon from splenic flexure to sigmoid.
- tranverse colectomy (hepatic to splenic flexure)
- Anterior resection (done for early and upper part of rectal tumours where the anal spinter can be spared).
- abdomino-perineal *resection* (sigmoid colon, rectum and anus, sigmoid mesentery,
- mesorectum and surrounding soft tissue). Done for low rectal and anal canal tumours; does not spare the anal spinter. Once the anus and rectum are removed, a colostomy is done which involves bringing a portion of the colon to an opening at the surface of the skin.
- A laparoscopic abdomino-perineal resection may also be performed.

In case of small intestine varying segments may be resected depending upon the lesion and site of involvement.

Non-neoplastic lesions.

- Measure the length and diameter of the resected segment.
- Describe the external surface - congestion, exudate, perforation, haemorrhage, stricture, tubercles, adhesions.
- If the specimen is fresh then the mesentery may be separated as close to the intestinal wall as possible and kept apart for ease of examination.
- Open by cutting longitudinally along the anti-mesenteric border as a general rule. If the lesions on the anti-mesenteric border the specimen may be opened along the opposite border
- Examine the contents for parasites.
- On opening describe the mucosal aspect - presence of oedema, haemorrage, ulceration,
- Give the length of the affected segment (i.e. gangrene (fig 3.16A), stricture or any other lesion) and its distance from the proximal and distal surgical margins.

- Mesenteric blood vessels are to be sectioned and examined in ischaemic lesions.
- Lymph nodes are to be sectioned and examined. The colour and appearance of the cut surface and size of the largest node are to mentioned.
- Examine surgical margins and comment on viability in case of ischaemic lesions.
- Examine and describe the appendix if present.
- In intussusception, the length of the intussusceptum and intussuscipiens is to be given with description of the segments involved (see fig 3.16 B).
- In cases of diverticulum, give the length and diameter of the diverticulum, with description of its wall and mucosa.

Bits

All bits in the small and large intestine are to be taken perpendicular to the mucosal folds, except surgical margins which may be taken circumferentially.

A – C - Multiple bits from lesions present in as many blocks as required approximately 1-2 bits per block depending upon the size.

D - 1-2 bits from uninvolved mucosa.

E - 2 bits proximal surgical margins (in cases of ishaemic colitis)

F - 2 bits distal surgical margin.((in cases of ishaemic colitis)

G - Lymph nodes

H - Several mesenteric blood vessels in ischaemic lesions

- In case of intussusception, sample both intussuscipiens and intussusceptum.
- In specimens of multiple polyposis three of the largest may be sampled. The sections are to include the base- of the polyp and adjacent mucosa. If a malignant change is suspected in the polyps a more generous sampling may be done.
- For specimens of diverticulum one bit is to be taken from the tip and one to include adjacent mucosa. In multiple diverticulosis at least three diverticula are to be sampled upto a total of 6 - 8 blocks.

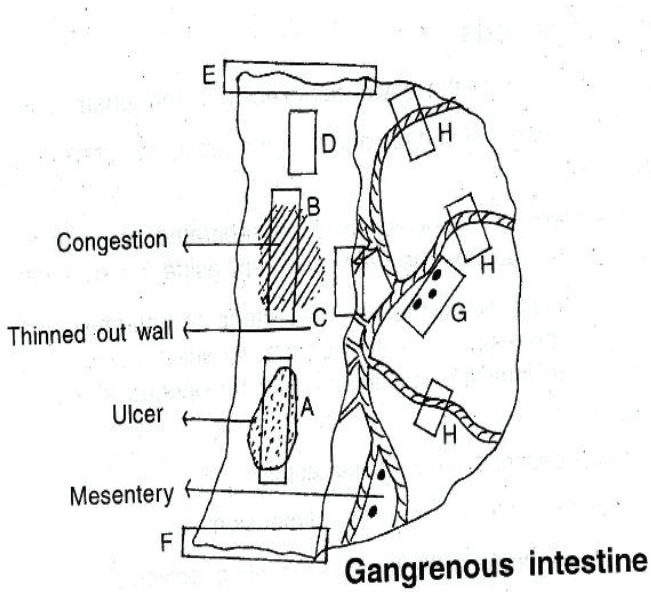


Figure 3.16A

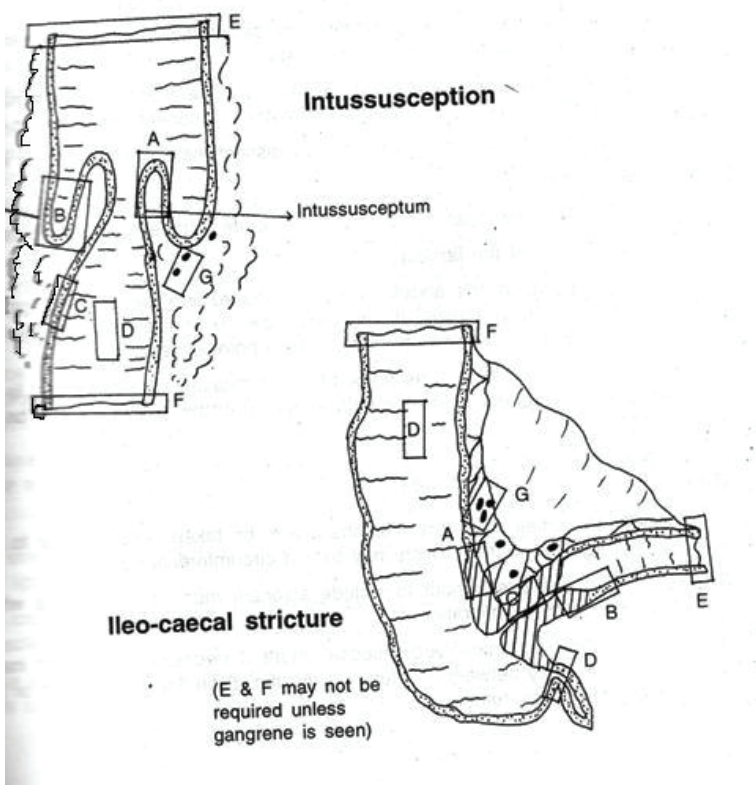


Figure 3.16B

B. Neoplastic lesions

Before going into the grossing of the neoplastic lesions it is essential to know the relationship of the peritoneum to the organs removed during the surgery performed.

The abdominal viscera are divided by their relationship to the covering peritoneum into two main groups: intraperitoneal and retroperitoneal organs. Intraperitoneal organs are enveloped by visceral peritoneum completely like the stomach, liver and spleen.

Retroperitoneal organs are covered by parietal peritoneum anteriorly, and can be further subdivided into two groups based on their embryological development: *Primarily retroperitoneal* organs which remain outside the parietal peritoneum like the oesophagus, rectum and kidneys.

- *Secondarily retroperitoneal* organs were that are initially intraperitoneal, suspended by mesentery but during embryogenesis, they became retroperitoneal as their mesentery gets with the posterior abdominal wall. Thus, in adults, only their anterior surface is covered with peritoneum (see fig 3.17). Examples of these include the ascending and descending colon .

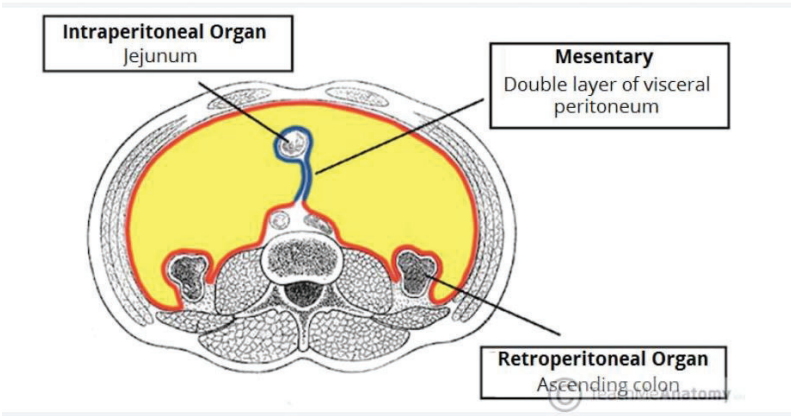


Fig 3.17

Points for Gross Examination & Grossing (Fig 3.18)

- Measure:
  - Length of specimen
  - Tumor size and distance from margins
- Identify:
  - Mesenteric attachment
  - Serosal involvement
- Examine:
  - Tumor type (polypoid, ulcerative, constricting)
  - Depth of invasion

- Lymph nodes:
- Search in mesocolon
- Describe the external surface and look for nodularity, strictures, perforation, adhesions or exudate.
- The mesentery or mesocolon may be separated as close to the intestinal wall as possible if the specimen is fresh and kept aside for examination.
- Cut longitudinally along the anti-mesenteric or anti-mesocolic border as a general rule, unless the lesion is on that border, in which case the opposite border may be chosen. Large intestine specimens may be opened along the taenia coli opposite the tumour.
- Ink the meso rectum and examine for complete/partial resection in cases of rectal resections.
- On opening describe the mucosal aspect:
    - Size of the lesion in two dimensions.
    - Appearance of the tumour (fungating, polypoid, stenosis, ulceration, necrosis,
    - haemorrhage)
    - Location with distance from the pectinate line in abdomino-perineal resections.
    - Maximum depth of infiltration on sectioning - look for involvement of serosa or in
    - the case of rectum, adipose tissue or adjacent organs. Inking at the maximum
    - depth of infiltration should be done.
    - Changes in the rest of the mucosa such as oedema, ulceration, haemorrhage or
    - polyps. If polyps are absent comment on the same.
- In case of deep penetration by tumour dissect the veins for tumour invasion.
- Lymph nodes
    - Number: total number, as well as the number of nodes grossly involved
    - Size of the largest
    - Location: the nodes can be separated into the following groups – around the tumour (level II), proximal (level I) and distal (level III) to the tumour and at the vascular pedicle (high point of resection).
        - Apical lymph node is the first node identified dissecting from the highest point of the vascular ligation. The help of a surgeon may be taken to identify this.
    - Prognosis wise only total lymph nodes involved matters rather than levels; but reporting the level involvement separately helps in planning the field for radiation.
    - The entire lymph node is to be embedded if it measures 5mm or less. In larger nodes one half is to be embedded and the half remaining in the specimen is to be marked with eosin.

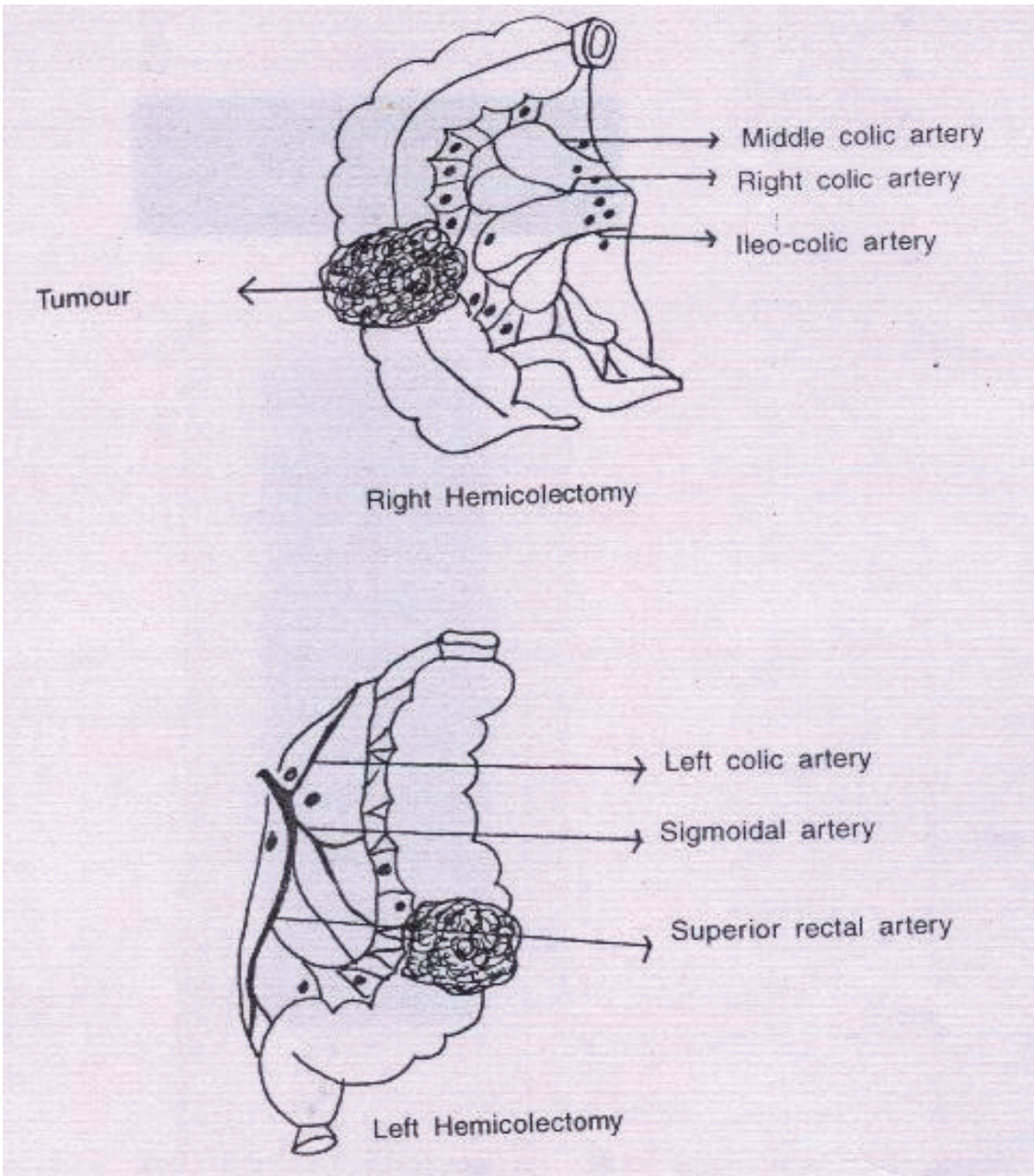


Fig 3.18

Bits (Fig 3.19)

All bits in the small and large intestine are to be taken perpendicular to the mucosal

folds, except surgical margin which may be cut circumferentially.

A, B, C : Three bits tumour to include adjacent mucosa and serosa with maximumdepth of infiltration.

D : One bit uninvolved mucosa (more if necessary). This bit may be taken midway between the tumour and the distal line of resection or 5 cms away from the tumour.

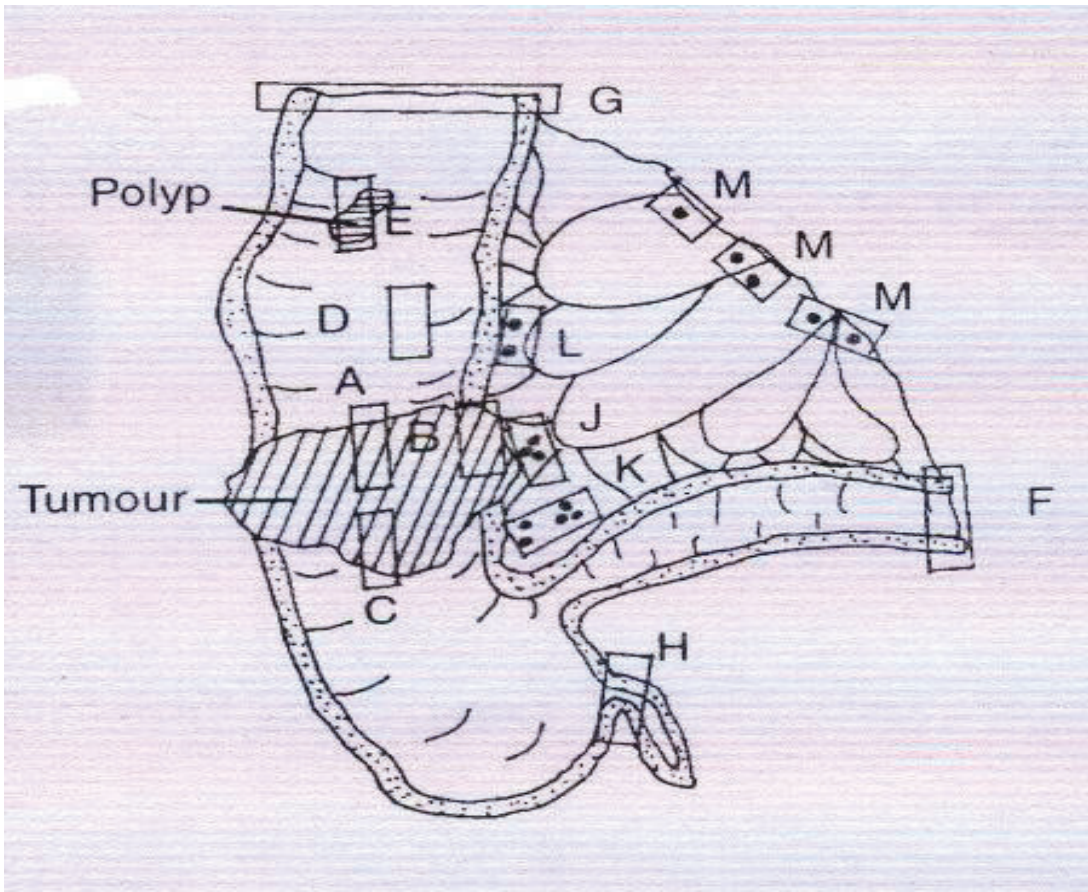


Figure 3.19 Carcinoma in colon, opened out

E : One bit any other lesion if present

F : Two bits proximal surgical margins.

G : Two bits distal surgical margins.

H :One bit appendix or ano-rectal junction whichever is present in the specimen

JKL & more : Lymph nodes

M : Vascular pedicle and lymph node at the high points of resection.

APPENDIX GROSSING TECHNIQUES

- Measure the length of the specimen
- Describe the external surface . fibrinous or purulent exudates,haemorrhage, perforation,
- adhesions or congestion.
- Cut the distal 2 cms of the specimen and divide this distal portion into longitudinal halves.
- The rest of the specimen is to be cross sectioned at 5 mm intervals.
- Examine the lumen for dilatation, ulceration, obliteration, stones or faecolith.
- Examine the wall for fibrosis, tumour etc.

Bits (3.20)

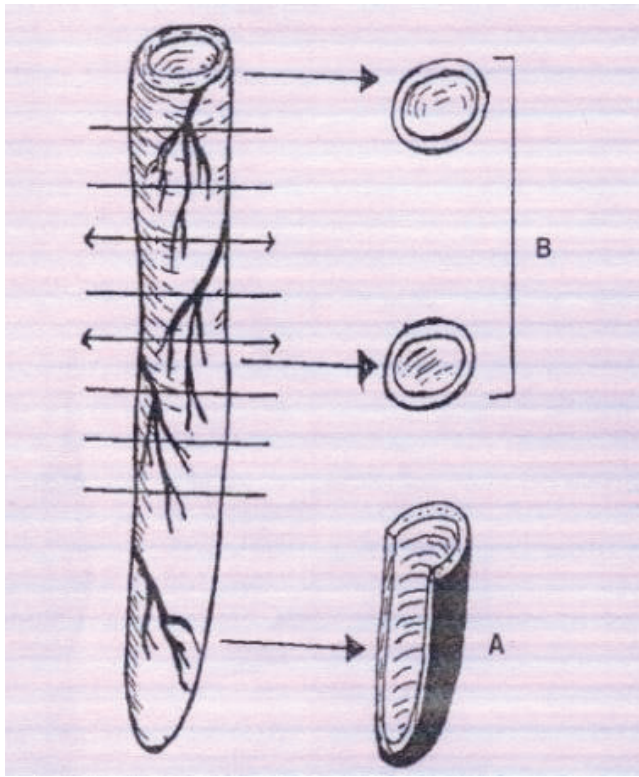


Figure 3.20

A: 1 bit longitudinal section from the tip of appendix

B: 2 bit cross sections - one each from the middle and the resected margins at suspected areas of inflamation or perforation.

Bits to be included from the adjacent soft tissue if involved. More bits to be taken if a neoplasm is suspected.

C III SMALL AND LARGE INTESTINE -GROSS PEARLS

Small Intestine

- *Tubular structure or spherical mass* adjacent to lumen, on closer examination muscle wall is common, lumen of tubular structure may or may not communicate with intestinal lumen : gut duplication.
- *Cap like tubular diverticulum* situated opposite to the mesenteric border, about 2 feet proximal to the ileo-caecal junction and 2 cm in length: Meckel's diverticulum (seen in 2% of population and predominantly in males).
- *Pin point erosions* in the mucosa "apthoid ulcers" : early Crohn's disease
- *Transverse ulcer causing constriction of the lumen:*ulcer of tuberlous etiology (it is transverse due to direction of lymphatic flow)

- *Linear or serpiginous long and deep ulcers :*parallel to one another or connected by short transverse ulcers : Crohn's disease.
- *Coblestone appearance of mucosa* : seen in Crohn's disease (due to lymphoid hyperplasia and submucosal oedema). Crohn's disease affects the terminal portion of the small bowel and shows fibrosis and narrowing of the lumen with dilatation and hypertrophy of the proximal portion ("eel" in rigor mortis appearance). The mesentery is also fibrosed and shortened with piling of the mesenteric fat and corrugated bowel contours. Skip areas are seen with seemingly normal bowel between involved segments.
- *Mucosal deep fissures* : seen in Crohn's and results in fistula formation.
- *Miliary white nodules on the serosa* : seen in tuberculosis and in Crohn's disease. It is due to granuloma formation on the serosa.
- *Prominent brown discoloration of the small bowel* : "Brown bowel syndrome" seen malabsorption due to accumulation of lipofuscin granules in the muscularis propria.
- *Segment in segment appearance* : Intussusception with sausage like appearance – a part of the bowel ie intususceipiens literally swallows up part of the bowel proximal to it. The swallowed portion is called the "intussusceptum"; occurs as a result of a triggering leading point in the bowel like a polyp, lymphoid follicle etc. which leads on due to the peristaltic activity.

Large bowel

The large intestine is identified grossly by the presence of taenia coli ie longitudinal fibrous bands and presence of appendices epiploicae ie fatty protruberances on the serosa.

- *Ulceration of the large intestinal mucosa, diffuse and superficial* ; giving appearance of Persian carpet : bacillary dysentery
- *Multiple flask shaped ulcers* (on cutting through) : Amoebic ulcers
- *Ulcerating and fungating mass* in the intestine : Carcinoma
- *"Napkin ring stricture"* : carcinoma left side of colon (right sided one is usually cauliflower like or polypoid growth).
- *Multiple pouches* in the large bowel, more commonly located in the sigmoid colon with communication with the colonic lumen : multiple diverticulosis
- *Mucosa is covered with mucus and blood, wet and hyperaemic :* acute stage of ulcerative colitis
- *Diffuse ulceration over a segment of large intestine with irregular ulcers* showing undermined edges; elevated sessile reddish nodules and pseudopolyps : established ulcerative colitis. Fibrotic segment , narrowed and shortened with mucosal atrophy : advanced stage of ulcerative colitis.

Appendix

- *Periappendiceal mass :*

  i. Periappendiceal abscess or fibrous induration (ligneous perityphlitis)- consequence of acute appendicitis

  ii. hyperplastic tuberculosis

  iii. Amoeboma

  iv. Carcinoma ileo-caecal region

- *Localised diffuse globular enlargement of the appendix*: lumen dilated and contains a large amount of gliary mucus- LAMN
- *Firm localized grey white tumour at the tip of appendix* or elsewhere in the appendiceal wall, turns yellow after formalin fixation: NET (formerly called carcinoid)

C IV SMALL AND LARGE INTESTINE – MICROSCOPY

INFLAMMATORY, ISHAEMIC AND MISCELLANEOUS LESIONS

1. ***Small intestinal biopsy assessment and interpretation :***
   - Avoid biopsy of the second part of the duodenum or duodenal bulb region where
     - presence of Brunner glands and acid peptic disease changes may distort the villous architecture.
   - For Gluten sensitive enteropathy (GSE) a distal duodenal or proximal jejunal biopsy is preferred;
   - a distal duodenal biopsy is preferred for Whipple's disease from areas which show yellow-white granular spots; changes of tropical sprue are equally prominent in the distal jejunum and ileum;
   - lymphangiectasia intestinalis also shows white spots on the mucosa where it occurs representing dilated lymphatic channels.
   - Giardia, stercoralis strongyloides and AIDS related micro-organisms (cryptosporidium, microsporidium, mycobacterium avium intracellulare) are seen in the duodenum.

i) Gross

All intestinal biopsies sent to the lab should be mounted onto pieces of filter paper with their mucosal side up. This enables fixation in an appropriate manner for proper cutting and orientation of villi.

Take measurements in 3 dimensions

Examine the biopsy under a dissecting microscope if available to determine the mucosal pattern and colour, without crushing or drying the tissue. The pattern is described as finger-like, leaf-like, cerebriform or flat.

Embed the biopsy such that the mucosal side is at right angles to the cutting surface.

ii) Microscopy: Assessment made on the following features:

a) Villous length and architecture : normal is finger-like with minor variations. They tend to be broad and leaf like in the duodenum, finger like and more numerous in the jejunum and club-shaped in the ileum. (see picture in section on grossing).

GSE results in villous blunting consisting of short widened villi or even a totally flat intestinal mucosa. A totally flat intestinal mucosa as seen in GSE is rare in **Tropical sprue.** Villous blunting can also be seen in **Whipple's disease** (club shaped villi), **lymphangiectasia**

(associated with dilated lymphatics in the lamina propria) and in **bacterial overgrowth syndrome.** (Total atropy is rare in these latter conditions).

Villous blunting can be graded as mild, moderate, severe **or** partial, subtotal and severe.

Length of the crypts should be noted and the villous to crypt ratio assessed. Actual measurements are rarely taken and the assessment done on the experience of the pathologist.

Normal villous to crypt ratio is > 2: 1 or 3:1. Overall thickness of the mucosa may remain the same in most conditions but the villous to crypt ratio is altered due to crypt hyperplasia and lengthening.

b) Intra-epithelial lymphocytes (IELs): Normally a small number of lymphocytes (T lymphocytes) inhabit the mucosal epithelium itself. For every 100 enterocytes there are less than 40 lymphocytes ie 1-3 per 10 enterocytes or < than 12 lymphocytes at the tip of each villous. Lymphocytes are increased in the epithelium in **GSE** , these are largely CD3+ve cells with a high proportion of gamma delta subtype. These are characterictically seen on the surface enterocytes of the villi in GSE, as compared to the other conditions like the **tropical sprue and blind loop syndrome** where the IELs may be increased but distributed along the enterocytes at the sides of the villi. While IELs may be mildly increased in the epithelium in **autoimmune enteropathy**, the intense surface lymphocytosis characteristic of GSE is not present and neutrophils are more commonly seen. IELs with neutrophils are also a feature of **bacterial overgrowth syndrome.** Increase in IELs can be seen in several other conditions – H pylori infections, food hypersensitivity and even use of non steroidal anti-inflammatory drugs.

c) Surface epithelial cells **:** Changes encountered are shortening of columnar cells to cuboidal, loss of normal basal nuclear polarity and loss of brush border and cytoplasmic basophilia. These can occur in **GSE, tropical sprue** . An increase in enterochromaffin cells has been reported in GSE whereas patients with autoimmune enteropathy may have a lack of goblet cells, enterochromaffin cells and Paneth cells on the biopsy.

Lipid vacuoles can be seen in the surface cells in **GSE**

d) Inflammatory cells (lymphocytes, plasma cells and eosinophils) in the lamina propria : an increase in the content of these cells is a feature of bacterial overgrowth syndrome, tropical sprue and acrodermatitis enteropathica (zinc deficiency).

e) Dilated lymphatics in lamina propria: can be seen in lymphantiectsia intestinalis and Whipple's disease.

f) Patchy distribution of lesions : lymphagiectasia intestinalis, bacterial overgrowth and whipples disease can show patchy lesions and therefore multiple biopsies may be needed for a diagnosis.

g) Micro-organisms- Special stains help in the identification of these. Modified trichrome stain and Warthin starry stains are used to pick up micro-organisms like microsporidia and cryptosporidium parvum. Giemsa and Trichrome stains pick up Giardiasis, AFB to exclude mycobacterium avium intracellulare and the PAS –d for Whipple's disease.

iii) Salient microscopic features of some common diseases giving rise to malabsorption

Gluten sensitivity enteropathy

Gluten-sensitive enteropathy otherwise known as Celiac disease, is an autoimmune inflammatory disorder of the small intestine that is precipitated by the ingestion of gluten, a component of wheat protein, in genetically susceptible individuals.

Serologic tests for antibodies against endomysium, transglutaminase, and gliadin identify most patients with the disease. Current theory suggests that ingested α-gliadin (a component of the gluten protein) binds with tissue transglutaminase (a ubiquitous intracellular enzyme) in the enterocytes. Deamidation of this enhances the immunogenicity of α-gliadin by creating epitopes that are recognized as foreign by host cell–mediated immunity.

Microscopy :

1) Villous blunting : of varying grades- mild to moderate, total villous atropy may be seen
2) Crypt hyperplasia :Increase in crypt depth with an attendent increase in enterocyte turnover. Overall thickness of the mucosa remains unchanged. Villous crypt ratio altered.
3) Lipid vacuoles in enterocytes – "lipid hang-up"
4) In severely affected cases surface epithelium shows loss of its normal columnar cells, mucin depletion and vacuolation.
5) Increase in the number of IELs > 3 per 10 enterocytes- CD3 cells. This may be the only change in early disease. The lymphocytes are seen characteristically in the surface enterocytes of the villi as compared to other conditions.
6) Changes most prominent in the distal duodenum
7) Increase in the inflammatory cells of the lamina propria

8) Picture is non-specific for diagnosis and should be correlated clinically with serology- increase antigliadin and anti tissue transglutaminase (tTG) anti bodies and response to administration of gluten free diet

9) The changes in the disease are classified by the "Marsh Classification" see fig 3.21.

The Classic pathologic changes of celiac disease in the small bowel are categorised by the "Marsh Classification"

Marsh stage 0 - normal mucosa

Marsh Stage1 --.↑ in intra-epithelial lymphocytes >20/100enterocytes

Marsh Stage 2 – proliferation of crypts of Lieberkuhn

Marsh stage 3 – partial or complete villous atrophy

Marsh stage 4 – hypoplasia of small bowel architecture

Upper Jejunal Mucosal Immunopathology

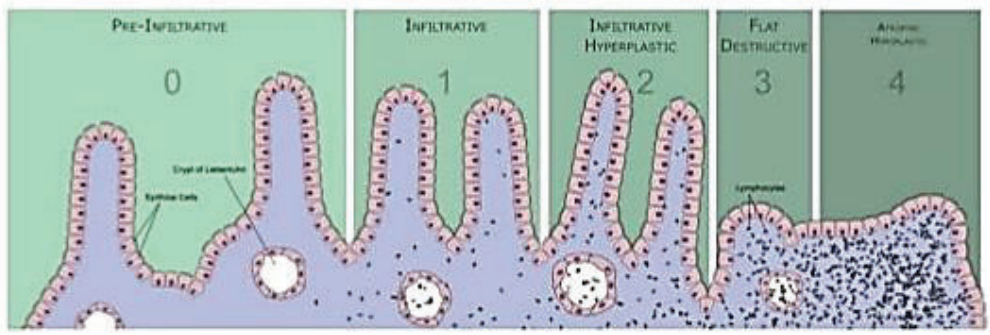


Fig 3.21 Marsh's Classification of mucosal changes in small bowel in Celiac disease (slide share. Net)

1) Collagenous sprue is presently considered a variant of GSE- has a thick collagen band beneath the mucosa which has changes of GSE

2) Look for association with a T cell lymphoma

Autoimmune enteropathy

Another autoimmune disorder which presents with diarrhoea and malabsorption. Biopsy shows :

1) Villous blunting and crypt hyperplasia similar to those seen in GSE but the intractable secretary diarrhoea is unresponsive to gluten free diet.

2) IELs may be increased in the intestinal epithelium but the intense surface

lymphocytosis of GSE is not present. Neutrophils are more commonly seen.

Lymphocytes are seen in the deeper mucosa along with apoptic debris.

3) Neutrophils and plasma cells in the lamina propria

4) Hallmark of disease is presence of anti-enterocyte and anti-goblet cell antibodies. Patients with anti goblet cell anti bodies have lack of goblet cells, enteroendocrine cells and Paneth cells.

Tropical sprue

1) Histologic findings are similar to those of GSE but not identical- totally flat mucosa is rare in TS.
2) Whereas changes of GSE are most predominant in the proximal small intestine the changes of TS are equally prominent in the ileum and proximal jejunum in well established cases
3) Characteristic geographic distribution – tropics. Carribean, northern South America, India and south east Asia supports an infective etiology though no specific causal organism has been elucidated. Broad spectrum antibiotic therapy results in dramatic improvement
4) Overgrowth of aerobic enteric bacteria seen in these patients which are toxin producing. This differs from the overgrowth of anaerobic bacteria such as occurs in the bacterial overgrowth syndrome or stasis syndrome
5) Concomitent folate and B12 deficiency may lead to mucosal atropy manifested by crypts lined by markedly enlarged (megaloblastic) nuclei.

Bacterial overgrowth syndrome :

1) Distal ileum has facultative anaerobic bacteria like Bacteroides, proximal small bowel has aerobes and bacteria ingested with food. This normal ecology can be disturbed in several conditions- stasis, blind loops, altered transit times (gastrectomy, Billroth procedures, AIDS, motility disorders etc) with overgrowth of anaerobes- this leads to diarrhoea and malabsorption.
2) Mucosal histology may be normal or shows villous blunting- mild or moderate
3) Increase in the chronic inflammatory cells of the lamina propria
4) Intraepithelial neutrophils and lymphocytes are increased
5) Findings are patchy- Biopsies taken at the same timefrom different levels may show varied appearances , this helps in distinguishing bacterial overgrowth from GSE
6) In advanced cases brush border damage may occur further compromising sugar, fat and aminoacid uptake
7) Correlation with clinical picture and response to antibiotic therapy confirms the condition.

Lymphangiectasia intestinalis

1) Grossly the intestinal mucosa has a spotted appearance with many white dots (the dilated lacteals) especially at the villous tips
2) Villi are blunted and there is edema of the lamina propria

3) Lymphatic channels are dilated through the bowel wall and lined by endothelial cells. This may extend into the mesenteric lymph nodes as well
4) Lymph channels contain foamy macrophages and proteinaceous debris
5) Lesions can be patchy multiple biopsies are needed for a diagnosis. Secondary lymphangiectasia (due to carcinoma, lymphoma and inflammatory bowel disease) may show diffuse dilation of the lymphatics.
6) Differentials include – Whipples disease, isolated lymphangioma and pneumatosis intestinalis.

Whipple's disease

Systemic infectious disease caused by a gram positive bacillus Tropheryma whipplei and may be related to an underlying defect in the host immune system.

1) Endoscopically the intestinal folds are thickened , sometimes yellow or white deposits are visible
2) Villi are rounded or blunted with many dilated lacteals
3) Lamina filled with pink coloured foamy macrophages. These contain small granules which are positive for d-PAS. The stain highlights the partially digested organisms within the macrophages. Intact rod shaped organisms are present free in the tissue as well and may be seen within other cell types including fibroblasts, plasma cells , lymphocytes, mast cells and epithelial cells.
4) Extracellular lipid is also seen
5) Distribution of lesions may be patchy
6) Differentials include- lymphangiectasia intestinalis, Mycobacterium avium complex infection of small bowel in AIDS patient

Giardiasis

1) Duodenal aspirates in this protozoal disease show the organisms best but they can be seen on routine duodenal biopsies with the Haematoxylin and Eosin stain. The trophozoite form is pear shaped and binucleate giving the appearance of a face with 2 "eyes" staring back at the observer when viewed ventrally; a sickle like shape is evident on lateral view. Trichome and Giemsa stains high light the organisms

2) Accompanying mucosal histology is variable and often normal

3) If abnormal- mild villous blunting is seen or even severe changes as seen in GSE particularly in patients with hypogammaglobulinemia

Acrodermatitis enteropathica

Is an autosomal recessive condition leading to malabsorption of zinc and associated with a variety of skin and mucosal abnormalities

1) Mucosa shows villous blunting and crypt hyperplasia

2) Increase in the lamina propria inflammatory cells
3) Edema may be seen- changes are non-specific

AIDS related infections

1) Immunodeficiency states are commonly associated with malabsorption and diarrhoea often resulting from secondary and opportunistic infections
2) Biopsies show inflammatory changes in the mucosa, decreased lymphoid tissue, altered lymphocyte populations and the presence of infectious organisms- viruses, bacteria, fungi and parasites

    a) protozoal- giardiasis

    b) Coccidial infections- cryptosporidium parvum most common in the small bowel. 2-5 micron basophilic spherical body protruding from the apex of the enterocyte- at the crypts or on the surface. Associated changes include villous blunting, crypt hyperplasia, mixed inflammatory cells and crypt abscesses. Giemsa stains may aid in diagnosis.

- Isospora belli and related species are most often seen in the small bowel but may also be seen in the colon. Histologic changes of mucosa are the same as above, tissue eosinophilia is seen. Intracellular organisms are large and banana shaped- 10 to 12 micron in size. Trophozoites are round with a prominent nucleus. GMS and Giemsa stains aid in diagnosis.
- Microsporidia : Enterocytozoon bieneusi and Encephalitozoon intestinalis are the most commonly seen human pathogens in this group. They are difficult to detect in H&E sections due to the minut size(1micron). A modified trichrome stain, Grams stain (are +ve), Warthin Starry and the Brown and Brenn stains help in picking them up.

2.Colonoscopic biopsy assessment & interpretation

Endoscopic colonic biopsies are usually performed in neoplasia; inflammatory bowel disease such as ulcerative colitis, Crohn's colitis ; in ishaemic colitis, pseudomembranous colitis, and amoebic colitis.

Fiberoptic scopes can reach as far as the terminal ileum. Step wise colonic biopsies in a patient with diarrhea can give important diagnostic information. Microscopic colitis, collagenous colitis and sometimes Crohn's colitis may have normal endoscopic appearances.

i) Ulcerative colitis (UC): - inflammation of rectum and distal colon, may reach a few inches from the caecum. Skip lesions in the appendix may be seen.

- ulcerative idiopathic proctitis or haemorrahgic proctitis is the same disease in a milder form. If rectal involvement is less in UC then it may mean previous treatment with rectal steroid enemas may have been given.
- typical exacerbation and remission

- *acute stage of the first attack* : oedema and vascular congestion of the lamina propria. Increase in the inflammatory cells- lymphocytes, plasma cells & eosinophils in the lamina propria (to be differentiated from infective colitis acute stage where there may be more number of neutrophils).

Polymorphs show margination to capillary endothelium and migrate to luminal surface- crypt abscesses (pus discharge can often be seen on the surface of the mucosa) superficial and crypt epithelium loses goblet cell component as an early feature-mucus depletion, replaced by simple basophilic epithelium.

Glandular tubular / crypt pattern becomes distorted due to inflammation & crypt abscesses.

The inflammation in ulcerative colitis is limited to the mucosa (though in very acute stage the submucosa may show oedema & vascular dilatation)

Ulceration may vary from superficial erosions to huge areas of total mucosal loss . In biopsies the mucosa may be covered by just granulation tissue.

A mild first attack may be followed by complete restoration of the mucosa, usually however there is residual abnormality ie the bases of the tubules have fallen short of the muscularis mucosae inspite of regaining a fairly normal appearance.

- *Recurrent attacks :* loss of tubules branching and irregularity in orientation (crypt distortion) goblet cell replacement occurs as the attack subsides

- *In remission :* tubules fall short of muscularis mucosae
- irregular in shape of tubules and their diameter
- dense collagenisation of the zone of lamina propria just above the muscularis mucosa where successfully regenerating tubules have fallen short of the muscularis mucosae
- *Some patients have the chronic persistent form* **:** mucosa never loses the features of acute inflammation. Features of mixtures of the acute phase & incompletely restored mucosa with chronic phase features of remission, persistent ulceration and attempted healing lead to pseudopolyp formation – evolve as areas of granulation tissue and over exuberant regenerative reaction of mucosal elements.

Sometimes the polyps become large leading to obstruction. Thses changes in a biopsy may cause confusion with malignancy due to the bizarre appearance of the stromal spindle shaped cells with large ganglion like nuclei mostly mesenchymal in origin.

Reactive hyperplasia of the mucosal tissue may result in a macroscopic polypoid state.

Ulcerative colitis in the elderly : onset is abrupt and severe, if the acute phase is overcome, then the further course is milder with a relapse rate. Some authors consider this to be ishaemic colitis.

Ulcerative colitis : chronic quiescent phase : goblet cell population is normal

Abnormal tubular pattern

Zone of collagen above muscularis mucosae is observed.

- *Chronic active ulcerative colitis :* Some tubules with a normal goblet cell population ; others with crypt abscesses; lymphoid hyperplasia with follicles.
- *Long standing ulcerative colitis* : i)Paneth cell metaplasia (more common on the right side of the colon but may be also seen in the rectum)

ii) Increase in the number of argentaffin and argyrophil cell population (using Grimelius stain) may lead to Carcinoid tumour

iii) Increase in eosinophils : this tissue eosinophilia may be used to differentiate UC from crohn's colitis.

- -*Carcinoma and Ulcerative colitis (UC) :* i)Patients with UC of long standing duration (>10years) and extensive or total colitis are prone for cancer.

ii) Patients with extensive colitis and having a genetic predisposition to colorectal cancer. Long standing inflammation is not necessarily of primary importance.

iii) The role of sequential rectal biopsies in evaluating the risk of development of cancer in UC is disputed. At what stage does dysplasia become precancer is difficult to decide for a pathologist to advocate colectomy.

iv)Microscopic change that cautions the onset of cancer as per Riddell, 1976 are

- adenomatous change in mucosa
- basal cell changes
- in situ anaplasia

  -clear cell change
- pancellular change

Adenomatous change is similar to the one that occurs in the colon, but here may occur in flat mucosa as well invillous and polypoid lesions. Increased dysplasia may be seen.

Basal cell change also may occur in flat mucosa; characterized by tubules formed by intensely hyperchromatic cells arranged in a linear fashion parallel to the basement membrane at the basal portion of the cell. Size and shape of cells vary slightly and there is absence of pleomorphism and changes of cell polarity but mitotic figures are increased in numbers. The cytoplasm is eosinophilic and goblet cells are scanty and poorly formed.

In situ anaplasia : occurs only in flat mucosa with basal cell change , single malignant cells are seen with or without signet ring forms.

Clear cell change : may appear innocuous. Macroscopically seen as a raised lesion with appearances similar to a metaplastic polyp.

Pancellular dysplasia also occurs in flat mucosa. Results in abnormal cells from all cell constituents- goblet cells, absorptive cells , Paneth cells and argentaffin cells. Argyrophilic cell dysplasia may predominate.

Squamous cell metaplasia may also occur in UC.

Dysplasia in UC : should be diagnosed in the absence of inflammation. As per the Dysplasia Morphology Study Group a 3 tier class for presence of dysplasia is suggested in inflammatory bowel disease : Positive for dysplasia; Negative for dysplasia and Indefinite for dysplasia. The positive for dysplasia is again subclassed into low grade and high grade. In the low grade dysplasia the nuclei are located towards the basal half of the cell. These same categories in the Ridell's system of classification of dysplasia are as follows – negative for dysplasia, indefinite for dysplasia, low grade dysplasia ro high grade dysplasia. All dysplasias should be diagnosed in the absence of inflammation. In the high grade variety the dysplastic nuclei occupy the luminal side.

Regenerative atypia should not be mistaken for dysplasia. Features favouring dysplasia are conversion of mucin to sialomucin; p53 +vity; and MIB 1 positivity extending through the entire height of the crypt as against seen only in the lower half in normal crypts.

Arguments exist as to the justification of colectomy in cases of dysplasia, as these could be followed up with regular biopsies of the rectal mucosa. Alternatively step wise biopsy of the colon at a distance of 10cm particularly of the flat mucosa may be done either annually, or as per protocols at various Centers and depending on available facilities.

Non –Hodgkin's lymphoma is another complication of long standing UC.

ii) Crohn's Disease :

Crohn's Disease (CD) commonly involves the terminal ileum but any part of the small intestine can be involved . It may involve the mouth, larynx, pharynx, oesophagus, stomach, small & large intestine, anus, musculoskeletal system and skin.

In 15 to 20% of cases it is limited only to the colon and only slightly less common than disease involving the small and large intestine. A diagnosis of CD of the skin, mouth or colon is made with confidence in the presence of small intestinal involvement. Colonic Crohn's alone poses a challenge to both the clinician & pathologist.

If a rectal biopsy is histologically normal and the proximal colon inflamed then the case is unlikely to be UC and more likely to be CD. Stepwise biopsies of the colon is the cornerstone to differentiate UC from CD.

Acute terminal ileitis or acute onset of Crohn's is difficult to diagnose and has to be differentiated from Yersinia enterocolitis.

Microscopic appearances of Crohn's Disease :

All mucosal biopsies should include submucosal tissue. Ideally ulcerative lesions should be biopsied as these yield superior information. Several biopsies from inflamed mucosa and ulcerated mucosa should be done particularly in suspected large intestinal involvement.

i) In the region of ulcers: polymorphs with occasional crypt abscess formation; not as much as in UC. The lining mucosa may show mild loss of mucus; again not as much as in UC. Both crypt abcesses and mucus depletion away from the ulcer are not a feature of CD.

ii) The lamina propria shows an increase in plasma cells and lymphocytes. Inflammation classically involves the submucosa.

iii) Granulomas : loose collections of epitheliod cells and Langhan's type of giant cells are seen in the vicinity of the lymphocytes, more in numbers in the submucosa , but may also occur in the mucosa adjacent to a crypt. Rarely granulomas may be located within crypts. The cell arrangement is not as compact as in sarcoid granulomas. Schaumann bodies may rarely be seen (remember sarcoidosis of the colon is rare but may be seen in the stomach).

iv) Micro-granulomas : collections of histiocytes and lymphocytes with or without giant cells accompanied with patchy mucosal inflammation are diagnostic of Crohn's if foreign body mucin granulomas are excluded. Micro-granulomas measure 80- 150ụ in diameter. and contain between 10-30 immature macrophages. If several biopsies are examined by serial sections then these can be found in 50 to 70% of cases.

v) Irregularity of crypts is mild and seen only near areas of ulceration

vi) Two types of ulcers are seen in CD : those situated on the surface of lymphoid aggregates and those which are linear / fissures. The latter are lined by granulation tissue and pale histiocytes. The first is the histological equivalent of apthoid ulcers seen macroscopically. The early lesion is a microabcess situated over a lymphoid aggregate. The ulcer may be seen at the base of the tubule.

vii) Inflammatory involvement of the rectal mucosa may occur in 45 to 99% of patients with sigmoidoscopically normal mucosa.

Indeterminate Colitis : term used when differentiation between UC & CD is not possible when deep ulceration and submucosal inflammation can occur in deep ulcerative colitis. Some of these may acyually be Crohn's Colitis.

Differences between UC and CD : fig 3.22 shows anatomical segmental distribution of lesions

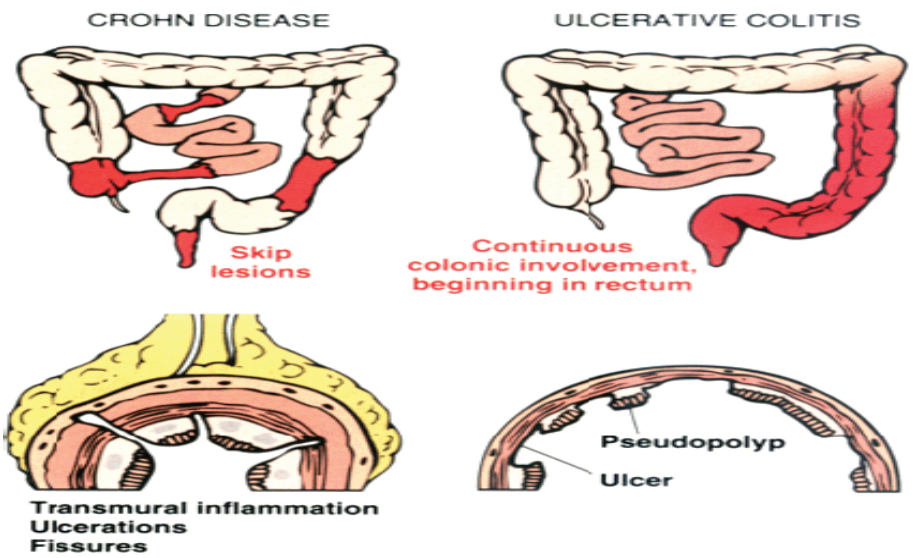


Fig 3.22

Criteria	Ulcerative Colitis	Crohn's Disease
Bleeding	+++	Inconspicuous
Pain abdomen	Left sided	Right sided
Sigmoidoscopy	Abnormal in 95%	Abnormal in < 50%
Perforation	In 12%	4%
Carcinoma colon	5-10%	Rare, can occur
Anal complications	Rare, minor	75%, fissures, fistulas, ulceration
Response to steroids	75%	25%
	Morphology – Gross	
Ileal involvement	Rare, backwash ileitis	Common, constricted
Strictures	Absent	Often present
Skip areas	Absent	Common
Internal fistulas	Absent	May be present
Long & transverse ulcers	Exceptional	Common
Fissuring	Absent	Comon
	Morphology -Microscopy	
Distribution	Diffuse preferably left sided, mucosal +submucosal	Focal, predominantly right sided, transmural
Inflammation	Mucosal	More in submucosa , Transmural
Eosinophils	++	Nil
Mucosal atrophy & regeneration	Marked	Minimal
Mucin	Decreased	Preserved
Lymphoid aggregates	Rare	Common
Edema	Minimal	Marked
Hyperemia	Extreme	Minimal
Granulomas	Absent	In 50 -70% of cases

Crypt abcesses	Common	Rare
Rectal involvement	Always	50%
Ileal involvement	Minimal dilated, not >10 cm	50% constricted, transmural inflammation
Lymph nodes	Reactive hyperplasia	May contain granulomas

iii) Ishaemic colitis : Two types of ishaemic colitis are identified ; *the first one* is due to mesenteric vessel thrombosis / atherothrombus formation resultig in ishaemic injury to that part of the intestine or colon. The most common area involved is the watershed zone between the supply of the superior mesenteric and inferior mesenteric blood vessels ie the splenic flexure. This is more common in the elderly, women on oral contraceptives and sometimes in young men. Also seen in instances of polyarteritis nodosa, surgical procedures on the abdominal aorta, inferior mesenteric vessels, iliac vessels etc.

Patients present with abdominal pain, and bloody diarrhea; the severity of symptoms is related to the extent of injury.

Gross : congested segment of the affected area.

Microscopy : - ishaemic necrosis of the superficial epithelium and a variable extent of the mucosa. If the base of the tubules is preserved then regeneration is complete in 5-7 days. - Microthrombi may be seen.

- Base of tubular involvement results in irregularity of tubules while regenerating.
- In more severe lesions secondary infection and ulceration occurs; the glands and tubules are more irregular in shape
- Fibrosis of the lamina propria occurs with devascularisation.
    - Creeping fibrosis beneath the muscularis mucosae occurs, which does not regenerate
    - Haemosiderin laden macrophages are seen

Second type : Ischaemic entero-colitis , haemorrhagic enterocolitis, uraemic entero

colitis or non occlusive intestinal infarction. Often a terminal condition with bloody diarrhea. May occur in post-operative shock.

No special localization to the splenic flexure; any part of the colon may be involved.

Pathogenesis : partial or complete block of the blood supply ; variations in blood flow due to bacteria, enzymes etc. Vacular thrombosis is secondary.

Microscopy – predominantly haemorrahgic necrosis with pseudomembrane formation. The relationship to pseudomembranous colitis is puzzling.

-mucosa is infarcted and mucosal and submucosal vessels are occluded by thrombi

-The neonatal necrotizing colitis also falls into this category

iv) Pseudomembranous colitis and antibiotic associated colitis :

-was found before the era of antibiotics

-has become more common with increasing use of antibiotics

-can present in many clinical settings after surgery, with diarrhea and mild fever to fulminant illness

Pathogenesis : Swartzman like reaction may be the basic mechanism with absorption of toxin of bacterial origin with or without intestinal flora.

- Clostridium Sordellini / Clostridium Difficale are involved.
- Clostridium difficale produces enterotoxin or toxin A , cytotoxin or toxin B.
- Microscopy : 3 stages of pseudomembrane formation occurs which
- may occur in continuum at the same time.
- type I : fibrin between 2 glands and ulceration of interglandular mucosa
- type II : a few glands may be involved. The base is filled with mucus and the surface shows a fibrinous exudates with polymorphs ie the 'cap lesion'
- type III : fully formed pseudomembrane with structured necrosis of the entire mucosa
- Prognosis : is poor with death occurring in most instances.

v) Collagenous colitis and lymphocytic colitis : -manifests as watery diarrhea in middle aged & older .

- Endoscopy may be normal
- Patchy increase in subepithelial collagenous plate. Normally measures 5µ but in this condition may increase to > 15µ
- lamina propria shows mild to moderate increase in lymphocytes.
- ↑ in intra-epithelial lymphocytes, epithelial degeneration & sloughing
- DD : acute colitis, non specific colitis, Inflammatory bowel disease, mucosal prolapse and ishaemic bowel disease.

Microscopic colitis: considered as a variant or early stage of lymphocytic colitis and represented by epithelial damage & ↑ in intra-epithelial lymphocytes.

3. Ulcerative Conditions of the Small & Large Bowel

Etiology	Location	Gross	Microscopy
Amoebic	Caecum, asending colon, appendix, sigmoid colon	Flask shaped ulcers with narrow neck & broad base. Solitary abscess in liver or multiple discrete abscesses 5-10% perforate	Inflammatory infiltrate. amoeba mimic macrophages, show erythrophagocytosis, PAS & iron Haematoxylin +ve
Typhoid ulcer Salmonella typhimureum, S paratyphi	Ileum & colon	Longitudinal ulcers over Peyer's parches	Mononuclear cell infiltrate(typhoid cells), blunting of villi, mucosal congestion & oedema
Tuberculous ulcer	Mostly Ileum, ileo-caecal junction (may or may not be associated with TB elsewhere)	Transverse ulcer(due to direction of lymphatics); leads to fibrosis causing stenosis & obstruction	Granulomas with or without necrosis composed of epitheliod cells, Langhans giant cells & lymphocytes, AFB may be seen
Hyperplastic tuberculosis	Ileo-caecal junction,	Thickening of ileal and colonic wall, mucosal ulceration, may lead to obstruction due to stenosis	Granulomas with or without necrosis composed of epitheliod cells, Langhans giant cells & lymphocytes, AFB may be seen
Bacillary dysentery Shigella organisms	Distal colon	Mucosa soft & friable, Persian carpet appearance. If severe denuded mucosa over large areas	Irregular ulcers with neutrophils & congestion
Bacillary dysentery Clostridium difficale (toxin)	Colon	Small plaque like lesions yellow white (cap lesion) of fibrino-purulent necrotic debris -Pseudomembrane formation	Mushroom / volcano fibrinous exudates over crypts. - fully formed pseudomembrane with structured necrosis of the entire mucosa in full blown lesion

Etiology	Location	Gross	Microscopy
Bacillary dysentery Campylobacter jejuni	Jejunum to ileum to anus	Multiple superficial ulcers of mucosa	Crypt abscess, ↓ villous to crypt ratio, congestion, ulceration
CMV , AIDS & transplant patients	Ileo-caecal area	Extensive ulceration or discrete mass (inflammatory)	Vasculitis
Behcet's colitis (syndrome)	Large bowel	Multiple ulcers of various sizes, shape & depth	Lymhpocytic vasculitis of the submucosal veins
GVHD	Gastri-intestinal tract	Crypt abscesses, inflammatory polyps, fibrosis	Inflammation
Ulcerative colitis	Recto-sigmoid area, ileum involved in 1/3rd cases	Irregular multiple ulcers, with petechial haemorrhages, pseudopolyps, sessile small & multiple	Crypt distortion, crypt abscesses, hyperemia & edema, mucus depletion, back wash ileitis
Crohn's Disease	Ileum, proximal colon	Early lesions- apthous ulcers, long serpentine linear ulcers connected with transverse ulcers, cobblestone appearance, fissures, skip lesions, fistulous tracts and fibrosis	Non caseating granulomas
Carcinoma	Rectosigmoid, any part of colon	Everted large cauliflower ulcer, usually single, firm	Adenocarcinomatous glands

Key : CMV : cytomegalo-virus; AIDS :acquired immunodeficiency syndrome; GVHD : graft versus host disease; TB : tuberculosis; AFB: acid fast bacillis

4. Eosinophilic gastritis (EG) and gastroenteritis (EGE) : is a condition with diffuse infiltration of eosinophils involving a segment or segments of the gastrointestinal tract. Specific causes of eosinophilic infiltration such as intestinal parasites, bacteria(H pylori), vasculitis, neoplasms, IBD, Hyper Eosinophilic Syndrome, myelo-proliferative disorders and drug reactions must be excluded.

Increased levels of IgE and food specific IgE levels have been found in patients with GE & EGE.

Clinical features depend on the primary site of involvement and the layer of the bowel wall affected. Mucosal, submucosal, muscular and serosal forms are known. Mucosal involvement presents as a inflammatory bowel disease whereas muscular involvement as an obstructive disease. Serosal involvement may give rise to ascitis and high peripheral eosinophilia. Atopic, non atopic & familial forms exist.

Microscopy : It must be remembered that there are no standard criteria for the histological diagnosis of EG & EGE.

- A tissue biopsy is essential to establish the diagnosis. There is an increase eosinophil infiltrate relative to the baseline normalcy for that particular location of the GIT particularly when cells are present in clusters or aggregates.
- increase of eosinophils may be patchy.
- eosinophils in the intra-epithelial compartments (crypts, glands, eosinophilic crypt abcesses
- infiltration of the mucosa associated lymphoid tissue
- eosinophils in the muscularis mucosae and other layers of the wall.
- all causes of tissue eosinophilia mentioned above must be excluded.

Elimination of the offending food , diet modification, anti-inflammatory drugs such as systemic and topical steroids represent the therapeutic approach. EG & EGE appears to be a waxing & waning disease and the involved GIT tract segments may vary from one to another.

Eosinophilic Colitis (EC) : Colon may be involved in isolation or associated with GE involvement . Allergic colitis of infancy also known as food protein induced / milk protein induced proctocolitis is a relatively common form of EC EC is usually a non IgE associated disease. The exact immunological mechanism has not been identified. Cow's milk and soya proteins are most often implicated in allergic colitis of infancy.

Clinical features : diarrhea, weight loss, abdominal pain, anorexia and blood in stools.

Microscopy : Increased eosinophils in clusters in the lamina propria and intra-epithelial compartment (surface & crypt epithelium and in the muscularis mucosae. The stain which makes eosinophils more prominent is the Sirius stain.

DD : ↑ eosinophils of UC and Crohn's disease.

Hypereosinophilic syndrome (HES) : The gastrointestinal tract may be involved in this syndrome. It's features are : i) persistent eosinophilia of >1500/ml for > 6 months

ii) idiopathic in nature (all known causes ruled out)

iii) Signs & symptoms of organ involvement or organ dysfunction attributable to eosinophilia. HES commonly involves the heart, skin, eye, CNS and GI tract.

5. Hirschsprung Disease : is a congenital bowel innervation disorder characterized by the absence of ganglion cells in the myesteric (Auerbach's plexus) and submucasal (Meissner''s plexus) in the distal colon, in it's classic form.

The disease prevalence is estimated at about 1 : 5000 live births. The disease more often afflicts males than females at a ratio of around 4 : 1, may be associated with several clinical syndromes.

Theories of pathogenesis include :

- Failure in migration of neural crest cells from caecum to rectum
- Lack of Meissner & Auerbach plexus in distal intestinal segment
- Absence of co-ordinated peristaltic contractions
- Results in functional obstruction

When the rectum is involved the proximal colon becomes massively distended [mega-colon].Mucosal inflammation & shallow ulcers are noted proximal to the diseased segment. Though imaging plays a role in the diagnosis of Hirshsprung's disease ; anal rectal biopsy has been considered an important diagnostic tool, with accuracy rate of 95%. Moreover, when additional immunohistochemical studies are carried out, the correct diagnosis can be arrived with a high sensitivity of upto 99.7%. Accurate diagnosis depends on the site of biopsy, the representative samples, the number of specimens, and finally the pathologist's reporting skill. If all the criteria are met, the diagnostic accuracy can even reach 100%. Suction is the most commonly used procedure of obtaining the biopsy ; it does not require general anaesthenia. Transmural rectal biopsy is performed if this fails. The diagnosis is confirmed by an area of reduced number or even lack of ganglion cells and significant hypertrophy of nerve fibres up to 1–2 cm from the edge of the pectinate line.

Suction biopsies are more difficult to interpret than conventional biopsies because they show only the surface submucosal nerve plexus. Transmural biopsies with intramuscular nerve plexus present in the specimen pose less difficulty in interpretation. Frozen sections with routine H&E stain should be adequate in experienced hands with the remaining tissue being processed routinely. Application of additional histochemical staining for acetylcholinesterase (AChE) done on frozen sections demonstrates a larger mesh of thick, dense, and irregular nerve fibres within muscularis mucosa of segments with the lesions and confirms the absence of ganglion cells on the frozen section report. Hyperactivity of AChE is pathognomonic of Hirschsprung's disease, enabling paediatric surgeons to proceed with surgery for HD on immediate diagnosis after frozen section. Therefore, histochemical staining together with H + E staining is the gold standard in HD diagnosis.

Neuron-specific enolase antibody demonstrates intensive expression in ganglions, which makes detection of small and immature cells easier, whereas S100 highlights the presence of ganglion cells by Schwann cell expression. Another recommended diagnostic method is histochemical staining with the Diff-Quik method. Ganglion cells show cytoplasm with a light blue colour. The method is easily accessible, inexpensive, and can be performed on frozen tissues directly. If the

characteristic findings of AChE staining are not met, clinicians should consider obtaining additional specimens and should observe the patient rather than doing definitive surgery for HD.

6. Mucosal prolapse Syndrome : Syn : Solitary rectal Ulcer Syndrome, Localised colitis cystica profunda, prolapsing mucosal folds in diverticular disease, inflammatory cloacogenic disease.

- Etiology is abnormal function of anal and pelvic floor muscles during defecation.
- presents with constipation and difficulty in defecation in the 3rd & 4th decade of life. Rectal bleeding ++
- Ulcers may be single or multiple
- Location : anal canal, rectum & sigmoid colon
- Ulcer is seen typically in the anterior / antero-lateral wall of rectum, irregular in shape , well demarcated.
    - Microscopy : some of the various features outlined during the process of

      it's development are :

        - mucosal architectural distortion with the mucosa adjacent to the ulcers showing fibromuscular obliteration of the lamina propria and polypoid appearance due to hyperplastic villiform appearance.
        - inflammation is mild or absent and mucosal capillaries are ecstatic
        - Erosions and acute inflammation with formation of inflammatory pseudomembrane.
        - ulcers show fibrino-purulent debris and granulation tissue
        - the colonic glands get misplaced into the muscularis mucosae or submucosa and may undergo dilatation ie localized colitis cystica profunda
        - the submucosal blood vessels are ecstatic and hyalinised
        - DD : mucinous carcinoma, pneumatosis cystoides intestinalis, chronic ulcerative colitis , Cowden's disease (the polyps are similar to mucosal prolapse syndrome histologically).

7. Polyps of the Intestine : A polyp is a tumorous mass that protrudes into the lumen of the gut. All polyps start as sessile lesions and the traction as it grows results in pedunculated lesions.

Polyps which form as a result of abnormal mucosal maturation, inflammation or abnormal architecture are non-neoplastic and do not have a malignant potential per se. eg hyperplastic polyps.

Those due to proliferation and dysplasia are termed adenomatous polyps / adenomas. They are truly neoplastic and hence precursors for cancer.

Non – neoplastic polyps are :

i) Hyperplastic polyps

ii) Hamartomatous polyps
iii) Inflammatory polyps
iv) Lymphoid polyps

i)Hyperplastic polyps: (regenerative, inflammatory, hyperplasiogenic hamartomatous) About 90% of all epithelial polyps of the stomach and large intestine are hyperplastic polyps.

Hyperplastic polyps in the stomach **:** Have already been described in the section on stomach and occur in a setting of chronic gastritis against a background of hypochlorhydria, low levels of pepsinogen and hypergastrinemia. They are small and sessile and are located commonly in the antrum. They are multiple (>20) in 20-25% of cases. Rarely (2-3.5 % undergo a maliganant change). Larger polyps are lobulated and pedunculated.

Microscopically they show elongation, tortuosity and dilation (often cystic) of the gastric foveolae with a component of pyloric/ fundic glands in the deeper portions. The stroma shows oedema, inflammatory cells, patchy fibrosis and scattered smooth muscle bundles from the muscularis mucosae. These are generally not related to dysplasia by themselves but may be associated due to related dysplastic change and intestinal metaplasia due to chronic gastritis.

In the colon : Found in the older age group, are small (<5mm in diameter), smooth moist protrutions of the mucosa positioned on top of the mucosal folds. May occur singly but more often multiple, more than half occur in the recto-sigmoid.

Classic hyperplastic polyps are non-neoplastic; besides occurring on the left side of the colon, can also occur in the appendix. Some hyperplastic polyps which are large and right sided measuring more than >1cm have an adenomatous change & been reported to have a significant association with right sided colo-rectal cancer (these are true neoplastic polyps and are dealt with later).

Microscopy of hyperplastic polyps : well formed glands with crypts are seen lined by non neoplastic epithelial cells most of which show differentiation into mature goblet/absorptive cells. *The latter predominate*. The delayed shedding of the surface epithelial cells and fusion of crypts creates a serrated epithelial profile akin to the saw toothed appearance of secretary endometrium. *Serration is seen prominently in the top half or top third of the elongated crypts.* Nuclei are basally located and bland in appearance. Paneth cells are identified at the base of the crypts. The columnar cells are eosinophilic. Thickening of the subepithelial collagen layer is seen and mitoses observed in the lower 2/3rds of the crypt.

Hyperplastic polyp type epithelium (serrated epithelium) can be seen in juvenile polyps, Peutz-Jeghers polyps, low grade appendiceal mucinous neoplasia and inflammatory cloacogenic polyps of the anal epithelium.

Fundic gland polyps: are a type of hyperplastic polyp of the stomach. They show the presence of attenuated oxyntic cells lining the dilated glands composing the polyp. They occur either sporadically, in association with long term treatment with proton pump inhibitors or in association with FAP or Z-E syndrome.

A combination of hyperplastic and adenomatous polyps are known as mixed polyps.

ii) Hamartomatous polyps :

Peutz-Jeghers (P-J) polyps*:* are hamartomous polyps which involve the mucosal epithelium, lamina propria and muscularis mucosae. May be single or multiple as part of Peutz –Jegher's syndrome. This is an autosomal dominant syndrome characterized by multiple hamartomatous polyps scattered throughout the gastro-intestinal tract with melanotic mucosal and cutaneous pigmentation around lips, oral mucosa, face genitalia and palmer surfaces of the hands. In the GIT the incidence of P-J polyps in the P-J syndrome is – stomach 25%; colon 30%; small bowel 100%. (Fig 3.23)

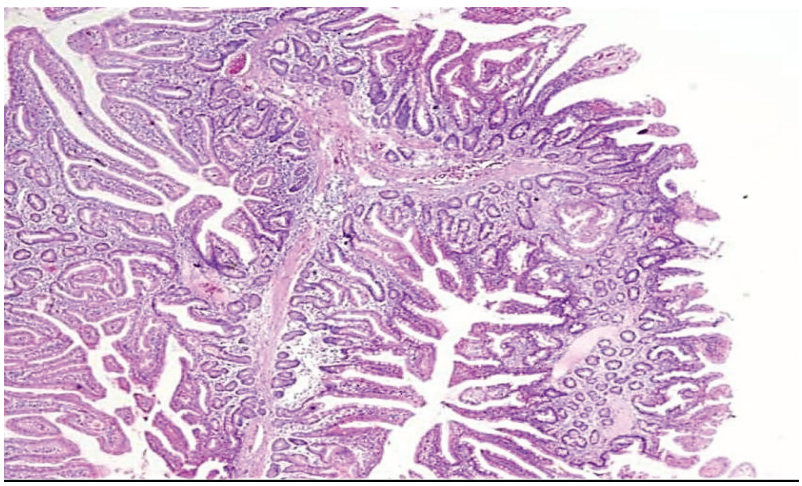


Fig 3.23 : Histology of Puetz-Jeghers polyp in small intestine. H&E x 100

Gross : They are large and pedunculated with firm lobulated contours. Histologically an arborising network of connective tissue and well developed smooth muscle extends into the polyp and surrounds abundant normal appearing glands lined by intestinal epithelium rich in goblet cells. Muscularis mucosa shows a characteristic splaying with muscle extending into the mucosa and surrounding individual crypts.

By themselves these polyps have no malignant potential. Patients with the syndrome have an increased risk of carcinomas of the pancreas, breast, lung, ovary and uterus, sex cord tumours of the ovary, adenoma malignum of the uterine cervix and Sertoli cell tumours of the testis. When GIT adenocarcinomas occur from concomitant adenomatous lesions the underlying genetic basis of the P-J syndrome is the mutation of the gene STK 11 (LKB1) located on chromosome 19.

Juvenile polyps: are polyps that represent focal hamartomatous malformations of the mucosal epithelium and lamina propria. Sporadic cases occur in children < than 5 years. Isolated hamartomatous polyps are identified in the colon of adults when they are called retention polyps. 80% of cases occur in the rectum but they may be scattered throughout the colon.

Gross : 1-3 cm in size, rounded smooth or slightly lobulated with short stalks. Retention polyps are < 1 cm in diameter.

Histology : shows abundant lamina propria with cystically dilated glands, numerous inflammatory cells and a congested and ulcerated surface. Single lesions show no malignant potential.

Rarely an autosomal dominant juvenile polyposis syndrome occurs with about 50-100 juvenile polyps in the GIT. This carries the risk of associated adenomas and thence adenocarcinomas. Mutations in SMAD 4 / DPC4 gene are responsible for juvenile polyposis syndrome.

Criteria for diagnosing juvenile polyposis syndrome : a) more than 5 juvenile polyps of the colon & rectum or b) juvenile polyps throughout the entire GIT or c) any number of polyps in a patient with a family history of juvenile polyposis. Adenomatous change is noted in 15 to 30% of juvenile polyps in a patient with juvenile polyposis.

Cowden's syndrome*:* Hamartomatous polyps are part of genetic syndromes such as the Cowden's syndrome which is an autosomal dominant genetic syndrome characterized by multiple hamartomas involving organs derived from all the three germ layers. Commonly involved sites are the GIT and mucocutaneous locations. Intestinal hamartomatous polyps (in 35% of cases), facial trichilemmomas, acral keratosis and oral papillomas are seen. The syndrome as a whole predisposes to breast and thyroid cancers. There is a germline mutation of PTEN gene located on chromose 10.

Canada Conkrite Syndrome *:* is an ectodermal non- hereditary disorder, constituted by alopecia, skin hyperpigmentation and dystrophic changes in the finger and toe nails associated with GI tract hamartomatous polyps.

iii) Inflammatory fibroid polyp syn : eosinophilic granuloma. These are mesenchymal proliferations composed of a mixture of stromal cells, small vascular channels and inflammatory cells particularly eosinophils. They are considered as pseudotumours and occur as a bulky submucosal tissue prominences, stretching the mucosa above and replacing the muscularis propria. The eosinophils seen infiltrating the polyp, are not associated with tissue eosinophilia.

The polyps can occur anywhere in the GI tract. In the stomach they are seen usually in the antrum. In the small intestine the polypoid masses may present as a cause of intussusception. Larger lesions lead to intestinal obstruction. IHC on the polyp shows : CD34+ve, CD64+ve, Vimentin +ve, α SMA +ve, desmin +ve and factor XIII A +ve.

iv) Lymphoid polyps : reactive lymphoid tissue presenting as pseudo-polyps should be distinguished from true neoplastic polyps and lymphomas of the gastro-intestinal tract.

Neoplastic polyps :

i) Adenomatous polyps
ii) Familial adenomatous polyposis
iii) Serrated lesions with and without dysplasia
iv) Mixed hyperplastic and adenomatous polyps
v) Hereditary non-polyposis colon cancer
vi) Lymphomatous polyposis

i) **Adenomatous polyps :** Intra-epithelial neoplasms that range from small often pedunculated lesions to large neoplasms that are usually sessile.

Prevalence: 20-30% before age 40 and 40-60% after age 60. The male to female ratio is equal. 90% of adenomas occur in the colon, constitute 5-10% of the polyps in the stomach and may also be seen in the small intestine (particularly in the vicinity of the Ampular of Vater). In the stomach they are common in the antrum, are sessile or pedunculated and 3-4 cm in size. They commonly arise in a background of intestinal metaplasia and show some degree of intestinal differentiation with absorptive cells, goblet cells, endocrine or even Paneth cells. They are therefore indistinguishable form colorectal adenomas and like them can be categorized into 3 groups.

By definition an adenoma contains proliferative dysplastic epithelium [which ranges from low grade to high grade (carcinoma in situ)] and thereby has malignant potential.. A well defined familial predisposition to sporadic adenomas is known and there is a 4 fold greater risk in first degree relatives with a 4 fold greater risk of colorectal cancer.

Three types of adenomas/ adenomatous polyps are seen :

a) Tubular adenomas - > 75% should have a tubular architecture

b) Villous adenomas > 50% should have a villous architecture.

c) Tubulo-villous adenomas 25-50% of the lesion should have a villous architecture.

Tubular adenomas are the most common, 5-10% are tubulo-villous and 1% are villous.

“Flat adenomas” can only be identified by histologic examination. These are also called “depressed adenomas” or “microscopic adenomas”. An invasive adenocarcinoma may be encountered in an adenoma of any subtype. The risk of malignancy is related to the size, degree of dysplasia and villosity of the growth pattern. Adenomas less than 1 cm in size have a low risk of malignancy whereas those greater than 2 cm are more frequently villous and a significant number of these (about 40%) contain invasive carcinoma. ‘Advanced adenoma’ is a term used for adenomas more than 1 cm in size, tubulo-villous or villous architecture and/or high grade dysplasia or intramucosal carcinoma. The excision of advanced adenoma is the best step in prevention of carcinoma. Advanced adenoma is also associated with synchronous (simultaneously occurring adenomas or those picked up 6 months after the diagnosis of primary adenoma or colorectal cancer) thereby altering the clinician towards a full surveillance of the colon.

Gross : Occur either singly or 2 or more distributed randomly. Smaller ones are smooth contoured and sessile. Larger ones are coarsely lobulated with slender stalks. Uncommonly they are > 2.5 cm in diameter. The stalk has a fibromuscular core covered at the sides by non-neoplastic mucosa, however in the stomach the adenomatous mucosa may extend down the stalk and into the adjacent area.

All polyps are composed of neoplastic (dysplastic) epithelium which lines glands as tall hyperchromatic disorderly epithelium that may or may not show mucin vacuoles. In a benign tubular adenoma the degree of dysplasia is low grade and the glands are separated by stroma.

High grade dysplasia may merge with areas of overt malignant change confined to the mucosa (intramucosal carcinoma). ***Invasion into the stalk constitutes invasive carcinoma.***

Villous adenomas: are larger and the more ominous of the epithelial tumours. They occur in older persons and are more common in the rectum and recto-sigmoid, though they can also occur elsewhere. Generally sessile, upto 10 cm, velvety or cauliflower like masses projecting 1-3 cm above the normal mucosa.

Histology shows frondlike villous extensions covered by dysplastic very disorderly columnar epithelium. Invasion leads to direct invasion into the wall of the colon either into the submucosa or deeper due to lack of a stalk.

Tubulo-villous adenomas : are intermediate between tubular and villous lesions in their

malignant potential. Invasive adenocarcinoma is found in approximately 5% of all adenomas. It is size and tumour type dependent, the highest incidence being in tumours larger than 2 cm and in the villous component.

ii) **Familial adenomatosis polyposis :** It is an autosomal dominant disorder with a high degree of penetrance. In this condition the large intestine is carpeted with adenomas (100 to 1000 or more in number.) Familial adenomatous polyposis (FAP) is a generalized growth disorder that includes intestinal polyposis as well as numerous extracolonic manifestations. Overlap exists among the different adenomatous polyposis syndromes, and the varied extraintestinal manifestations of FAP have led to confusion in their nomenclature.

Gardner syndrome was the term applied to patients with colonic polyposis, epidermoid cysts, osteomas, and desmoids.

Turcot syndrome was diagnosed if the patient had colonic polyposis with brain tumors. The current understanding of these disorders indicates that FAP, Gardner syndrome, and Turcot syndrome all represent variations of the same disease rather than distinct entities, and result from defects in a single pleiotropic gene with variable expressivity**.** Diagnostic criteria for FAP have been outlined and include either one of the following : i) 100 or more polyps ii) germline mutation in APC (adenomatosis polyposis coli) gene on chromosome 5q21. iii) family history of adenomatosis polyposis coli iv) at least one of the following- epidermoid cyst, osteoma, fibromas, super-numery teeth, congenital hypertrophy of retinal epithelium, dermoid tumours. K-ras mutations are present in one fourth of cases. The average age of the patient when the syndrome is detected is the second decade. A PCR technique diagnoses the pre-symtomatic disorder. Abut 90% of patients develop small bowel adenomas (in duodenum) as well as gastric polyps (fundic gland polyps and adenomas).

Carcinomas arising in the background of familial polyposis occur on an average 20 years earlier than colorectal carcinomas presenting in early 30s. Prophylactic colectomy is performed at 20-25 years of age latest. A close monitoring is required after surgery as polyps and carcinomas can arise in the rectal stump if ileo-proctoscopy is performed.

An attenuated form of the disease is associated with relatively few adenomas that are typically right-sided and flat. Cancers developing in these are also right sided and later in onset than the classical form.

iii) Serrated Lesions : Serrated polyps are a heterogenous group of polyps with morphological features characterized by a serrated or **" Saw tooth "** appearance of the surface of mucosa (similar to secretory endometrium).

The subtypes of serrated lesions of the colorectal region are :

i) Hyperplastic polyp, microvesicular type (MVHP)
ii) Hyperplastic polyp, goblet cell -rich type (GCHP)
iii) Sessile serrated lesion (SSL)
iv) Sessile serrated lesion with dysplasia
v) Traditional serrated adenomas (TSA)
vi) Serrated adenoma unclassified

Hyperplastic polyps : were formally perceived as harmless lesions without any potential for malignant progression mainly because hyperplastic polyps lacked cytological atypia. However it was recognized that some of these lesions, represented a heterogeneous group of polyps with characteristic serrated morphology which showed a significant risk of neoplastic progression. These serrated lesions showed characteristic epigenetic alterations not commonly seen in colorectal adenomas and progressed to colorectal carcinoma via the so-called serrated pathway (CpG-island-methylation-phenotype pathway).

Of the serrated lesions the hyperplastic polyps constitute 80-90%; sessile serrated adenomas constitute 15-20%; and traditional serrated adenomas are rare and constitute only 1-2%.

Hyperplastic polyps are of 2 types _ Microvesicular hyperplastic polyps and Goblet cell rich hyperplastic polys.

Location : The majority of hyperplastic polyps, upto 90%, both the MVHPs and GCHPs are found in the distal colon and rectum

Microscopy : Described above; and also given in the table below are the microscopic features of the 2 different types of hyperplastic polyps.

*Sessile serrated lesions***:** The WHO 2019 classification of gastro-intestinal neoplasia recommends the term 'serrated lesions' instead of 'serrated adenomas' The preferred name in the recent WHO classication is serrated lesion, as these may be flat rather than polypoid, and the association with BRAF or KRAS mutation delineates two separate neoplastic pathways. MVHP is considered a precursor to SSL.

Localization: right colon including appendix ; 70-80 % of SSLs particularly those with dysplasia have a predilection for the proximal colon.

Gross : Sessile ; are larger , diameter >5 mm

Microscopy : At low power the lesions have elongated and dilated crypts, are more Villiform and have a more complex branching pattern as compared to hyperplastic polyps.

Columnar cells predominate over goblet cells with a highly eosinophilic cytoplasm. They lack the Thickened subepithelial collagen layer seen in hyperplastic polyps. Nuclei show elongation, an increase in nucleo-cytoplasmic ratio, nucleoli and atypia. Features do not meet criteria to match either an adenomatous or hyperplastic polyp. In a nut shell serrated adenomas show -

a. hyper serration : serration in the lower third of the crypt as compared to serrations in the upper part as seen in hyperplastic polyps.
b. more villiform with a more complex branching pattern as compared to hyperplastic polyps dilatation of crypts most prominent at the base.
c. horizontally oriented dilated crypts just above the musularis mucosae (classically L-shaped (boot shaped) and T- shaped.
d. nuclear atypia with vesicular round to ovoid nuclei with nucleoli focal mucin over-production that resembles LAMN of the appendix eosinophilia of the columnar cell cytoplasm. Columnar cells predominate over goblet cells, though mature goblet cells are at the base of the crypts.
e. lack the thickened subepithelial collagen layer seen in hyperplastic polyps nuclei are elongated to oval to round, with an increased nucleo-cytoplasmic ratio, nucleoli and atypia.

In serrated lesions with dysplasia, the dysplasia may take the form of *conventional adenoma* like dysplasia, or *serrated dysplasia*. The dysplastic area is well demarcated from the adjacent non-dysplastic mucosa.

Serrated adenomatous polyposis : WHO criteria for hyperplastic polyposis : a) atleast 5 histologically diagnosed hyperplastic polyps proximal to the sigmoid colon of which 2 are larger than 10mm or b) any number of hyperplastic polyps occurring proximal to the sigmoid colon in someone who has a first degree relative with hyperplastic polyposis; or c) more than 30 hyperplastic polyps of any size that are distributed throughout the colorectum. The number of polyps have varied from numerous to hundreds. Patients with hyperplastic polyposis have associated colorectal carcinoma & family history of colorectal carcinoma. Serrated adenomas and classic tubular adenomas may also be present.

Traditional serrated adenomas : Constitute 0.6 to 1.3 % of polyps examined histologically.

70 % of TSAs occur in the distal colon and rectum. Can arise denovo; GCHPs and a few MVHPs give rise to TSA. Reported in FAP who have < 100 polyps with a mutation at 3' and 5' end of APC protein (attenuated APC). Arise through high frequency MSI (MSI-H) and low frequency MSI (MSI-L) pathways serving as MSI-H & MSI-L cancers.

Serrated adenomas can have areas of traditional adenomas and high grade dysplasia.

Gross : pedunculated or semi-pedunculated /sessile with a mean size of 8.6 mm (2mm to 10mm)

Microscopy :

- Prominently Serrated surface similar to SSLs
- Diffuse eosinophilic cytoplasm and centrally placed palisaded penicillate nuclei
- Ectopic crypt formation (ECF) in the upper part. (Ectopic crypts are those that do not reach the base ie do not reach upto the muscularis mucosae
- Dysplasia / IEN may accompany the above changes.

Salient features of various serrated lesions :

Lesion	Crypt architecture	Prolifera-tion zone of crypts	Cell morpho-logy	Mucin type	BRAF Mut	KRAS Mut	GNAS Mut	Cp-G island methylation
MVHP	Funnel shaped crypts with serrations limited to upper third or half	Basal portions of the crypts	Small basally located nuclei; no dysplasia	Microvesicular & goblet cell type	70-80%	-	-	+
CGHP	Elongated and enlarged crypts; little or no serration	Basal portions of the crypts	Small basally located nuclei; no dysplasia	Goblet cells predominant	-	50%	-	-
SSL	Dilatation of lower crypt base extending horizontal above mus Mucosae. See description above	Located away from crypt base, Extends up, variable from crypt to crypt	Small basally located nuclei; occasional large nuclei; no dysplasia	Mixed microvesicular & goblet cell Type	>90%	-	-	++
SSLD	Complex architecture in the dysplastic component – crypt crowding, branching and cribriform-ing; villous architecture	Like SSL but more proliferation in dysplastic component	Varied morphology of dysplastic component (serrated and conventional dysplasia)	Varied type	>90%	-	-	+++

TSA	Slit like serrations; ectopic crypts that fail to reach the mus mucosae	In ectopic crypts and crypt bases	Columnar cells with eosinophil-lic cytoplasm. Elongate penicillate nuclei with nuclear stratification.	Occasional scattered goblet cells; rare goblet cell type described	20-40%	50-70%	-	+
LAMN	Serrations/ undulating mucosa ; absent mus m;	Low grade neoplasm; No obvious proliferatinoted	Elongated nuclei; Bland morphology		+		+	-

Molecular events in serrated neoplastic pathway (excluding LAMN) :

- Two pathways : BRAF and KRAS (see fig 3.24)
- SSLs develop from normal mucosa (denovo) or from MVHP. They have BRAF mutations.
- In SSLs (serrated dysplasia), CIMP* ie 'CpG island methylation phenotype' leads to MLH1 methylation (MLH1 silencing). This MLH1 methylation progresses to mismatch repair deficient colorectal carcinoma.

  Alternatively TP53 mutation in SSL (conventional dysplasia) leads to mismatch proficient colorectal carcinoma.

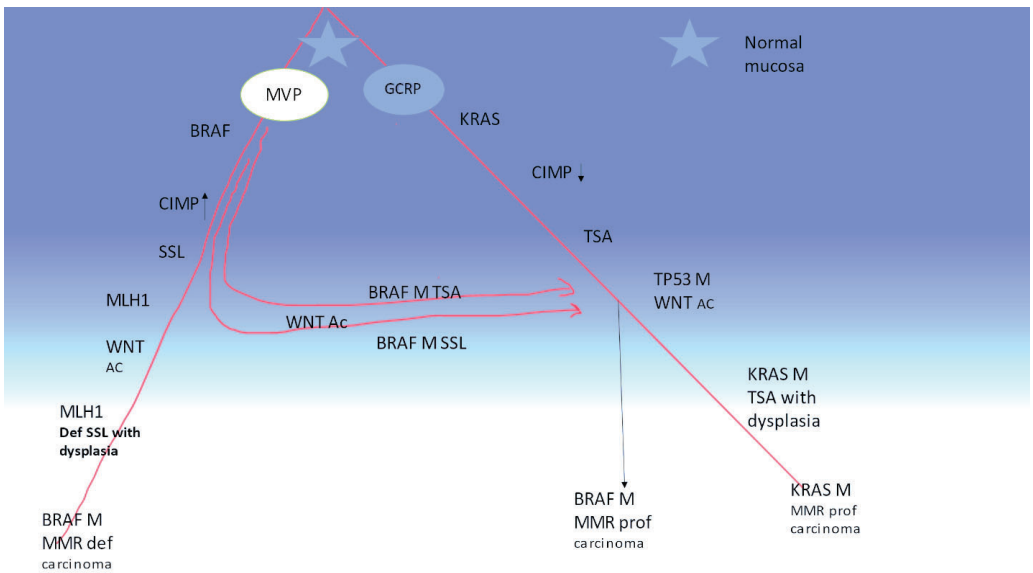


Fig 3.24 : Left limb – BRAF mutation (SSL); right limb KRAS mutation (TSA.) Horizontal limb shows BRAF mut TSA and some BRAF mut SSL leading to BRAF mut MMR prof carcinoma.

Key: WNT Ac : WNT activation; CIMP : CpG island methylation phenotype

- TSA develop donovo from normal mucosa, or from SSL, or from GCHP. These have KRAS mutations. Those derived from SSLs show BRAF mutations. Both BRAF mutated and KRAS mutated TSA lesions undergo TP53 mutation and progress to high grade dysplasia leading to mismatch proficient colorectal carcinomas.
- WNT signalling pathway activation through different mechanisms in transition to dysplasia.

CIMP* : CpG islands are dinucleotide islands located within promotor regions. CIMP (CpG island methylator phenotype) is defined as widespread hypermethylation of CpG islands seen in clinically distinct subset of cancer patients [25]. Hypermethylation of CpG nucleotides results in reinforcement of repressive characteristics & steric occlusion of transcription factor binding reducing gene expression. Repression results in 'critical hit' eg BRCA1 silencing in breast carcinoma, MLH1 silencing in colorectal carcinomas.

MLH1 silencing as seen above in SSls inhibits DNA repair leading to microsettelite (MSI) instability and cascades into abnormal functional consequences.

CIMP levels of methylation are described as CIMP high (seen in SSls) and CIMP low (as seen in TSA).

iv) **Mixed hyperplastic and / or adenomatous polyps** : Polyps with distinct areas of classic adenomas, hyperplastic polyps or serrated adenomas occur.

v) **HNPCC :** Lynch syndrome, often called hereditary nonpolyposis colorectal cancer (HNPCC), is an inherited disorder that increases the risk of developing several carcinomas, colon , as well as endometrial carcinoma, ovary, stomach, small intestine, hepatobiliary tract, upper urinary tract, brain, and skin.

vi) **Lymphomatous polyposis (LP)** is generally thought to be an expression of non-Hodgkin's lymphoma (NHL) of follicular mantle cell (MC) origin or germinal cell origin .

It occurs as multiple polyps and is a distinct clinicopathological entity. It is distinct from the recently described clinicopathological forms of centrocytic lymphoma and intermediate lymphocytic lymphoma, both of which show extensive peripheral lymphadenopathy and splenomegaly, but it is probably closely related to them. The differences are probably attributable to distinct cell tropism or homing properties rather than to cellular histogenesis or degree of maturation resulting in their gastro-intestinal location.

8.Vienna classification of Gastro – intestinal epithelial neoplasia:

The Vienna Classification of Gastrointestinal Epithelial Neoplasia is the first attempt at the standardization of the histologic criteria in various anatomic locations of the GIT. Most important advantage of the Vienna Classification is that the various categories in whichever segment of the tract they may occur are associated with different recommendations for further therapeutic indications. The proposed classification is a five categories system.

CATEGORY 1: NEGATIVE FOR NEOPLASIA/DYSPLASIA

This category encompasses either completely normal gastric mucosa or various gastritides (Helicobacter pylori gastritis) or gastropathies (bile reflux gastritis/non-steroidal anti-inflammatory drug (NSAID) related gastropathy) as well as intestinal metaplasia

CATEGORY 2: INDEFINITE FOR NEOPLASIA/DYSPLASIA

This diagnosis should be rendered when there is uncertainty about the nature of the lesion, whether it represents a regenerative/reactive process or a neoplastic lesion.

The cause of the diagnostic difficulty might be secondary to inflammation (sometimes related to H pylori gastritis) or reactive chemical changes (bile reflux gastritis, NSAID gastropathy) or because of the inadequacy of the specimen (small size, crushing artefact).

CATEGORY 3: NON-INVASIVE NEOPLASIA, LOW GRADE (LOW GRADE ADENOMA/DYSPLASIA)

This represents an unequivocal neoplastic process with low risk of malignant degeneration. It roughly corresponds to the generally previously accepted low grade dysplasia.

CATEGORY 4: NON-INVASIVE NEOPLASIA, HIGH GRADE (HIGH GRADE ADENOMA/DYSPLASIA)

These are a group of lesions with features more severe than those seen in low grade dysplasia but without unequivocal invasion. This category has been subdivided into three groups:

- 4.1 high grade adenoma/dysplasia;
- 4.2 non-invasive carcinoma (carcinoma in situ);
- 4.3 suspicious for invasive carcinoma.

The grouping of those three different types of lesions is important in order to achieve a high interobserver agreement among pathologists, whether under Japanese or Western influence in terms of diagnosis of gastric dysplasia. The word “non-invasive” stands for the absence of evident invasion.

It places together a lesion that Japanese pathologists would classify as carcinoma based on the presence of notable cytological alterations (carcinoma in situ) but that most Western pathologists would interpret as high grade dysplasia because of the absence of invasion.

CATEGORY 5: INVASIVE NEOPLASIA

This category encompasses:

- 5.1 intramucosal carcinoma
- 5.2 submucosal carcinoma or beyond
    - 5.2.a “Non-invasive” stands for absence of evident invasion.
    - 5.2.b “Intramucosal” stands for invasion into the lamina propria or muscularis mucosae.

Intramucosal carcinoma corresponds to adenocarcinoma with invasion limited to the lamina propria or the muscularis mucosa.

It was also hoped that this classification could be applied throughout the gastrointestinal tract.

Several working groups have determined the feasibility of this system in different segments of the gastrointestinal tract including squamous oesophagus, Barrett's oesophagus, stomach, small bowel, large bowel, and anus.

a) that there is consistency of terminology in different organs within the gastrointestinal tract,
b) its acceptability by the pathology and gastroenterology communities at large if all of these problems are resolved.

9. WHO CLASSIFICATION OF SMALL INTESTINE AND AMPULLA TUMOURS (5th edition)

I. EPITHELIAL TUMOURS

Benign epithelial tumours and precursors :

Adenomatous polyp low grade

Adenomatous polyp high grade

Intestinal adenoma low grade

Intestinal adenoma high grade

Serrated dysplasia low grade

Serrated dysplasia high grade

Non invasive pancreatico-biliary papillary neoplasm with low grade dysplasia

Non invasive pancreatico-biliary papillary neoplasm with high grade dysplasia

Intra-ampullary papillary -tubular neoplasm.

Malignant epithelial tumours

Adenocarcinoma, NOS

Mucinous adenocarcinoma

Signet ring cell carcinoma

Medullary carcinoma

Adenocarcinoma intestinal type

Pancreatico-biliary type carcinoma

Tubular adenocarcinoma

Neuro-endocrine tumour, NOS

Neuro-endocrine tumour grade 1

Neuro-endocrine tumour grade 2

Neuro-endocrine tumour grade3

Gastrinoma , NOS

Somatostatinoma, NOS

Enterochromaffin cell carcinoid

Extra-adrenal paraganlioma NOS

Neuroendocrine carcinoma, NOS

Large cell neuroendocrine carcinoma

Small cell neuroendocrine carcinoma

Mixed neuroendocrine-non -neuroendocrine neoplasm

(MiNEN)

POLYPS

Hyperplastic

Peutz-Jeghers

Juvenile

Tumours of the small intestine and Ampulla :

Neoplasms/Carcinoma of the small intestine are rare as compared to those of the stomach and colon. They constitute < 5 % of the total carcinomas in the gut. Among the segments of small intestine involved, the duodenum is highest, followed by the jejunum and then ileum. In the duodenum most of them arise from the ampulla.

Ampullary adenoma : Is a rare polyp consisting of dysplastic glandular epithelium within the anatomical duodenal ampulla. Subtypes include intestinal type adenoma, low grade; intestinal type adenoma high grade; non invasive pancreatico-biliary papillary neoplasm with low grade dysplasia; non invasive pancreatico-biliary papillary neoplasm with high grade dysplasia; and intra ampullary papillary-tubular neoplasm.

Location and histology : those arising within the ampullary duct show a 'pancreatico-biliary type carcinoma' and are called ampullary ductal carcinomas. The histology of these varies from differentiation towards gastric, intestinal or pancreatico-biliary. Most show mixtures of papillary-tubular pattern. Those arising from the duodenal mucosa over the ampulla are called 'peri-ampullary duodenal' and show an intestinal type morphology; their prognosis is better than the ampullary-ductal carcinomas. May be sporadic or part of the familial polyposis syndrome.

Non ampullary adenoma : is a polyp of the intestine which shows dysplastic glandular epithelium with intestinal type or pyloric gland differentiation. Subtypes include intestinal type adenoma, lowgrade; intestinal type adenoma high grade; serrated dysplasia low grade; serrated dysplasia high grade.

Location and histology : neoplasms can arise any where in the intestinal mucosa but majority arise in the duodenum (detected by endoscopy). Pyloric gland adenomas (PGAs) are situated mostly in the upper duodenum. Adenomas located in the jejunum and ileum present late sometimes after progression to cancer.

Intestinal type adenomas show intestinal type differentiation with a tubule-papillary pattern, lowgrade dysplasia and may show Paneth, goblet or endocrine differentiation. PgAs consist of closely packed pyloric type glands lined by cuboidal to low columnar cells with a foamy cytoplasm. These lesions may be associated with foveolar or Brunner gland hyperplasia and have been used synonymously with Brunner gland adenoma.

10. Neuroendocrine tumours of the GIT

The WHO recognises a new classification of the neuroendocrine tumours of the GIT as being divided into 2 main categories- the neuroendocrine tumours (NETs) which were previously designated as carcinoid tumours of the GIT and poorly differentiated neuroendocrine carcinomas (NECs) which share with the NETs the expression of neuroendocrine markers. This categorisation is supported by genetic, clinical, behavioural and prognostic differences between the two groups. Therefore Neuroendocrine neoplasms (NENs) of the gastrointestinal tract (are epithelial neoplasms with neuroendocrine differentiation and) include well differentiated neuroendocrine tumours (NETs) and poorly differentiated neuroendocrine carcinomas (NECs).

Mixed neuroendocrine and non-neuroendocrine neoplasms are called ‘MiNENs’ and are mixed epithelial neoplasms with a neuroendocrine and non neuroendocrine component (such as a carcinoma), each of which is morphologically and immunohistochemically recognisable as a discrete component and constitutes *at least 30% of the neoplasm.* They can occur anywhere along the gastrointestinal tract. *The neuroendocrine component is usually a NEC*. Mixed neuroendocrine - non neuroendocrine neoplasms have an exocrine component (typically adenocarcinomas but could also be squamous cell carcinoma or any other) and neuroendocrine component each accounting for > or equal to 30% of the neoplasm. The exocrine component follows the molecular pathway of a conventional adenocarcinoma (intestinal type).

This group includes the previous group of tumours labelled as ‘MANECs’ where the exocrine component referred to is only an adenocarcinoma.

Poorly differentiated neuroendocrine carcinomas (NEC) are high grade tumours, closer to carcinomas; and are subtyped as small cell NECs (SCNEC) and large cell NECs (LCNEC).

These neoplasms share neuroendocrine differentiation based only on the common factor of immunostaining for chromogranin A and/or synaptophysin (the 2 most reliable markers of neuroendocrine differentiation).

Goblet cell adenocarcinoma is no longer considered a neuroendocrine neoplasm and is now grouped as carcinoma.

Neuroendocrine Tumours (NETs) (formerly termed 'carcinoids' of the gut. : These arise from the entero-chrommaffin cells (EC) located at the base of the crypts or the subepithelial neuroendocrine cells. These are epithelial neoplasms with well differentiated neuroendocrine features. They occur more commonly in the small intestine than in the rectum, pancreas, stomach and appendix. In the stomach NETs occur as asymptomatic gastric polyps, or as multiple lesions of the gastric corpus arising on a background of hypergastrinemia induced endocrine cell hyperplasia, chronic atrophic gastritis of the corpus or Zollinger –Ellison Syndrome particularly when this is associated with MEN type I. The latter is associated with multiple gastrinomas of the duodenum. In the small intestine the classical location is in the duodenum, more than 95% of duodenal NETs are in part 1 or 2 of the duodenum with those in part '2' are located in the ampullary region. They also occur in the jejunum and ileum. In the appendix NETs occur close to the tip where they are detected as incidental findings in specimens resected for acute appenditicis. In the colorectal area, NETs are more common in the rectum.

Functional NETs are those associated with clinical manifestations of the abnormal hormone production eg insulinoma, gastrinoma, glucaganoma etc. Non functioning NETs also produce hormones which can be detected in the serum or by immunohistochemistry within the tumour cells.

Gross : NETs are well circumscribed and the tumour turns yellow on exposure to formalin. Most neuro -endocrine tumours of the small intestine and appendix are less than 2 cm in size, some are polypoid.

Microscopically : Arise from the endocrine cell population within the crypts (enterochromaffin cells) and those infiltrating the mucosa superficial to the muscularis mucosae before invading the submucosa. The overlying epithelium becomes elevated and may ulcerate. The classical variant consists of cells arranged in islands in which the peripheral cells show palisading. The cells are small with uniform round nuclei with salt and pepper chromatin showing infrequent mitoses. The cytoplasm shows marked granularity. Other histologic patterns include trabecular, diffuse sheets, glandular and mixtures.

Cells stain black with argentaffin reaction, brick red with diazo method and blue with lead haematoxylin. "Argentaffin reaction" -is the ability of a silver complex solution to blacken a tissue element such granules within cells *without the need of a reducing bath*-. It is most commonly used to describe the brown black granularity observed in the NET (carcinoid) tumours. (see fig – second edition fig 5.16 page 207) The tumours arise from the Kulchitsky cells or neuroendocrine cells and these cells manufacture the 5-HT (5 hydroxytryptamine) from tryptophan –and are responsible for the argentaffin reaction.

Mid gut NETs (carcinoids) –(appendix and small intestine) almost always react with silver solutions and -are argentaffin +ve. Foregut and hindgut carcinoids are only argyrophillic. i.e. react only when a reducing substance is added. eg. NETs from rectum, stomach, –bronchus, more

frequently fail to give argentaffin reaction but are argyrophilic. All -argentaffin tumours are also argyrophilic but not vice versa.

NETs are also positive for IHC markers such as synaptophysin and chromogranin A.

NETs are graded as G1, G2, G3 (low, intermediate and high grade) based on their proliferative activity. Mitotic rate and Ki-67 proliferative index are taken into account and assessed in the areas showing the highest mitotic activity. Mitotic rates are expressed as number of mitoses per 2 mm^2 which equals to 10 high power fields (40x) with an ocular field of 0.5 mm. Ki-67 index is calculated by counting 500 cells in the region of highest labelling. Grade 3 NETs are considered well differentiated but with a higher grade and should be differentiated from a NEC which is also a high grade neoplasm. A definite differentiation point is the mutational profile of these neoplasia. NETs are associated with mutations in MEN1, DAXX and ATRX particularly when occurring in the pancreas. High grade NECs show mutations in TP53 and RB1. The IHC markers for chromogranin and synaptophysin may be more pronounced in NETs as compared to NECs .

Table to show various types of neuroendocrine tumours and their morphology relating to grade, mitoses and KI-67 activity.

Tumour type	**Differentiation**	**Grade**	**Mitotic rate (mitoses/2mm^2)**	**Ki-67 index**
NET G1	Well differentiated	Low	<2	<3%
NET G2		Intermediate	2-20	3-20%
NET G3		High	>20	>20%
NEC, small cell type	Poorly differentiated	High grade by default	>20	>20%
NEC, large cell type				
MiNEN	Well or poorly differentiated	Variable, usually high grade	Variable, usually same as NECs	Variable, usually same as NECs

Prognosis : Tumours limited to the intestinal wall are associated with a 85% survival. The more aggressive NETs are tumours are large, and show extensive local spread with ulceration and necrosis.

*Neuroendocrine carcinomas :*These are genetically separate for NETs. These are generally considered high grade with > 20 mitoses per 10 HPFs and proliferative index of > 20%; and respond differently to treatment regimes. They are poorly differentiated neoplasms with morphological and immunohistochemical features of neuroendocrine differentiation in that they answer for neuroendocrine markers -either synaptophysin or chromogranin A, in a significant

proportion of cells. They can be small cell in type (SCNEC) with fusiform nuclei, finely granular chromatin and scanty cytoplasm with features of nuclear moulding. The other subtype is the large cell type (LCNEC) with round nuclei showing prominent nucleoli and moderate amount of cytoplasm. Either type can show necrosis. These neoplasms can occur in any part of the GIT and maybe either pure or mixed with other types of carcinomas – adenocarcinomas or even squamous cell carcinomas. NECs occur in similar anatomical areas as conventional adenomas and colorectal carcinomas and NECs of the appendix can occur anywhere in the organ. They also show TP53 and RB1 mutations. NECs are highly aggressive neoplasms, more than even carcinomas arising in the colon.

Mixed neuroendocrine-non neuroendocrine neoplasms (MiNENs): are neoplasms wherein a neuroendocrine component is combined with a non neuro-endocrine component each constituting least 30% of the neoplasm. . Previously these neoplasms were categorised as mixed adeno-neuroendocrine carcinomas (MANEC); however in recognition of the fact that the non-neuroendocrine component may not always be an adenocarcinoma they have been renamed as MiNENs. Different types of MINENs arise in different parts of the GIT eg mixed adenocarcinoma-NEC (small cell or large cell); mixed adenocarcinoma-NET; etc in the stomach, oesophagus or colon. The more common combination is a non neuroendocrine adeno-carcinoma with a neuroendocrine carcinoma and not a NET. The neuroendocrine component should be proven by demonstration of a distinct synaptophysin or chromogranin positivity.

11. Microscopy of appendix

Acute appendicitis

Inflammation of the appendix and subsequent appendicectomy is the most common cause for surgery performed for an acute abdomen, particularly when no other cause for the acute abdomen has been detected clinically. It can occur at any age.

Cause for acute appendicitis are varied: from obstruction, alteration of intestinal flora, abuse of the mucosa due to mechanical reasons, spices. A decrease in blood supply contributes to the inflammation and ulceration. The obstruction may be due to a foecolith, other food particles resulting in an increase in the intra -luminal pressure and subsequent ishaemia.

***Grossly*,** the common finding seen in most cases is just a dullness of the serosa. In others the appendix appears grossly normal. At times in full blown cases the organ is gangrenous, black and distended.

Microscopy *:* While reporting on a case of appendicitis the pathologist has to correlate the clinical history and complete picture- usual presentation of appendicitis with fever , colicky pain around the umbilicus (referred pain) which later migrates to the right lower quadrant, nausea and vomiting; tenderness on palpation, leukocytosis, elevated erythrocyte sedimentation rate and C-reactive protein. Presence of at least some of these signs helps in correlating when histological findings are minimal.

The most common finding looked for and observed is neutrophils within the mucosa as well as congestion. Gradually the neutrophils are seen in all layers dissecting through the muscle fibres. Acute appendicitis may also show mucosal ulceration besides the acute inflammation. Oedema and hyperemia, with variably present fibrinous or purulent exudates covering the serosa is noted. Intraluminal acute inflammatory exudate with transmural inflammation and necrosis are observed in well established cases. The inflammation may involve part or most segments of the appendix.

In instances the inflammation may extend from the outside milieu and only a peri-appendicitis is seen with acute inflammation, congestion and fibrinous exudate covering the serosa.

The most dreaded complication of acute appendicitis is perforation; to avoid which the surgery is performed. Perforation may lead to the formation of a peri-appendiceal abscess ("ligneous peri-typhlitis").

Chronic appendcitis : The term is disputed and used by some authors when acute inflammatory cells are not detected and only lymphoid hyperplasia with a round cell infiltrate in the submucosa is evident. Lymphoid hyperplasia may also be part of an acute inflammation. This term is used more to support the removal of the appendix rather than outlining a pathological cause.

Possible causes of chronic appendicitis may be any of those which result in acute appenditis with a more insidious onset - accumulation of fecal matter, calcified deposits, tumors, enlarged lymphoid follicles, worm infestation, trauma, accumulation of foreign objects, like stones, marbles, or pins etc

Granulomatous inflammation of the appendix however may be part of a systemic disorder such as Crohn's disease / sarcoidosis, and infections such as mycobacterium tuberculosis, yersinia, parasites and fungi. Ileo-caecal tuberculosis gives rise to granulomas in the appendix and granulomatous inflammation of the appendix can also be part of an amoebic infection of the large bowel. Rarely an amoebic infection of the large intestine can result in the formation of a hard mass called as an amoeba, in the appendiceal region, mimicking a neoplasm. Similarly a tuberculous infection may result in an indurated mass known as an tuberculoma.

Sometimes the lumen of the appendix is replaced by spindle cells /fibrous tissue with a few chronic inflammatory cells (including eosinophils). This is an incidental finding and at times associated with hypertrophied nerve bundles, and adipose tissue .

Appendicular neoplasm: Neoplasms of the appendix are commonly epithelial and neuroendocrine in origin.

The following is the classification of the epithelial lesions of the appendix: (based on the WHO classification)

- appendiceal epithelial neoplasms
  - Hyperplastic polyp
  - Sessile serrated lesion without dysplasia
  - Serrated dysplasia low grade

        - Serrated dysplasia high grade
    - mucinous neoplasms
        - low-grade appendiceal mucinous neoplasm (LAMN)
        - high-grade appendiceal mucinous neoplasm (HAMN)
        - appendiceal mucinous adenocarcinoma
        - Signet ring cell type
        - Undifferentiated
    - non-mucinous neoplasms (colonic-type)
        - appendiceal adenoma
        - appendiceal adenocarcinoma
- Goblet cell adenocarcinoma of the appendix
- Appendiceal neuroendocrine tumors
    - Neuroendocrine tumours grade 1
    - Neuroendocrine tumour grade 2
    - Neuroendocrine tumour grade 3
    - L-cell tumour
    - Glucagon like peptide producing tumour
    - PP/PYY producing tumour
    - Enterochromaffin -cell carcinoid
    - Serotonin producing carcinoid
- Neuroendocrine carcinoma NOS
    - Large cell neuroendocrine carcinoma
    - Small cell neuroendocrine carcinoma
- appendiceal lymphoma

- Among the epithelial neoplasms, mucinous tumours are more common as compared to non-mucinous (intestinal type) neoplasms. The term ‘serrated lesion’ is preferred for ‘serrated adenomas’ or ‘serrated polyps’. ‘Low grade appendicular mucinous neoplasm’ (LAMN) replaces ‘mucinous tumours of uncertain malignant potential’ / ‘mucinous cystadenocarcinoma’ / ‘mucinous cystadenoma’. ‘High grade appendicular mucinous neoplasm’ (HAMN) lies within the spectrum of mucinous tumours. ‘Goblet cell carcinoid / carcinoma’ is replaced by ‘goblet cell adenocarcinoma’ which is grouped with and staged as carcinomas instead of neuro-endocrine tumours. The term mucocoele is descriptive in as much as it only conveys a localised collection of mucin ignoring the cause for it, and should no longer be used[3]. Some authors use the term mucinous cystadenoma when the mucin collection is within the mucosa, within muscularis mucosae[3], however the most recent concept (including WHO) is that any mucinous tumour of the appendix, however bland the morphology falls under the category of LAMN and when the entire appendix is

examined, there will be foci of thinning of the wall leading to dissecting mucus across leading to pseudomyxoma peritonei.

The following table summarises the main features of the appendicular neoplasms [11] :

Neoplasm	Sex incidence	Location in appendix Clinical features	Mutations	Microscopy/	Grade
Hyperplastic polyp. Serrated lesion without dysplasia/with dysplasia	Equal	Follows acute inflammation/ Diverticulosis Incidental findings	Occasional ones show KRAS/BRAF mutations & maybe neoplastic. KRAS are more frequent with serrated dysplasia.	Polyp characterised by serrated architecture of the crypt lumen.	Benign
LAMN		Within appendix May present as a swollen appendicial mass.	KRAS +, GNAS+ APC, TP53, BRAF, MSI , SMAD all - ve GNAS may lead to excessive mucin secretion	Villous tumour , slender villi lined by bland hyper-mucinous epithelium, pushing margin against a fibrotic jagged appendiceal wall, no invasion, dissecting mucin through the wall Undulating epithelium+	3-tier grading Grade1
HAMN Rare, Less commonly encountered than LAMN		Within appendix; may present with features like LAMN	GNAS less common	Similar to LAMN but high grade cytology with pushing margins, micro-papillary features, cribriforming, piling up of epithelial cells; no invasion	3 tier grading Grade 2 or 3

Invasive mucinous carcinoma	Female preponderance	Within appendix Peritoneal spread may result in progressive distension of abdomen	KRAS +ve GNAS +ve BRAF and MSI rare	Invasion+, >50% of tumour with extracellular mucin	3 tier grading
Invasive mucinous carcinoma signet ring type	Female preponderance	Within appendix	KRAS +ve GNAS +ve , but less frequently BRAF and MSI rare	Invasion+ >50% of signet ring cells Dissecting through the wall.	3-tier grading, Usually grade 3
Invasive mucinous carcinoma Undifferentiated type	Female preponderance	Within appendix	KRAS+ve GNAS +ve BRAF and MSI rare	Poorly differentiated morphology; extracellular pools of mucin (>50% of tumour) with single, clusters and strips of neoplastic epithelium	Garde 3
Non-mucinous adenocarcinoma (Intestinal type)	Slight male preponderance	Within appendix Rare compared to mucinous variant	MSI +++	Infiltrating glands with desmoplastic stroma. Similar to colonic carcinoma with glands	2 tier grading
Goblet cell adenocarcinoma	Males and females equal	Distal appemdix; may present as appendicitis		Exocrine tumour with Infiltrating of goblet cells; endocrine cells and Paneth-like cells	To be graded as conventional adenocarcinoma

LAMN : was a term introduced in the WHO 2010 classification; it is the commonest cause for pseudo myxoma peritonei. A clinical suspicion is of LAMN is made when on imaging the following features are detected - a focal well-defined cystic mass, with slightly higher than water attenuation, thickened cystic wall with ring mural enhancement and a characteristic progressive

contrast enhancement in CT imaging, particularly occurring in females with non-specific symptoms similar to appendicitis [30].

The characteristics of LAMN and other neoplasms of the appendix are outlined in the table :

Differential diagnosis of LAMN :

- Diverticular disease with rupture : resembles LAMN when LAMN presents as a diverticulum sending mucin into the muscularis propria with extension of it onto the appendicial surface. However hyperplastic changes of mucosa, but maintained crypt architecture separated by lamina propria and schwann cell proliferation in lamina propria differentiate diverticular disease.
- Serrated polyps : show complex serrations, lack the filiform villous or undulating pattern typical of LAMN; maintain the mucosal architecture and are not associated with dissecting mucus through the wall or pseudomyxoma peritonei. Also serrated polyps may show thinning and loss of muscularis mucosae.
- Mucinous carcinoma : shows at least focal infiltration with desmoplastic response.

HAMN ; shares the same features as LAMN but the morphology is of high grade cytology. LAMN and HAMN if confined to the submucosa and muscularis propria are staged as pTis.

Mucinous adenocarcinoma of the appendix : Mucinous adenocarcinomas[31] occurring anywhere in the body are characterised by an epithelial carcinoma associated with an abnormal mucin secretion; the WHO characterises these neoplasia as secretion by the tumour cells of abundant amounts of extracellular mucin involving more than 50% of the tumour volume.

Mucinous adenocarcinomas (MACs) are known to occur in colo-rectal region (including appendix) constituting 10-15% of the colo-rectal carcinomas and occur in other anatomical sites such as the breast, stomach, ovary and lungs. It has been observed that MACs of the stomach, appendix, ovary, pancreas, lung and bronchus are associated with more advanced tumour stage and MAC of the stomach with a higher grade. Whether these neoplasms in the various anatomic locations have common prognostic factors is still not established; however tumours in the breast have a distinctly better prognosis than tumours in the colon. The incidence of tumours in the appendix, lung and bronchus is on the rise in the last decade or two.

Pseudomyxoma Peritoneii (PMP) : is the clinical term applied to a neoplastic condition characterised by relentless accumulation of grossly evident mucinous tumour deposits in the peritoneal cavity. It almost always originates from a mucinous neoplasm of the appendix . This theory is supported by the fact that MUC 2 and MUC 5AC are gel-forming mucins seen in PMP and MUC 5AC is overexpressed in ovarian neoplasms and MUC 2 is overexpressed in appendicular tumours. In most cases of PMP, CK20, CDX2 and MUC 2 are diffusely positive lending support to an appendiceal origin of PMP. CK 20 is expressed in all colonic neoplasms (CK 7 on the other hand is expressed in all ovarian tumours and rarely in PMP) and CDX2 is a gene that encodes an intestinal specific transcription factor which is expressed in the nuclei of epithelial cells of the intestine.

A small percentage of PMP cases arise from pancreas, urachus or appendiceal mucinous tumours arising from ovarian teratomas [31].

The tendency is for the malignant cells from the appendix to produce abundant extracellular mucin to infiltrate the peritoneum and underlying tissues with a low tendency for nodal or haematogenous spread. Within the peritoneal cavity the mucin secreting tumour cells continue to proliferate and give rise to the typical "jelly belly".

The excessive mucin produces disease in the pelvis , paracolic gutters, greater omentum and around the liver, spleen and diffusely infiltrate the ovaries. There is relative sparing of the serosa of the peristaltically active small bowel, as a result of what is known as the 'redistribution phenomenon' put forth by Sugar baker in 1994 which says that 'large volumes of disease will be found in certain predetermined anatomic sites within the peritoneal cavity but will be absent or greatly reduced in others'.

Mucinous neoplasms of the appendix represent a spectrum of malignant potential, and are the most common cause of pseudomyxoma peritonei. In patients with PMP the appendix may either show a low grade mucinous neoplasm with normal appearances of the appendix or may be grossly absent due to total involvement by the neoplasm. Such low grade neoplasms as stated above are termed as LAMNs because of their propensity for subtle invasion of the wall with low grade cytology and broad pushing front/margin. Such benign appearing cases were termed as "disseminated peritoneal adenomucinosis" (DPAM) by Ronnett etal (1995) in contrast to "peritoneal mucinous carcinomatosis" (PMCA) which applied to cases with more classic malignant features.

Others favoured a uniform terminology instead of varied terms such as DPAM and PMCA (Bradley, 2006). They advocated "mucinous carcinoma peritonei" and graded these as low grade (MCP-L) and high grade (MCP-H) the latter including moderate and high grade tumours. CARR et al have suggested the following grades, these are to be currently used :

- PMP/MCP low grade is reserved for cases with minimal or low cytologic atypia, low cellularity, and no evidence of destructive invasion. ie PMP grade 1=MCP-L
- PMP grade 2 ie MCP-H is reserved for cases that are moderately and poorly differentiated (grades 2-3); ie PMP grade 2 =MCP-H and
- PMP grade 3= MCP-H with signet ring cells.

All cases should have a predominance of extra cellular mucin.

KRAS mutations are identified in grades 1 and 2 but not in grade 3 and in the grade 3 cases, the appendix is often obliterated, being consumed by the neoplasm [31].

It is important to note that in cases of peritoneal spread, the histologic grade of the peritoneal disease may be different than the primary appendiceal tumor. In these case, the peritoneal histology is considered more important for prognostication.

12. Carcinoma Colon :

Carcinoma of the Colon and Rectum : are malignant epithelial neoplasms of the large bowel with glandular or mucinous features. They may be located in any part of the colon and for purposes of convenience are grouped as right sided, left sided and rectal tumours. Commonest site is recto-sigmoid followed by caecum and ascending colon then transverse and descending colon

- Rt sided colonic tumours are –polypoid, exophytic masses, no obstruction
- Lt sided- annular/ napkin ring, narrowing of lumen, beaded, heaped up margin with central ulceration.

13. WHO Classification of Tumours of the Colon and Rectum

Benign epithelial tumours and precursors

Serrated dysplasia, low grade

Serrated dysplasia high grade

Hyperplastic polyp , microvesicular type

Hyperplastic polyp , goblet cell

Adenomatous polyp, low grade dysplasia

Adenomatous polyp, high grade dysplasia

Tubular adenoma, low grade

Tubular adenoma, high grade

Villous adenoma ,low grade

Villous adenoma high grade.

Tubulo-villous adenoma, low grade

Tubulo-villous adenoma, high grade

Glandular intra epithelial neoplasia, low grade

Glandular intraepithelial neoplasia, high grade

Malignant Epithelial Tumours

Adenocarcinoma, Nos

Serrated adenocarcinoma

Adenomalike adeno carcinoma

Micro-papillary adenocarcinoma

Mucinous adenocarcinoma

Poorly cohesive adenocarcinoma

Signet ring cell adenocarcinoma

Medullary adenocarcinoma

Adenosquamous adenocarcinoma

Carcinoma undifferentiated, Nos

Carcinoma with sarcomatoid component

Neuroendocrine tumour , NOS

Neuroendocrine tumour grade 1

Neuroendocrine tumour grade 2

Neuroendocrine tumour grade 3

L -cell tumour

Glucagon-like peptide producing tumour

PP/PYY producing tumour

Enterochromaffin cell carcinoid

Serotonin producing tumour

Neuroendocrine Carcinoma, NOS

Large Cell neuroendocrine carcinoma

Small cell neuroendocrine carcinoma

Mixed neuroendocrine non neroendocrine neoplasm (MiNEN)

Pathogenesis of Carcinoma Colon :

- Occurs with increased incidence in older males. When in younger age group is usually associated with a pre-existing Ulcerative colitis or familial adenomatous polyps.
- Dietory factors- Excess calorie intake ;Low fiber diet predisposes to it ; Refined carbohydrates and red meat are implicated.
- Genetic : Several pathways are implicated in the development of colorectal carcinomas

1) Most tumours (about 84%) develop via the conventional adenoma-carcinoma pathway through genetic and epigenetic abnormalities involving the APC, KRAS, TP53, SMAD4 mutations.

- APC / β catenin : the APC gene- normally regulates ß catenin by forming macromolecules and degradation. When APC is mutated
- ß catenin translocates to the nucleus and activates TGF and upregulates proliferation. This is involved in both sporadic as well as cancer associated with FAP.
- Lack of SMAD 4 and SMAD 2 involved in TGF beta signaling leads to carcinogenesis (normally it inhibits cell cycle & growth of colonic epithelial cells)
- Loss of p53 or p53 mutation is also associated with colorectal carcinoma.

1) About 13% of carcinomas occur from mismatch repair gene (MLH1 and MSH2) pathway; these are mostly the serrated adenocarcinomas with precursor lesions being serrated lesions, hyperplastic polyps and Traditional serrated polyps. These lesions show BRAF and KRAS mutations and CpG island methylator phenotype (see fig 'A'..above).
2) About 3 % of adenocarcinomas have mutations affecting the proof reading domain of POLE ie considered as ultramutants. Exome sequencing[27] has used to identify a novel mutation, c.1373A>T (p.Tyr458Phe), in the gene for DNA polymerase epsilon catalytic subunit (POLE). This mutation is located in the active site of the exonuclease domain of the enzyme
3) Mutation in mismatch repair genes MSH 2/ MLH1 leads to micro satellite instability causing HNPCC (HNPCC stands for hereditary non polyposis colonic carcinoma) or Lynch Syndrome. Micro satellites are located in the MSH2 and MLH1 genes in majority of the cases. Microsatellite instability leads to HNPCC syndrome where repair of instability is not possible because of mutation in repair genes. This results in familial clustering of cancers at several sites : colorectum, endometrium, stomach, ovary, uterus, brain, small bowel, hepatobiliary tract.

Microscopy of colonic Carcinoma : Majority of the adenocarcinomas are categorised histologically as adenocarcinomas NOS, and characterised by invasion atleast through muscularis mucosa into submucosa and beyond. Other histological subtypes include -mucinous carcinomas, signet ring cell carcinoma, medullary carcinoma, serrated adenocarcinomas, adenosquamous carcinoma, micropapillary adenocarcinoma and carcinoma with sarcomatoid features.

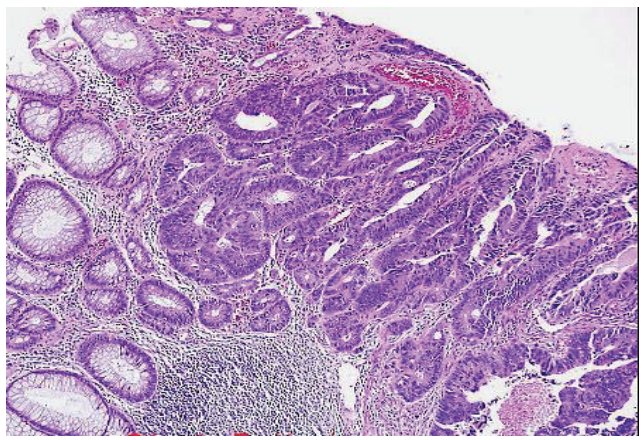


Fig 3.25 : Adenocarcinoma colon with neoplasm arising from the mucosa and infiltrating into underlying mucosa. H&E x 100

14. WHO Classification of Tumours of the Anal Canal

Benign Epithelial Tumours and Precursors

Squamous intraepithelial neoplasm low grade

Squamous intraepithelial neoplasm High grade

Malignant epithelial tumours

Squamous Cell Carcinoma, NOS

Verrucous squamous ell Carcinoma

Adenocarcinoma, NOS

Neuroendocrine tumour , NOS

Neuroendocrine tumour grade 1

Neuroendocrine tumour,grade 2

Neuroendocrine tumour grade 3

Neuroendocrine Carcinoma, NOS

Large Cell Neuroendocrine Carcinoma

Small Cell Neuroendocrine Carcinoma

Mixed neuroendocrine non neroendocrine neoplasm (MiNEN)

15. WHO 5th Edition (2019) – Key Updates in GIT Tumours

1. Conceptual Shift (Very Important)

- Strong integration of morphology + molecular pathology
- Classification increasingly biology-driven rather than purely histologic
- Emphasis on:
  - Predictive biomarkers (MSI, HER2, PD-L1, etc.)
  - Genetic pathways of tumorigenesis
  - **2. Neuroendocrine Neoplasms (Major Revision)**

2.Unified Classification Across GIT

- NENs now classified in **each organ system** (not separate endocrine volume)

Key Changes

- Division into:
  - NET (well-differentiated)
  - NEC (poorly differentiated)
- Introduction of:
  - NET G3 (well-differentiated high grade) → NEW ENTITY
- Mixed tumours renamed:
  - MiNEN (Mixed Neuroendocrine–Non-Neuroendocrine Neoplasm)

Molecular Correlates

- NET: MEN1, DAXX, ATRX
- NEC: TP53, RB1

Practical takeaway:
Always specify **differentiation + grade + Ki-67**

3. Precursor Lesions & Dysplasia Terminology

- Standardization:
  - "Dysplasia" preferred (GI tract)
  - Avoid "carcinoma in situ"
- Two-tier grading increasingly used

Colon/Appendix

- Term "serrated lesion" preferred over polyp
- Molecular pathways emphasized:
  - BRAF pathway
  - KRAS pathway

4. Appendiceal Tumours – Major Reclassification

- Goblet cell carcinoid → Goblet cell adenocarcinoma
- Recognized as:
  - Predominantly adenocarcinoma with minor neuroendocrine component

Clinical impact: behaves aggressively → treat as adenocarcinoma

HIGH-YIELD "EXAM PEARLS"

- NET G3 = new entity
- MiNEN replaces mixed endocrine tumours
- Goblet cell tumour = adenocarcinoma, not carcinoid
- Serrated lesions = non-polypoid + molecular pathway driven
- Dysplasia terminology standardized (no CIS)
- Pancreatic precursor lesions → 2-tier system
- ICC → small duct vs large duct

Strong molecular integration in classification

C V SMALL AND LARGE INTESTINE SYNOPTIC REPORTING

Reporting of small intestinal endoscopic biopsies

Gross

Type of biopsy : endoscopic Yes □ □No

Site of biopsy : Second part of duodenum □ □Distal duodenum □Jejunum

Ileum □ Other , specify…….

Measurements : ……..x…….x……mm

Mounted on paper/gel foam : Yes □ □No

Examined on a dissecting microscope : yes □ □No

Villous pattern

finger-like □

leaf- like□

cerebriform □

flat □

Microscopy

Biopsy adequate Yes □ □No

Muscularis mucosae present Yes □ □No

Villous architecture : Normal □

□Mild blunting

□Moderate blunting

□Severe blunting

□Total atropy

Villous : crypt ratio : Altered Yes □ Normal □(3:1)

Ratio…… : ….

Overall mucosal thickness : Maintained □ □Decreased

Surface Enterocytes : Columnar □ Cuboidal □ Basophilic □cytoplasm

□Loss of basal nuclear polarity □Loss of brush border

□Lipid vacuoles

Intra-epithelial lymphocytes >3/10 enterocytes □Yes □No

Immunohistochemistry : CD3 Normal □ □Increased

Intrapithelial neutrophils : Normal □ □Increased

Crypt cell hyperplasia : Yes □ No □

Subepithelial collagenenous band : Present Absent

Goblet cells : Normal □ Decreased □

Paneth cells : Normal □ Decreased □

Argentaffin cells : Normal □ Decreased □ Increased □

Lamina propria components :

Lymphocytes Normal □ Increased □

Plasma cells Normal □ Increased □

Eosinophils Normal □ Increased □

Neutrophils Normal □ Increased □

Foamy macrophages : Present □ PAS +ve □Yes No □

Absent □

Dilated lecteals : Present □ Absent □

Other features :

Granulomas : Present □ Absent □ Type…….Location …….

Lymphoid aggregates/ follicles: □Present Absent □

Pneumatosis intestinalis: Present □ Absent □

Parasites :

Giardia lamblia : Present □ Absent □

Strongyloides stercoralis : Present □ Absent □

Schistosoma : Present □ Absent □

Others (coccidia, M. Avium intracellulare, etc): ……..

Neoplasms :

Adenocarcinoma : Present □ □Absent

Epithelial dysplasia : Present □ Absent □

Lymphoma : Present □ Type…….. Absent □

Other round cell infiltrates : □Yes No □ Specify…….

Metastatic : Specify…….

SNOMED Codes.

T……..

M……

Pathologist's signature …… Date……

Reporting of resected specimen of small Intestine (applies to specimens of duodenum, jejunum and ileum)

Gross

Length of intestine …….cms

Specimen includes : of small bowel □……..cms

of large bowel □ ……..cms

appendix □

Serosa : Congested □ dull □ tubercles □

Presence of perforation Yes □ No □

Presence of ulcers Yes □ No □

Number of ulcers 1□ 2□ 3□ >3 □

Presence of strictures Yes □ No □ No of strictures …….

Intussusception Yes □ No □ Length of intussucepiens ……..mm Length of intussusceptum ….mm

Presence of tumour Yes □ No □ Polypoid □ Ulcerative □ Infiltrative □

Size of tumour ….cms x …cms

Location from closest resection margin ……..cms

Margin involved Yes □ No □

Location from farthest resection margin……..cms

Lymph nodes : No detected ……

No grossly involved……

Microscopy

Non tumour

Granulomatous inflammation consistent with tuberculosis □

Findings consistent with intussusception □

Crohn's disease □

Typhoid □

Parasites □ Specify…….

Other , specify ……

Comments ……

……..

Tumour

Segment involved : Duodenum□Jejunum□Ileum □ Not specified □

Histological subtype: Adenocarcinoma □

NET (Carcinoid) □

Neuroendocrine carcinoma □

Mixed; Specify type…….

Lymphoma □ Specify type……

Differentiation : well □ mod □ poor □

Local Invasion

☐ No evidence of primary tumour (pT0)

☐ carcinoma in situ (pTis)

☐ lamina propria, muscularis mucosae, (pT1a) submucosa (pT1b)

☐ Muscularis propria (pT2)

☐ Beyond muscularis propria, subserosa, or non peritonealised perimuscular tissue (for duodenum where serosa is lacking, for jejunum & ileun it can be mesentery) without breaching serosa (pT3).

☐ Tumour cells have breached the visceral peritoneal surface or adjacent structures (pT4)

Resection margins : Both clear □

One margin involved □

(Specify proximal or distal if possible)……..

Closest margin is ….cms away. (Specify which if possible)…….

Both margins involved □

Regional lymph nodes (6 or more nodes to be examined) :

pN0 -no lymph node metastasis

pN1 – metastasis in 1-2 lymph nodes

pN2 – metastasis in 3 or more nodes.

pM1 – distance metastasis microscopically confirmed.

Colorectal biopsy histopathology report (non –tumour & tumour)

Gross

Type of biopsy : endoscopic Yes □ □No Other (Specify)…….

Site of biopsy : A) Caecum □

B) Ascending colon □

C)Transverse colon □

D) Descending colon□

E) Sigmoid □

F) Rectum □

Other , specify…….

Measurements : ……..x…….x……mm each

Mounted on paper/gel foam : Yes □ □No

Inadequate □

Microscopy :

Crypt architecture : Preserved □ Distorted □

Inflammation in lamina propria : Nil □

Mild □ Mod □ Severe□

Crypt abcesses **:** Nil □ Occasional □ Several □

Mucus depletion **:** Nil □ Present □

Hyperemia **:** Nil □ Mild □Mod □Severe□

Regenerative atypia □ Nil □

Features are consistent with active phase of UC □

Features are consistent with quiescent phase of UC □

Features are not consistent with UC □

Dysplasia Negative □

Positive for dysplasia low grade □

Positive for dysplasia high grade □

Non –specific colitis □

Infective colitis □

Amoebic colitis □

Invasive carcinoma is identified □, in

Invasive carcinoma is not seen □, in …..

Caecum : □……..

Ascending colon □……

Transverse colon□ ……

Descending colon ……..

Sigmoid colon□ ……..

Rectum □……..

Either report separately or combine report of 2 or more areas.

Colorectal cancer resection histopathology report

Gross Description

Length of resected segment (large intestine) : …….cms

Includes caecum : yes ☐ No ☐

Includes small intestine yes ☐ ……cms No ☐

Site of tumour ……

Maximum tumour diameter …….

Distance of tumour to nearer margin (cut end) …….

Presence of tumour perforation (pT4) ☐ Yes ☐ No

For rectal tumours Tumour is ☐ above ☐ at ☐ below the peritoneal reflection

Distance from the dentate line …….cms

Histology Type :

Adenocarcinoma ☐ Yes ☐ No

Mucinous and signet ring adenocarcinomas ☐ Yes ☐ No

Other.....

Differentiation by predominant area

☐ Well/moderate ☐ Poor

Local Invasion

☐ No evidence of primary tumour

☐ Carcinoma in situ , invasion of lamina propria (pTis)

☐ Submucosa (pT1)

☐ Muscularis propria (pT2)

☐ Beyond muscularis propria (pT3)

☐ Tumour cells have breached the peritoneal surface or invaded adjacent organs (pT4)

Margins

Tumour involvement	N/A	Yes	No
Doughnut	☐	☐	☐
Margin (cut end)	☐	☐	☐

For rectal tumours

Circumferential margin involvement	☐	☐	☐

Histological measurement from tumour to circumferential margin …….cms

Metastatic Spread

No of lymph nodes examined ……..

No of positive lymph nodes (should examine atleast 12 or more nodes)

(pN1 1-3 nodes, pN2 4+nodes involved; N2b is more than 7 nodes)

The presence of satellite nodules in the pericolrectal adipose tissues does not alter the 'T' stage but is taken as node positivity and changes the stage to pN1c.

Metastasis : Distant metastasis microscopically confirmed (pM1) ☐

Apical node positive (Dukes C2) Yes ☐ No ☐

Extramural vascular spread yes ☐ No ☐

Background Abnormalities

	Yes	No
Adenoma(s)	☐	☐
Dysplasia –high grade	☐	☐
low grade	☐	☐
Ulcerative colitis	☐	☐
(Dysplasia –high grade	☐	☐
low grade	☐	☐
Crohn's disease	☐	☐
Dysplasia –high grade	☐	☐
low grade	☐	☐
Familial adenomatous polyposis	☐	☐
Synchronous carcinomas(s	☐	☐

(Complete a separate form for each cancer)

Pathological staging

Complete resection at all margins ☐Yes ☐ No

TNM : T ☐ N ☐ M ☐

Dukes staging :

Dukes A (Growth limited to wall, nodes negative) ☐

Dukes B (Growth beyond muscularis propria, nodes negative ☐

Dukes C1 (Nodes positive and apical node negative) ☐

Dukes C2 (Apical node positive) ☐

Histologically confirmed liver metastases ☐ Yes ☐ No

Signature Date…../…../…… SNOMED Codes……/……

Notes : 1. Patients who have lymph node involvement (Dukes stages C1 & C2, TNM stages pN1, pN2) are likely to receive adjuvant chemotherapy which is of possible benefit.

2. Patients with rectal adenocarcinoma and circumferential margin involvement are at

high risk of local recurrence and may receive post-operative radiotherapy +/-,

chemotherapy which is toxic and costly but may decrease the likelihood of death. The frequency of circumferential margin involvement found may indicate the quality of rectal cancer surgery being performed!.

Distance of tumour to nearest margin

Measured from the nearest cut end of the specimen, not the circumferential margin. It is only necessary to examine the margins histologically if tumour extends macroscopically to within 30mm of one of these. For tumours further than this it can be assumed that the cut ends are not involved. Exceptions to this recommendation are adenocarcinomas that are found on subsequent histology to have an exceptionally infiltrative growth pattern, show extensive vascular or lymphatic permeation, or are pure signet ring carcinomas, small cell carcinomas, or undifferentiated carcinomas.

Presence of tumour perforation

If the tumour has perforated into the peritoneal cavity this should be recorded. Such cases are

always regarded as pT4 in the TNM staging system. If perforation does not involve the tumour the “No” box should be marked.

For Rectal Tumours

Relationship to the Peritoneal Reflection

The crucial landmark for recording the site of rectal tumours is the peritoneal reflection. This is identified from the exterior surface of the *anterior* aspect of the specimen.

Rectal tumours are classified according to whether they are:

a) entirely above the level of the peritoneal reflection anteriorly

b) astride (or at) the level of the peritoneal reflection anteriorly

c) entirely below the level of the peritoneal reflection anteriorly

Tumours below the peritoneal reflection have the highest rates of local recurrence.

Margins: Tumour involvement:

Doughnuts Strictly speaking, it is not necessary to examine doughnuts histologically if the main tumour is >30mm from the cut end of the main specimen or in other rare cases described above but this is a decision to be made locally.

When doughnuts from stapling devices are examined histologically the presence or absence of tumour is recorded. If doughnuts are not sectioned because it is deemed unnecessary locally, or if no doughnuts are submitted for examination by the surgeon, this item should be recorded as not applicable.

Margin (cut end) When cut ends are examined histologically (see criteria above) the presence or absence of tumour should be recorded. If margins are not examined histologically this should be recorded.

Circumferential Margin (Rectal cancers only)

Accurate assessment of the circumferential (radial) margins of these rectal tumours is very important because it influences post-operative therapy.

Note that the circumferential margin is reported only for rectal cancers; for tumours at other sites the "not applicable" box is marked. It represents involvement of the surgical margins of the connective tissues around the rectum in an area where there is no peritoneal covering.

Hence involvement of this margin is different from, and quite unrelated to, serosal involvement.

Synoptic Reporting for Appendix : See section on Large intestine (same reporting)

TNM stage of Intestinal Tumours

TNM Stage	Description
Tumor	
T1	Tumor invades submucosa
T2	Tumor involves muscularis propria but does not cross it
T3	Tumor extends beyond muscularis propria into mesorectal or pericolic fat
T4	Tumor invades adjacent organs or perforates the visceral peritoneum
Node	
N0	No nodal metastasis
N1	1–3 perirectal or pericolic nodes
N2	4 or more perirectal or pericolic nodes
Metastasis	
MX	Cannot be assessed
M0	No metastasis
M1	Distant metastasis

TNM for Appendix

Varies by the following points :

Primary tumour :

- pT0 : No evidence of primary tumour
- pTis : Intraepithelial or invasion into lamina propria; no extension through muscularis mucosae into submucosa.
- pTis LAMN : acellular mucin or mucinous epithelium that may extend into muscularis propria.
- pT1 : tumour invades submucosa (not applicable to LAMN)
- pT2 : tumour invades muscularis propria (not applicable to LAMN)
- pT3 : tumour invades subserosa or mesoappendix
- pT4 : tumour penetrates visceral peritoneum; includes acellular mucin; invades other organs/structures.

Metastasis :

- M1 : Distant metastasis
- M1a : Intraperitoneal acellular mucin only
- M1b : Intraperitoneal metastasis including mucinous epithelium
- M1c : Non – peritoneal metastasis

ASSESS YOURSELF – GASTROINTESTINAL TRACT (Oesophagus, Stomach, Intestine)

Essay Questions (10 marks each)

1. Discuss the pathogenesis, morphology and complications of Barrett's oesophagus, including its role as a precursor lesion.
2. Describe the gross and microscopic features of carcinoma of the oesophagus, including staging and prognostic factors.
3. Write an account of inflammatory bowel disease, highlighting differences between ulcerative colitis and Crohn disease.

Short Answer Questions (5 marks each)

1. Microscopic features of reflux oesophagitis
2. Grossing technique of oesophagectomy specimen
3. Histological features of gastric adenocarcinoma
4. Types and morphology of intestinal polyps
5. Features of coeliac disease

Short Notes / Point Questions (3 marks each)

1. Goblet cells in Barrett's oesophagus – significance
2. Lymphatic spread of oesophageal carcinoma

MCQs

1. **Barrett's oesophagus is characterised by:**
   A. Stratified squamous epithelium
   B. Intestinal metaplasia with goblet cells
   C. Keratinised epithelium
   D. Neuroendocrine proliferation
2. **The most common carcinoma in the lower oesophagus is:**
   A. Squamous cell carcinoma
   B. Adenocarcinoma
   C. Small cell carcinoma
   D. Lymphoma

3. **Crohn disease most commonly involves:**
   A. Rectum only
   B. Entire colon continuously
   C. Terminal ileum with skip lesions
   D. Stomach alone

Answer Key for MCQs

1. B
4. 2.B
5. C

Chapter 4
Salivary Glands

Chapter outline I Salivary Glands Anatomy; II Salivary Glands Grossing Techniques; III Salivary glands Gross Pearls; IV Salivary Glands Microscopy; V Salivary glands Synoptic Reporting

I SALIVARY GLANDS ANATOMY

- Salivary glands are exocrine glands **(ie** they have a duct system to flow their secretions)
- The saliva that is produced by these glands is brought to the oral cavity by a duct system
- The main function of the saliva is the lubrication of the food that is being chewed and to assist in its deglutition
- Saliva also performs digestion of the carbohydrates by the enzyme that it has
- Types of salivary glands : (Fig 4.1)

Major salivary gland

parotid glands

submandibular glands

sublingual glands

Minor salivary glands

Labial & buccal glands

Glosso-palatine glands

Palatine glands

Lingual glands

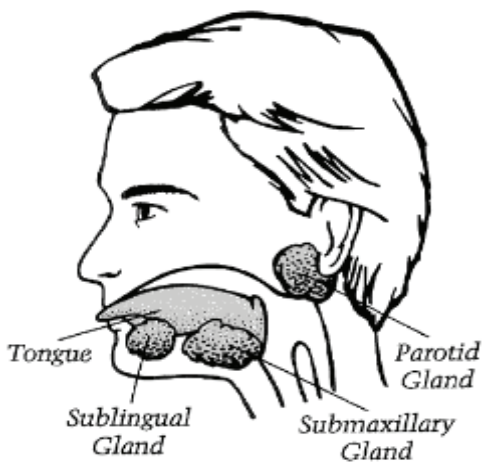


Fig 4.1

- The parotid salivary gland : weight of parotid salivary gland is 14 – 28 g (broad superficial lobe, small deep lobe). It is enclosed in a well defined connective tissue capsule with its superficial part lying in front of the ear and the deeper part lying in the retromandibular fossa. Its secretory duct is the Stenson's duct which opens in the buccal mucosa opposite to the 2nd maxillary molar. It's a predominantly serous gland. All the five terminal branches of the facial nerve pass through the parotid gland.
- Submandibular salivary gland : This gland is also enveloped by a well developed capsule. It is located in the submandibular triangle behind and below the free border of the mylohyoid muscle with its small extension lying above the mylo-hyoid. Normal weight of submandibular (sub-maxillary) salivary gland is 7-8g (mixed salivary; serous acini predominate). Its has a Wharton's duct, which opens at the lingual freenum on the floor of the mouth
- Sublingual salivary gland : It lies in between the floor of the mouth and the mylohyoid muscle, it is composed of large main part and small numerous glands. It is also a mixed gland but predominantly mucus. Normal weight of sublingual salivary gland 3 g.
- Of the major salivary glands, the submandibular gland secretes the largest amount of saliva. Along with the sublingual duct, the main duct (Wharton's duct) is known to open into the sublingual caruncula. About 85% of sialolith occurrences in humans is in the submandibular gland and duct, which causes local pain during swallowing in most cases.
- Parotid duct (Stenson duct): links the parotid gland to the buccal mucosa, beside the maxillary second molar. Submandibular duct (Wharton duct): links the submandibular gland to the floor of the mouth. Ducts of Rivinus: the sublingual gland, drains pipes via many small ducts all which open up into the floor of the mouth and are collectively called the ducts of Rivinus; the biggest is the main duct of the sublingual salivary gland called Bartholin duct.
- Minor salivary glands : -labial & buccal glands: These are present on the lips and the cheeks and are of the mixed type
- Glosso-palatine glands: They are pure mucous glands, their location is the isthmus of the glosso-palatine fold.

II SALIVARY GLANDS – GROSSING TECHNIQUES

The type of surgeries that may be performed for salivary gland tumours in the parotid include:

- Superficial parotidectomy (includes the superficial lobe of the parotid & spares the facial nerve.
- Total parotidectomy (includes both the superficial & deep lobes and the facial nerve).
- Total maxillectomy.

Sometimes the submandibular salivary gland may also be resected in case of tumours and chronic sialadenitis

Procedure :

1. Measure & weigh the specimen
2. Ink the surgical margins (in suspected tumour)
3. Cut parallel slices 4-5 mm thick and examine the cut surfaces
4. Look for intra parenchymal lymph nodes, and bisect them if present
5. Describe - the cut surface, presence of tumour- size, shape & consistency, whether solid or cystic, mucoid or firm white, solitary or multiple, encapsulated or well circumscribed, infiltrating. State the distance of the tumour from the surgical margins of resection, in particular the closest margin.
   - adjacent salivary gland
   - cut surface of any intraparenchymal (intra-parotid) nodes.
6. If a major nerve has been resected (ideally this should be marked by the surgeon), the proximal & distal margins should be marked & blocked separately.

Bits (Fig 4.2)

A, B, C – two to three bits tumour with adjacent salivary gland tissue. Closest surgical margin may be included with one of the bits if it is close.

D – surgical margins

E- lymph nodes if present (not shown in the figure 4.2)

F & F – nerve margins- proximal & distal if marked (not shown)

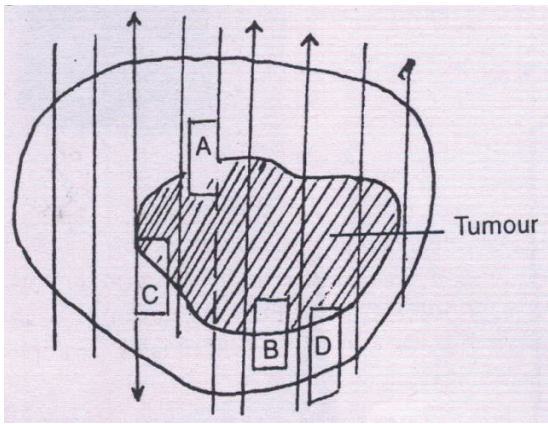


Figure 4. 2

If no tumour is identified grossly then take 2 or more representative bits from the salivary gland from representative areas as per findings.

III SALIVARY GLANDS GROSS PEARLS

Fibrotic hard gland : cut surface shows fibrosis, parenchymal atropy, a few dilated ducts and microliths : Chronic calculus sialadenitis. Calculi are more commonly seen in the submaxillary salivary gland as compared to the parotid salivary gland. (Fig 4.3)

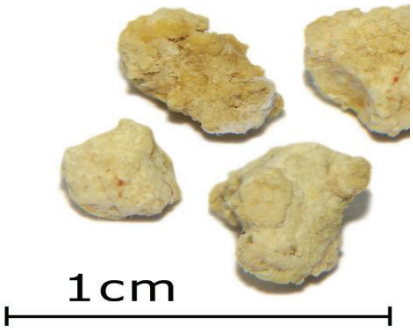


Figure 4.3 shows calculi varying from 0.3 to 0.5 cm

Firm to hard gland : Pseudotumour : C/S homogenous white : amyloidosis; if fibrotic inflammatory.

Kuttner's tumour : Unilateral chronic sclerosing sialadenitis of the submandibular salivary gland, presenting as a palpable mass.

Cysts : keratinous cysts; salivary duct cyst; lymphoepithelial cysts; ranula.

Cystic tumour : Warthin's tumour (may appear multiloculated with grayish septa and occurs bilaterally.); sometimes sebaceous adenoma and lymph adenomas, intraductal papilloma., cystadenoma & cystadenocarcinoma

Enlarged gland, rubbery and resilient with a bosselated surface, small to large.

C/S : well circumscribed, cartilaginous, glistening & translucent : Pleomorphic adenoma (benign mixed tumour). May have tongue like tumour extensions into the surrounding normal tissue.

Solid tan tumour : Oxiphilic adenoma, myoepithelioma, adenoid cystic carcinoma, salivary duct carcinoma., epithelial-myoepithelial carcinoma.

Other solid circumscribed tumours : adenomas ; myoepithelioma

Solid friable mass usually < 3 cm : Acinic cell carcinoma

Infiltrative solid tumours : Adenoid cystic carcinoma; carcinoma ex pleomorphic adenoma; muco-epidermoid carcinoma.

Haemorrhagic infarction : occurs in Warthin's tumour.

Bilateral tumour : Warthin's tumour, Oncocytomas (Miculicz's diease bilateral – tumour like).

Minor salivary gland tumours : canalicular adenoma; basal cell adenoma; adenoid cystic carcinoma; terminal duct carcinoma.

IV SALIVARY GLANDS MICROSCOPY

1. **Sialadenitis :** -*Acute sialadenitis* : may be bacterial – as a result of infection by streptococci & staphylococcus aureus. – this may result in abscess formation . Acute

sialadenitis also results from viral infections such as paramyxoma virus (mumps); EBV; Coxsackie virus & Influenza & Parainfluenza viruses.

- *Chronic sialadenitis* : may be of obstructive or non-obstructive type.
- Leads to parenchymal atrophy, fibrosis. Microliths are found in the obstructive variety. Periductal lympho-plasmacytic infiltrates lead eventually to sclerosis.
- *Granulomatous sialadenitis* : etiologic factors could be tuberculosis, mycotic, sarcoidosis, xanthogranulomatous etc. Some of these may actually involve the intraparotid lymph nodes.
- *Autoimmune sialadenitis* : may occur as an isolated involvement of the salivary gland (Mikulicz's disease) or part of a systemic involvement. The latter is termed as Sjogren's syndrome and has as components xerostomia (dryness of mouth), keratoconjunctivitis sicca (dryness of eyes) with bilateral enlargement of the parotid salivary glands. Rheumatoid arthritis and SLE may also be associated. Histology shows a diffuse infiltrate of lymphocytes throughout the salivary gland parenchyma with cells infiltrating the ductal epithelium (lympho-epithelial lesions). Such lymphoid proliferation over several years may lead to lymphoma.

San-Diego criteria for diagnosis of Sjogren's syndrome :

a) keratoconjunctivitis sicca
b) xerostomia
c) extensive lymphoctytic infiltrate on minor salivary gland biopsy
d) laboratory evidence of systemic autoimmune disorder

Kuttner's tumour : a term specifically used for unilateral sialadenitis of the submandibular salivary gland with dense fibrosis; presents grossly as a tumour. Microscopically, the lobular architecture is usually preserved but a lymphoplasmacytic infiltrate surrounds the ducts with accompanying periductal fibrosis.

2. **Non inflammatory conditions** : - *Sialadenosis* : is hyperplasia of the salivary gland tissue. It may be recurrent and results in enlargement of the salivary glands usually bilateral and associated with an underlying systemic disorder. It mainly occurs in the parotid gland. A few cases with unilateral involvement have been reported.

   Microscopy shows hyperplastic salivary gland tissue with a normal architecture. Slight enlargement of the nuclei and acini may be observed.

   Oncocytosis : is oncocytic transformation of ductal & acinar epithelium. It is unilateral and often occurs in the elderly in the parotid gland. The multifocal nodular & oncocytic foci may measure upto 1 cm in diameter and show a solid & trabecular growth pattern. This adenomatous hyperplasia may progress to Oncocytoma. The process may also involve minor salivary glands.

   Necrotising sialometaplasia : is an ishaemic and reactive lesion of the minor salivary glands of the palate. Occurs in white men around a mean age of 46 years. The salivary duct epithelium located centrally exhibits squamous metaplasia. Peripheral to this,

necrotic acini are found with acute & chronic inflammatory cells. Yet peripheral to this intact & viable salivary gland tissue is seen giving a zonal & lobular distribution. Goblet cell metaplasia may also be seen with the squamous metaplasia. Histologically should be differentiated from a muco-epidermoid carcinoma and squamous cell carcinoma. The former has no lobular architecture, shows 3 types of cells (squamoid, intermediate and mucus, the latter forming glandular cysts), lacks inflammation and is infiltrative; squamous cell carcinoma also shows infiltration with cellular and nuclear pleomorphism of the squamoid elements.

3. **Cysts of the salivary gland** : -*mucocele (Fig 4.4)* : may result from obstruction of an salivary gland excretory duct as a result of which it gets distended by mucus. Classic example is a ranula occurring on the floor of the mouth as a translucent cyst and deriving its name from the latin word "rana" which means "little frog"- the pouch at the throat of the animal bearing a resemblance to this cyst!. (see fig 5). A mucocele may also result from extravasation and collection of mucus due to any trauma to the duct/ salivary gland tissue. This lacks a true lining in contrast to the former.

Figure 4.4

- *salivary duct cysts* may also develop within the gland and show a lining by metaplastic goblet cells, clear cells or squamous cells and exhibit varying degrees of proliferation. The lumen contains calcospherules or crystalline deposits in addition to retained mucus.
- *Lympho-epithelial cysts* : branchiogenic cysts- occur rarely in the parotid. A flattened stratified epithelium is surrounded by a lymphoid stroma.

4. **Histogenesis and differentiation of Salivary gland tumours :**

Normal structure of salivary gland acini and ducts .

- Salivary glands contain a mixture of mucus or serous acini, surrounded by myoepithelial cells. The secretions from acini move to the intercalated ducts, then to striated ducts to interlobular ducts, which empty the contents into the oral cavity or sinonasal tract. The intercalated ducts are lined by cuboidal cells, with occasional myoepithelial cells noted. The luminal cells however are considered separate from the myoepithelial cells. Striated ducts are lined by columnar cells generally lacking myoepithelial cells. Finally, the interlobular ducts have both cuboidal and columnar cells, but nearly always lack myoepithelial cells. (fig 4.5)

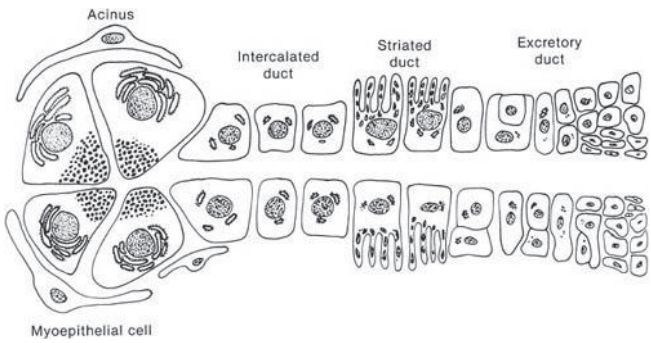


Figure 4.5 : Cells lining the salivary gland acini and duct.

Salivary gland tumors are classified on the basis of the differentiation properties of the tumor cells. Of less significance is the histogenesis from which the tumour cells arise, be it the terminal part of the ducts leading into acini or the reserve cells giving rise to various cell types. For a histo-pathologist, it is this differentiation process and the architectural arrangement of the tumor cells that are the keys to classifying a particular salivary gland tumor added to which is the matrix and it's incorporation within tumour, secreted by the cells, in particular the myo-epithelial cells. Therefore it becomes immaterial to attempt to predict from what segment of the duct system a particular tumor arose.

Tumours are seen in salivary glands with either luminal (ductular) or abluminal (myoepithelial) differentiation the latter with varying degrees of stromal matrix secretion.

Tumours with dual luminal and abluminal differentiation

Pleomorphic adenoma

Basal cell adenoma and basal cell adenocarcinoma

Adenoid cystic carcinoma

Epithelial-Myoepithelial carcinoma

Tumours with only ductular differentiation

Acinic cell carcinoma

Muco-epidermoid carcinoma

Salivary duct carcinoma

Tumours with only abluminal (myoepithelial) differentiation.

Myoepithelioma

Immunohistochemistry (IHC): Markers such as P-63, high molecular weight cytokeratin (CK-14) are positive for myoepithelial cells. Other myoid markers are calponin, actin, myosin, S-100 and Glial Fibro Acidic Protein (GFAP).

Basal cells differ ultastructurally from myoepithelial cells in the absence of myofilaments. They have the capacity of multidirectional differentiation and play an important role in regeneration

and metaplasia. They are reactive for p-63 and high molecular weight cytokeratin, but are negative for myoid markers like S-100 and GFAP.

The luminal cells are readily highlighted by immunostaining for cytokeratin, carcinoembryonic antigen (CEA), and epithelial membrane antigen (EMA). CD117/c-kit is negative in the normal salivary gland cells, however, is interestingly positive in the luminal (glandular) cells of various types of salivary gland tumors.

WHO Histological Classification of Salivary gland Tumours.

Incidence of Salivary gland tumours is around 2% of head and neck cancers. The WHO classification of 2017 has undergone a few significant changes as compared to the one outlined in 2005, and then subsequently the 2022 classification. A comparison of 2005 and 2017 is given below :

Table 1: Comparison of WHO Classifications of Salivary Gland Tumours (2005 vs 2017)

2005 WHO	2017 WHO (4th Edition)	Remarks / Changes
MALIGNANT TUMOURS		
Mucoepidermoid carcinoma	Mucoepidermoid carcinoma	Unchanged
Adenoid cystic carcinoma	Adenoid cystic carcinoma	Unchanged
Acinic cell carcinoma	Acinic cell carcinoma	Unchanged
—	Secretory carcinoma	New entity (ETV6::NTRK3)
Polymorphous low-grade adenocarcinoma	Polymorphous adenocarcinoma	Name simplified; includes CAMSG
Epithelial–myoepithelial carcinoma	Epithelial–myoepithelial carcinoma	Unchanged
Clear cell carcinoma, NOS	Clear cell carcinoma	Defined as hyalinizing type
Basal cell adenocarcinoma	Basal cell adenocarcinoma	Unchanged
Sebaceous carcinoma	Sebaceous adenocarcinoma	Name corrected
Low-grade cribriform cystadenocarcinoma	Intraductal carcinoma	Reclassified
Cystadenocarcinoma	Cystadenocarcinoma	Unchanged
Mucinous adenocarcinoma	Mucinous adenocarcinoma	Unchanged

Oncocytic carcinoma	Oncocytic carcinoma	Unchanged
Adenocarcinoma, NOS	Adenocarcinoma NOS	Retained category
Carcinosarcoma	Carcinosarcoma	Unchanged
Metastasising pleomorphic adenoma	—	Deleted
—	**Salivary duct carcinoma**	Added
—	**Myoepithelial carcinoma**	Added
—	**Carcinoma ex pleomorphic adenoma**	Added
—	Poorly differentiated carcinoma	Added: neuroendocrine / non-neuroendocrine
—	**Lymphoepithelial carcinoma**	Added
—	**Squamous cell carcinoma**	Added
—	**Oncocytic carcinoma**	Retained
Borderline tumour: Sialoblastoma	**Sialoblastoma**	Retained
BENIGN TUMOURS		
Pleomorphic adenoma	Pleomorphic adenoma	Unchanged
Myoepithelioma	Myoepithelioma	Unchanged
Basal cell adenoma	Basal cell adenoma	Unchanged
Warthin tumour	Warthin tumour	Unchanged
Oncocytoma	Oncocytoma	Unchanged
Lymphadenoma (sebaceous / non-sebaceous)	Lymphadenoma	Simplified
Cystadenoma	Cystadenoma	Unchanged
—	**Sialadenoma papilliferum**	Added
Duct papillomas – inverted, intraductal, sialadenoma papilliferum	Ductal papillomas	Grouped
Sebaceous adenoma	Sebaceous adenoma	Unchanged

Canalicular adenoma	Canalicular and other ductal adenomas	Grouped
OTHER EPITHELIAL LESIONS		
—	Sclerosing polycystic adenosis	New lesion
Nodular oncocytic hyperplasia	**Nodular oncocytic hyperplasia**	Retained
Lymphoepithelial lesions	**Lymphoepithelial lesions**	Retained
Intercalated duct hyperplasia	**Intercalated duct hyperplasia**	Retained
SOFT TISSUE LESIONS		
Haemangioma	Haemangioma	Unchanged
Lipoma / Sialolipoma	Lipoma / Sialolipoma	Unchanged
Nodular fasciitis	Nodular fasciitis	Unchanged
HAEMATOLYMPHOID LESIONS		
Extranodal marginal zone lymphoma (MALT type)	**Extranodal marginal zone lymphoma (MALT type)**	Retained
Hodgkin lymphoma	—	Deleted
Diffuse large B-cell lymphoma	—	Deleted

Table 2: WHO Classification of Salivary Gland Tumours (5th Edition, 2022)

Category	Tumour Type / Lesion	Comments / Molecular Features
MALIGNANT EPITHELIAL TUMOURS		
Mucoepidermoid carcinoma		Unchanged
Adenoid cystic carcinoma		MYB::NFIB or MYBL1::NFIB fusion
Polymorphous adenocarcinoma	Includes cribriform adenocarcinoma of minor salivary glands	PRKD1, PRKD2, PRKD3 mutations

Secretory carcinoma	(ETV6::NTRK3 fusion–positive)	NTRK or RET fusion; targetable
Acinic cell carcinoma		Retained
Salivary duct carcinoma		HER2 amplification, AR positivity
Adenocarcinoma, NOS		Retained category
Myoepithelial carcinoma		HRAS, PIK3CA, AKT1 mutations
Epithelial–myoepithelial carcinoma		Biphasic (ductal and myoepithelial) pattern
Basal cell adenocarcinoma		CYLD mutation (Brooke-Spiegler association)
Clear cell carcinoma	Hyalinizing type	EWSR1::ATF1 fusion
Mucinous adenocarcinoma		Retained
Oncocytic carcinoma		Retained
Sebaceous adenocarcinoma		Retained
Intraductal carcinoma	Low- and high-grade variants	HRAS, PIK3CA alterations
Carcinoma ex pleomorphic adenoma		PLAG1, HMGA2, plus TP53, HRAS
Sclerosing microcystic adenocarcinoma	NEW entity	EWSR1::CREM fusion, oral cavity predilection
Poorly differentiated carcinoma	Includes neuroendocrine (large/small cell) and undifferentiated types	Retained umbrella category
Lymphoepithelial carcinoma		EBV-associated in some cases
Squamous cell carcinoma		Retained
BORDERLINE / PAEDIATRIC TUMOURS		
Sialoblastoma		Embryonal-type neoplasm

BENIGN EPITHELIAL TUMOURS		
Pleomorphic adenoma		PLAG1, HMGA2 rearrangements
Basal cell adenoma		CYLD mutation possible
Canalicular adenoma and other ductal adenomas		Common in upper lip
Myoepithelioma		Retained
Warthin tumour		Bilateral; smoking association
Oncocytoma		Retained
Sebaceous adenoma		Retained
Lymphadenoma (sebaceous and non-sebaceous)		Retained
Cystadenoma		Retained
Sialadenoma papilliferum		HRAS or BRAF mutations; HPV-negative
OTHER EPITHELIAL LESIONS		
Sclerosing polycystic adenosis		Benign; possibly neoplastic
Intercalated duct hyperplasia		May mimic adenoma
Nodular oncocytic hyperplasia		Retained
Lymphoepithelial lesions		Autoimmune association
Chronic sclerosing sialadenitis (Küttner tumour)		Chronic inflammatory lesion
MESENCHYMAL AND HAEMATOLYMPHOID LESIONS		
Haemangioma		Retained
Lipoma / Sialolipoma		Retained

Nodular fasciitis		Retained
Extranodal marginal zone lymphoma (MALT)		Main primary lymphoma
Other non-Hodgkin lymphomas		Rare
TUMOUR-LIKE LESIONS		
IgG4-related sialadenitis		Newly included
Reactive lymphoid hyperplasia		Retained
Cystic lesions (mucous retention, ductal, lymphoepithelial)		Retained

Table 3 : Recent Molecular and Immunohistochemical Advances in Salivary Gland Tumours

Tumour Type	Key Molecular Alteration	Diagnostic / Clinical Significance
Pleomorphic adenoma	PLAG1, HMGA2 rearrangements	Confirms diagnosis; precursor to carcinoma ex pleomorphic adenoma
Carcinoma ex pleomorphic adenoma	PLAG1, HMGA2, with TP53, HRAS	Indicates malignant transformation
Secretory carcinoma	ETV6::NTRK3, ETV6::RET	NTRK-targeted therapy possible
Adenoid cystic carcinoma	MYB::NFIB, MYBL1::NFIB	Diagnostic marker; not prognostic
Polymorphous adenocarcinoma	PRKD1, PRKD2, PRKD3 mutations	Helps differentiate from adenoid cystic carcinoma
Clear cell carcinoma	EWSR1::ATF1	Distinguishes from mucoepidermoid carcinoma
Basal cell adenoma / carcinoma	CYLD mutation	Brooke–Spiegler syndrome link
Epithelial–myoepithelial carcinoma	HRAS, PIK3CA, AKT1	Molecular overlap with other biphasic tumours
Salivary duct carcinoma	HER2 amplification, AR positivity	Predicts benefit from anti-HER2 or anti-androgen therapy
Sclerosing microcystic adenocarcinoma	EWSR1::CREM	Newly defined; oral mucosal origin

SOME SALIENT FEATURES OF THE NEOPLASIA :

MUCOEPIDERMOID CARCINOMA (MEC)

(~15.5 % of all salivary gland tumours)

Epidemiology: The commonest malignant salivary gland tumour in both adults and children, and the most frequent malignant tumour of minor salivary glands.

Sites: Parotid > minor glands > submandibular; intra-osseous ("central") MEC may arise in the mandible or maxilla, often from malignant transformation of odontogenic cyst lining.

- Clinical features: Presents as a slowly enlarging, painless mass; age range from 1st to 9th decade (peak 4th); slight female predominance.

Gross Pathology

Ill-defined, firm to hard mass, sometimes partially encapsulated.

Cut surface grey-white with mucin-filled cysts or blood-stained areas.

Microscopy

- **Architecture:** Haphazardly arranged mucin-filled cysts and irregular nests or solid islands of tumour cells. (Fig 4.6)
- **Cell types:**
  - *Mucous cells* – columnar/goblet shaped, with abundant intracytoplasmic mucin.
  - *Epidermoid (squamous) cells* – polygonal with eosinophilic cytoplasm.
  - *Intermediate cells* – small, basaloid or non-descript, linking the two extremes.
- **Stroma:** Often fibrotic; borders are irregular and invasive.

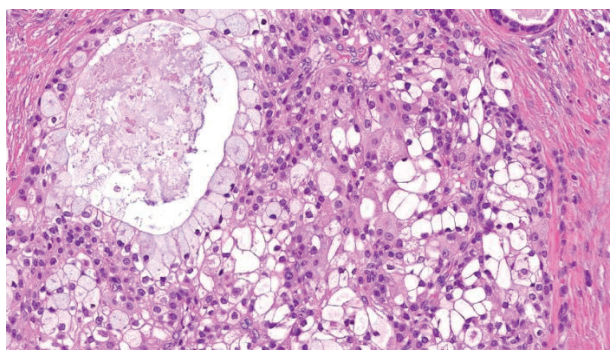


Fig 4.6 Mucin filled cysts in a muco-epidermoid carcinoma. Mucin filled cells and squamoid cells are seen. H & E x 100

Histologic Grading

Although WHO (4th ed.) was less inclined to formal grading, most authors still use the **three-tier system (low, intermediate, high)** based on cystic component, cellular atypia, mitotic activity, perineural/vascular invasion, and necrosis.

Grade	Features
Low	Predominantly cystic; lined by mucous cells; bland nuclei, rare mitoses.
Intermediate	Mixed cystic and solid; moderate atypia.
High	Predominantly solid; squamoid cells; pleomorphism, hyperchromasia, brisk mitoses, perineural and vascular invasion common. *Keratin pearls absent.*

Note: Grading correlates closely with prognosis.

Immunohistochemistry

- Cytokeratin: Positive (CK AE1/AE3, CK7, CK8/18).
- EMA, CEA: Variable positivity.
- MUC 1 / MUC 4: MUC 4 expression → favourable prognosis; MUC 1 → more aggressive behaviour.
- p63 / p40: Highlight epidermoid and intermediate cells.
- MAML2-fusion protein**:** can be detected by FISH or RT-PCR.
- May show variable S-100 and actin positivity.

Molecular and Genetic Features

Characteristic translocation: *t(11;19)(q21;p13)* resulting in CRTC1-MAML2 fusion (seen in ~60–80 % of cases). Associated with *low/intermediate-grade tumours* and favourable prognosis.

- Variants:
  - *CRTC3-MAML2 fusion* (t(11;15)) – seen in a smaller subset, especially parotid MEC.
  - *TP53 mutations* and *CDKN2A (p16) deletions* correlate with high-grade and poor outcome.
- Prognostic note: MAML2-positive tumours have 5-year survival > 90 %, versus ~50 % when negative.

Recent Advances (WHO 2022, 5th Edition)

- MEC continues as a distinct malignant epithelial tumour, with emphasis on fusion-status-based diagnosis rather than purely morphologic grading.
- MAML2 fusion testing is now recommended for confirmation in challenging or cystic cases.
- Recognition of oncocytic and sclerosing variants; both may mimic other salivary gland lesions but share MAML2 fusion.
- Immuno-profile and molecular pattern help distinguish MEC from *adenosquamous carcinoma* or *salivary duct carcinoma.*

Diagnostic Summary Box – Mucoepidermoid Carcinoma (MEC)

Feature	Key Points
IHC Profile	CK7+, CK5/6+, p63+, MUC1+, MUC4+ (good prognosis), EMA+, CEA variable, S-100 and actin focal; mucin stains (PAS/Alcian blue) highlight mucous cells.
Molecular Hallmark	t(11;19)(q14–21;p12–13) → CRTC1–MAML2 fusion (~75% cases). Rare fusions: *CRTC3–MAML2*.
Diagnostic Utility	CRTC1–MAML2 fusion confirms diagnosis and distinguishes MEC from adenosquamous carcinoma.
Prognostic Indicator	Fusion-positive tumours have better prognosis and lower recurrence/metastasis risk; high-grade MEC lacks fusion and shows increased p53 expression.

ADENOID CYSTIC CARCINOMA (ADCC)

(~10% of all salivary gland malignancies)

Definition:

A biphasic, invasive malignant epithelial neoplasm composed of basaloid and ductal cells with predominant myoepithelial/basal cell differentiation.

Epidemiology:

Usually presents in the 4th–6th decades with a slight female predilection.

Sites:

1. Minor salivary glands – most frequently involved (palate > other oral sites).
2. Among major glands, the parotid is most often affected, followed by the submandibular gland.
3. Can also occur in the upper aerodigestive tract – sinonasal cavity, nasopharynx, oropharynx, tracheobronchial tree, and lacrimal gland.

Clinical features:

Slow-growing but relentlessly progressive tumour with a tendency for perineural invasion, multiple local recurrences, and delayed distant metastasis (lung, bone, brain). Long-term prognosis remains guarded despite indolent course.

Gross Pathology

- Poorly circumscribed, firm to hard, grey-white or tan infiltrative tumour.
- Cut surface is solid and fibrous; may show small cystic foci.

Microscopy

- Three main characteristic architectural patterns may coexist — cribriform, tubular, and solid.

Pattern	Features
Cribriform	Most characteristic; discrete to confluent nests of uniform basaloid cells punctuated by round, rigid pseudocystic spaces filled with eosinophilic or basophilic hyaline material ("Swiss cheese" appearance). The hyaline material is PAS-positive, diastase-resistant, and alcian blue positive – corresponds ultrastructurally to basement membrane and glycosaminoglycans.
Tubular	Elongated tubules lined by inner ductal epithelial cells and an outer layer of basaloid/myoepithelial cells; stroma is hyalinised.
Solid	Sheets or nests of basaloid cells with hyperchromatic nuclei and scant cytoplasm; necrosis, perineural and vascular invasion frequent; correlates with high-grade behaviour.

1. Stroma: Collagenous to myxohyaline.
2. Absence of "melting" of tumour cells into the stroma (as seen in pleomorphic adenoma) helps distinction.

Grading Systems :

Two-tier: *Low grade* – no solid component; *High grade* -.– any presence of solid component

Three-tier (Szanto et al.):

Grade I: Pure cribriform/tubular pattern (no solid component); *Grade II:* 1–29% solid component; *Grade III:* ≥30% solid component.

Presence and extent of **solid pattern** strongly predict poor outcome.

Immunohistochemistry

- Basaloid/myoepithelial cells: CK (weak), vimentin, S-100 (patchy), smooth muscle actin, calponin, p63/p40, and GFAP variably positive.
- Ductal epithelial cells: Strongly positive for CK7, CAM 5.2, CEA, EMA.
- Myoepithelial markers confirm dual cell differentiation; c-KIT (CD117) shows diffuse membranous staining in most cases.
- MYB nuclear expression (IHC or FISH) serves as a surrogate marker for MYB gene fusion.

Molecular Genetics

- Characteristic gene fusions:

- *t(6;9)(q22–23;p23–24)* → MYB–NFIB fusion (most common, ~60–70%).
- *t(8;9)(p12;p23)* → MYBL1–NFIB fusion (alternative in MYB-negative tumours).

- These fusions activate MYB/MYBL1 transcriptional pathways, upregulating genes involved in cell cycle, apoptosis resistance, and angiogenesis.
- Loss of 1p36, 6q23, and 12q12–15 reported in high-grade and recurrent tumours.
- TERT promoter mutations and NOTCH1 pathway alterations are associated with tumour progression and poor prognosis.
- Prognostic note: Tumours with MYB or MYBL1 fusions generally have better differentiation and longer survival than those without identifiable fusions.

Recent Advances (WHO 2022, 5th Edition)

- AdCC remains a distinct entity under malignant epithelial tumours of salivary glands.
- Emphasis on fusion-status confirmation (MYB/MYBL1–NFIB) for diagnosis, especially in small biopsies and unusual sites.
- Solid and high-grade variants are now recognised as biologically aggressive subtypes.
- New variants described: Dedifferentiated/High-grade transformation type and hyalinised stroma–rich variant.
- Molecular testing (FISH or NGS) recommended for differentiating AdCC from morphologic mimics such as polymorphous adenocarcinoma or basal cell adenocarcinoma.

Diagnostic Summary Box – Adenoid Cystic Carcinoma (AdCC)

Feature	Key Points
IHC Profile	Dual cell population: Luminal cells – CK7+, EMA+, CEA+; Abluminal/myoepithelial cells – CK14+, S-100+, SMA+, calponin+, p63+. CD117 (c-KIT) diffuse positive. MYB nuclear positivity supports diagnosis.
Molecular Hallmark	MYB–NFIB fusion (t(6;9)); alternative MYBL1–NFIB fusion. Associated with activation of MYB transcriptional pathway.
Diagnostic Utility	Confirms AdCC vs polymorphous adenocarcinoma or basal cell adenocarcinoma; MYB IHC or FISH helpful on small biopsies.
Prognostic Indicator	Presence of solid pattern, NOTCH1, or TERT promoter mutations → poor prognosis; MYB fusion–positive tumours generally more indolent.

ACINIC CELL CARCINOMA (ACICC)

(~6–8% of all salivary gland tumours)

Definition: A low- to intermediate-grade malignant epithelial neoplasm showing serous acinar differentiation with characteristic cytoplasmic zymogen granules.

Epidemiology: Most frequently affects the parotid gland (majority), rarely submandibular or minor salivary glands. Slight female predilection; wide age range (2nd–7th decades).

Clinical Features

- Slow-growing, painless parotid mass; may occasionally cause facial nerve weakness in large tumours.
- Local recurrence occurs in 10–20% of cases; metastases are uncommon but may involve regional lymph nodes or lungs in dedifferentiated/high-grade cases.

Gross Pathology

- Well-circumscribed, often partially encapsulated; cut surface is tan to grey, soft to firm, sometimes cystic.
- May have haemorrhagic or microcystic areas.

Microscopy

- **Architectural patterns:** Solid, microcystic, papillary-cystic, or follicular; often mixed in a single tumour.
- **Cell types:**
  - Serous acinar cells – abundant basophilic or granular cytoplasm; basophilic zymogen granules highlight serous differentiation.
  - Intercalated duct-like cells – small cuboidal with bland nuclei.
  - Clear cells – may be present; occasionally vacuolated.
- **Stroma:** Fibrous, sometimes hyalinized; capsule may be incomplete.

Grading / Prognostic Features

- Most AciCCs are low-grade, but high-grade variants show:
  - Increased solid growth pattern, nuclear pleomorphism, necrosis, mitotic activity.
  - Dedifferentiation is rare but associated with poor prognosis.
- Perineural invasion is uncommon but, when present, increases recurrence risk.

Immunohistochemistry

- Positive markers: DOG1+, CK7+, EMA+, α1-antichymotrypsin+, amylase+, SOX10+.
- Negative markers: Usually negative for S-100 and p63 (helps differentiate from myoepithelial or basal cell tumours).
- PAS-D staining: Highlights cytoplasmic zymogen granules.

Molecular Genetics

- Characteristic alteration: NR4A3 gene rearrangements, commonly t(4;9)(q13;q31) fusions (partners: *HTN3, SCPP, STMN2*).

- Detection: NR4A3 overexpression detectable by IHC or RNA in-situ hybridization; helps distinguish AciCC from secretory carcinoma, oncocytic carcinoma, and clear cell carcinoma.
- High-grade transformation correlates with additional genetic hits (TP53 mutations, copy number alterations).

Prognosis

- Generally favourable; 10-year survival ~80–90% for conventional low-grade AciCC.
- High-grade or dedifferentiated tumours show higher recurrence and metastasis rates.

Diagnostic Summary Box – Acinic Cell Carcinoma (AciCC)

Feature	Key Points
IHC Profile	DOG1+, CK7+, EMA+, α1-antichymotrypsin+, amylase+, SOX10+; usually negative for S-100 and p63 (helps exclude myoepithelial tumours). PAS-D highlights cytoplasmic zymogen granules.
Molecular Hallmark	t(4;9)(q13;q31) → NR4A3 gene rearrangement (fusion partners: *HTN3, SCPP, or STMN2*). NR4A3 overexpression detectable by IHC or RNA-ISH.
Diagnostic Utility	NR4A3 positivity helps distinguish AcCC from secretory carcinoma and oncocytic neoplasms.
Prognostic Indicator	Generally indolent; dedifferentiated/high-grade transformation confers aggressive course and increased recurrence/metastasis risk.

SECRETORY CARCINOMA (SALIVARY GLAND)

Definition / synonyms

Newly introduced as a distinct entity in the 2017 WHO classification. Formerly called mammary analogue secretory carcinoma (MASC) and historically considered an "acinic cell carcinoma — zymogen-poor / intercalated-cell predominant variant." It is morphologically and genetically related to secretory carcinoma of the breast.

Epidemiology / common sites

Occurs in adults across a wide age range. Sites include the parotid gland, submandibular gland, minor salivary glands and sinonasal area. Unlike classic acinic cell carcinoma, sex predilection is approximately equal and minor salivary gland location is relatively more common.

Clinical

Slow-growing mass; often indolent but can rarely show nodal or distant spread or undergo high-grade transformation.

Gross
Well-circumscribed to focally infiltrative, often tan-yellow and may be cystic with eosinophilic colloid-like secretions.

Microscopy (typical features)

1. Glands/microcysts/tubules/papillae/solid areas lined by cuboidal to eosinophilic vacuolated cells, lumina filled with eosinophilic secretions — morphology mirrors secretory carcinoma of the breast.
2. Papillary structures are commonly seen (contrast to classic acinic cell carcinoma).
3. Cells may show intraluminal and intracytoplasmic mucin. True zymogen granules (PAS-D resistant) are absent.

Immunohistochemistry

- Typically positive: S100, mammaglobin, CK7 (variable SOX10).
- Typically negative: DOG1 (helps separate from acinic cell carcinoma).
- Other: CEA may be positive; ER usually negative.
- Practical **test**: pan-TRK (pan-TRK IHC) is a useful screening tool for NTRK fusions (see Molecular).

Molecular genetics

- The canonical and most common abnormality is t(12;15)(p13;q25) — ETV6::NTRK3 fusion, the same fusion seen in breast secretory carcinoma. Detection may be by FISH, RT-PCR or targeted NGS. (User material + refs).
- An expanded molecular spectrum is now recognized: other ETV6 fusion partners (e.g., RET, MAML3), MET and rare non-NTRK partners have been reported — therefore a negative NTRK result does not entirely exclude variant ETV6 fusions. Confirmatory molecular testing is recommended when management hinges on fusion status.

Differential diagnosis

- Acinic cell carcinoma (zymogen-rich vs zymogen-poor), low-grade mucoepidermoid carcinoma, polymorphous adenocarcinoma, other low-grade salivary tumours. IHC (S100 / mammaglobin / DOG1 / pan-TRK) and molecular testing (ETV6::NTRK3 by FISH/NGS) help resolve difficult cases. (User material + refs).

Prognosis & treatment

- Generally indolent with good long-term outcome after complete excision. Rare cases show nodal/distant spread or high-grade transformation and behave aggressively.
- Targeted therapy: For ETV6::NTRK3-positive advanced, unresectable or metastatic disease, TRK inhibitors (e.g., larotrectinib, entrectinib) have produced rapid and durable responses in multiple reports and series; in some cases they have downstaged disease to

permit conversion to surgery. Be aware that variant fusions (e.g., ETV6::RET) will **not** respond to TRK inhibitors and require different targeted strategies.

Recent advances

Pan-TRK IHC is a widely used, effective screening test for NTRK fusions in suspected secretory carcinoma; positive staining should be confirmed by molecular methods for therapeutic decisions.

- Molecular heterogeneity: besides *ETV6::NTRK3*, other ETV6 fusions (RET, MET, MAML3) and rare partners have been identified — test strategy should be flexible (FISH/NGS/fusion panel) depending on local availability.
- TRK inhibitor therapy has become a clinically relevant option for advanced NTRK-fusion positive cases, with case series showing meaningful response and occasionally enabling conversion to surgery.

Diagnostic summary box — Secretory carcinoma (key points)

- Morphology: glands/microcysts/papillae of vacuolated cuboidal cells with eosinophilic luminal secretions. (Matches breast secretory carcinoma).
- IHC: S100 +, mammaglobin +, DOG1 −, consider pan-TRK screening.
- Molecular hallmark: ETV6::NTRK3 (confirm with molecular testing).
- Treatment: surgery; consider TRK inhibitor for advanced **NTRK-fusion positive** disease.

POLYMORPHOUS ADENOCARCINOMA (PAC) — (FORMERLY PLGA / CRIBRIFORM ADENOCARCINOMA / CATS / CAMSG WHEN SITE-SPECIFIC)

Definition / synonyms

A low-grade malignant epithelial tumour of mainly minor salivary glands characterized by pronounced architectural diversity (polymorphous growth) with cytologic uniformity. Covers entities historically called polymorphous low-grade adenocarcinoma (PLGA), cribriform adenocarcinoma of minor salivary glands (CAMSG), cribriform adenocarcinoma of the tongue/minor salivary glands (CATS), etc. (User material)

Epidemiology / site

Second most common intra-oral salivary malignancy (after mucoepidermoid carcinoma). Strong predilection for minor salivary glands of the palate; rare in the parotid (<5%). Female preponderance reported; wide adult age range. (User material)

Clinical

Painless, slow-growing submucosal mass in the palate/oral cavity. CAMSG (cribriform/cribriform-papillary variants) shows a higher propensity for early nodal metastasis (notwithstanding an overall indolent clinical course).

Gross

Firm, poorly circumscribed submucosal mass; may ulcerate.

Microscopy (typical features)

- Architectural diversity: tubular, fused tubules, trabeculae, single-cell files, solid nests, fascicles, cribriform, papillary and papillary-cystic patterns.
- Cytology: relatively uniform round/pale nuclei, fine chromatin, indistinct nucleoli; necrosis and mitoses generally rare.
- Perineural invasion is common. (User material)

Immunohistochemistry

- Cytokeratins and EMA positive. S100 and SOX10 often positive (SOX10 frequently strongly positive). BCL-2 overexpression reported; CEA variable. (User material + refs).

Molecular genetics

- The majority of conventional PACs harbour the PRKD1 E710D hotspot mutation (activating).
- A clinically and morphologically distinct subset — classically cribriform/cribriform-papillary tumours including CAMSG/CATS — more often harbour PRKD family gene fusions (PRKD1/2/3 fusions) rather than the hotspot mutation. This molecular split correlates with certain clinicopathologic differences and helps subclassify ambiguous tumours.

Differential diagnosis

- Adenoid cystic carcinoma (more aggressive) — compare cytology, EMA/CEA reactivity in luminal cells, peripheral p63 positivity in ACC; both may share some chromosome 12 abnormalities but differ in behaviour and molecular drivers. Also consider metastasis, low-grade mucoepidermoid carcinoma, pleomorphic adenoma (in limited biopsies).

Prognosis & treatment

- Overall favourable; behaves as a low-grade carcinoma. Reported recurrence rate ≈20%, nodal metastasis <10% and distant metastasis <2% (your figures). Cribriform/papillary-predominant tumours (CAMSG/CATS) have higher nodal risk and may require closer nodal assessment. Complete excision with clear margins is the mainstay of treatment.

Recent advances — practical points

- Molecular classification is now practical: PRKD1 E710D hotspot marks many classic PACs, while PRKD family fusions are found in the cribriform/cribriform-papillary spectrum — testing (targeted sequencing or fusion panels) clarifies cases with overlapping morphology.
- Prognostic thresholds (validated in multiple series): ≥10% papillary component or ≥30% cribriform component correlate with worse behaviour and higher nodal risk — these percentages can be used to guide reporting and consideration of nodal evaluation.

Diagnostic summary box — Polymorphous adenocarcinoma (key points)

- Key morphology: polymorphous architecture with bland uniform nuclei; perineural invasion frequent.
- IHC**:** CK/EMA +; S100 and SOX10 commonly +; BCL-2 often overexpressed; CEA variable.
- Molecular split**:** PRKD1 E710D hotspot (classic PAC) vs PRKD fusions (cribriform/cribriform-papillary variants).
- **Clinical:** generally indolent; excision with clear margins; consider nodal evaluation for cribriform/papillary-predominant tumours.

EPITHELIAL–MYOEPITHELIAL CARCINOMA (EMC)

Definition / synonyms

A biphasic low-grade malignant salivary gland neoplasm composed of inner duct-lining epithelial cells and outer clear myoepithelial cells, typically forming duct-like structures. First described as a distinct entity in the 1991 WHO classification.

Epidemiology / sites

- Represents ~1% of all salivary gland tumours.
- Predominantly arises in the parotid gland, but also occurs in submandibular and minor salivary glands.
- Usually affects middle-aged and older adults; slight female predominance reported.

Clinical features

- Slow-growing, painless mass; occasionally multinodular.
- Locally recurrent potential is notable; distant metastasis is rare but may occur in high-grade or dedifferentiated cases.

Gross

- Often a well-circumscribed, encapsulated or partially encapsulated mass (2–4 cm), tan-white to gray, sometimes multinodular.
- Cut surface may be solid, cystic or lobulated.

Microscopy (typical features)

- Classic *biphasic pattern* with inner eosinophilic ductal epithelial cells and outer clear polygonal myoepithelial cells.
- Arranged in tubular, solid, trabecular or papillary patterns; stroma may be hyalinized or myxoid.
- The clear outer cells often form a continuous rim around the ducts.

- Variants include:
  - Sebaceous, oncocytic, apocrine, and solid clear cell variants.
  - Dedifferentiated (high-grade) EMC, showing abrupt transition to high-grade carcinoma (loss of biphasic pattern, necrosis, increased mitoses).

Immunohistochemistry

Component	Immunoprofile
Inner epithelial cells	Cytokeratin (CK7, CK8/18), EMA, CEA positive
Outer myoepithelial cells	S-100, p63, p40, calponin, SMA, GFAP positive
Other	Ki-67 low in low-grade EMC; higher in dedifferentiated areas

Molecular genetics

- Recurrent HRAS, PIK3CA, and FGFR3 mutations have been described; HRAS Q61R is most frequent (~70% of tested cases).
- PLAG1 and HMGA2 rearrangements are rare, helping separate EMC from carcinoma ex pleomorphic adenoma.
- High-grade / dedifferentiated EMC may acquire TP53 or CDKN2A alterations.

Prognosis & treatment

- Low-grade malignant; 30–40% may recur locally, particularly if incompletely excised.
- Nodal metastasis: ~10–15%; distant metastasis: uncommon (~5%).
- High-grade transformation (dedifferentiation) portends aggressive behavior and poorer outcome.
- Treatment**:** wide local excision with clear margins; adjuvant radiotherapy considered for high-grade, recurrent, or margin-positive cases.

Recent advances — practical points

- HRAS mutations (especially Q61R) identified as a molecular signature for many EMCs; IHC surrogate (HRAS Q61R-specific antibody) can aid diagnosis in challenging clear-cell lesions.
- Recognition of dedifferentiated / high-grade EMC as a distinct, more aggressive subset with loss of biphasic pattern and high proliferation index; should be specifically mentioned in reports.
- Molecular testing helps distinguish EMC from mimics such as clear cell carcinoma (EWSR1 fusions) and myoepithelial carcinoma (PLAG1/HMGA2 negative).

Diagnostic summary box — Epithelial–Myoepithelial Carcinoma (key points)

- Biphasic tumour: inner ductal epithelial cells + outer clear myoepithelial cells.
- IHC: inner CK7/EMA+, outer S100/p63/p40/SMA+.
- Mutation: frequent *HRAS Q61R*; may show PIK3CA/FGFR3 mutations.
- Behaviour**:** low-grade, but recurrences common; high-grade transformation → poor prognosis.
- Management**:** complete excision; radiotherapy for high-risk/dedifferentiated cases.

INTRADUCTAL CARCINOMA

(Low grade cribriform cystadenocarcinoma / Low grade salivary duct carcinoma)

Definition:

A neoplasm with intracystic or intraductal growth of neoplastic ductal epithelial cells.

Synonyms:

- Low grade cribriform cystadenocarcinoma (WHO 2005)
- Intraductal carcinoma, low grade
- Low grade salivary duct carcinoma

General Features:

- A rare cystic proliferative carcinoma that resembles the spectrum of breast lesions ranging from atypical ductal hyperplasia to micropapillary and cribriform low grade ductal carcinoma in situ.
- Low grade salivary duct carcinoma is the older terminology.
- The tumour is unencapsulated, generally displaces rather than truly invades surrounding salivary gland tissue.
- Multiple cysts and solid structures of varying sizes are composed of small bland ductal cells with regular nuclei and clear to eosinophilic cytoplasm.
- Cells proliferate to form papillary and cribriform structures, but comedonecrosis or necrosis is absent.

Differential Diagnosis:

- Salivary duct carcinoma (usual type), particularly its in situ component.

Behaviour:

- Non-aggressive clinical course.

Molecular Findings:

- A subset of tumours show NCOA4–RET and TRIM27–RET gene fusions.

Immunohistochemistry (IHC):

Marker	Result	Diagnostic Note
S100	Strongly positive	
AR (Androgen receptor)	Negative	
HER2/neu	Negative	
Contrast: Salivary duct carcinoma is S100–, AR+, HER2/neu+		

SALIVARY DUCT CARCINOMA

Definition:

A highly aggressive malignant tumour composed of solid, papillary-cystic, and cribriform growth patterns that closely resemble in-situ and invasive ductal carcinoma of the breast.

Epidemiology:

- Predominantly affects the elderly.
- Male : Female = 3:1 to 6:1.
- Parotid gland (≈ 80%) is most commonly involved; others arise in the submandibular or minor salivary glands.
- May arise de novo or from a pre-existing pleomorphic adenoma.

Gross Features:

- Poorly circumscribed, predominantly solid, tan-coloured tumour.
- Necrosis common.
- ~70% show extracapsular extension beyond the salivary gland.

Microscopy:

- Striking resemblance to invasive ductal carcinoma of the breast.
- Infiltrative component composed of cords, nests, glands, and single cells with abundant eosinophilic cytoplasm, pleomorphic vesicular nuclei, and prominent nucleoli.
- Apocrine change is often seen.
- Mitotic activity is evident.
- In situ components: cribriform, papillary, and solid patterns.

Variants:

- Micropapillary – shows *inside-out* EMA staining (similar to breast carcinoma).

1. Sarcomatoid
2. Mucin-rich – with abundant extracellular mucin.
3. Oncocytic

Immunohistochemistry (IHC):

Marker	Result	Diagnostic Note
AR (Androgen receptor)	Positive in >90%	Characteristic marker
GATA3, AE1/AE3, CK7, EMA, GCDFP-15	Positive	Support epithelial and apocrine differentiation
HER2/neu	Distinct membrane positivity in most cases	
ER / PR	Usually negative	Unlike breast carcinoma

Molecular Findings:

- TP53 mutations – 23%
- HRAS mutations – 23%
- PIK3CA mutations – 23%
- ERBB2 amplification – 35%
- About 75% show alterations in MAP kinase (BRAF/HRAS/NF1) or ERBB2 pathways.

Behaviour:

- Highly aggressive with frequent recurrence and metastasis.

Differential Diagnosis:

- Salivary duct carcinoma (usual type), especially its *in situ* component.

Diagnostic Summary Box -between intraduct carcinoma & salivary duct carcinoma

Feature	Intraductal Carcinoma	Salivary Duct Carcinoma
Grade / Behaviour	Low grade, indolent	High grade, aggressive
Encapsulation / Invasion	Unencapsulated, non-invasive (displacing growth)	Poorly circumscribed, invasive
Architecture	Cystic, papillary, cribriform; no necrosis	Solid, papillary, cribriform; frequent necrosis

Feature	Intraductal Carcinoma	Salivary Duct Carcinoma
Cell Features	Bland nuclei, clear/eosinophilic cytoplasm	Pleomorphic nuclei, apocrine change
IHC – S100	Positive	Negative
IHC – AR	Negative	Positive (>90%)
IHC – HER2/neu	Negative	Positive (membranous)
Molecular Findings	RET fusions (NCOA4–RET, TRIM27–RET)	TP53, HRAS, PIK3CA mutations; ERBB2 amplification
Clinical Course	Non-aggressive	Aggressive, often metastasizing

Adenocarcinoma, Not Otherwise Specified (NOS) :

This entity encompasses a spectrum of malignant epithelial neoplasms composed of ductal and glandular structures, with or without cystic formation, that lack the defining morphological features of the more common and specific salivary gland carcinomas.
It may present as a cystic or papillary-cystic neoplasm, mucinous carcinoma, or even an intestinal-type adenocarcinoma — patterns which do not conform to any recognized salivary gland tumour type.

SIALOBLASTOMA

Definition:
A rare congenital or perinatal salivary gland tumour that usually presents as a parotid mass at or soon after birth.
It is considered a neoplasm of low malignant potential showing primitive salivary gland differentiation.

WHO Classification (2017 / 2022):

- Recognized under “Epithelial tumours of uncertain or low malignant potential.”
- Previously termed *embryoma* or *basaloid adenoma of infancy.*

Gross Features:

- Usually well-circumscribed or partially encapsulated mass in the parotid region.
- Cut surface: solid, tan-white or gray, occasionally cystic or lobulated.

Microscopy:

- Composed of primitive basaloid or epithelioid cells arranged in ductular, organoid, or cribriform patterns within a fibrous to partly myxoid stroma.
- Duct-like structures may have eosinophilic secretions.

- Mitoses and pleomorphism are minimal or absent.
- Rarely shows foci resembling fetal salivary gland differentiation.

Behaviour:

- May be locally infiltrative but rarely metastasizes.
- Occasional reports of recurrence after incomplete excision.
- Some cases associated with congenital anomalies or Beckwith–Wiedemann syndrome.

Prognosis:
Generally favourable with complete excision; malignant transformation is exceptionally rare.

PLEOMORPHIC ADENOMA

Definition:
Also known as *Mixed tumour,* this is a benign salivary gland neoplasm showing epithelial (luminal) and myoepithelial (abluminal) differentiation with a characteristic stromal component. It is the most common salivary gland tumour, constituting 28–74% of all salivary gland neoplasms.

Epidemiology:

- Occurs at any age, most frequent in the 3rd–5th decades.
- Female predominance.
- 75% occur in the parotid gland (90% of these in the superficial lobe).
- 5–10% arise in the submandibular gland and ~10% in minor salivary glands (palate, cheek, tongue, floor of mouth).
- Rarely synchronous, metachronous, or familial cases occur.

Molecular Features:

- Recurrent chromosomal translocations involving 8q12 (PLAG1) and 12q13–15 (HMGA2) are characteristic.
- These rearrangements lead to overexpression of oncogenic transcription factors.

Histogenesis:

- Originates from ductal and myoepithelial cells.
- The stromal component (myxoid, chondroid, or fibrous) is produced by modified myoepithelial cells, hence the tumour is epithelial in nature.

Gross Features:

- Well-circumscribed, often thinly encapsulated mass, varying from a few mm to several cm.

- Cut surface: rubbery, gray-white, glistening or mucoid.
- Capsule may be deficient with tongue-like extensions into surrounding glandular tissue — the cause of recurrence after excision.

Microscopy: (Fig 4.7)

- Highly variable architecture with ductal, trabecular, solid, and myxochondroid areas.
- Ductal structures lined by cuboidal or columnar epithelial cells surrounded by myoepithelial mantles that merge with a chondromyxoid stroma.
- Ribbons, cords, and nests of tumour cells may be seen.
- Stroma may be chondroid, myxoid, chondromyxoid, fibrous, or rarely osseous/adipose.
- Crystalloids (collagenous, tyrosine "daisy heads," or oxalate) may occur.

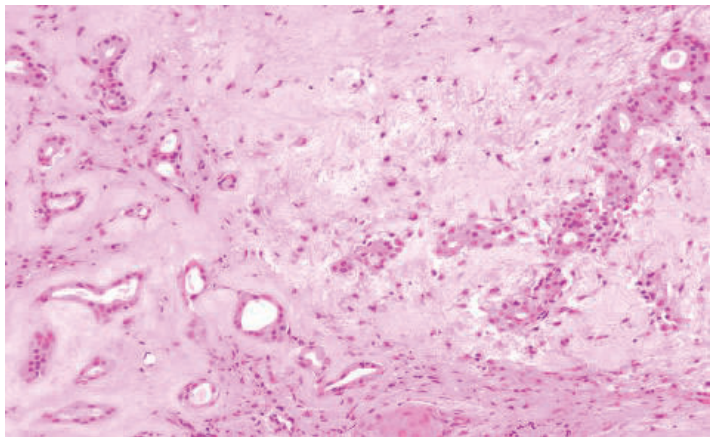


Fig 4.7 shows ductal structures against a myxoid stroma. H & E x 100

Special Variant:

- **Metastasizing Pleomorphic Adenoma:**
  - Histologically benign but shows distant metastase**s** (bone, lung, lymph nodes, skin, kidney).
  - Usually follows prior surgical excision, appearing years later (1.5–51 years).
  - Generally indolent, but rarely aggressive.

Prognosis:

- Excellent with complete excision.
- Recurrences result from capsular breach or tumour spillage.
- Risk of malignant transformation to *Carcinoma ex Pleomorphic Adenoma* increases with tumour duration and recurrence.

Carcinoma ex Pleomorphic Adenoma (CXPA)

Definition / synonyms

A malignant epithelial neoplasm arising from a pre-existing pleomorphic adenoma (PA).
The malignant component may be any type of salivary carcinoma, most commonly salivary duct carcinoma, myoepithelial carcinoma, or less often adenocarcinoma NOS, epithelial–myoepithelial carcinoma, or mucoepidermoid carcinoma.
Synonyms: *Malignant mixed tumour; carcinoma arising in pleomorphic adenoma.* (3, 13)

Epidemiology / sites

- Accounts for ~3–6 % of all salivary gland tumours and ~12 % of salivary malignancies.
- Most frequent site: parotid gland (~75 %). Also occurs in submandibular and minor salivary glands.
- Typically presents in middle-aged to elderly adults (decade later than benign PA).

Clinical features

- Long-standing, slow-growing parotid or submandibular mass that suddenly enlarges rapidly, becomes painful or fixed, or shows facial nerve involvement.
- May develop skin ulceration or regional nodal metastasis.
- Duration of pre-existing PA ranges from months to decades.

Gross

- Irregular, infiltrative, often multinodular tumour with residual PA areas.
- Cut surface: tan-gray to yellow with zones of necrosis and hemorrhage; sometimes encapsulated in early lesions.

Microscopy (typical features)

Two distinct components:

1. **Residual benign pleomorphic adenoma:**
    - Areas showing ductal and myxochondroid stroma typical of PA.
2. **Malignant epithelial component:**
    - Most often salivary duct carcinoma (type with apocrine cells and comedonecrosis).
    - Others: myoepithelial carcinoma, adenocarcinoma NOS, rarely epithelial–myoepithelial carcinoma pattern.
    - Interface between benign and malignant areas may be abrupt or gradual.
    - Perineural and vascular invasion common in frankly invasive lesions.

Histologic grading / subtypes

CXPA is classified by extent of invasion beyond the capsule of the original PA:

Category	Definition	Prognosis
Non-invasive / intracapsular	Carcinomatous change confined within the PA capsule	Excellent (near-benign)
Minimally invasive	≤ 1.5 mm invasion beyond capsule	Favourable
Widely invasive	> 1.5 mm invasion beyond capsule	Aggressive; risk of recurrence / metastasis

Immunohistochemistry

- Ductal / salivary duct carcinoma-type: CK7, AR, HER2, GCDFP-15, mammaglobin positive.
- Myoepithelial carcinoma-type: S100, p63, calponin, SMA positive.
- Residual PA: PLAG1 and HMGA2 positive (markers of PA origin).
- Ki-67 / p53**:** high proliferative index and p53 over-expression in malignant component.

Molecular genetics

- PLAG1 and HMGA2 rearrangements retained from the parent PA (evidence of clonal evolution).
- Secondary TP53, HER2 amplification, PIK3CA, HRAS, and CTNNB1 mutations correlate with malignant transformation.
- HER2 amplification / AR expression in salivary duct carcinoma-type CXPA predicts possible benefit from targeted therapy (trastuzumab, anti-androgen).
- Occasional copy-number gains of 8q (MYC) and 12q (PLAG1 region) in high-grade tumours.

Differential diagnosis

Differential	Key distinguishing feature
Benign PA	No invasion; bland myxochondroid stroma.
De-novo salivary duct carcinoma	Lacks residual PA; occurs *de novo*; often pure high-grade morphology.
Myoepithelial carcinoma (de novo)	Entirely myoepithelial; no pre-existing PA component.
Metastatic carcinoma	Clinical history and absence of residual PA help.

Prognosis & treatment

- Prognosis largely depends on extent of invasion and histologic grade of the malignant component.
  - *Non-/minimally invasive:* excellent prognosis (5-year survival > 95 %).
  - *Widely invasive:* poor prognosis (5-year survival ≈ 30–50 %).
- Recurrence rate: ~30 %; metastasis: 20–25 %.
- Treatment: wide excision (parotidectomy) with margin clearance; neck dissection if nodes involved; adjuvant radiotherapy for high-grade or invasive cases.

Recent advances — practical points

- Molecular progression model: benign PA → accumulation of TP53, PIK3CA, HER2 alterations → CXPA. Recognizing residual PA with these secondary changes confirms origin.
- Targeted therapy opportunities: HER2-amplified or AR-positive salivary duct carcinoma-type CXPA may benefit from anti-HER2 or anti-androgen therapy.
- p53 / Ki-67 IHC assists in differentiating early malignant transformation within PA.
- MRI features: ill-defined margins, perineural spread, or infiltrative enhancement suggest malignant transformation pre-operatively.

Diagnostic Summary box — Carcinoma ex Pleomorphic Adenoma (key points)

- Arises in pre-existing PA; may contain benign and malignant components.
- Common malignant types: salivary duct carcinoma, myoepithelial carcinoma.
- Molecular: PLAG1/HMGA2 rearranged → secondary TP53, HER2, PIK3CA changes.
- Prognosis: depends on invasion depth (> 1.5 mm = poor).
- Management**:** complete excision ± neck dissection; consider HER2/AR-targeted therapy for positive cases.

WARTHIN'S TUMOUR

Synonyms: Adenolymphoma, Papillary Cystadenoma Lymphomatosum

Epidemiology:

- ~15% of all epithelial salivary gland tumours
- Strong association with smoking
- Predominantly men in 6th–7th decades
- Bilateral/multifocal in <10% of cases

Gross Features:

- Well-circumscribed, doughy to cystic mass, ~2 cm average
- Cut surface: cystic, with mucous/proteinaceous material

Microscopy:

- Irregular cystic structures with papillary folds
- Double-layered epithelium: superficial oncocytic cells (eosinophilic, mitochondria-rich) + cuboidal basal cells
- Separated from lymphoid stroma by a basement membrane, with follicles present
- Epithelium may show squamous or mucous metaplasia
- Lumen: eosinophilic debris

Clinical Notes:

- Rare malignant transformation (<0.1%): adenocarcinoma, SCC, undifferentiated carcinoma
- Lymphoid stroma may rarely give rise to lymphoma

BASAL CELL ADENOMA

Definition:

- Rare benign salivary gland tumour (1–3% of salivary neoplasms) composed of basaloid cells sharply delineated from the stroma by a basement membrane.

Epidemiology:

- Slight female preponderance
- Peaks in 6th–7th decade
- 70% parotid, next most common: upper lip

Gross Features:

- Well-circumscribed, with or without capsule
- Cut surface: tan or light brown
- Membranous variant: multifocal, multinodular, may recur; also called dermal analogue tumour, associated with cutaneous adnexal tumours

Microscopy:

- Small basaloid cells: round basophilic nuclei, scant cytoplasm
- Growth patterns: solid, trabecular, tubular, membranous
- Solid: peripheral palisading

- Tubular: single or double-layered tubules
- Membranous: abundant **thick eosinophilic PAS-positive hyaline basal lamina** around smooth tumour islands; higher recurrence risk

Immunohistochemistry:

- Ductal cells: CEA, EMA, CK, α1-antichymotrypsin
- Basaloid cells: vimentin, actin, calponin, p63, focal S100

Clinical Notes:

- Usually benign; membranous variant may recur and rarely transform malignantly

BASAL CELL ADENOCARCINOMA

Definition:

- Malignant counterpart of basal cell adenoma, occurring mainly in elderly, predominantly in the parotid gland

Morphology / Microscopy:

- Similar growth patterns to basal cell adenoma (solid, trabecular, tubular, membranous)
- Infiltrative growth, perineural invasion, intravascular spread

Differential Diagnosis:

- Basal cell adenoma (may show capsular invasion)
- Solid variant of Adenoid Cystic Carcinoma

Clinical Notes:

- Malignant behaviour with local invasion; rare metastasis

ONCOCYTOMA

Definition:

- Benign salivary gland tumour composed almost entirely of oncocytes (large eosinophilic granular cells rich in mitochondria)

Epidemiology:

- Rare, mostly parotid gland
- Occurs in 6th–7th decades, slight female predominance

Gross Features:

- Well-circumscribed, tan-brown, solid, firm nodules
- Usually <4 cm

Microscopy:

- Sheets, nests, or trabeculae of oncocytes with granular eosinophilic cytoplasm
- Minimal stroma, well-circumscribed
- Capsule usually present, occasional capsular invasion

Clinical Notes:

- Slow-growing, usually asymptomatic
- Rare recurrence if excised completely
- Malignant counterpart: Oncocytic carcinoma**ONCOCYTIC CARCINOMA** ⁝ Very rare and exhibits marked nuclear pleomorphism.

MYOEPITHELIOMA: (1–2.9%)

Definition:

- Benign tumour composed exclusively or almost exclusively of myoepithelial cells.
- Up to 5–10% ductal component may be allowed by some authors.
- Chondromyxoid stroma absent, but mucinous (hyaluronic acid) stroma is permissible.

Epidemiology:

- Frequently involves parotid gland and palate
- Peak age: 3rd–5th decade

Gross Features:

- Thin capsule, solid, tan or yellow cut surface

Microscopy:

- Cell types: spindly, plasmacytoid-hyaline, epithelioid, or clear
- Spindle cells: storiform, swirling, or herringbone patterns
- Plasmacytoid-hyaline cells: similar to pleomorphic adenoma, with loose myxoid stroma
- Epithelioid cells: polygonal, eosinophilic cytoplasm, bland nuclei
- Clear cells: glycogen-rich
- Collagenous crystalloids may be present

Immunohistochemistry:

- Pan-cytokeratin, CK14, EMA (focal)
- Myoepithelial markers: calponin, S100, GFAP, actin, vimentin

Differential Diagnosis:

- Pleomorphic adenoma
- Malignant myoepithelioma
- Mesenchymal tumours (schwannoma, nodular fasciitis)

MYOEPITHELIAL CARCINOMA

Definition:

- Malignant counterpart of myoepithelioma, demonstrating cytologic atypia and infiltrative growth.
- Borderline cases: "myoepithelial neoplasm of uncertain malignant potential."

Epidemiology:

- Peak age: 6th decade (about 10 years older than benign)
- May arise de novo or from pre-existing pleomorphic adenoma or myoepithelioma

Gross Features:

- Usually unencapsulated, may show necrosis or cystic degeneration
- Size: 2.1–5.5 cm

Microscopy:

- Infiltrative, multinodular growth
- Varied cell types: spindle, epithelioid, plasmacytoid, clear cells
- Mitotic count: >7/10 HPF
- Necrosis and myxoid areas may be present
- mitoses >7/10 HPF
- Outcome**:** Local recurrence, distant metastasis possible

Immunohistochemistry:

- Same as benign counterpart, EMA positive
- CEA and HMB-45 negative

Clinical Outcome:

- Variable: local recurrence, distant metastasis, tumour-related death

LYMPHADENOMA

Definition:

- Rare tumour (<1% of salivary gland neoplasms), including sebaceous adenoma and sebaceous carcinoma in modern classification
- Occurs exclusively in parotid

Epidemiology:

- Average age: ~60 years

Gross Features:

- Solid, well-encapsulated or demarcated
- Occasionally cystic

Microscopy:

- Well-differentiated epithelial cells with minimal atypia
- Adenomas: no infiltration
- Sebaceous lymphadenoma: sebaceous cell nests + lymphoid stroma with follicles
- Sebaceous carcinoma: rare, usually parotid, 1–6 cm, may involve facial nerve; nests/sheets of pleomorphic sebaceous cells

Clinical Notes:

- Recurrence rare for adenomas
- Carcinomas extremely rare; aggressive behaviour possible

DUCT PAPILLOMAS

Definition

- are papillary neoplastic projections with stroma and covering epithelia. Various morphological terms are – 'inverted duct papilloma', 'sialadenoma papilliferum' & 'intraductal papilloma'; they are a group of rare benign salivary gland tumours which arise from the excretory ducts or the junction between ductal or mucosal epithelium and characterized by a papillary growth pattern.

Clinical notes and features

- Present as small and asymptomatic intraoral masses in the adults.
- *Inverted duct papilloma* is an inward growth into the underlying stroma with projecting epithelial islands composed of non keratinizing squamous cells or transitional type cells with occasional columnar and goblet cells.

- *Intraductal papilloma* develops in a deeply situated salivary duct often presenting as a unicystic dilatation of the duct. A single to double layer of cuboidal to columnar cells forms the epithelial lining supported by thin cores of fibrovascular tissue.

SIALADENOMA PAPILLEFERUM :

Definition

- Exophytic papillary or verrucous growth from the buccal mucosal surface with a pedunculated base and ductal proliferation.
- The analogous lesion in the skin is the syringocystadenoma papilleferum
- **Epidemiology:** Extremely uncommon; most cases arise in minor salivary glands of the palate.
- **Gross**: Small, exophytic, verrucous or papillary lesion, often less than 2 cm.
- **Microscopy**: Papillary squamous epithelium overlying ductal structures with complex branching; resembles inverted papilloma but with salivary ductal component.
- **Prognosis:** Excellent; complete excision is curative, recurrence is rare.
- Differentials: Squamous papilloma, inverted ductal papilloma, papillary cystadenoma.
- **Molecular Markers**: Limited data; occasional BRAF mutations reported.

CYSTADENOMA

- **Definition**: Benign salivary gland neoplasm composed of cystic spaces lined by epithelial cells.
- **Epidemiology**: Rare; occurs in both major and minor salivary glands, most often parotid.
- **Gross**: Well-circumscribed, multicystic lesion, often translucent or mucoid.
- **Microscopy**: Multiple cystic spaces lined by cuboidal, columnar, or oncocytic epithelium; may show papillary projections.
- **Prognosis**: Excellent; complete excision is curative.
- **Differentials**: Warthin tumor, papillary cystadenoma, low-grade mucoepidermoid carcinoma, duct ectasia, cystadenocarcinoma, and polycystic disease
- **Molecular Markers**: No consistent markers identified.

CYSTADENOCARCINOMA

- **Definition**: Malignant salivary gland tumor with predominant cystic architecture and invasive growth.
- **Epidemiology**: Very rare; most cases in parotid gland, wide age range.
- **Gross**: Poorly circumscribed, cystic and solid areas, may show hemorrhage or necrosis.
- **Microscopy**: Multicystic spaces lined by atypical epithelial cells with papillary or micropapillary growth; infiltrative borders.

- **Prognosis**: Variable; low-grade tumors have favorable outcome, high-grade show recurrence and metastasis.
- **Differentials**: Papillary cystadenoma, mucoepidermoid carcinoma, adenocarcinoma NOS.
- **Molecular Markers**: Limited; occasional HER2 amplification or p53 abnormalities reported.
- **IHC :** Ductular cells show CK+ve; EMA +ve ; MAM6; MAM3; lysozyme +ve

CANALICULAR ADENOMA

- Definition: Benign salivary gland tumor characterized by cords of columnar epithelial cells forming canal-like structures.
- Epidemiology: Rare; accounts for <1% of salivary gland tumors. Predominantly arises in minor salivary glands, especially upper lip. Peak incidence in elderly patients, with female predominance.
- Gross: Small, well-circumscribed, soft, nodular lesion, usually <2 cm.
- Microscopy: Strands and cords of columnar epithelial cells arranged in branching, canal-like patterns within a loose vascular stroma. Cells have bland nuclei, eosinophilic cytoplasm; no atypia or mitoses.
- Prognosis: Excellent; complete excision is curative. Recurrence is rare.
- Differentials: Basal cell adenoma, pleomorphic adenoma, adenoid cystic carcinoma (cribriform pattern), mucoepidermoid carcinoma (low-grade).
- Molecular Markers: No consistent genetic alterations identified; considered a distinct benign entity without known recurrent molecular drivers.

ADENOMATOUS LESIONS (NON-NEOPLASTIC)

- **Definition**: Hyperplastic or hamartomatous proliferations of salivary gland tissue, not true neoplasms.
- **Epidemiology**: Rare; often incidental findings in minor salivary glands.
- **Gross**: Small, nodular, well-circumscribed lesion resembling adenoma. Maybe *Intercalated duct hyperplasia (IDH); oncocytic nodular hyperplasia, lymphoepithelial lesions etc*
- **Microscopy**: Proliferation of normal-appearing salivary gland acini and ducts in lobular arrangement; lacks atypia or infiltrative growth.
- **Prognosis**: Benign; no recurrence after excision.
- **Differentials**: Pleomorphic adenoma, basal cell adenoma, sialadenosis.
- **Molecular Markers**: None identified; considered reactive rather than neoplastic

SCLEROSING POLYCYSTIC ADENOSIS :

- **Definition** : This is a benign salivary gland lesion introduced in the 2017 WHO classification in 'other epithelial lesions' bears a close resemblance to fibrocystic disease of the breast and sclerosing adenosis (SPAN). -Synonyms : Sclerosing adenosis; sclerosing polystic sialadenopathy; polystic adenosis; sclerosing polycystic adenoma. The lesion is considered clonal and therefore most likely an adenoma.
- **Clinical features** : The age range varies, with a slight female predilection; 1.3 : 1
- **Gross** :Most cases involve the parotid though it can occur elsewhere in the oral and nasal cavities..
- **Microscopy** : Duct morphology ranges from intercalated duct like to apocrine and cells may how characteristic red granules in the cytoplasm.
- **Prognosis** : good

Comparative Table: Benign Salivary Gland Adenomas

Lesion	Epidemiology / Site	Gross Features	Microscopy Highlights	Prognosis	Molecular Markers
Pleomorphic Adenoma	Most common; parotid > minor glands	Firm, lobulated, encapsulated	Epithelial + myoepithelial cells in myxoid/chondroid stroma	Excellent; recurrence if capsule violated	PLAG1, HMGA2 rearrangements
Warthin Tumor	Older males; parotid, smokers	Cystic, papillary, brown fluid	Papillary oncocytic epithelium + lymphoid stroma	Excellent; rare recurrence	Mitochondrial DNA mutations
Oncocytoma	Rare; parotid	Well-circumscribed, tan/brown	Sheets of oncocytes with granular eosinophilic cytoplasm	Excellent	Mitochondrial abnormalities
Basal Cell Adenoma	Parotid, elderly	Firm, encapsulated	Basaloid cells in solid, trabecular, tubular, membranous patterns	Excellent; membranous type may recur	β-catenin mutations (occasional)
Canalicular Adenoma	Upper lip, elderly women	Small, soft, nodular	Columnar cells in cords forming canal-like structures	Excellent	None consistent
Cystadenoma	Parotid, minor glands	Multicystic, translucent	Cystic spaces lined by cuboidal/columnar epithelium	Excellent	None consistent

Lesion	Epidemiology / Site	Gross Features	Microscopy Highlights	Prognosis	Molecular Markers
Sialadenoma Papilliferum	Palate, rare	Small, exophytic papillary mass	Papillary squamous epithelium with ductal proliferation	Excellent	Occasional BRAF mutations
Adenomatous Lesions	Minor glands, incidental	Small, nodular	Hyperplastic lobules of normal acini/ducts	Benign, non-neoplastic	None

SALIVARY GLAND TUMOURS AS PER THE GRADE:

Low grade salivary gland tumours :

Sialoblastoma

Basal cell adecarcinoma

Low grade cystadenocarcinoma

Muco-epidermoid carcinoma Grade I

Adenoid cystic carcinoma with no solid component

Epithelial-myoepithelial carcinoma

Polymorphous adenocarcinoma

Clear cell carcinoma NOS, low grade

High grade salivary gland tumours

Salivary duct carcinoma & variants

Oncocytic carcinoma

High grade adenocarcinoma NOS

Adenoid cystic carcinoma with > 30% solid component

Muco-epidermoid carcinoma Grade III

Intermediate grade

Mucinous adenocarcinoma

Muco-epidermoid carcinoma Grade II

Adenoid cystic carcinoma with 1-29% solid component

Clear cell carcinoma , NOS, high grade

Acinic cell carcinoma

High grade transformation : introduced in the recent concept of the 4th edition is the preferred terminology (over dedifferentiation) for progression of a lower grade carcinoma with conventional morphology into a pleomorphic high-grade carcinoma . The criteria for recognition of this morphology lies in its aggressive behavior and therefore a potential difference in clinical management. Tumors for which this phenomenon is well characterized include acinic cell carcinoma, adenoid cystic carcinoma, and epithelial-myoepithelial carcinoma. For documenting this transformation, a side by side comparison with conventional low grade lesion confirms a more aggressive high grade behavior. Secretory carcinoma and PAC have also been reported to rarely undergo this high-grade transformation, which was part of the reason for dropping the term "low-grade" from polymorphous low grade adenocarcinoma.

Among the immunohistochemical markers, Ki-67 is the one that helps distinguish between the conventional and transformed elements, keeping in mind morphological criteria. Also overexpression of p53 is seen in most transformed components.

V SALIVARY GLAND SYNOPTIC REPORTING

Gross:

Site: Parotid □ Submandibular □ Sublingual □

Left □ Right □ Not specified □

Other □ Please specify ……..……

Size : ……..cm x ……cm

Type of biopsy

Tru cut □ Wedge □ Resection □

Superficial parotidectomy □ Total parotidectomy □

Tumour present Yes □ No □

Maximum tumour dimension …… (mm)

Cut surface : Solid □ Fibrotic □ Cystic change □

Necrosis Yes □ No □

Haemorrhage Yes □ No □

Macroscopic extra glandular extension: Yes □ No □ …….mm into soft tissue.

Closest surgical margin ……mm

Microscopy :

Non tumour

Sialadenitis Acute □ Chronic □ Granulomatous □ Tuberculosis □

Other □ (specify)……..

Duct dilation Yes □ No □

Lithiasis Yes □ No □

Miculitz disease Yes □ No □

Lympho epithelial lesion seen Yes □ No □

Necrotising sialometaplasia Yes □ No □

Cyst Yes □ No □ Type, specify…….

Comment ……

Tumour

Benign □ Malignant □

Histological sub type ……..

Bilateral □ Unilateral □

Histological grade : Not applicable □

Gx Cannot be assessed □

G1 well differentiated □

G2 mod differentiated □

G3 Poorly differentiated

Other……..

Surgical resection margins Clear □ Distance of closest margin from tumour □ Involved □ Specify which (if possible)……

Lymphovascular invasion Absent □ present □ indeterminate □

Perineural invasion Absent □ present □

Lymph nodes :

Right neck dissection : Yes □ No □

Comprehensive □ Selective □

Node levels present: I □ II □ III □ IV □ V □ VI □ Other □

Total number of nodes…… (in each category)

Number positive nodes……... (in each category)

Levels with metastases: I □ II □ III □ IV □ V□ VI □

Other □

Largest metastasis …..…….. (mm)

Extracapsular spread (ECS/Extranodal) Yes □ No □

Levels with ECS……

Left neck dissection: Yes □ No □

Comprehensive □ Selective □

Node levels present: I □ II □ III □ IV □ V □ VI □ Other □

Total number of nodes……(in each category)

Number positive nodes……...(in each category)

Levels with metastases: I □ II □ III □ IV □ V □ VI□ Other □

Largest metastasis …..…….. (mm)

Extracapsular spread (ECS/Extranodal) Yes □ No □

Levels with ECS……

Tumour site (only in case of node biopsy)…….……

New primary □ Recurrence □ Not known □

Tumour type……

pTNM stage pT….. pN……. pM…..…

SNOMED codes

Topography…… Morphology…….

Resection of primary tumour clear □ close □ involved □

Signature …… Date ……

TNM Staging of Salivary Gland Tumours – AJCC 8th Edition

Primary Tumour (pT):

- **pTx:** Cannot be assessed
- **pT0:** No evidence of primary tumour
- **pT1:** ≤ 2 cm, no extraparenchymal extension
- **pT2:** > 2 cm but ≤ 4 cm, no extraparenchymal extension
- **pT3:** > 4 cm or any size with extraparenchymal extension, **without facial nerve involvement**
- **pT4a:** Invades skin, mandible, ear canal, or facial nerve
- **pT4b:** Invades skull base, pterygoid plates, or carotid artery

Note: Extraparenchymal extension is clinical or macroscopic invasion of soft tissues.

Regional Lymph Nodes (pN):

- **pNX:** Cannot be assessed
- **pN0:** No regional lymph node metastasis
- **pN1:** Single ipsilateral node ≤ 3 cm, no ENE
- **pN2a:** Single ipsilateral node ≤ 3 cm with ENE, or > 3–6 cm without ENE
- **pN2b:** Multiple ipsilateral nodes ≤ 6 cm, no ENE
- **pN2c:** Bilateral or contralateral nodes ≤ 6 cm, no ENE
- **pN3a:** Node > 6 cm, no ENE
- **pN3b:** ENE in any node (single ipsilateral > 3 cm, contralateral, or multiple nodes)

Notes:

- Selective neck dissection: ≥10 nodes; Comprehensive (radical/modified) ≥15 nodes
- Midline nodes = ipsilateral

- ENE: tumour extends beyond node capsule into surrounding tissue, may have stromal reaction

Distant Metastasis (pM):

- **pMX:** Cannot be assessed
- **pM0:** No distant metastasis
- **pM1:** Distant metastasis (specify site)

AJCC Stage Grouping:

- Stage 0: Tis N0 M0
- Stage I: T1 N0 M0
- Stage II: T2 N0 M0
- Stage III: T3 N0 M0 or T0–3 N1 M0
- Stage IVA: T4a N0–1 M0 or T0–4a N2 M0
- Stage IVB: Any T N3 M0 or T4b any N M0
- Stage IVC: Any T any N M1

ASSESS YOURSELF SALIVARY GLANDS

Essay-Type Questions (10 marks each)

1. Describe the histopathology, molecular features, and clinical behaviour of Pleomorphic Adenoma of salivary glands. Include gross features, recurrence, and risk of malignant transformation.
2. Outline the classification, morphology, and clinical significance of Adenoid Cystic Carcinoma and its variants. Include perineural invasion and prognostic factors.
3. Discuss Sialoblastoma and Adenocarcinoma NOS in salivary glands, emphasizing diagnostic criteria, differential diagnosis, and the impact of molecular studies on their classification.

Short Answer (5 marks each)

1. List the key immunohistochemical markers used to differentiate Salivary Duct Carcinoma from other high-grade salivary gland neoplasms.
2. Explain the significance of MAML2 and ETV6-NTRK3 gene rearrangements in salivary gland tumours. Which tumours are they associated with?
3. Briefly describe the morphological patterns and clinical relevance of Secretory Carcinoma (Mammary Analogue Secretory Carcinoma) of salivary glands.

Point Questions (3 marks each)

1. Name the most common site and age group for Pleomorphic Adenoma.
2. List three tumours that may present with a cystic or papillary-cystic pattern in salivary glands.
3. Identify two histologic features that help distinguish low-grade Adenocarcinoma NOS from other salivary gland malignancies.

MCQs

1. **Which of the following translocations is characteristic of Secretory Carcinoma of the salivary gland?**
   A) t(11;19) MAML2–CRTC1
   B) t(12;15) ETV6–NTRK3
   C) t(8;14) MYC
   D) t(9;22) BCR–ABL
   Answer: B) t(12;15) ETV6–NTRK3
2. **The most common site for Pleomorphic Adenoma is:**
   A) Submandibular gland
   B) Minor salivary glands of palate
   C) Parotid gland, superficial lobe
   D) Sublingual gland
   Answer: C) Parotid gland, superficial lobe
3. **Which of the following statements about Sialoblastoma is TRUE?**
   A) Occurs predominantly in adults
   B) Shows high mitotic activity and pleomorphism
   C) Considered a low malignant potential tumour in infants
   D) Frequently metastasizes to lymph nodes
   Answer: C) Considered a low malignant potential tumour in infants

Chapter 5
Respiratory System

Chapter outline A Upper Repiratory tract; B lungs; C Pleura .

AI Upper respiratory tract Anatomy; A II Upper Respiratory tract Grossing Techniques ; A III Upper Respiratory tract Gross Pearls; A IV Upper Respiratory tract Microscopy; A V Upper Respiratory Tract Synoptic Reporting.

B I Lungs Anatomy; C I Pleura Anatomy B II Lungs Grossing Techniques; C II Pleura Grossing Techniques; B III Lungs Gross Pearls; ; C III Pleura Gross Pearls ;B IV Lungs and Pleura (CIV) Microscopy ; B V Lungs Synoptic Reporting ;C V Pleura Synoptic Reporting .

A I UPPER RESPIRATORY TRACT ANATOMY

Nasal Cavity and Paranasal Sinuses

The nasal cavity is the uppermost part of the respiratory tract, divided by the nasal septum into two passages, while the paranasal sinuses are air-filled extensions of the nasal cavity within surrounding bones. Together, they are clinically relevant in grossing because of their anatomical boundaries, drainage pathways, and surgical landmarks.

Nasal Cavity

- **Boundaries:**
  - **Roof:** Formed by nasal bones, cribriform plate of ethmoid, and sphenoid body.
  - **Floor:** Hard palate (maxilla and palatine bones).
  - **Medial wall:** Nasal septum (vomer, perpendicular plate of ethmoid, septal cartilage).
  - **Lateral wall:** Contains turbinates (superior, middle, inferior conchae) and meatuses.
- **Functions:**
  - Air conditioning (warming, humidifying).
  - Filtration of particles.
  - Olfactory epithelium in superior region.
  - Drainage of paranasal sinuses and nasolacrimal duct.
- **Applied Anatomy- Grossing relevance:** Septal deviations, turbinate hypertrophy, and mucosal changes are common findings in surgical specimens.

Paranasal Sinuses

These are paired, air-filled cavities lined by respiratory mucosa, communicating with the nasal cavity.

Sinus	Location	Drainage Pathway	Grossing Relevance
Maxillary	In maxilla, largest sinus	Middle meatus via semilunar hiatus	Frequent site of sinusitis; mucosal thickening and cysts often sampled
Frontal	In frontal bone above orbit	Middle meatus via frontonasal duct	Important in chronic sinus disease; surgical margins may be examined
Ethmoidal	Between nose and orbit	Anterior → middle meatus; Posterior → superior meatus	Close relation to orbit and skull base; delicate lamina papyracea
Sphenoidal	In sphenoid body	Sphenoethmoidal recess	Proximity to optic nerve and pituitary; relevant in trans-sphenoidal approaches

Drainage patterns: Most sinuses drain into the middle meatus, except posterior ethmoids (superior meatus) and sphenoid (spheno-ethmoidal recess).

Surgical landmarks: Middle turbinate and uncinate process are key in endoscopic sinus surgery.

Pharynx

- **Divisions:**
  - *Nasopharynx:* Behind nasal cavity, above soft palate.
  - *Oropharynx:* Behind oral cavity, extending from soft palate to epiglottis.
  - *Laryngopharynx (hypopharynx):* From epiglottis to cricoid cartilage, continuous with esophagus.
- **Walls:** Muscular tube formed by constrictor muscles; lined by mucosa.
- **Functions:** Passage for air and food; resonance in speech; lymphoid tissue (tonsils) for immune defense.

Applied Anatomy - Grossing Relevance

- Specimen orientation: Identify nasopharynx, oropharynx, and hypopharynx.
- Common sites of pathology:
  - *Nasopharynx:* Adenoid tissue, nasopharyngeal carcinoma.
  - *Oropharynx:* Palatine tonsils, base of tongue, soft palate — frequent sites of squamous cell carcinoma.
  - *Hypopharynx:* Pyriform sinus, posterior pharyngeal wall, postcricoid region — prone to late-presenting carcinomas.

- Spaces and spread: Tumours often extend into parapharyngeal space, skull base, or larynx; grossing should document involvement.
- Lymphoid tissue: Tonsillar specimens require careful orientation and assessment of mucosal surface and crypts.
- Adjacent structures: Close relation to cervical lymph nodes; neck dissection specimens often accompany pharyngeal resections.

Larynx

For the grossing of the specimen of larynx, one needs to have a good knowledge of the surgical anatomy of the larynx which is as follows:

The larynx is the tubular structure that lies between the pharynx and the trachea (see Fig 5.1 & 5. 2).

The superolateral border is marked by the tip of the epiglottis and the aryepiglottic folds. The lower border is formed by the inferior rim of the cricoid cartilage.

The posterior boundary includes the posterior commissure mucosa, the mucous. membrane covering the cricoid cartilage, the arytenoid region and the interarytenoid space.

The anterior wall (see Fig 5. 2) is formed by the lingual surface of the epiglottis, the thyrohyoid membrane, the anterior commissure, the thyroid cartilage, cricothyroid membrane and the anterior arch of the cricoid cartilage.

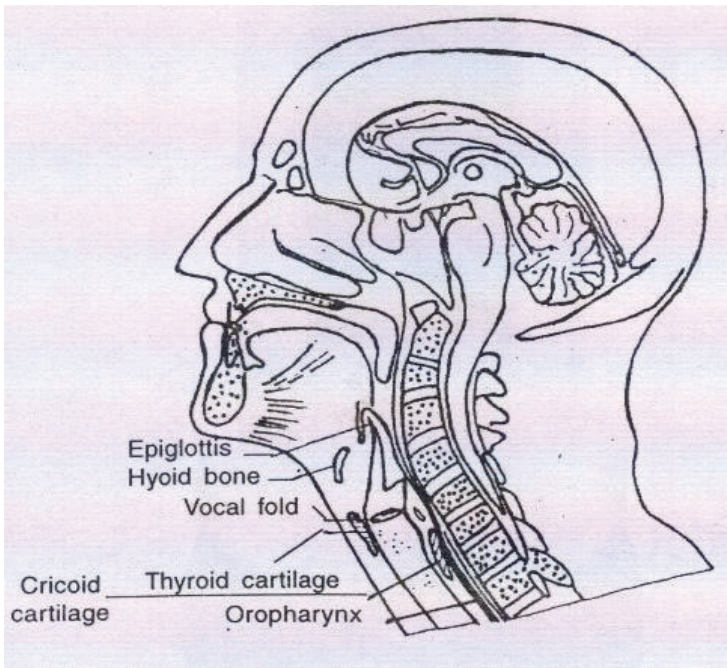


Fig 5.1 : Sagittal section through larynx

The larynx extends from the epiglottis superiorly to the lower border of the cricoid cartilage inferiorly and is divided into three regions:

- Supraglottis : Epiglottis, false cords, aryepiglottic folds
- Glottis : True vocal cords + commissures
- Subglottis : : Below cords to cricoid

  The supraglottic larynx (see Fig 5. 3) includes the epiglottis (both its lingual and laryngeal surfaces), the false vocal cords (or ventricular bands), aryepiglottic folds, arytenoid cartilages and ventricles. It separates the rest of the larynx from the pharynx (where it is subject to the noxious stimuli that attack both the respiratory and digestive systems).

"The tips of the epiglottis and the aryepiglottic folds form the superior margin of the supraglottis. The inferior margin is an imaginary horizontal plane passing through the apex of the ventricle (see Fig 5.4).

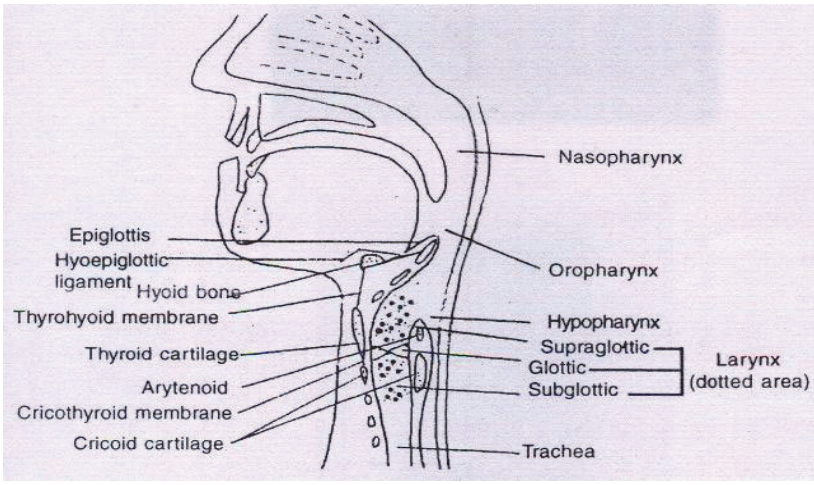


Fig 5 .2 : shows sagittal section through levels of larynx

The glottic portion comprises the true vocal cords, with the anterior and posterior commissures. The vocal cords average 14 to 16 mm in length in the adult male and 10 to 12 mm in the female. At their midpotnt, the cords are approximately 5 mm in height while anteriorly they are only 1 to 3 mm.

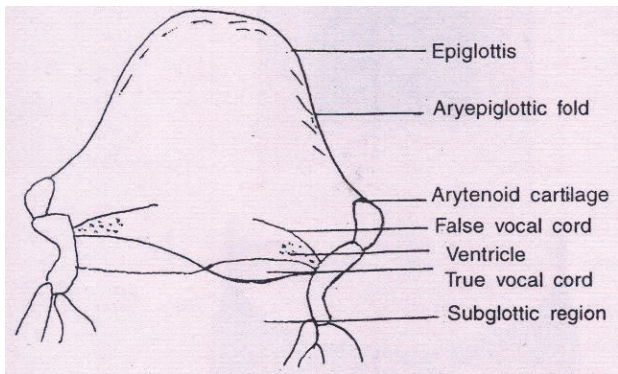


Fig. 5.3: shows upper view of supraglottic larynx

The superior border of the glottis is a horizontal plane passing through the apex of the ventricle and the inferior border is situated at a horizontal plane one centimeter below the apex of the ventricle (See Fig 5. 4).

The Subglottic region is not easily defined, but it comprises the area at which the larynx merges with the trachea. It extends from the lower border of the glottis (which is an imaginary horizontal plane one cm below the apex of the ventricle) to the inferior rim of the cricoid cartilage (see Fig 5.1). It measures approximately 26 mm in men and 23 mm in women.

The laryngeal framework (see Fig 5. 4 & 5.5) comprises of thyroid and cricoid cartilages. The thyroid cartilage is the shield shaped structure forming the anterior surface of the larynx and provides the origin and insertion to the intrinsic musculature. The cricoid cartilage is the only complete ring in the tracheobronchial tract and its broadest part forms the posterior wall of the larynx (see Fig 5. 1).

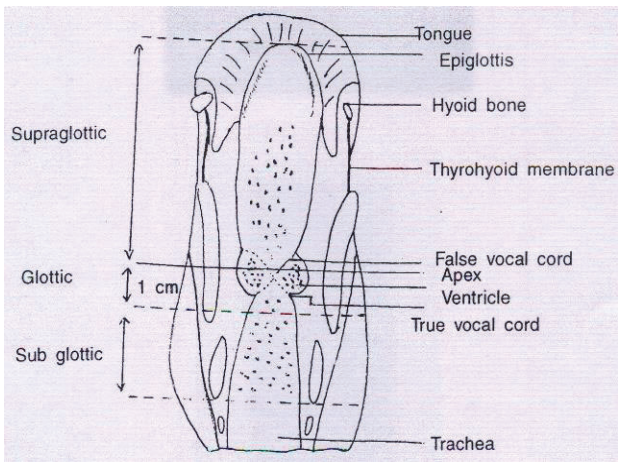


Fig. 5.4 : shows the superior and inferior limits of larynx

The epiglottis which forms the superolateral border of the larynx is a thin leaf-like flexible elastic fibrocartilage that projects upward behind the tongue and the body of the hyoid bone. The islands of fibrocartilage are separated by fenestrae. The cartilage is attached by thyoepiglottic ligament to the inner surface of the thyroid cartilage just above the anterior commissure and its sides are attached to the arytenoid cartilages by the aryepiglottic folds.

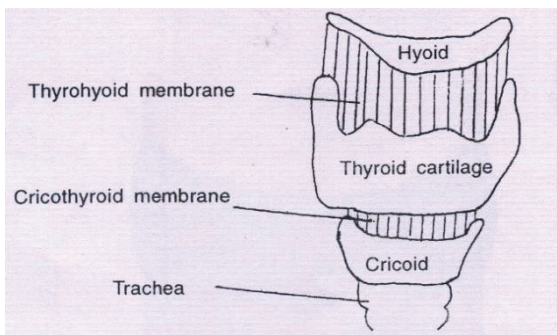


Fig 5.5 : shows the cartilaginous limits of larynx

The *hyoid bone* is not strictly a part of the larynx, but it supports the thyrohyoid and hypo-epiglottic ligaments which form the superior and anterior boundaries of the pre-epiglottic space (see Fig 5. 6).

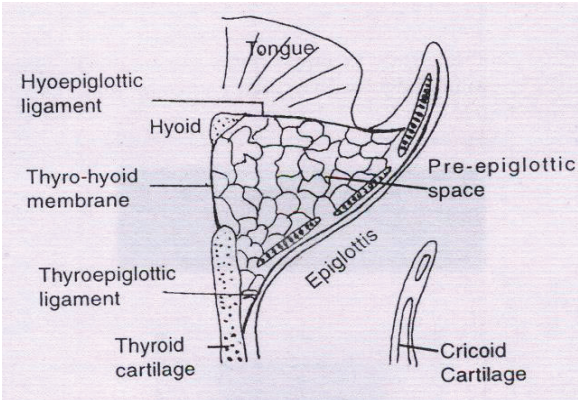


Fig 5. 6 : shows the pre-epiglottic space.

Three anatomial structures have prognostic value and need to be defined in the surgical anatomy of the larynx: pre-epiglottic space, paraglottic space and Broyles ligament.

The *pre-epiglottic space* (see Fig 5. 6) is a roughly triangular space filled with adipose and loose connective tissue situated in front of the anterior surface of the lower part of the epiglottis. It is bounded anteriorly by the thyroid cartilage and thyrohyoid membrane, posteriorly by the epiglottis and thyroepiglottic ligament and superiorly by the hyoepiglottic ligament.

The *para glottic space* (see figure 5.7) is not actually a space but rather an area external to the ventricles and saccules, filled with adipose and loose connective tissue. It is bounded by the conus elasticus (which extends from the lower edge of the thyroid cartilage to the anterior and superior border of the cricoid cartilage) inferiorly, the thyroid cartilage laterally, the quadrangular membrance (the membrance that extends from the epiglottis to the arytenoid cartilage) medially and pyriform sinus posteriorly.

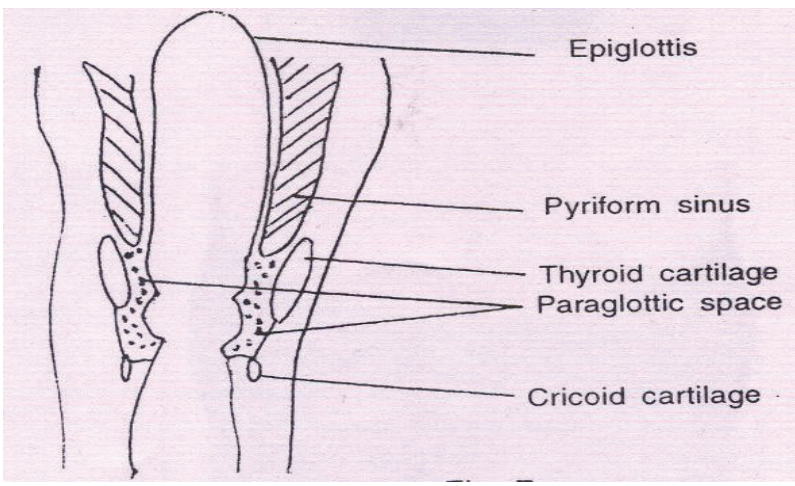


Fig 5.7 : shows the para glottic space

Both the spaces contain lymphatics and blood vessels but lack lymph nodes.

Broyles ligament (anterior commissure tendon) is a band of fibrous tissue one mm in width and 10 mm in length that extends from the vocal ligaments to the midline of the inner surface of the upper thyroid cartilage. Its importance lies in the fact that it not only contains lymphatics and blood vessels, but at its attachment to the thyroid cartilage there is no perichondrium, thus eliminating an important tumour barrier. It gives attachment to the thyroepiglottic, vestibular and vocal ligaments which converge to form the conus elastious and it is here that the thyroarytenoid muscles, the only muscles of the true vocal cordS insert. This anterior commissure tendon pr;,ovides the anterior attachment for the true vocal' cords, separating the right from the left.

The supraglottic larynx is closely related to the oral cavity and the oropharynx as it is derived from the 3rd and the 4th branchial arches. The glottis and subglottis are more intimately related to the lungs as they arise from the 6th branchial arch and are more susceptible to inhaled carcinogens. Bocca et al (1968) have stated that "the larynx consists of two hemilarynges, each of them with its own different derivation and with its largely independent lymphatic circulation. This means that each of these upper and lower hemilarynges may become invaded by cancer independent of one another and that the extension of cancer is limited within the boundaries of embryological demarcation". Therefore, whatever the nature of the tumour, a definite barrier exists between the glottic and supraglottic portions of the larynx, so that resection of a surgical margin of even 2 to 3 mm below a supraglottic carcinoma is adequate.

The small length of larynx shows marked variation in the lining epithelium. The epiglottis is covered by stratified squamous epithelium similar to that of the oral cavity. Underlying this squamous epithelium are modified salivary glands that secrete a particularly thick mucus. Over the false vocal cords, the epithelium changes to a ciliated columnar epithelium that extends to a variable extent into the ventricle with submucosal modified salivary glands. The true vocal cords are covered by stratified squamous epithelium with no (or very few) submucosal glands. The mucous membrane inferior to the true vocal cords is essentially the same as that of the trachea and major bronchi -. a ciliated columnar epithelium with numerous submucosal glands.

- **Glottic tumours present early (hoarseness) → better prognosis**
- **Supraglottic tumours present late → nodal spread common**

Larynx – Applied anatomy for Grossing -

- **Specimen orientation:** Identify supraglottic, glottic, and subglottic regions; margins must be carefully inked for surgical pathology.
- **Cartilaginous framework:** Thyroid, cricoid, and arytenoid cartilages are landmarks for tumor spread and resection margins.
- **Mucosal surfaces:** Note involvement of vocal cords, ventricular folds, and aryepiglottic folds; these are critical for staging.
- **Spaces:** Pre-epiglottic and paraglottic spaces are frequent routes of tumor extension; grossing should document invasion.

- **Adjacent structures:** Close relation to thyroid gland, trachea, and pharynx — specimens may include these for margin assessment.
- **Common pathology:** Squamous cell carcinoma is the predominant lesion; grossing requires attention to ulceration, exophytic growth, and cartilage invasion.

A II UPPER RESPIRATORY TRACT – GROSSING TECHNIQUES

Nasal Cavity and Paranasal Sinuses

Nasal Polyps

- Description: Usually multiple, pale gray-pink, soft, glistening, sessile or pedunculated masses.
- Measurement: Record the largest dimension; note if multiple fragments are present.
- Orientation: Separate fragments if necessary; avoid crushing the tissue.
- Processing**:** Submit entire polyp for histology; larger polyps can be sliced longitudinally to include both surface epithelium and stromal core.
- Specimen handling: In grossing, note mucosal changes, polyps, cysts, and bony involvement. Orientation is critical for pathology correlation.
- Gross examination should note mucosal thickening, polyps, cysts, and bony involvement. Orientation is essential for correlation with clinical disease and surgical outcome.
- Grossing requires careful attention to mucosal pathology, drainage pathways, and anatomical landmarks.
- Septal deviations, turbinate hypertrophy, mucosal pathology are common findings in specimens.

Inverted Papillomas / Neoplastic Lesions

- Note size, location, and involvement of adjacent structures.
- Orientation is critical: specify lateral vs. medial, superior vs. inferior aspects.
- Submit entire lesion if small; if large, serially section perpendicular to the surface for maximum depth of epithelial proliferation.

Nasopharynx

- Biopsy Specimens: Describe the fragment number, size, and color. Submit all tissue for histology.
- Resection Specimens (rare): Measure dimensions of nasopharyngeal mucosa, note involvement of fossa of Rosenmüller, adjacent skull base, or soft tissue.
- Margins: Mark mucosal margins and submit sections for histology.
- Orientation**:** Superior-inferior and anterior-posterior aspects should be noted.

Larynx

Specimen Types

- **Laryngoscopic Biopsies:**
  - Note number and color of tissue fragments.
  - Measure greatest and smallest fragments.
  - Submit all tissue for histology.
- **Laryngectomy Specimens:**
  Three types: Hemilaryngectomy, Supraglottic Laryngectomy, Total Laryngectomy.

Laryngectomy Specimens : General Principles (Applicable to All Laryngectomy Specimens)

- Orient the specimen accurately (superior–inferior, anterior–posterior, right–left).
- Identify and describe all anatomical components included.
- Carefully assess tumour location, extent, depth of invasion, cartilage involvement, and margins.
- Ink all relevant surgical margins before sectioning.

A. Hemilaryngectomy Specimen

Indication:
A voice-conserving procedure performed for selected glottic carcinomas.

Specimen Components:

- One half of thyroid cartilage
- Corresponding true and false vocal cords
- Ventricle

Grossing Procedure

1. **Orientation**
   - Identify superior/inferior, anterior/posterior surfaces and right or left side (see fig 3.8) .
2. **Measurements**
   - Record length, horizontal dimension, and thickness of the specimen.

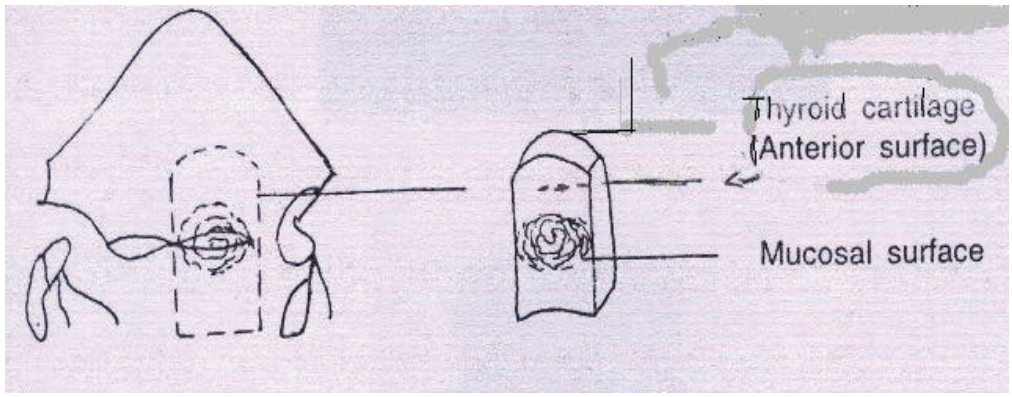


Fig 3.8 : Demonstration of hemi-laryngectomy specimen.

3. **Tumour Description**
    - Size: Three dimensions
    - Configuration: Exophytic / Endophytic
    - Location:
        - Glottic, with documentation of supraglottic or subglottic extension
        - Measure subglottic extension from the superior border of the true vocal cord
    - Extent:
        - Focal or multicentric
        - Length of vocal cord involved
        - Involvement of:
            - Anterior commissure
            - Posterior commissure
            - Ventricle
            - Aryepiglottic fold
            - Pyriform sinus (if involved)
    - **Margins:**
        - Distance from superior, inferior, medial, and lateral margins
4. **Inking**
    - Ink all surgical margins.
5. **Uninvolved Mucosa**
    - Describe colour, thickness, and surface characteristics.
6. **Sectioning**
    - Make thin parallel postero-anterior slices (“bread-loafing”).(see fig 5.9)

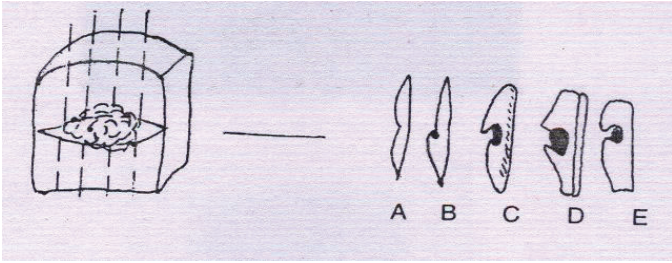


Fig 5.9 : parallel cuts in hemilaryngectomy

- If cartilage cutting facilities are available, sections should pass through the thyroid cartilage.
- At least one section must include 'maximum depth of tumour infiltration with cartilage'.

Bits

- Submit all parallel sections sequentially.
- Clearly indicate the block showing maximum depth of invasion and cartilage involvement.

B. Supraglottic Laryngectomy Specimen

Indication:
Tumours involving the **epiglottis and false vocal cords**.

Procedure:
Excision of the upper half of the larynx through the ventricles.

Grossing Procedure

1. **Orientation**
   - Identify superior, inferior, anterior, and posterior aspects.
2. **Measurements**
   - Record length and maximum diameter.
3. **Sectioning**
   - Bisect the specimen in the midline sagittally.
4. **Tumour Description**
   - **Size:** Two dimensions
   - **Configuration:** Exophytic / Endophytic
   - **Location:**
     - One or both sides of supraglottis
     - Crossing or approaching midline
     - Supra- or infra-hyoid (if hyoid bone present)

- **Extent:**
  - False cords
  - Pre-epiglottic space
  - Aryepiglottic folds
  - Pyriform sinus (measure medial wall involvement)
- **Margins:**
  - Superior (vallecula/base of tongue)
  - Inferior (transection at ventricles)
  - Anterior (pre-epiglottic soft tissue)

5. **Inking**
   - Ink superior, inferior, and anterior margins.
6. **Uninvolved Mucosa**
   - Describe separately.
7. **Tumour Thickness**
   - Identify and measure maximum depth of infiltration.

Bits

- Tumour with pre-epiglottic space and anterior mucosal margin
- Tumour with maximum depth of invasion
- Tumour with closest inferior margin
- Right and left aryepiglottic folds (sections perpendicular to fold)
- Superior mucosal margin (linear sampling if not flush with hyoid)
- See Diagram for instance (fig 5.10)

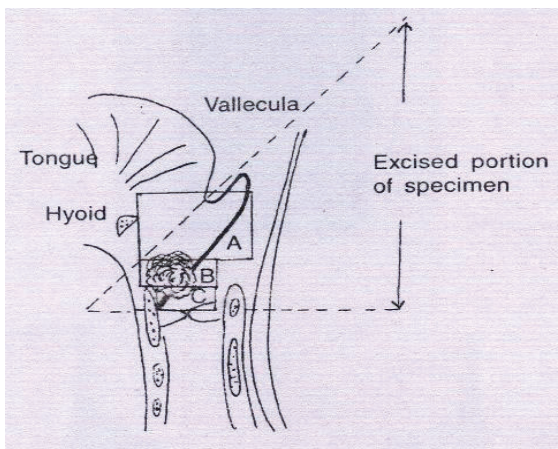


Fig 5.10 : Sagittal section

A. 1 bit tumour with pre-epiglottic space and portion of the cut margin of the mucous membrane on the anterior surface of the epiglottis.

B. 1 bit tumour with maximum depth of infiltration.

C. 1 bit tumour with the closest distal (inferior) margin.

D. 1 bit tumour with right aryepiglottic fold.(The cut is perpendicular to the fold)

E. 1 bit tumour with left aryepiglottic fold. (The aryepiglottic fold forms the medial wall of the pyriform fossa)

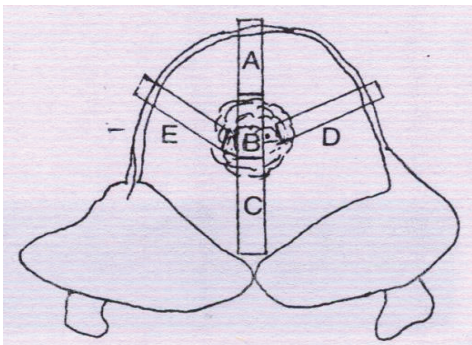


Fig. 11 End on view

F. 2 bits superior cut margin of the mucous membrane covering the anterior surface of the epiglottis in a linear fashion. (apart from that given in A)

This bit need not be taken if the mucosal margin is flush with the hyoid bone.

C. Total Laryngectomy Specimen

Indication:
Advanced tumours not amenable to conservative surgery.

Grossing Procedure

1. **Measurements**
   - Length from epiglottis to inferior border of cricoid cartilage
   - Length of trachea and number of tracheal rings
2. **Structures Identified**
   - Hyoid bone
   - Thyroid and parathyroid glands (if present)
   - Pyriform sinuses
   - Skin and tracheostomy site (if present)
   - Radical neck dissection components (if attached)
3. **Dissection**
   - Separate larynx from neck dissection specimen.

4. **Opening**
    - Open larynx along the posterior midline and fix in an open position.
5. **Tumour Description**
    - **Size:** Two dimensions
    - **Configuration:** Exophytic / Endophytic / Ulcerated
    - **Location:**
        - Supraglottic
        - Glottic
        - Subglottic
        - Transglottic (involving all three regions with fixed cord)
    - **Extent:**
        - One or both cords
        - Focal or multicentric
        - Length of cord involvement
        - Degree of subglottic extension
        - Involvement of:
            - Anterior commissure
            - Posterior commissure
            - Ventricle
            - Aryepiglottic folds
            - Pyriform sinus (measure involvement)
        - Extra-laryngeal spread
    - **Margins:**
        - Inferior margin
        - Tracheal margin
        - Tracheostomy site (if present)
6. **Tracheostomy Site**
    - Measure size, tract length, distance from tumour, and distal margin.
7. **Inking**
    - Ink lingual, pharyngeal, mucosal, and tracheal margins.
8. **Sectioning**
    - Serial sagittal or coronal sections.
    - At least one section must include tumour, thyroid cartilage, and deepest invasion.
9. **Additional Structures**
    - Thyroid: record weight, size, and cut surface

- Neck dissection: gross and sample as per neck dissection protocol

Bits

- Tumour with pre-epiglottic space and anterior mucosal margin
- Tumour with maximum depth of invasion
- Tumour with thyroid cartilage
- Tracheostomy stoma
- Right and left aryepiglottic folds
- Tracheal margin
- Posterior laryngeal wall/pharyngeal wall
- Thyroid (one bit from each lobe)
- Lymph nodes as per neck dissection protocol
- See figs below given as sample. (Fig 5.12 & 5.13)

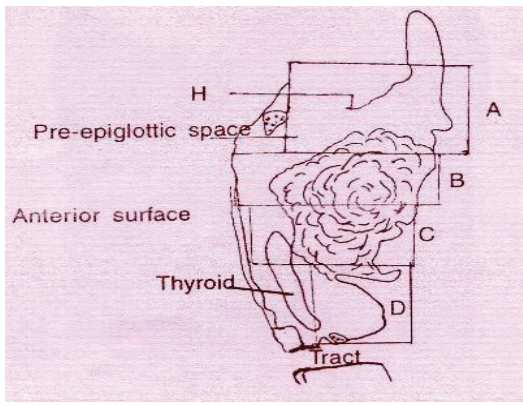


Fig. 5.12 Sagittal Section

A. One bit tumour with the pre-epiglottic space and central portion of the cut margin of the mucous membrane on the anterior aspect of the epiglottis.
B. One bit tumour with maximum depth of infiltration
C. One bit tumour with thyroid cartilage
D. One bit tumour with the tracheostomy stoma
E. One bit right aryepiglottic fold with or without tumour.

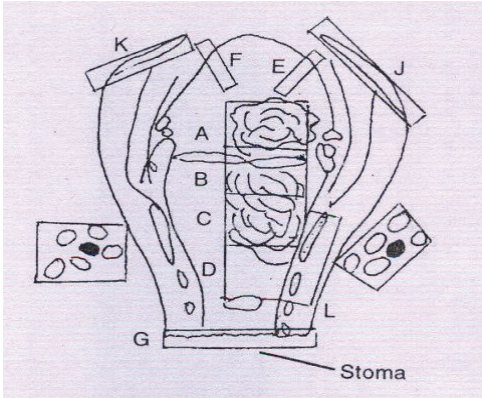


Fig 5.13 : Posterior view

One bit left aryepiglottic fold with/without tumour. (The cuts should be made perpendicular to the folds.)

F. One bit tracheal surgical margin.

G. Two bits cut margin (of the mucous membrane covering the anterior aspect of the epiglottis) taken in a linear fashion if it is not flush with the hyoid bone.(Fig 5.12). If the margin is flush with the hyoid. this bit need not be taken.

H. One bit right latero-posterior mucous membrane margin taken Horizontally (mucosal margin of the lateral wall of pyriform sinus).

I. One bit left latera-posterior mucosal margin taken horizontally.

J. One bit posterior wall of larynx (inclusive of the anterior wall of pharynx).

More bits need to be taken for the following:

One bit thyroid from each lobe

Lymph nodes as per the chapter on Radical neck dissection.

Note the following during grossing of Larynx

- Hoarseness with small glottic lesion → **Early squamous cell carcinoma**
- Ulcero-infiltrative or exophytic growth → **Squamous cell carcinoma**
- Supraglottic / hypopharyngeal tumors → **Late presentation, midline crossing**
- Multifocal, warty lesions in children → **Recurrent respiratory papillomatosis (HPV 6,11)**

Alveolar Ridge and Hard Palate

Specimen types: incisional biopsy, partial maxillectomy, segmental resection

Grossing guidelines:

1. **Orientation:** Identify anterior-posterior, medial-lateral aspects; note adjacent teeth, alveolar bone, and maxillary sinus floor.

2. **Measurements:** Record specimen dimensions and lesion size (length × width × depth).
3. **Lesion description:**
    - Morphology: exophytic, ulcerative, nodular, infiltrative
    - Surface: smooth, friable, verrucous, or ulcerated
    - Color: white, gray, pink, necrotic, or hemorrhagic
4. **Margins:** Ink all soft tissue and bony margins; record distance of lesion to margins.
5. **Sampling:**
    - Include tumor center and periphery
    - Base attachment, bone involvement, and any adjacent mucosa
    - Areas of ulceration or necrosis

Special considerations: For maxillectomy specimens, include sinus floor, nasal cavity floor, and palate if involved. *Maxillectomy* may be performed for tumours involving the maxillary sinus, upper alveolar border, or hard palate.

The upper alveolar ridge includes the alveolar process of the maxilla and its covering mucosa which extends from the line of attachment of mucosa in the upper gingival buccal gutter to the junction of the hard palate. Its posterior margin is the upper end of the pterygopalatine arch. Fig 5.13 shows cut section of maxilla to show normal relatioships.

The hard palate is the semilunar area between the upper alveolar ridge and the mucous membrane covering the palatine process of the maxillary bones. It is bounded in front and at the sides by the alveolar ridge, at the back; it is continuous with the soft palate and separates the oral from the nasal cavity. The lymphatic drainage of the hard palate is mainly to level I and level II nodes, and it may also be bilateral.

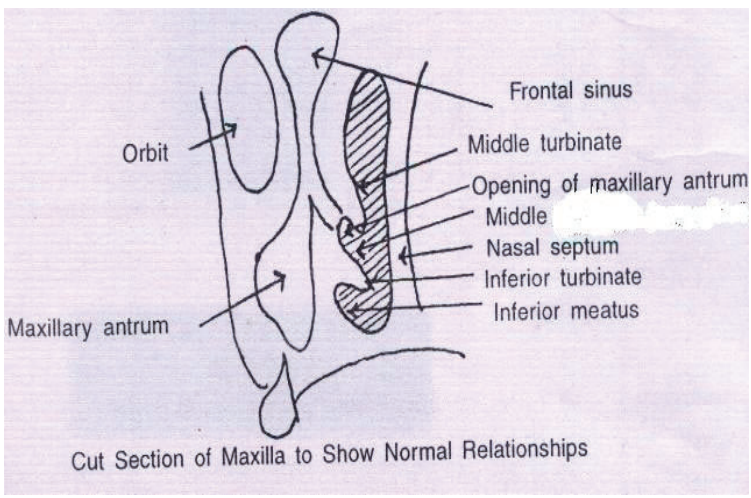


Fig 5.13. Cut Section of Maxilla to Show Normal Relationships

Bits (Fig 5.14)

A,B,C - Three bits tumour

D - One bit tumour with 'underlying bone

E,F,G,H - Four bits mucosal surgical margins; these include anterior, lateral, posterior, medial (brought forward in figure).

J,K,L - Three bits bony surgical margins; these include anterior, posterior and superior bony surgical margins.

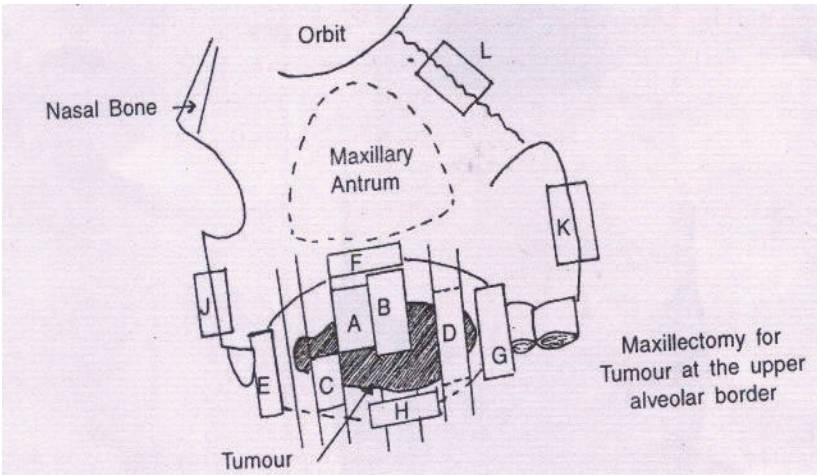


Fig 5.14 Bits in upper alveolar ridge tumour.

Common lesions:

- Squamous cell carcinoma (most common)
- Verrucous carcinoma
- Odontogenic tumors

Note: for All Upper Respiratory Specimens

- **Orientation:** Essential for correlation with imaging and surgical margins
- **Margins:** Always ink and measure distances to tumor
- **Sampling:** Submit all small biopsies; for larger resections, sample tumor center, periphery, margins, and areas of necrosis or unusual appearance
- **Documentation:** Photograph specimen if possible for records and multidisciplinary discussion.

A III UPPER RESPIRATORY TRACT- GROSS PEARLS

(Nasal cavity, paranasal sinuses, nasopharynx, larynx)

- **Multiple, bilateral, pale, translucent polypoid lesions** in the nasal cavity are usually **inflammatory nasal polyps**; always sample the **base** to exclude inverted papilloma or associated carcinoma.
- **Unilateral nasal masses**, especially when firm or hemorrhagic, should raise suspicion of **neoplasia** (inverted papilloma, carcinoma, or vascular tumors).
- **Papillary or cerebriform surface** in sinonasal lesions is characteristic of 'inverted sinonasal papilloma'; extensive sampling is essential due to risk of synchronous or metachronous carcinoma.
- **Friable, hemorrhagic masses** in the nasopharynx of adolescent males are characteristic of juvenile nasopharyngeal angiofibroma. (H/o Profuse epistaxis + vascular mass, avoid excessive manipulation due to risk of bleeding.)
- **Ill-defined mucosal thickening** or subtle irregularity in the **fossa of Rosenmüller** may represent nasopharyngeal carcinoma, often diagnosed only after cervical lymph node metastasis.(often presents with cervical lymph node metastasis; not primary mass)
- **Ulceroinfiltrative or exophytic lesions** of the larynx, particularly on the true vocal cords, are most often squamous cell carcinomas.
- **Glottic tumors** tend to be smaller at presentation due to early hoarseness, whereas **supraglottic and hypopharyngeal tumors** often present late and are more likely to cross the midline.
- **Cartilage involvement** (thyroid or cricoid) in laryngeal specimens signifies **advanced disease** and must be carefully assessed and sampled.
- **Black pigmented areas** in sinonasal or nasopharyngeal masses suggest 'malignant melanoma'.
- **Bone destruction** in paranasal sinus tumors is a key gross indicator of malignancy and should be documented and extensively sampled.

A III UPPER RESPIRATORY TRACT – MICROSCOPY

1. Nasal Cavity and Paranasal Sinuses

A. Non-neoplastic Lesions

1. **Inflammatory Polyps**

- Microscopy: Edematous stroma with loose connective tissue, dilated vascular channels, scattered inflammatory cells (lymphocytes, plasma cells, occasional eosinophils).
- Surface Epithelium: Respiratory type, often hyperplastic; squamous metaplasia may be seen.

- Special Features: Abundant eosinophils suggest allergic etiology; fibroblast proliferation in long-standing lesions.
- Pearl: Large polyps may mimic low-grade neoplasms; careful evaluation of epithelial proliferation is critical.

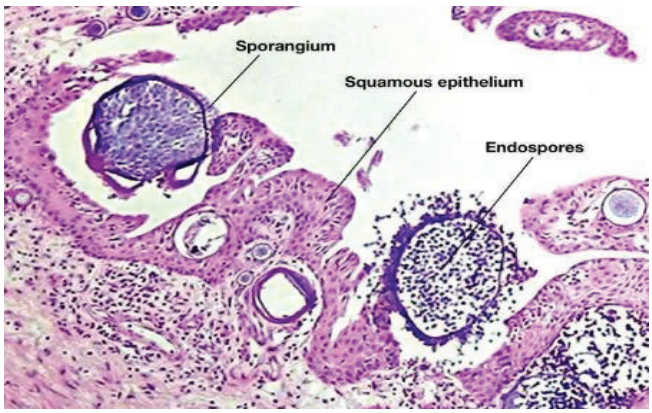


Fig 5.15 Nasal mucosa with epithelium showing rhinosporidiosis. Sporangia are filled with spores in the nasal mucosa.

2. **Fungal Sinusitis**

- Non-invasive: Fungal hyphae within mucin, minimal tissue invasion.
- Invasive: Hyphae within mucosa, submucosa, vessels; necrosis may be prominent.
- Special Stains**:** PAS, GMS highlight hyphae.

B. Benign Neoplasms

1. **Inverted Sinonasal Papilloma**

- Microscopy: Endophytic growth of thickened squamous or respiratory epithelium into underlying stroma.
- Features: Basal cell hyperplasia, occasional microabscesses.
- Pitfall: Foci of invasive carcinoma can occur in ~3% of cases; thorough sampling essential.
- IHC / Molecular**:** p16 may be positive in HPV-associated lesions; EGFR mutations can be seen.

C. Malignant Lesions

1. **Squamous Cell Carcinoma (SCC)**

- Microscopy: Keratinizing and non-keratinizing variants.
  - Keratinizing: Eosinophilic keratin pearls, intercellular bridges.
  - Non-keratinizing: Sheets or nests of basaloid cells, scant keratin.
- IHC**:** p40/p63 for squamous differentiation; cytokeratin (CK5/6).
- Poorly differentiated carcinomas require exclusion of:

- Small cell neuroendocrine carcinoma
- Sinonasal undifferentiated carcinoma (SNUC)

2. **Olfactory Neuroblastoma** (Esthesioneuroblastoma)

- Microscopy: Small uniform round blue cells, scant cytoplasm, round nuclei, fibrillary background (neuropil). Homer-Wright rosettes may be present.
- Round nuclei with fine chromatin; inconspicuous nucleoli
- Stroma: Fibrovascular, highly vascular.
- IHC: Synaptophysin+, Chromogranin+, NSE+; S100+ sustentacular cells at periphery.
- Molecular**:** Lacks EWS-FLI1 fusion gene (differentiates from Ewing sarcoma).
- Can mimic small cell neuroendocrine carcinoma; differentiation by location, IHC, and rosettes is key.
- Sustentacular cells highlighted by **S-100** at periphery; positive clue

D. Nasopharyngeal Lesions

1. Nasopharyngeal Carcinoma

- Often subtle or inapparent grossly
- Two major histologic patterns:

a) Non-keratinizing carcinoma

- Sheets or syncytial aggregates of malignant epithelial cells
- Vesicular nuclei with prominent nucleoli
- Dense lymphoid infiltrate in the background
- Strong association with **EBV**

b) Undifferentiated carcinoma (Lymphoepithelioma)

- Tumour cells dispersed among lymphocytes
- Regaud pattern: epithelial nests surrounded by lymphoid stroma
- Schmincke pattern: diffuse growth with intimate lymphoid admixture

IHC: Cytokeratin+, p63+; EBV detection by EBER-ISH in non-keratinizing/undifferentiated type Undifferentiated NPC may mimic lymphoma; nuclear features and cytokeratin positivity are differentiating.

2. **Nasopharyngeal Angiofibroma**

- Microscopy: Fibrocollagenous stroma with variable vascular channels (thin-walled, stellate, or staghorn pattern). Endothelial cells flat to plump; smooth muscle may be discontinuous.
- IHC: Vimentin+, Testosterone receptor+, Ki-67 low.

- Nuclear pleomorphism and multinucleated stromal cells may be seen but do not indicate malignancy.

3. **NK/T-cell Lymphoma (Angiocentric)**

- Microscopy: Atypical small-to-large lymphoid cells, angiocentric/angiodestructive pattern, necrosis.
- IHC: CD45+, CD3ε+, CD56+, EBV-encoded RNA (EBER) positive by ISH.
- Differential includes other midline destructive lesions; EBV positivity and angiocentricity are diagnostic.

2. Larynx

A. Infectious Lesions

1. **Tuberculosis**

- Necrotizing granulomas with epithelioid cells, Langhans giant cells, central caseation.
- Occasional acid-fast bacilli visible (Ziehl-Neelsen).
- Laryngeal TB is rising in immunocompromised patients, particularly HIV/AIDS.

2. **Actinomycosis**

- Colonies of filamentous bacteria surrounded by acute inflammation.
- Stains: Gram+, silver stains.

3. **Fungal Infections**

- Histoplasmosis, blastomycosis, coccidioidomycosis: fungal organisms within submucosa, necrotic debris.
- Stains: PAS, GMS.

B. Non-neoplastic Lesions

1. **Vocal Cord Polyps**

- Microscopy: Loose edematous/myxoid stroma; vascular spaces with fibrin deposition; overlying squamous epithelium may be intact or slightly hyperplastic.
- Must be distinguished from fibrin-rich reactive lesions.

2. **Localized Laryngeal Amyloidosis**

- Microscopy: Extracellular eosinophilic deposits in submucosa; may surround vessels and fat cells.
- Special Stain: Congo red; apple-green birefringence under polarized light.
- Pearl**:** Differentiate from plasma cell-rich inflammatory lesions and systemic amyloidosis.

C. Benign Neoplasms

1. **Squamous Papilloma**

- Microscopy: Finger-like fronds of hyperplastic squamous epithelium over fibrovascular cores; minimal keratinization; rare dysplasia.
- IHC/Molecular: HPV types 6 and 11; p16 may be focally positive.
- Pearl**:** Solitary adult papillomas and verrucous carcinoma must be distinguished; latter shows prominent keratin and dysplasia.

2. **Paraganglioma**

- Microscopy: Zellballen pattern of chief cells with peripheral sustentacular cells; vascular stroma prominent.
- IHC: Chief cells – Chromogranin+, Synaptophysin+; Sustentacular cells – S100+.
- Pearl**:** Distinguish from carcinoid/neuroendocrine carcinoma by S100+ sustentacular cells.

D. Malignant Neoplasms

1. **Squamous Cell Carcinoma (SCC)**

- Microscopy: Keratinizing or non-keratinizing; nests, cords, sheets of atypical squamous cells.
- Variants: Verrucous carcinoma, spindle cell carcinoma, basaloid SCC.
- Pearl**:** Premalignant lesions include keratinizing dysplasia; often correlates with invasive SCC.

2. **Neuroendocrine Carcinoma :**

- **Classification:**
  - Well-differentiated (typical carcinoid)
  - Moderately differentiated (atypical carcinoid)
  - Poorly differentiated (small cell/oat cell carcinoma)
- **Microscopy:** Small-to-medium neuroendocrine cells in nests, trabeculae, or sheets; necrosis and mitoses correlate with grade.
- **IHC:** Synaptophysin+, Chromogranin+, NSE+; Ki-67 proliferation index for grading.

A IV UPPER RESPIRATORY TRACT SYNOPTIC REPORTING (SITE-SPECIFIC TEMPLATES)

1. NASAL CAVITY

a. Specimen details

- Procedure: Biopsy / Polypectomy / Excision

- Laterality: Right / Left / Midline
- Anatomic Subsite: Septum / Turbinate / Floor / Lateral wall

b. Microscopy

- Surface Epithelium: Respiratory / Squamous / Transitional
- Epithelial Changes: Normal / Dysplasia (grade) / Carcinoma in situ
- Lesion Type: Inflammatory / Benign neoplasm / Malignant neoplasm
- Histologic Type:
- Growth Pattern: Exophytic / Endophytic / Infiltrative
- Stromal Features: Edema / Fibrosis / Desmoplasia
- Inflammation: Absent / Mild / Moderate / Severe (type)
- Necrosis: Present / Absent
- Lymphovascular Invasion: Present / Absent
- Perineural Invasion: Present / Absent

C. Margins (if applicable)

- Mucosal Margins: Free / Involved / Not applicable
- Closest Margin: ____ mm

d. Final diagnosis

- Definitive Diagnosis:
- Grade (if applicable):

2. NASOPHARYNX

A. Specimen details

- Procedure: Biopsy / Excision
- Site: Roof / Posterior wall / Fossa of Rosenmüller

B. Microscopy

- Surface Epithelium: Respiratory
- Tumor Type: Non-keratinizing carcinoma / Keratinizing SCC / Other
- Architectural Pattern: Syncytial / Nests / Sheets
- Lymphoid Stroma: Prominent / Scant / Absent
- Cytologic Atypia: Mild / Moderate / Severe
- Necrosis: Present / Absent
- Lymphovascular Invasion: Present / Absent

- Perineural Invasion: Present / Absent

C. Ancillary studies

- EBV (EBER in-situ hybridization): Positive / Negative
- IHC (if performed):

d. Final diagnosis

- Definitive Diagnosis:
- WHO Type (if applicable):

3. OROPHARYNX

a. Specimen details

- Site: Tonsil / Base of tongue / Soft palate / Pharyngeal wall
- Procedure: Biopsy / Tonsillectomy / Excision
- Laterality: Right / Left / Bilateral

b. Microscopy

- Surface Epithelium: Squamous
- Dysplasia: Absent / Mild / Moderate / Severe
- Tumor Type: Squamous cell carcinoma / Other
- Keratinization: Present / Absent
- Growth Pattern: Infiltrative / Pushing
- Stromal Reaction: Desmoplastic / Minimal
- Lymphovascular Invasion: Present / Absent
- Perineural Invasion: Present / Absent

c. Ancillary studies

- p16 IHC: Positive / Negative

d. Final diagnosis

- Definitive Diagnosis:
- HPV-associated: Yes / No

4. LARYNX

a. Specimen details

- Subsite: Supraglottic / Glottic / Subglottic

- Procedure: Biopsy / Partial laryngectomy / Total laryngectomy
- Laterality: Right / Left / Midline

b. Microscopy

- Surface Epithelium: Squamous
- Dysplasia: Absent / Present (grade)
- Tumor Type: Squamous cell carcinoma / Other
- Grade: Well / Moderately / Poorly differentiated
- Depth of Invasion: ____ mm
- Cartilage Invasion: Present / Absent
- Lymphovascular Invasion: Present / Absent
- Perineural Invasion: Present / Absent

c. Margins

- Mucosal Margins: Free / Involved
- Deep Margin: Free / Involved

d. Final diagnosis

- Definitive Diagnosis:
- Pathologic Stage (pT):

5. PARANASAL SINUSES

a. Specimen details

- Sinus Involved: Maxillary / Ethmoid / Frontal / Sphenoid
- Procedure: Biopsy / Excision / Craniofacial resection
- Laterality: Right / Left / Bilateral

b. Microscopy

- Tumor Type: SCC / Adenocarcinoma / Sinonasal undifferentiated carcinoma / Other
- Architectural Pattern: Nests / Glands / Sheets
- Cytologic Grade: Low / High
- Bone Invasion: Present / Absent
- Orbital / Dural Invasion: Present / Absent
- Necrosis: Present / Absent
- Lymphovascular Invasion: Present / Absent
- Perineural Invasion: Present / Absent

c. Margins

- Margins: Free / Involved / Cannot be assessed
- Closest Margin: ____ mm

d. Final diagnosis

- Definitive Diagnosis:
- Grade:
- Pathologic Stage (pT):

TNM CLASSIFICATION OF CARCINOMA OF THE LARYNX (UPDATED, AJCC-ALIGNED)

This classification applies to carcinomas of the larynx.

ANATOMICAL SITES AND SUBSITES

1. Supraglottis

- Suprahyoid epiglottis (including tip, lingual/anterior and laryngeal surfaces)
- Aryepiglottic fold (laryngeal aspect)
- Arytenoid
- Infrahyoid epiglottis
- Ventricular bands (false vocal cords)

2. Glottis

- True vocal cords
- Anterior commissure
- Posterior commissure

3. Subglottis

T – PRIMARY TUMOUR

- **TX**: Primary tumour cannot be assessed
- **T0**: No evidence of primary tumour
- **Tis**: Carcinoma in situ

SUPRAGLOTTIS

- **T1**: Tumour limited to one subsite of supraglottis with normal vocal cord mobility
- **T2**: Tumour invades mucosa of more than one adjacent subsite of supraglottis or glottis, or region outside the supraglottis (e.g. mucosa of base of tongue, vallecula, medial wall of pyriform sinus), **without fixation of the larynx**

- **T3**: Tumour limited to the larynx with vocal cord fixation and/or invasion of **postcricoid area, pre-epiglottic space, or deep (intrinsic) muscles of base of tongue**
- **T4**: Tumour invades through thyroid cartilage and/or extends into extralaryngeal soft tissues (e.g. soft tissues of neck, thyroid gland, oesophagus)

GLOTTIS

- **T1**: Tumour limited to vocal cord(s) (may involve anterior or posterior commissure) with normal mobility
  - **T1a**: Tumour limited to one vocal cord
  - **T1b**: Tumour involves both vocal cords
- **T2**: Tumour extends to supraglottis and/or subglottis and/or with impaired vocal cord mobility
- **T3**: Tumour limited to the larynx with vocal cord fixation
- **T4**: Tumour invades through thyroid cartilage and/or extends beyond the larynx (e.g. trachea, soft tissues of neck, thyroid gland, pharynx)

SUBGLOTTIS

- **T1**: Tumour limited to subglottis
- **T2**: Tumour extends to vocal cord(s) with normal or impaired mobility
- **T3**: Tumour limited to the larynx with vocal cord fixation
- **T4**: Tumour invades through cricoid or thyroid cartilage and/or extends beyond the larynx (e.g. trachea, soft tissues of neck, thyroid gland, oesophagus)

N – REGIONAL LYMPH NODES

- **NX**: Regional lymph nodes cannot be assessed
- **N0**: No regional lymph node metastasis

(Histological examination of a selective neck dissection specimen should ordinarily include ≥6 lymph nodes; radical or modified radical neck dissection specimens should include ≥10 lymph nodes.)

- **N1**: Metastasis in a single ipsilateral lymph node ≤3 cm in greatest dimension
- **N2**: Metastasis in a single ipsilateral lymph node >3 cm but ≤6 cm, **or** multiple ipsilateral lymph nodes none >6 cm, **or** bilateral or contralateral lymph nodes none >6 cm
  - **N2a**: Single ipsilateral lymph node >3 cm but ≤6 cm
  - **N2b**: Multiple ipsilateral lymph nodes, none >6 cm
  - **N2c**: Bilateral or contralateral lymph nodes, none >6 cm
- **N3**: Metastasis in a lymph node >6 cm in greatest dimension

Note: Midline lymph nodes are considered ipsilateral.

M – DISTANT METASTASIS

- **MX**: Distant metastasis cannot be assessed
- **M0**: No distant metastasis
- **M1**: Distant metastasis present

B I LUNG ANATOMY

The lungs are paired respiratory organs occupying the pleural cavities of the thorax on either side of the mediastinum. Their principal function is gas exchange, but from a surgical standpoint they are important because: These anatomical units allow segmental resections with preservation of uninvolved lung.

Each lung has an independent broncho-vascular supply.

The hilum and root structures are key during lobectomy and pneumonectomy.

Independently each lung has (Fig 5.16 A & B) –

- Is covered by visceral pleura ; Each lung is conical in shape ; has an apex, base, surfaces, borders , a hilum and root.
- The right lung has 3 lobes and through it courses the oblique and horizontal fissures. It has 10 segments . Each segment: has an independent bronchus , Segmental artery, the veins are intersegmental.
- The left lung has 2 lobes and an oblique fissure separating them (see fig 5. 15A); it has 8–10 segments .
- The lingular extension of the left upper lobe corresponds to the middle lobe of the right lung. Lobectomies follow fissure planes.
- The apex of the lung extends 2–3 cm above the medial third of the clavicle
- The apex can be involved by a Pancoast tumour which may inturn involve the brachial plexus, subclavian vessels and sympathetic chain leading to Horner syndrome.

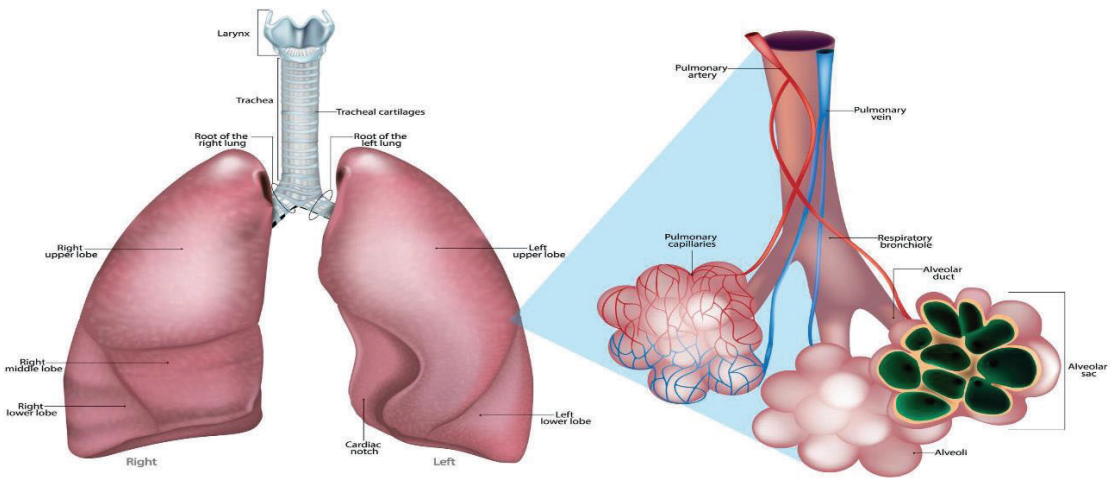


Fig 5.16 A & B

- The base of the right lung rests on the liver and right kidney. The Left lung base is related to the Stomach, Spleen and Left kidney. Of surgical importance here is the occurrence of a subphrenic abscess, diaphragmatic hernia and collection of a pleural effusion which maybe picked up on imaging.
- The costal surfaces are convex and related to ribs and intercostal spaces. Thoracotomy approaches are through this surface.
- The diaphragmatic surface is concave and in relationship to diaphragm
- The hilum of the lung is the depression where the following structures enter or leave the lung- main bronchus, pulmonary artery, pulmonary veins, bronchial vessels, lymphatics and nerves.
- The root of the lung is the collection of structures connecting the lung to mediastinum and is composed of the bronchus, pulmonary artery, two pulmonary veins, bronchial arteries and veins, lymphatics and the autonomic nerves
- The lung has dual blood supply-Pulmonary arteries carry the deoxygenated blood and the bronchial arteries from thoracic aorta carry the oxygenated blood to the lungs.
- Lymphatics are th superfial and deep lymphatics and drin into the bronchial and tracheal nodes. The lung has both sympathetic and parasympathetic nerve supply.
- Fig 5.15 B shows a terminal bronchiole with air sacs at the periphery.

C I PLEURA ANATOMY

The pleura is best understood as a thin serous membrane with two layers—parietal and visceral—whose gross features (smoothness, thickness, nodularity, puckering, or discoloration) are critical to describe. The pleura's relationship to lung parenchyma, diaphragm, and mediastinum must be carefully noted during specimen handling.

Anatomy of the Pleura

Layers

- **Visceral pleura**
  - Covers the lung surface.
  - Thin, transparent, and tightly adherent to lung parenchyma.
  - Normally smooth and glistening; thickening or nodularity suggests pathology (e.g., mesothelioma, fibrosis).
- **Parietal pleura**
  - Lines thoracic cavity (chest wall, diaphragm, mediastinum).
  - Thicker than visceral pleura, easier to dissect.
  - Often sampled in pleurectomy specimens.

B II LUNG GROSSING TECHNIQUES

- Important Surgical Procedures on the lung are Segmentectomy ; Lobectomy ; Pneumonectomy; Wedge resections ; Bullectomy and Decortication. The types of specimens generally received in a surgical pathology lab are Transbronchial biopsy ; Transthoracic core (Tru-cut) biopsy; Open lung / wedge biopsy ; Lobectomy specimen or Pneumonectomy specimens for tumoural and non-tumoural conditions.
- Lung cancer follows segmental lymphatic spread.
- Bronchiectasis -Often localized to lower lobes
- Aspiration pneumonia involves usually the right lower lobe superior segment.(The right bronchus is more vertical)
- Pulmonary embolism lodges in pulmonary artery branches
- Pancoast tumor involves the lung apex
- Bronchopulmonary segment is the surgical unit of lung.
- Pulmonary veins run in intersegmental planes.
- Segmental bronchi and arteries run together.
- Segmentectomy is possible due to anatomical independence.

SMALL BIOPSIES

(Transbronchial, Transthoracic core, Open lung / wedge biopsies)

Transbronchial biopsy via flexible fibre-optic bronchoscope is the most commonly received specimen. Usually **4–6 tissue fragments**, each **1–2 mm** in size, are obtained.

GROSSING STEPS

1. Measure the specimen (aggregate and largest fragment) in **three dimensions**.
2. Describe:
    - Colour
    - Consistency
    - Presence of mucus, blood or necrotic material
3. If ≤4 mm, submit the tissue entirely.
4. If >4 mm, describe the cut surface and submit entirely.
5. Multiple step / serial sections should be examined on microscopy.

LOBECTOMY & PNEUMONECTOMY – NON-TUMOURAL CONDITIONS

1. SPECIMEN HANDLING

- State whether specimen is fresh or fixed, intact or previously sectioned.
- Identify and record the lobe; in pneumonectomy specimens, mention the side.

2. INFECTION CONTROL

- For **tuberculosis or other contagious diseases** (suspected or proven):
  - Fix in formalin for at least 24 hours (preferably 48 hours) before grossing.
  - Send contaminated instruments for appropriate sterilization.

3. MEASUREMENTS

- Record weight and three-dimensional measurements.

4. EXTERNAL SURFACE (PLEURA)

- Thickness
- Fibrosis
- Fibrinous or purulent exudate
- Nodularity or adhesions

5. BRONCHUS

- Identify the main bronchus and record its **diameter**.
- Open longitudinally with scissors and inspect the **luminal surface**.

6. SECTIONING

- Section along the greatest dimension of the lung/lobe.
- The first slice should pass through the hilum, from medial to lateral.
- Make 1 cm thick parallel slices.
- Arrange slices sequentially and examine carefully.

7. PARENCHYMAL FINDINGS

Describe and measure lesions, including:

- Consolidation (measure extent in two dimensions)
- Cavities: number, location, size, and nature of wall
- Fibrosis
- Haemorrhage
- Necrosis
- Bronchiectasis
- Emphysema

8. RELATIONSHIP TO BRONCHUS

- State the relationship of lesions to the bronchial tree.
- Examine cut surfaces of bronchi for:

- Dilatation
- Inflammation
- Bronchopneumonia

- Open bronchi and bronchioles towards the periphery as far as possible using fine scissors.

9. HILAR LYMPH NODES

- Record number, size and appearance.
- Nodes >5 mm should be sliced and the cut surface described.

TISSUE SAMPLING (NON-TUMOURAL- see fig 5.17)

- A, B, C: 3 blocks from lesion (more if required)
- D, E: 2 blocks from uninvolved lung (preferably from different lobes)
- F: Hilar lymph nodes
- G: Pleura (2 blocks)

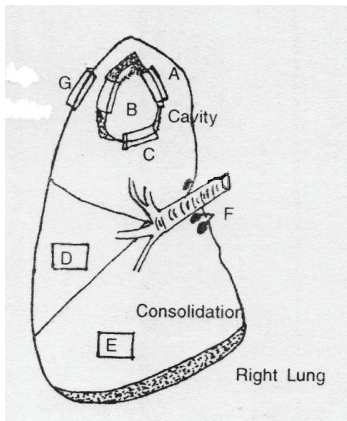


Fig 5.17, : Grossing of resected lung non tumour

SPECIAL INSTANCES

1. **Suspected Asbestosis**
    - Vigorously scrape the cut surface of lung.
    - Smear on glass slide, air-dry, stain lightly with toluidine blue, mount and examine for asbestos bodies.
    - Alternatively, a wet mount may be examined.
2. **Rib Submitted with Lung**
    - Cut rib into 2 cm pieces.
    - Crush to express marrow.
    - Process marrow routinely for histology.

3. **Inflation (Wet Fixation) Technique**
    - Formalin injected through main bronchus to inflate lung.
    - Clamp bronchus and fix overnight (preferably 48 hours).
    - Gravitational infusion of 10% formalin may also be used.
    - This method provides superior fixation and preserved alveolar morphology.

LUNG RESECTION FOR TUMOUR

GROSSING STEPS

1. Specify type of resection: lobectomy or pneumonectomy.
2. Record weight and measurements.
3. Remove hilar lymph nodes en bloc and set aside for separate dissection.
4. Sample the bronchial resection margin.
5. Open the bronchus and section lung parenchyma as described above.

TUMOUR DESCRIPTION

- Location (lobe)
- Relationship to bronchus; intraluminal extension
- Size (maximum dimension; additional dimensions if required)
- Appearance: solid / cystic
- Necrosis and haemorrhage
- Satellite nodules
- Pleural involvement
- Distance from bronchial and other surgical margins

TISSUE SAMPLING (TUMOUR)(Fig 5.18)

- **A, B, C**: Tumour (one block showing relationship to bronchus; more if needed)
- **D, E**: Non-neoplastic lung including pleura
- **F, G**: Hilar lymph nodes
- **H**: Bronchial surgical margin

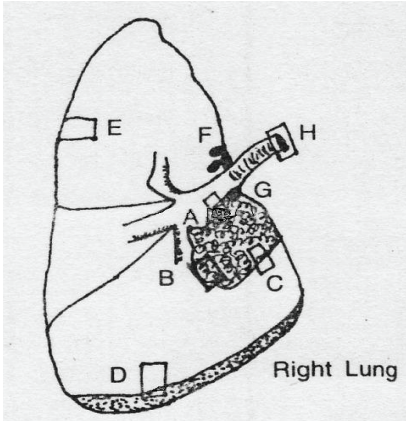


Fig 5.18

C II PLEURA GROSSING TECHNIQUES

Gross Features to be noted

- Surface appearance: Smooth vs. granular, dull, or puckered.
- Thickness: Uniform vs. focal thickening.
- Color changes: Tan-pink (normal), black spots (anthracotic pigment), white-tan (fibrosis), or hemorrhagic.
- Nodules or plaques: Size, distribution, and distance from margins.
- Relationship to adjacent structures:
    - Distance of lesions from pleura.
    - Involvement of diaphragm or peritoneum (important in mesothelioma resections).
    - Adhesions to lung parenchyma.

DECORTICATION SPECIMEN

(Received in empyema, tuberculosis, and neoplasms such as mesothelioma)

1. Measure largest and smallest fragments in three dimensions.
2. Describe surface:
    - Exudate
    - Necrosis (especially in tuberculosis)
    - Nodularity
    - Fibrosis
3. Slice all pieces linearly at 0.5 cm intervals.
4. Lay out slices sequentially and examine all cut surfaces carefully.

SAMPLING (Fig 5.19)

- Take representative blocks from different areas, guided by gross appearance and clinical suspicion.

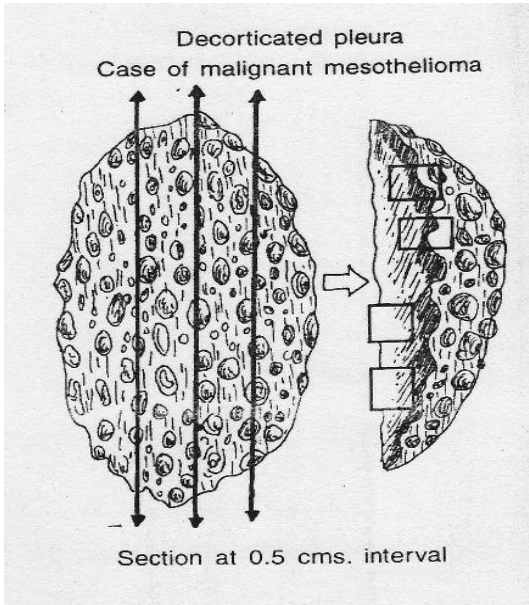


Fig 5.19 Shows grossing of pleura.

TNM-ORIENTED GROSS SAMPLING POINTS – LUNG AND PLEURA

Gross examination of lung resection specins is critical for **accurate TNM staging**, prognostication, and therapeutic decision-making.

Primary Tumour (T)

- **Measure the tumour in three dimensions**, recording the *maximum diameter* precisely.
    - Tumour size directly determines **T category** in lung carcinoma.
- **Note tumour location**:
    - Central (hilar/endobronchial)
    - Peripheral (subpleural or parenchymal)
- **Assess relationship to bronchi**:
    - Main bronchus, lobar bronchus, or segmental bronchus
    - Measure distance of tumour from bronchial resection margin.
- **Evaluate tumour extent**:
    - Invasion into:
        - Visceral pleura
        - Chest wall
        - Pericardium
        - Diaphragm

        - Mediastinal fat
    - Pleural invasion should be correlated with elastic stains on microscopy.
- **Document tumour appearance**:
    - Solid, necrotic, cavitary, mucinous, or haemorrhagic
    - Presence of satellite nodules in the same lobe or other lobes.
- **Sample generously**:
    - Tumour centre
    - Tumour periphery
    - Tumour–normal lung interface
    - Areas suspicious for pleural or vascular invasion

Pleural Involvement

- **Inspect visceral and parietal pleura** for:
    - Nodules
    - Diffuse thickening
    - Plaques
- **Sample pleura** when:
    - Tumour abuts or involves pleura
    - Pleura is thickened or nodular
    - There is haemorrhagic pleural effusion
- **Distinguish**:
    - Visceral pleural invasion (affects T stage)
    - Parietal pleural or chest wall invasion (higher T stage)

Margins

- **Bronchial margin**:
    - Submit *en face or perpendicular sections*
    - Essential in central tumours
- **Vascular margins**:
    - Pulmonary artery and vein margins should be sampled
- **Parenchymal margin**:
    - Especially important in wedge resections and segmentectomies
- **Chest wall margin** (if present):
    - Must be sampled separately and labelled clearly

Regional Lymph Nodes (N)

- **Identify and separately submit lymph nodes** from:
    - Hilar

    - Interlobar
    - Peribronchial
    - Mediastinal stations (if included)
- **Record**:
    - Number of lymph nodes examined
    - Number involved by tumour
    - Size of largest metastatic node
- **Note extranodal extension**, if present.

Distant Metastasis (M)

- Although usually assessed clinically and radiologically, note **gross evidence** of:
    - Satellite nodules in a different lobe of the same lung
    - Pleural nodules
    - Pericardial involvement
- Such findings should be **documented and sampled**, as they may upstage disease.

Special Situations

- **Multifocal tumours**:
    - Sample each lesion separately
    - Record size and location of all nodules
- **Post-therapy specimens**:
    - Document fibrosis, necrosis, cavitation
    - Sample areas of residual viable tumour
- **Pleural tumours (suspected mesothelioma)**:
    - Extensive sampling from:
        - Nodular areas
        - Thickened pleura
        - Tumour–lung interface

Accurate TNM staging in lung cancer begins at the gross table. Tumour size, pleural invasion, margin status, and lymph node assessment must be systematically documented and sampled.

B LUNG GROSS PEARLS

- **Brown induration**:
  Seen in *chronic venous congestion* of lung.
- **Black pigment seen as spots of varying sizes (2–3 mm) throughout the cut surface**:
  Carbon pigment (anthracosis).

- **Solid appearance of lobe, hepatization (liver-like)**:
  Consolidation in pneumonia; may also be seen in *lepidic-predominant adenocarcinoma (formerly bronchiolo-alveolar carcinoma)*.
- **Solid, reddish appearance**:
  Red hepatization – second stage of pneumonia.
- **Solid, grey appearance**:
  Grey hepatization – third stage of pneumonia.
- **Miliary pattern (millet-like nodules)**:
  Result of *haematogenous spread*, classically *miliary tuberculosis*.
- **Cavitary lesion**:
  Seen in *fibrocaseous tuberculosis*, *lung abscess*, *cavitating carcinoma* (especially squamous cell carcinoma), and *advanced bronchiectasis*.
- **Dilated bronchi extending to the pleural surface**:
  Bronchiectasis.
- **Exaggerated spongy cut surface with subpleural bullae**:
  Emphysema.
- **Rigid, spongy lung with honeycomb appearance**:
  Interstitial lung diseases, especially *usual interstitial pneumonia*.
- **Hilar mass**:
  Commonly *small cell carcinoma* and *squamous cell carcinoma*.
- **Endobronchial lesion**:
  Carcinoid tumour, *early squamous cell carcinoma*; rarely *papillary adenocarcinoma*.
- **Combined endobronchial and parenchymal mass (dumbbell appearance)**:
  Suggestive of *carcinoid tumour*.
- **Peripherally located solitary tumour**:
  Adenocarcinoma most common; occasionally *peripheral carcinoid*.
- **Peripheral diffuse tumour encasing pleura**:
  Malignant mesothelioma; rarely *peripheral adenocarcinoma with pleural spread*.
- **Multiple rounded nodules throughout lung parenchyma (cannon-ball appearance on imaging)**:
  Metastatic carcinoma.
- **Tumour with brown-black cut surface**:
  Haemorrhage within tumour; consider *metastatic malignant melanoma*.
- **Firm, grey-white tumour with necrosis and cavitation**:
  Suggestive of *squamous cell carcinoma*.
- **Mucoid, gelatinous cut surface**:
  Mucin-producing adenocarcinoma.

C III. PLEURA GROSS PEARLS

- **Nodular pleural thickening**:
  Malignant mesothelioma; rarely adenocarcinoma with pleural metastasis.
- **Diffuse pleural thickening without nodularity**:
  Empyema, chronic pleuritis, or fibrothorax.
- **Pleural plaques (chalky white, well-circumscribed)**:
  Seen in asbestos exposure (non-neoplastic).
- **Haemorrhagic pleural effusion with tumour deposits**:
  Strongly suggests *malignancy*.

B & C IV LUNG AND PLEURA -MICROSCOPY

1. Lung Abscesses

The commonest locations of lung abscesses -are at the right lower lobe, right upper lobe, and left lower lobe. These result from:

a) Aspiration of foreign material
b) Sequelae of pneumonia
c) Secondary infection in lung carcinoma
d) Overgrowth of fungi (Mucor, Aspergillus) over other etiologies

Microscopy

- Central liquefactive necrosis with abundant neutrophils
- Peripheral granulation tissue and fibrosis in chronic lesions
- Adjacent bronchial wall destruction

Complications

- Haemorrhage
- Bronchopleural fistula
- Empyema
- Brain abscess

Recent advances / added pearls

- In immunocompromised patients, fungal abscesses may show angioinvasion (especially Aspergillus, Mucor) with thrombosis and infarction.
- CT-guided biopsy material may show only necrotic debris—sampling from the periphery of the lesion is essential.
- Secondary abscess in carcinoma should prompt diligent search for viable tumour at the abscess wall.

2. Granulomatous Inflammation of the Lung

A wide variety of granulomatous inflammations involve the lung.

a) Tuberculosis

Morphologic forms

i) Ghon's focus – primary focus in lower part of upper lobe or upper part of lower lobe
ii) Fibrocaseous tuberculosis – commonest reactivation form with cavitation
iii) Miliary tuberculosis – millet-sized granulomas from haematogenous spread
iv) Tuberculous bronchopneumonia – granulomas around inflamed bronchioles
v) Tuberculoma – localized granulomatous mass

Microscopy

- Epithelioid cell granulomas
- Langhans giant cells
- Central caseous necrosis (variable)

Diagnostic pearl

- Acid-fast bacilli demonstrable in only 50–65% of cases
- Absence of AFB does not exclude tuberculosis

Atypical mycobacteria

- Occur mainly in immunocompromised hosts
- Organisms are longer, thicker, more coarsely beaded and bent
- Disease tends to be more aggressive

Recent advances

- PCR-based MTB detection and GeneXpert have increased sensitivity in paucibacillary biopsies.
- Necrotizing granulomas without organisms should raise differential of TB, fungal infection, GPA, and sarcoid-like reactions to malignancy or drugs.

b) Sarcoidosis

Microscopy

- Well-formed non-caseating "naked" granulomas
- Epithelioid cells and Langhans giant cells
- Sparse lymphocytic rim

Characteristic inclusions

- Asteroid bodies
- Schaumann bodies

Distribution

- Along lymphatic routes (bronchovascular bundles, septa, pleura)

Biochemical associations

- Elevated ACE
- Hypercalcemia

Recent advances

- Necrosis may be present (fibrinoid or eosinophilic) and does not exclude sarcoidosis
- Sarcoid-like granulomas are now well recognized in association with malignancy and immune checkpoint inhibitors

c) Fungal Infections

Includes histoplasmosis, blastomycosis, cryptococcosis, aspergillosis, mucormycosis.

Microscopy

- Granulomatous inflammation ± necrosis
- Organisms demonstrated by GMS and PAS stains

Aspergillosis

- Aspergilloma (fungus ball within cavity)
- Invasive and necrotizing forms

Recent advances

- Angioinvasion is the key diagnostic feature in invasive fungal infections
- Galactomannan and β-D-glucan assays aid diagnosis but tissue morphology remains gold standard

d) Granulomatosis with Polyangiitis (Wegener's)

Microscopy

- Necrotizing granulomatous inflammation
- Necrotizing vasculitis
- Mixed inflammatory infiltrate with giant cells

Clinical correlation

- Upper respiratory tract + lung + kidney involvement

Recent advances

- Strong association with PR3-ANCA (c-ANCA)
- Limited GPA may involve lung alone and mimic infection or malignancy

e) Eosinophilic Granulomatosis with Polyangiitis (Churg–Strauss)

Microscopy

- Eosinophil-rich granulomatous inflammation
- Necrotizing vasculitis
- Asthma history and peripheral eosinophilia

Recent advances

- Often p-ANCA positive
- Can overlap morphologically with hypereosinophilic syndromes

3. Interstitial Pneumonias

Current classification follows the **ATS/ERS guidelines**

Clinical entity	Histologic pattern
Idiopathic Pulmonary Fibrosis	UIP
Smoking related ILD	DIP, RB-ILD
ARDS	DAD
Acute Interstitial Pneumonia	Organizing DAD
NSIP	NSIP
COP	Organizing pneumonia
Sjögren's-associated ILD	LIP

a) Usual Interstitial Pneumonia (UIP)

Microscopy

- Patchy interstitial fibrosis
- Temporal heterogeneity
- Fibroblastic foci
- Honeycomb change

Prognosis

- Poor
- Limited steroid response

Recent advances

- UIP pattern ≠ IPF always; can be seen in CTD-ILD and drug-related ILD
- Presence of fibroblastic foci correlates with worse prognosis

b) Desquamative Interstitial Pneumonia (DIP)

Microscopy

- Alveolar macrophage accumulation
- Minimal fibrosis
- Type II pneumocyte hyperplasia

Recent advances

- Now considered part of smoking-related ILD spectrum
- Excellent response to smoking cessation + steroids

c) Respiratory Bronchiolitis–ILD

Microscopy

- Bronchiolocentric pigmented macrophages
- Peribronchiolar fibrosis

Pearl

- Must distinguish from incidental RB in smokers

d) Diffuse Alveolar Damage (DAD)

Phases

- Exudative: oedema, hyaline membranes
- Organizing: fibroblastic proliferation

Recent advances

- Seen in **COVID-19 lung injury**, drug toxicity, immune therapy
- Atypical pneumocyte hyperplasia may mimic carcinoma

e) Acute Interstitial Pneumonia

- Fulminant form of DAD
- High mortality

f) Organizing Pneumonia (BOOP / COP)

Microscopy

- Masson bodies
- Preserved lung architecture

Recent advances

- Secondary OP increasingly recognized after viral infections and radiation

g) NSIP (Non-steroidal Interstitial Pneumonia)

Key feature

- Uniformity of involvement

Prognosis

- Better than UIP

h) Lymphoid Interstitial Pneumonia (LIP)

Microscopy

- Diffuse interstitial lymphoplasmacytic infiltrate

Recent advances

- Must exclude low-grade MALT lymphoma using clonality studies

4. Pulmonary Langerhans Cell Histiocytosis

Microscopy

- Langerhans cells with nuclear grooves
- Eosinophils
- CD1a+, Langerin+, S-100+

Recent advances

- Strong smoking association
- BRAF V600E mutations reported in some cases

5. Pneumoconiosis

- defined as a non neoplastic reaction of lungs to inhaled mineral or organic dust.
- **Anthracosis** : i) refers to the presence of carbon particles in the lung. It is not a disease entity as carbon is inert; and is routinely seen in all lungs.

ii) When reaction occurs it leads to "coal workers pneumoconiosis" which may results in massive fibrosis.

- **Silicosis :** i)occurs due to silica particles, quartz, silicon dioxide.

ii) Micro-nodular scars along lymphatic network, particularly along bronchovascular bundles.

iii) silicosis is often complicated by tuberculosis

iv) Cellular nodules composed of fibroblasts & histiocytes with silica particles.Nodules may fuse to produce large masses which undergo necrosis & cavitate.

v) The edges of nodules have a characteristic stellate shape.

vi) Silica particles can be demonstrated under polarized light: birefringent spicules with pointed ends 5μ or less seen within and outside cells.

Mixed dust fibrosis : is pneumoconiosis resulting from mixed dust exposure including silica & quartz.

- **Asbestosis :**results in interstitial pneumonis leading in later stages to honeycomb lung. Diagnosis rests on identification in asbestosis of asbestos bodies *Asbestos bodies* **:** Asbestos fibres can be divided into serpentines (chrysolites) & amphiboles (amosite & crocidolite). Use of these in the industrial area & inhalation results in the presence of asbestos bodies in the lung smears.

Pleural manifestation of asbestos exposure are pleural plaques & mesotheliomas.

- pleural plaques are associated with amosite & crocidolite fibres.They are made up of hyalinised fibrous tissue & found where the visceral & parietal pleura approximate during respiratory excursions. With time they become calcified.
- The prevalence of mesothelioma ranges from 2-10% with exposure times upto 20 years or more. Amosite & crocidolite are the fibres found in most cases of mesothelioma. These are detected by conventional microscopy & electron micrscopy. Asbestos bodies can be seen in sputum & bronchioalveolar lavage as long thin symmetric beaded structures with bulbous ends. The core is translucent and the coat brown due to its iron content. A Perl's Prussian blue stain helps in picking them up better (ferruginous bodies)

6.CYSTIC CONDITIONS OF THE LUNG : A cyst is defined as a parenchymal space

Lined by either an epithelium (true cyst) or by a fibrous wall of varying thickness

(pseudocyst).

Cysts of the lung in the paediatric age group include pulmonary sequestration, congenital

lobar emphysema, bronchogenic cyst & cystic adenomatoid transformation.

In adults they include- bronchiectasis, Langerhan's cell histiocytosis,

lymphangioleiomyomatosis, emphysema, interstitial lung disease & hydatid cyst.

7.BRONCHIECTASIS :

Bronchiectasis is a chronic, irreversible dilatation of bronchi and bronchioles resulting from a vicious cycle of airway infection, inflammation, impaired mucociliary clearance, and progressive destruction of the bronchial wall. Pathologically, there is transmural inflammation with loss of smooth muscle and elastic tissue, leading to saccular or cylindrical dilatation, luminal mucus plugging, and adjacent parenchymal fibrosis. Traditionally classified as post-infective, congenital, or obstructive, current understanding emphasizes **etiology-based phenotyping**, including post-tuberculous disease, primary ciliary dyskinesia, immunodeficiency-associated bronchiectasis, and allergic bronchopulmonary aspergillosis. **High-resolution CT (HRCT)** has replaced bronchography as the diagnostic gold standard, with features such as signet-ring sign, lack of bronchial tapering, and visualization of bronchi in the peripheral lung fields. Recent advances include recognition of the **airway microbiome** and chronic neutrophilic inflammation driven by protease–antiprotease imbalance, the role of **biofilm-forming organisms** (especially *Pseudomonas aeruginosa*) in disease severity, and the use of **biologic and targeted anti-inflammatory therapies** in selected phenotypes. Molecular studies have also highlighted overlap with COPD and asthma, leading to the concept of **treatable traits**, which is increasingly guiding individualized management.

8. MICROSCOPY OF LUNG TUMOURS

The terminology of lung tumors has changed significantly in the 2015 WHO classification (Travis: WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart, 4th Edition, 2015) as compared to the 1999 WHO classification and the subsequent 2011 changes.

The 1999 WHO/IASLC Histological Classification of Lung and Pleural Tumours.

1 . EPITHELIAL TUMOURS

a) Benign

Papillomas

Squamous cell papilloma

Exophytic

Inverted

Glandular papilloma

Mixed squamous cell & glandular papilloma

Adenomas

Alveolar adenoma

Papillary adenoma

Adenomas of salivary gland type

Mucous gland adenoma

Pleomorphic adenoma

Other

Mucinous cystadenoma

Others

b) Preinvasive lesions

Squamous dysplasia/carcinoma in situ

Atypical adenomatous hyperplasia

Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia

c) Invasive Malignant

Squamous cell carcinoma

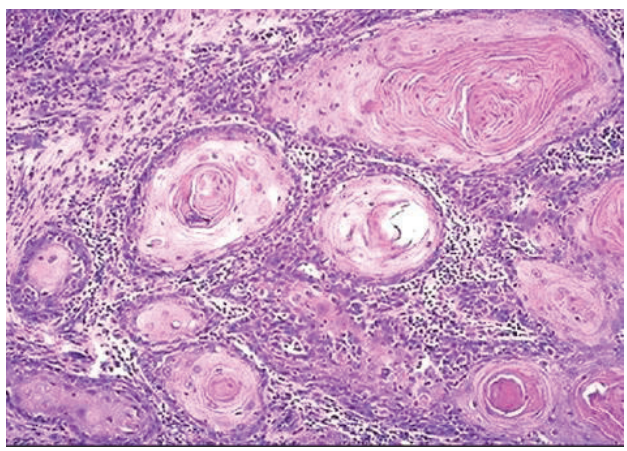


Fig5.20 SC carcinoma

with Squamous pearls infiltrating stroma. H7E x100

Variants - Papillary

Clear cell

Small cell

Basaloid

Small cell carcinoma

Variant - combined small cell carcinoma

Adenocarcinoma

Acinar

Papillary

Bronchiolo-alveolar carcinoma

non mucinous

mucinous

mixed mucinous & non mucinous or indeterminate

Solid adenocarcinoma with mucin production

Mixed

Variants - Well differentiated fetal adenocarcinoma

- Mucinous (colloid)
- Mucinous cystadenocarcinoma
- Signet ring
- Clear cell

Large cell carcinoma

Variants – Large cell nueroendocrine carcinoma

Combined large cell neuroendocrine carcinoma

-Basaloid carcinoma

- Lymphoepithelioma like carcinoma

- Clear cell carcinoma

-Large cell carcinoma with rhabdoid phenotype

Adenosquamous carcinoma

Carcinoma with pleomorphic, sarcomatoid or sarcomatous elements

Carcinoma with spindle cells &/or giant cells

-pleomorphic carcinoma

- Spindle cell carcinoma

- giant cell carcinoma

Carcinosarcoma

Blastoma (Pulmonary blastoma)

Others

Carcinoid tumour

Typical carcinoid

Atypical carcinoid

Carcinomas of Salivary gland type

Mucoepidermoid carcinoma

Adenoid cystic carcinoma

Others

Unclassified carcinoma

2. SOFT TISSUE TUMOURS

Localised fibrous tumour

Epitheliod haemangioendothelioma

Pleuropulmonary blastoma

Chondroma

Calcifying fibrous pseudotumour of the pelvis

Congenital peribronchial myofibroblastic tumour

Diffuse pulmonary lymphangiomatosis

Desmoplastic small round cell tumour

Other

3. MESOTHELIAL

Benign

-adenomatoid tumour

Malignant

-epithelioid mesothelioma

-sarcomatoid mesothelioma

desmoplastic mesothelioma

-biphasic mesothelioma

-other

4. MISCELLANEOUS TUMOURS

Harmartoma

Sclerosing haemangioma

Clear cell tumour

Germ cell neoplasms

-teratoma, mature or immature

-malignant germ cell tumour

Thymoma

Melanoma

Others

5. LYMPHOPROLIFERATIVE DISEASES

Lymphoid interstitial pneumonia

Nodular lymphoid hyperplasia

Low grade marginal zone B-cell lymphoma of the Mucosa

Associated Lymphoid Tissue (MALT)

Lymphomatoid granulomatosis

6. SECONDARY TUMOURS

7. UNCLASSIFIED TUMOURS

8. TUMOUR LIKE LESIONS

Tumourlet

Multiple meningothelioid nodules

Langerhan's cell histiocytosis

Inflammatory pseudotumour (inflammatory myofibroblastic tumour)

Organising pneumonia

Amyloid tumour

Hyalinising granuloma

Lymphangiomyomatosis

Multifocal micronodular pneumocyte hyperplasia

Endometriosis

Bronchial inflammatory polyp

Others

The Current WHO classification recommends molecular testing in all lung adenocarcinomas and in selected non-adenocarcinomas, particularly in small biopsy specimens.

In the older classification it was –

Morphology first , then molecular testing; presently modern thinking is "Molecular first" morphology later .

Molecular Classification of Lung Carcinomas (Has Therapeutic Relevance)

Advances in molecular pathology have transformed the classification and management of lung carcinomas, particularly **adenocarcinoma**, with identification of actionable genetic alterations that predict response to targeted therapy.

- **EGFR (Epidermal Growth Factor Receptor) mutations**
  Seen predominantly in adenocarcinomas, especially in **non-smokers, females, and East Asian populations**. Common mutations include exon 19 deletions and exon 21 L858R substitution. Tumours show sensitivity to EGFR tyrosine kinase inhibitors and typically display lepidic, acinar, or papillary patterns.
- **ALK (Anaplastic Lymphoma Kinase) rearrangements**
  Occur in a subset of lung adenocarcinomas, often in younger patients and non-smokers. Frequently associated with solid growth pattern and signet-ring cells. These tumours respond to ALK inhibitors and are usually mutually exclusive with EGFR and KRAS mutations.
- **KRAS mutations**
  Commonly associated with **smoking-related adenocarcinomas**. Mutations, especially KRAS G12C, are linked with aggressive behaviour and relative resistance to EGFR-targeted therapy, though KRAS-directed therapies have recently emerged. KRAS mutations are frequent in invasive mucinous adenocarcinoma.
- **PD-L1 expression**
  Programmed death ligand-1 expression is assessed by immunohistochemistry and serves as a predictive biomarker for **immune checkpoint inhibitor therapy**. High PD-L1 expression is more commonly seen in poorly differentiated carcinomas and tumours with high tumour mutational burden.

WHO (2015)

WHO classification of tumors of the lung (**Travis: WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart, 4th Edition, 2015**) :

Epithelial tumors ICD-O codes

- Adenocarcinoma
  - Lepidic adenocarcinoma
  - Acinar adenocarcinoma
  - Papillary adenocarcinoma
  - Micropapillary adenocarcinoma
  - Solid adenocarcinoma
  - Invasive mucinous adenocarcinoma
    - Mixed invasive mucinous and nonmucinous adenocarcinoma
  - Colloid adenocarcinoma
  - Fetal adenocarcinoma
  - Enteric adenocarcinoma
  - Minimally invasive adenocarcinoma
    - Nonmucinous
    - Mucinous
  - Preinvasive lesions
    - Atypical adenomatous hyperplasia8250/0
    - Adenocarcinoma in situ
    - Nonmucinous
    - Mucinous
- Squamous cell carcinoma
  - Keratinizing squamous cell carcinoma
  - Nonkeratinizing squamous cell carcinoma
  - Basaloid squamous cell carcinoma
  - Preinvasive lesion
    - Squamous cell carcinoma in situ
- Neuroendocrine tumors
  - Small cell carcinoma
    - Combined small cell carcinoma
  - Large cell neuroendocrine carcinoma
    - Combined large cell neuroendocrine carcinoma
  - Carcinoid tumors
    - Typical carcinoid
    - Atypical carcinoid
  - Preinvasive lesion
    - Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia

- Large cell carcinoma
- Adenosquamous carcinoma
- Pleomorphic carcinoma
- Spindle cell carcinoma
- Giant cell carcinoma
- Carcinosarcoma
- Pulmonary blastoma
- Other and unclassified carcinomas
    - Lymphoepithelioma-like carcinoma
    - NUT carcinoma
- Salivary gland type tumors
    - Mucoepidermoid carcinoma
    - Adenoid cystic carcinoma
    - Epithelial myoepithelial carcinoma
    - Pleomorphic adenoma
- Papillomas
    - Squamous cell papilloma
        - Exophytic
        - Inverted
    - Glandular papilloma
    - Mixed squamous and glandular papilloma8560/0
- Adenomas
    - Sclerosing pneumocytoma
    - Alveolar adenoma
    - Papillary adenoma
    - Mucinous cystadenoma
    - Mucous gland adenoma

Mesenchymal tumors

- Pulmonary hamartoma
- Chondroma
- PEComatous tumors
    - Lymphangioleiomyomatosis
    - PEComa, benign
        - Clear cell tumor
    - PEComa, malignant

- Congenital peribronchial myofibroblastic tumor
- Diffuse pulmonary lymphangiomatosis
- Inflammatory myofibroblastic tumor
- Epithelioid hemangioendothelioma
- Pleuropulmonary blastoma
- Synovial sarcoma
- Pulmonary artery intimal sarcoma
- Pulmonary myxoid sarcoma with *EWSR1-CREB1* translocation
- Myoepithelial tumors
    - Myoepithelioma
    - Myoepithelial carcinoma

Lymphohistiocytic tumors

- Extranodal marginal zone lymphoma of mucosa associated lymphoid tissue (MALT lymphoma)
- Diffuse large B cell lymphoma
- Lymphomatoid granulomatosis
- Intravascular large B cell lymphoma
- Pulmonary Langerhans cell histiocytosis
- Erdheim-Chester disease

Tumors of ectopic origin

Metastatic tumors

Important Features of Preinvasive Lesions

i) Squamous Cell Dysplasia and Carcinoma in Situ

- Represents a stepwise progression from squamous metaplasia → mild, moderate, severe dysplasia → carcinoma in situ, analogous to cervical intraepithelial neoplasia.
- Severe dysplasia and carcinoma in situ show full-thickness epithelial atypia, increased mitoses, loss of maturation, and an intact basement membrane.
- A helpful diagnostic feature is micropapillary capillary protrusion into the dysplastic epithelium.
- Diagnosis should be made with caution in the setting of active inflammation, ARDS, prior radiotherapy or chemotherapy, as reactive atypia may mimic dysplasia.
- Important mimics include reserve cell hyperplasia and immature squamous metaplasia.

- Molecular studies demonstrate early alterations in TP53, CDKN2A, and SOX2, supporting its role as a true precursor lesion.

ii) Atypical Adenomatous Hyperplasia (AAH)

- Recognized as a preneoplastic lesion and precursor of lung adenocarcinoma, particularly the lepidic spectrum.
- Defined by WHO as a small (≤5 mm), localized proliferation of mildly to moderately atypical cuboidal to low columnar epithelial cells (type II pneumocytes or club cells) lining alveolar walls and respiratory bronchioles.
- Cells show slightly increased nuclear-cytoplasmic ratio, hyperchromatic nuclei, inconspicuous nucleoli, and absent mitoses.
- Often an incidental finding in resected lungs, frequently multicentric; multicentric AAH is associated with increased risk of adenocarcinoma.
- Molecular overlap with adenocarcinoma includes EGFR mutations and KRAS alterations, supporting a multistep progression from AAH → AIS → invasive adenocarcinoma.
- The main differential diagnosis is adenocarcinoma in situ, distinguished by size, degree of atypia, and invasion.

iii) Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia (DIPNECH)

- Characterized by diffuse proliferation of pulmonary neuroendocrine cells confined to the bronchiolar epithelium, often associated with constrictive bronchiolitis.
- Considered a preneoplastic lesion for typical and atypical carcinoid tumours, but not for small cell carcinoma or LCNEC.
- Neuroendocrine cells are immunoreactive for chromogranin, synaptophysin, and CD56 (NCAM).
- Cells secrete bioactive peptides such as bombesin, contributing to peribronchiolar fibrosis.
- Increasingly recognized due to improved imaging and awareness, particularly in middle-aged, non-smoking women.

AAH vs AIS vs MIA vs Invasive Adenocarcinoma

Feature	AAH	AIS	MIA	Invasive Adenocarcinoma
Nature	Preneoplastic lesion	Preinvasive carcinoma	Early invasive carcinoma	Fully invasive carcinoma
Size	≤ 5 mm	≤ 3 cm	≤ 3 cm	Variable, often > 3 cm

Feature	AAH	AIS	MIA	Invasive Adenocarcinoma
Growth pattern	Atypical cuboidal cells lining alveoli	Pure lepidic growth	Predominantly lepidic	Acinar, papillary, micropapillary, solid ± lepidic
Invasion	Absent	Absent	≤ 5 mm invasion	> 5 mm invasion
Cytological atypia	Mild	Mild to moderate	Mild to moderate	Moderate to severe
Mitoses	Absent	Rare	Rare	Frequent
Necrosis	Absent	Absent	Absent	May be present
Stromal / vascular / pleural invasion	Absent	Absent	Absent	May be present
Molecular features	EGFR, KRAS (early events)	EGFR common	EGFR common	EGFR, KRAS, ALK, others
Prognosis after resection	Excellent	100% disease-free survival	Near 100% survival	Variable, stage-dependent

Important pearls -

- BAC is obsolete → use AAH–AIS–MIA–Invasive adenocarcinoma spectrum
- AIS and MIA have near-100% survival after complete resection
- Micropapillary and solid patterns indicate poor prognosis
- Molecular testing is now part of routine diagnosis
- Squamous dysplasia and squamous carcinoma in situ
- Atypical adenomatous hyperplasia (AAH)
- Adenocarcinoma in situ (AIS) *(new entity replacing non-mucinous BAC)*
- Minimally invasive adenocarcinoma (MIA) *(new)*
- Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH)
- *Bronchiolo-alveolar carcinoma (BAC) has been deleted* from WHO classification.

Detail on some of the Tumours and recent advances in their Classification :

a) Benign epithelial tumours

(Largely unchanged, but now considered rare and of limited clinical significance)

- Papillomas
    - Squamous cell papilloma (exophytic, inverted)
    - Glandular papilloma
    - Mixed squamous and glandular papilloma
- Adenomas
    - Alveolar adenoma
    - Papillary adenoma
    - Salivary gland–type adenomas
        - Mucous gland adenoma
        - Pleomorphic adenoma
- Mucinous cystadenoma
- Others

b) Adenocarcinoma

i) Incidence is gradually increasing; being the predominant subtype in some countries. It is the most common type of lung carcinoma and shows marked histologic and molecular heterogeneity, with frequent EGFR, ALK, and KRAS alterations that have significant therapeutic implications.

ii) grossly poorly circumscribed gray yellow lesions, single or multiple with a mucoid glairy appearance if mucin secreting.

iii) 65% of cases are peripherally located & about 77% involve the visceral pleura (pseudomesotheliomatous carcinoma)

iv) high percentage of adenocarcinomas arise in association with peripheral scar or honey combing & show foci of AAH in the neighbouring air spaces.

v) frequent heterogeneity at histology. Mixtures of patterns are more common as compared to single patterns outlined below.

vi) Microscopy shows formation of tubules, papillae and secretion of mucin, so depending on the features the neoplasm is categorized as

-acinar : glandular & acinar pattern

-papillary : to be differentiated from thyroid papillary carcinoma by immunohistochemistry.

-solid with mucin production

- Presence of cilia are rare & should suggest a reactive process. Eosinophilic intranuclear inclusions are a feature.

Variants of adenocarcinoma are – well differentiated fetal adenocarcinoma (such terms reflect the fetal appearance of tissue in these tumours which are thought to recapitulate the developing

lung during the 9th to eleventh week of gestation), mucinous (colloid) adenocarcinoma , mucinous cystadenocarcinoma, signet ring adenocarcinoma & clear cell adenocarcinoma.

(Key modifications to the 1999 WHO/IASLC classification) **WHO (2015 → 2021/2023)** ***concepts of*** **Adenocarcinoma .**

This is *most extensively revised category.*

Predominant pattern–based classification (instead of mixed types):

- Lepidic
- Acinar
    - Papillary
    - Micropapillary
    - Solid

Variants

- Invasive mucinous adenocarcinoma *(formerly mucinous BAC)*
- Colloid (mucinous) adenocarcinoma
- Fetal adenocarcinoma (low and high grade)
- Enteric adenocarcinoma

◈ **AIS** (≤3 cm, pure lepidic growth, no invasion) ; ◈ **MIA** (≤3 cm, ≤5 mm invasion) ◈ Bronchiolo-alveolar Carcinoma deleted from the WHO Classification.

Differences between **conventional adenocarcinoma lung** and **metastatic colorectal carcinoma at immunohistochemistry:**

	Conventional Adenocar lung	**Metastatic Colorectal Car**
CK 7	+ve	-ve
CK 20	-ve	+ve
TTF- 1	+ve	-ve
α-1 antitrypsin	-ve	-ve
PE-10		
MUC 3	-ve	
MUC 6	-ve	
MOC -31	+ve	+ve in 80%

Squamous cell carcinoma :

i) predominatly involves major & segmental bronchi; could also be seen peripherally & subpleurally

ii) Microscopy shows- tumour cells with evidence of keratin production in the form of pearls & individual cell keratinisation

- other features include oncocytic appearance of cells, giant cell foreign body reaction to keratin , palisaded granulomas, inflammatory cell infiltrates, lipedic growth into air spaces at the periphery.
- 10% of hilar tumours but 60% of peripheral tumours may show evidence of mucin production.
- Only when substantial amounts of other elements are seen then they could be termed carcinomas.

Variants : a)Papillary – well differentiated intrabronchial papillary lesion

- no necrosis

Evidence of keratinisation seen (clear cell change is more commonly

seen in adenocarcinoma) b) Non-keratinising

c) Basaloid type – periphery of tumour cell nests show palisading.

Evidence of squamous differentiation within nests

- aggressive tumour similar to those in the upper aero-digestive tract.

IHC : Low as well as high mol. wt cytokeratin +ve; Involucrin +ve

Some cases – vimentin, EMA , HMF6-2 (human milk fat globulin)

S-100, Leu M1 & CEA +ve

p63 (p53 homologous nuclear protein) +ve

consistently (--ve in small cell carcinoma)

(Key modifications to the 1999 WHO/IASLC classification) WHO (2015 → 2021/2023) concepts

Squamous Cell Carcinoma (Invasive malignant epithelial tumours)

- Keratinizing
- Non-keratinizing
- Basaloid

(Papillary, clear cell(change due to glycogen), and small cell–like patterns are now regarded as morphologic patterns, not true variants.)

Differences between squamous cell and adenocarcinoma the lung at immunohistochemistry:

	Squamous CC	Adenocarcinoma
CK	High mol wt +ve Low mol wt +ve	Low mol wt +ve CK7+ve CK20 -ve
34 β E 12 (Basal cell keratin) (High mol wt)	+ve	+ve
Involucrin	+ve	-ve
Vimentin (+ve where spindle cell diff seen)	Sometimes +ve	Rarely
EMA	Sometimes +ve	+ve
CEA	Sometimes +ve	+ve
Human milk fat globulin	Sometimes +ve	+ve
Leu M 1	Sometimes +ve	
S-100 protein	Sometimes +ve	+ve in Langerhan's cells in stroma
TTF-1	-ve	+ve
Surfactant apoprotein (PE-10)	-ve	+ve (50%)
P_{63}(p_{53} homologous protein)	+ve	
NSE, Leu 7, chromogranin Synaptophysin	-ve	-ve

· Human milk fat globulin;· Leu-M1;· Involucrin; · Surfactant apoprotein (PE-10);

· NSE / Leu-7 (non-specific)- these markers are not as important and more of historical importance now .

Recent Update (IHC):
p40 is more specific than p63 for squamous cell carcinoma.
TTF-1 and Napsin A are the most reliable markers for lung adenocarcinoma.

Neuroendocrine Tumours of the Lung

Neuroendocrine tumours (NETs) of the lung represent a distinct spectrum of epithelial neoplasms characterized by neuroendocrine differentiation and a wide range of biological behaviour, from indolent to highly aggressive. In the current WHO classification, pulmonary neuroendocrine tumours are unified into a single group comprising typical carcinoid, atypical carcinoid, large cell neuroendocrine carcinoma (LCNEC), and small cell carcinoma, reflecting shared lineage but differing degrees of differentiation and malignancy.

Typical and atypical carcinoid tumours are well-differentiated neuroendocrine neoplasms, commonly arising in central airways, showing organoid, trabecular, or rosette-like architecture. Typical carcinoids are low-grade tumours with low mitotic activity and absence of necrosis, whereas atypical carcinoids show increased mitoses and focal necrosis and exhibit more aggressive behaviour. At the opposite end of the spectrum are small cell carcinoma and LCNEC, which are poorly differentiated, high-grade neuroendocrine carcinomas characterized by high mitotic rates, extensive necrosis, and early metastasis.

Immunohistochemically, neuroendocrine tumours express chromogranin, synaptophysin, and CD56, with variable expression of TTF-1, particularly in high-grade tumours. Molecularly, high-grade neuroendocrine carcinomas show frequent TP53 and RB1 inactivation, high tumour mutational burden, and alterations in MYC family genes, whereas carcinoid tumours lack common actionable driver mutations seen in adenocarcinoma. Clinically, pulmonary neuroendocrine tumours may be associated with paraneoplastic syndromes, particularly ectopic hormone production in small cell carcinoma.

An important preneoplastic condition is diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH), which is recognized as a precursor lesion for typical and atypical carcinoid tumours, but not for small cell carcinoma or LCNEC. Accurate classification of pulmonary neuroendocrine tumours is essential due to marked differences in prognosis and therapeutic approach, ranging from surgical resection in carcinoid tumours to systemic chemotherapy and immunotherapy in high-grade neuroendocrine carcinomas.

"Thus, pulmonary neuroendocrine tumours constitute a biologically unified yet clinically diverse group, necessitating precise histologic and immunohistochemical classification."

Carcinoid Tumours of the Lung

Pulmonary carcinoid tumours are well-differentiated neuroendocrine neoplasms arising from bronchial neuroendocrine (Kulchitsky) cells and account for a small proportion of primary lung tumours. They are subdivided into typical carcinoid and atypical carcinoid based on mitotic activity and presence of necrosis. Typical carcinoids are low-grade tumours characterized by fewer than 2 mitoses per 2 mm^2 and absence of necrosis, whereas atypical carcinoids show increased mitotic activity (2–10 mitoses per 2 mm^2) and focal necrosis and display more aggressive behaviour.

Grossly, carcinoid tumours often present as well-circumscribed, polypoid endobronchial masses, commonly located in central airways. Histologically, they exhibit organoid, trabecular, nested, or rosette-like growth patterns, composed of uniform cells with moderate eosinophilic cytoplasm and round nuclei with finely granular "salt-and-pepper" chromatin. Immunohistochemically, tumour cells express chromogranin, synaptophysin, and CD56. Carcinoid tumours may be associated with diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH), which is regarded as a precursor lesion. Prognosis is excellent for typical carcinoids and intermediate for atypical carcinoids following surgical resection.

Large Cell Neuroendocrine Carcinoma (LCNEC)

Large cell neuroendocrine carcinoma is a high-grade, poorly differentiated neuroendocrine carcinoma of the lung, sharing biologic features with small cell carcinoma but showing large cell cytologic features. It typically occurs in older smokers and presents as a peripheral lung mass. LCNEC is recognized as a distinct entity in the WHO classification due to its aggressive behaviour and neuroendocrine differentiation.

Histologically, LCNEC is composed of large polygonal cells with abundant cytoplasm, vesicular nuclei, and prominent nucleoli, arranged in organoid, trabecular, palisading, or rosette-like patterns, along with high mitotic activity and extensive necrosis. Immunohistochemistry demonstrates expression of neuroendocrine markers, including chromogranin, synaptophysin, and CD56. Molecularly, LCNEC frequently shows TP53 alterations, with or without RB1 inactivation, placing it biologically between non-small cell carcinoma and small cell carcinoma. The prognosis is poor, and optimal therapeutic strategies remain an area of active investigation.

Small Cell Carcinoma of the Lung

Small cell carcinoma is a high-grade malignant neuroendocrine carcinoma characterized by rapid growth, early metastasis, and strong association with cigarette smoking. It most commonly arises as a central hilar mass and is often disseminated at the time of diagnosis, making surgical resection uncommon.

Grossly, the tumour appears as a soft, friable mass with extensive necrosis. Microscopically, it is composed of small cells with scant cytoplasm, hyperchromatic nuclei, finely granular chromatin, absent or inconspicuous nucleoli, and characteristic nuclear moulding. Mitotic activity is extremely high, and necrosis is widespread. Tumour cells express neuroendocrine markers and frequently show TTF-1 positivity.

Molecularly, small cell carcinoma shows near-universal inactivation of TP53 and RB1, high tumour mutational burden, and MYC family gene alterations. There are no recognized preneoplastic lesions, and DIPNECH does not precede small cell carcinoma. Clinically, small cell carcinoma is often associated with paraneoplastic syndromes, and current management relies on systemic chemotherapy with emerging roles for immunotherapy.

In the recent WHO classifications, small cell carcinoma is grouped under pulmonary neuroendocrine tumours, along with:

- Typical carcinoid
- Atypical carcinoid
- Large cell neuroendocrine carcinoma (LCNEC)
- Small cell carcinoma

So, it is no longer viewed as a variant of squamous or large cell carcinoma, but as a high-grade neuroendocrine carcinoma.

Small Cell Carcinoma vs Large Cell Neuroendocrine Carcinoma

Feature	Small Cell Carcinoma (SCLC)	Large Cell Neuroendocrine Carcinoma (LCNEC)
WHO category	High-grade neuroendocrine carcinoma	High-grade neuroendocrine carcinoma
Cell size	Small cells	Large cells
Cytoplasm	Scant	Moderate to abundant
Nuclei	Hyperchromatic, finely granular	Vesicular with prominent nucleoli
Nuclear molding	Prominent	Absent or minimal
Mitotic rate	Very high	Very high
Necrosis	Extensive	Extensive
Growth pattern	Sheets, ribbons	Organoid, trabecular, palisading
Neuroendocrine markers	Strong positivity	Positive
TTF-1	Positive in most cases	Variable
Ki-67 index	Extremely high (>70–80%)	High (usually >40–50%)
Smoking association	Strong	Strong
Molecular features	TP53, RB1 loss	TP53 ± RB1 loss
Clinical behaviour	Very aggressive	Aggressive
Treatment approach	Chemotherapy ± immunotherapy	Often treated like NSCLC or SCLC (grey zone)

"Small cell carcinoma and large cell neuroendocrine carcinoma represent the two high-grade neuroendocrine carcinomas of the lung, differing mainly in cytologic features but sharing aggressive clinical behaviour."

Comparison between Small Cell Carcinoma and Squamous Cell Carcinoma of Lung

Feature	Small Cell Carcinoma	Squamous Cell Carcinoma
WHO category	High-grade neuroendocrine carcinoma	Non-small cell carcinoma
Cell of origin	Neuroendocrine (Kulchitsky) cells	Bronchial squamous epithelium
Smoking association	Very strong	Strong
Common location	Central / hilar	Central (may extend peripherally)
Gross appearance	Soft, friable mass with necrosis	Firm, grey-white mass; may show cavitation
Cell size	Small cells	Large polygonal cells
Cytoplasm	Scant	Moderate to abundant
Nuclei	Hyperchromatic, finely granular	Vesicular
Nucleoli	Absent or inconspicuous	Prominent
Nuclear moulding	Characteristic	Absent
Keratinization	Absent	Present (keratin pearls)
Intercellular bridges	Absent	Present
Mitotic activity	Very high	Moderate
Necrosis	Extensive	Common
Neuroendocrine markers	Positive	Negative
p40 / p63	Negative	Positive
TTF-1	Positive in most cases	Usually negative
Molecular features	TP53 & RB1 loss	TP53 mutations common
Paraneoplastic syndromes	Common (SIADH, Cushing)	Rare
Metastasis	Early and widespread	Later
Surgical resection	Usually not done	Often feasible
Treatment	Chemotherapy ± immunotherapy	Surgery ± chemo / radiotherapy
Prognosis	Very poor	Better than SCLC

High-yield Pearls

- **Nuclear moulding + scant cytoplasm = Small cell carcinoma**
- **Keratin pearls + intercellular bridges = Squamous cell carcinoma**
- **Paraneoplastic syndromes → think small cell**
- **p40 positivity confirms squamous differentiation**

Current Immunohistochemical (IHC) Approach in Small Lung Biopsies

Small lung biopsies and cytology specimens require a minimal yet highly informative immunohistochemical panel, as tissue conservation is essential for subsequent molecular testing. The primary aim is to subclassify non-small cell carcinoma into squamous cell carcinoma or adenocarcinoma and to identify neuroendocrine differentiation, if present.

Step 1: Distinguish adenocarcinoma from squamous cell carcinoma

- TTF-1 and Napsin A → markers of lung adenocarcinoma
- p40 (preferred) or p63 → markers of squamous cell carcinoma

A limited panel of TTF-1 and p40 is usually sufficient in most cases.

Step 2: Identify neuroendocrine tumours

- Synaptophysin
- Chromogranin
- CD56

Neuroendocrine markers are indicated when morphology suggests small cell carcinoma or large cell neuroendocrine carcinoma.

Step 3: Exclude metastatic tumours when indicated

- CK7 / CK20 profile
- CDX2 (colorectal origin)
- TTF-1 (lung origin)

Step 4: Preserve tissue for molecular testing

- Excessive immunohistochemistry should be avoided to allow testing for EGFR, ALK, KRAS, and PD-L1, particularly in adenocarcinoma.

Current guidelines emphasize that in small biopsies, accurate subtyping with minimal IHC is preferable to extensive staining that compromises molecular analysis. IHC performed can be integrated with clinical data and radiologic imaging.

Large Cell Carcinoma

- Diagnosis of exclusion
- Includes:
  - LCNEC (moved to neuroendocrine group)

    - Basaloid carcinoma : large cells with peripheral palisading – tumour nests should show no squamous cell differentiation (differentiates it from basaloid variant of SCC)
- Lymphoepithelioma-like carcinoma: large cells intermingled with lymphocytes as seen in the nasopharynx , EBV +ve.
- Clear cell carcinoma : tumour with large cell morphology with >90% of cells showing clear cytoplasm but no mucin.
- Large cell carcinoma with rhabdoid phenotype : eosinophilic globular cytoplasmic inclusions.

Adenosquamous carcinoma : Constitutes 3.5 % of surgically resected lung cancers. A neoplasm containing significant proportion of squamous cell and adenocarcinoma components (each component should be atleast 10% of the whole tumour in order to make this diagnosis & both elements should be malignant.). More common in peripheral than central tumours (about 90% of these tumours are peripheral).

 In the recent WHO classifications, adenosquamous carcinoma is defined as a **single clonal tumour**, with both squamous and glandular components sharing **common driver mutations,** supporting a monoclonal origin.

 These tumours often harbour **KRAS or EGFR mutations**, particularly in the adenocarcinoma component, which may have therapeutic implications.

NUT Carcinoma of the Lung

NUT carcinoma is a rare, highly aggressive, poorly differentiated carcinoma defined by rearrangement of the NUTM1 (nuclear protein in testis) gene. Although initially described in midline structures of the head, neck, and mediastinum, the lung is now recognized as an important primary site. NUT carcinoma affects both children and adults and has no strong association with smoking.

Histologically, NUT carcinoma is composed of monomorphic sheets of undifferentiated or poorly differentiated cells with scant cytoplasm, vesicular nuclei, and prominent nucleoli. A characteristic feature is abrupt focal squamous differentiation, manifested by sudden keratinization within otherwise undifferentiated tumour, which serves as an important diagnostic clue. Extensive necrosis and high mitotic activity are common.

Immunohistochemically, tumour cells show strong and diffuse nuclear positivity for NUT protein, which is highly specific and diagnostic. The tumours often express p40 and p63, reflecting squamous differentiation, but are typically negative for TTF-1 and neuroendocrine markers. Molecularly, the most common genetic abnormality is BRD4–NUT fusion, followed by other BRD family rearrangements.

Clinically, NUT carcinoma of the lung is characterized by rapid progression, early metastasis, and extremely poor prognosis, with median survival measured in months. Recognition of this entity is critical, as it may be mistaken for poorly differentiated squamous cell carcinoma or small cell

carcinoma on small biopsies. Emerging targeted therapies, including bromodomain and extra-terminal (BET) inhibitors, have shown promise in early studies, making accurate diagnosis increasingly relevant.

High-yield exam pearls

- **Abrupt squamous differentiation in an undifferentiated carcinoma → suspect NUT carcinoma**
- **NUT nuclear positivity is diagnostic**
- **TTF-1 negative, p40 positive**
- **Defined by NUTM1 gene rearrangement**

Carcinomas with pleomorphic elements (Sarcomatoid carcinoma) : Categorised on light microscopy. Includes a wide spectrum with foci of squamous carcinoma in 8%, large cell carcinoma in 25%, and adenocarcinoma in 45%. The remaining 22% of tumours were spindle cell carcinomas or giant cell carcinomas. The spindle cells vary from epithelioid in appearance to slender cells with elongated cytoplasm. Mixed patterns with spindle cells & pleomorphic giant cells may be seen.

Recent WHO concept

- These tumours are now grouped under Pulmonary Sarcomatoid Carcinomas, a distinct category of poorly differentiated non-small cell carcinomas and includes:
  - Pleomorphic carcinoma
  - Spindle cell carcinoma
  - Giant cell carcinoma
  - Carcinosarcoma
  - Pulmonary blastoma
- Molecular studies show frequent TP53 mutations, high tumour mutational burden, and high PD-L1 expression, explaining emerging responses to immune checkpoint inhibitors.

Carcinosarcoma : included in this category is defined as a biphasic tumour with carcinomatous & sarcomatous components only if the latter demonstrate heterologous elements such as malignant bone, cartilage & skeletal muscle.

Carcinosarcoma is now regarded as a **monoclonal epithelial tumour with sarcomatoid transformation**, rather than a true collision tumour. Sarcomatous components often show **epithelial marker expression,** supporting epithelial origin.

Pulmonary blastoma: is a rare adult sarcomatoid carcinoma composed of both epithelial and mesenchymal components resembling primitive or embryonal structures. Histologically, the epithelial component forms glandular proliferations with tubular structures of varying sizes, separated by a densely cellular spindle-celled stroma. Adult pulmonary blastoma often harbors **β-catenin (CTNNB1) mutations** and is distinct from pediatric pleuropulmonary blastoma.

-Salivary Gland–Type Carcinomas

- Mucoepidermoid carcinoma
- Adenoid cystic carcinoma

Others

Mesothelioma

i) Benign : adenomatoid tumour : tubules lined by cuboidal epithelium in a fibrous stroma.

ii) Malignant mesothelioma : Can be best diagnosed by a thorough evaluation of all existent clinical and radiologic data, progressive nature of the disease with recurrent pleural effusions, cytologic appearance of the pleural fluid, absence of significant bronchoscopic findings , typical thoracotomy observation, gross specimen appearance, confirmation by light microcsopy, histochemistry & immunohistochemistry.

iii) Gross : Numerous studded nodules, both well defined & poorly defined in a thickened decorticated pleura. C/S of nodules shows slit like spaces corresponding to glandular lumina.

iv) **Microscopy:**
Malignant mesothelioma shows neoplastic mesothelial proliferation arranged in papillary, tubulopapillary, pseudoacinar, or solid patterns. Tumour cells exhibit varying degrees of cytologic atypia. A key feature distinguishing malignant mesothelioma from reactive mesothelial hyperplasia is **invasive growth**, including infiltration into underlying fat, skeletal muscle, lung parenchyma, or chest wall structures. Necrosis and increased mitotic activity may be present, particularly in non-epithelioid subtypes.

Histologic subtypes (WHO) include :

- Epithelioid mesothelioma (most common, best prognosis
- Sarcomatoid mesothelioma
- Biphasic mesothelioma
- Desmoplastic mesothelioma (variant of sarcomatoid)

Cytology : Features were laid down as far back as 1962, by Klempman known as "Klempman's criteria"; when a high suspicion could be made based on this in the days when immunohistochemistry was no where on the scene. Some of the salient points of these criteria are : - Increased cellularity of the pleural fluid

- Obvious resemblance to mesothelial cells (differentiated malignant cells) with some cells atleast showing microvilli.
- Binucleat & multinucleate forms
- Cell in cell appearance (cell engulfment or cannibalism)
- Peripheral knobs on cell nest borders (not seen in adenocarcinoma).

This suspicious has to be confirmed on biopsy, the closest differential being a peripheral adecarcinoma. The chief differences are outlined below :

Adenocarcinoma vs Mesothelioma :

Adenocarcinoma	Mesothelioma
Clinical features- Mass, localized thickening, pleural effusion	**Clinical features –**Pleura studded with nodules on imaging , massive pleural effusion, reacumulates rapidly on tapping
Gross	**Gross**
Periphery of lung	Along pleura- visceral or parietal
Involvement of lung and pleura	Involvement of pleura-may infiltrate lung
More commonly localised	Diffuse
Nodules are large	Fine to large nodules 1mm to a few cms
Cut surface grey white	Cut surface grey white with well demonstrable slit like spaces
Microscopy Glands ++ Sheets of cells in poorly differentiated forms	**Microscopy** Glands ++ in epithelial variant. Poorly formed basement. Lining cells seemingly rest directly on stroma. Glands and stroma in mixed variant. Only stroma in sarcomatoid variant
Mucin Neutral mucin (PAS and Mucicarmine +ve)	**Mucin** Acid mucin- hyaluronic acid (Alcian blue +ve at pH 2.5 digested by hyaluronidase) Very Rare cases of glycogen rich mesothelioma may be PAS +ve

Immunohistochemistry	**Immunohistochemistry**
Calretinin –ve (sometimes +ve)	+ve
Cytokeratin 5/6 -ve	+ve*
WT 1 –ve	+ve
Thrombomodulin -ve	+ve
Vimentin -ve	+ve
CEA +ve	-ve
B72.3 +ve	-ve
Ber Ep4 +ve	-ve
MOC 31 +ve	-ve
TTF +ve	-ve
Pan keratin +ve	+ve
EMA +ve	+ve
Basement membrane component +ve	+ve
D2 40 –ve	+ve

* also +ve in SCC & Transitional CC

Calretinin, cytokeratin 5/6 and WT 1 at present are the best +ve markers for mesothelioma and CEA, B 72.3 and MOC 31 are the best –ve markers. A panel pf 4 markers – calretinin, cytokeratin 5/6(or WT1) , CEA and MOC 31(or B72.3) enables a correct diagnosis in most cases (Ordonez, NG : Immunohistochemical diagnosis of mesothelioma. Am J Surg Pathol 2003, 27 : 1031-1051).

Loss **of BAP1 expression** on immunohistochemistry and **homozygous deletion of CDKN2A (p16)** by FISH strongly support a diagnosis of malignant mesothelioma and are not seen in reactive mesothelial proliferations. **MTAP loss** may serve as a surrogate marker for CDKN2A deletion.

Solitary fibrous tumour : Was formerly called as solitary fibrous mesothelioma. **Now classified (WHO 2021/2023)** as a **mesenchymal tumour of fibroblastic origin**, unrelated to mesothelioma. **SFT is characterized by a NAB2–STAT6 gene fusion**, resulting in nuclear STAT6 expression.

- **Gross :** Size may reach 6cm, well circumscribed, firm lobulated. Cut surface is grey white to yellow white with whorling and fasiculation. It may also present as a mural nodule within a large pleural lined cyst. May be found attached to either the visceral or sometimes the parietal pleura.

Other sites of occurrence of a neoplasm with identical features are the peritoneum, retro- peritoneum, mediastinum, nasopharynx, orbit, breast etc .

- **Microscopy :** Tangled network of fibroblast-like cells with abundant collagen fibres many of which have a keloid like quality. Haemangiopericytoma like areas are observed. Others may have prominent myxoid features. Differential diagnosis for fibrous dominant lesions are fibromatosis and calcifying fibrous tumours. Differential diagnosis for myxoid dominant lesions are low grade myxofibrosarcoma and low grade fibromyxoid tumours.

- IHC shows - **STAT6 (nuclear): strongly positive (most sensitive and specific marker)** ; CD34: positive (often diffuse); BCL2: positive ;CD99: often positive; Keratin: negative; Desmin and vimentin : usually negative (occasional focal positivity may occur)

Though most of these are benign, malignant variants are also seen with increased cellularity, atypia, mitotic activity and necrosis. Features suggesting malignancy include increased cellularity, nuclear atypia, mitotic activity (>4/10 HPF), necrosis, infiltrative margins, and tumour size >10 cm.

It is negative for keratin and therefore not considered a true type of mesothelioma but a sub-mesothelioma arising from the sub mesothelial fibrous connective tissue. It is also CD34+ve and bcl 2 +ve. Most SFTs are indolent, but a subset shows aggressive behaviour with local recurrence or metastasis, sometimes after long intervals; about 12% cause death due to extensive intrathoracic growth.

Pulmonary epithelioid hemangioendothelioma: these are multiple slow growing lung tumours with occasional metastasis to lymph nodes & distant organs. They are considered endothelial in origin because of presence of immunoreactive factor VIII & Weibel-Palade bodies. It is also called malignant angioendothelioma or epitheliod haemangioendothelioma or sarcoma.

IHC shows Endothelial markers: **CD31, CD34, ERG;** Characteristic gene fusions:

- **WWTR1–CAMTA1** (most cases)
- **YAP1–TFE3 (**subset)

PEComatous tumours : (formerly called sugar tumour) **:** a benign peripheral located tumour with clear cells & fine vascular stroma. Cells have small uniform nuclei & abundant glycogen in the cytoplasm. Cells are considered to show melanocytic differentiation due to presence of pre-melanosomes in the cytoplasm & HMB +tivity. IHC shows : HMB-45 positive ;Melan-A positive; Keratin negative. It is generally benign, very rare malignant behavior

B V REVISED SYNOPTIC REPORTING TEMPLATE (WHO / IASLC – CURRENT)

Lung Biopsies and Resection Specimens

A. Specimen Details

- Type of specimen:
  - Transbronchial biopsy
  - Core needle (CT-guided) biopsy
  - EBUS-guided lymph node biopsy
  - Wedge resection
  - Segmentectomy
  - Lobectomy
  - Bilobectomy
  - Pneumonectomy
  - Pleural biopsy
- Laterality: Right / Left
- Lobe(s): RUL / RML / RLL / LUL / LLL
- Pleura included: Yes / No
- Lymph nodes submitted:
  - Hilar: Number examined ___
  - Mediastinal: Number examined ___

B. Non-Neoplastic Lung Findings (if present)

- Pneumonia: Lobar / Bronchopneumonia / Interstitial
- Granulomatous inflammation
  - Tuberculosis (AFB: Yes / No)
  - Sarcoidosis
  - Fungal (specify)
- Emphysema
- Bronchiectasis
- Interstitial lung disease (specify pattern)
- Pulmonary oedema / Congestion
- Vasculitis
- Other significant findings: _____

C. Tumour

- Tumour present: Yes / No
- Location: Central / Peripheral
- Bronchial involvement: Yes / No
- Tumour size (invasive component): ___ cm
- Focality: Single / Multiple

D. Histological Type (WHO classification)

- **Adenocarcinoma**
  - Adenocarcinoma in situ (AIS)
  - Minimally invasive adenocarcinoma (MIA)
  - Invasive adenocarcinoma – predominant pattern:
    - Lepidic ___ %
    - Acinar ___ %
    - Papillary ___ %
    - Micropapillary ___ %
    - Solid ___ %
- **Squamous cell carcinoma**
- **Neuroendocrine tumours**
  - Typical carcinoid
  - Atypical carcinoid
  - Large cell neuroendocrine carcinoma
  - Small cell carcinoma
- **Adenosquamous carcinoma**
- **Sarcomatoid carcinomas**
  - Pleomorphic carcinoma
  - Spindle cell carcinoma
  - Giant cell carcinoma
  - Carcinosarcoma
  - Pulmonary blastoma
- **Salivary gland–type carcinoma**
  - Mucoepidermoid carcinoma
  - Adenoid cystic carcinoma
- **Mesothelioma**
  - Epithelioid
  - Sarcomatoid

    - Biphasic
- **Metastatic tumour** (probable primary: _____)
- **Other / Unclassified** (specify)

E. Histological Grade

- Well differentiated
- Moderately differentiated
- Poorly differentiated
- Not applicable / Cannot be assessed

F. Invasion and Spread

- Lymphovascular invasion: Present / Absent
- Spread through air spaces (STAS): Present / Absent / Cannot be assessed
- Pleural invasion:
    - PL0 – No pleural invasion
    - PL1 – Beyond elastic layer
    - PL2 – To pleural surface
    - PL3 – Parietal pleura / chest wall

G. Margins

- Bronchial margin: Free / Involved
- Vascular margin: Free / Involved
- Parenchymal margin: Free / Involved

H. Lymph Nodes

- Number examined: ___
- Number involved: ___
- Nodal stations involved: _____

I. Biomarker / Molecular Studies (as applicable)

- EGFR: Mutated / Wild type / Pending
- ALK: Positive / Negative / Pending
- ROS1: Positive / Negative / Pending
- KRAS: Mutated / Wild type
- BRAF: Mutated / Wild type
- PD-L1 expression: ___ %
- Other relevant markers: _____

J. Pathologic Staging (IASLC 8th Edition)

- pT: ___
- pN: ___
- pM: ___
- Final Pathologic Stage: _____

K. Comment

(Include correlation with radiology, limitations of biopsy, need for molecular testing, or clinically relevant notes.)

Signature: _____

Date: _____

TNM Staging of Lung Cancer – IASLC 8th Edition (Summary Box)

T – Primary Tumour

TX
Primary tumour cannot be assessed, or tumour proven by malignant cells in sputum/bronchial washings but not visualized by imaging or bronchoscopy.

T0
No evidence of primary tumour.

Tis
Carcinoma in situ (includes adenocarcinoma in situ).

Tumour Size–Based Categories

T1 – Tumour ≤3 cm, surrounded by lung or visceral pleura, without invasion proximal to lobar bronchus

- **T1a**: ≤1 cm
- **T1b**: >1 cm to ≤2 cm
- **T1c**: >2 cm to ≤3 cm

T2 – Tumour >3 cm to ≤5 cm *or* tumour with any of the following features:

- Involves main bronchus (without carinal involvement)
- Invades visceral pleura
- Associated with atelectasis or obstructive pneumonitis extending to the hilar region
- **T2a**: >3 cm to ≤4 cm
- **T2b**: >4 cm to ≤5 cm

T3 – Tumour >5 cm to ≤7 cm *or* tumour that:

- Invades chest wall (including superior sulcus), phrenic nerve, parietal pericardium

- Has separate tumour nodule(s) in the same lobe
- Associated with atelectasis or obstructive pneumonitis of the entire lung

T4 – Tumour >7 cm *or* tumour that invades:

- Mediastinum, heart, great vessels
- Trachea, carina, oesophagus
- Vertebral body
- Has separate tumour nodule(s) in a different ipsilateral lobe

N – Regional Lymph Nodes

NX
Regional lymph nodes cannot be assessed.

N0
No regional lymph node metastasis.

N1
Metastasis in ipsilateral peribronchial, ipsilateral hilar, or intrapulmonary lymph nodes (including direct extension).

N2
Metastasis in ipsilateral mediastinal and/or subcarinal lymph nodes.

N3
Metastasis in contralateral mediastinal or hilar lymph nodes, or ipsilateral/contralateral scalene or supraclavicular lymph nodes.

M – Distant Metastasis

M0
No distant metastasis.

- **M1a**
- Separate tumour nodule(s) in contralateral lung
- Pleural or pericardial nodules
- Malignant pleural or pericardial effusion

M1b
Single extrathoracic metastasis in a single organ.

M1c
Multiple extrathoracic metastases in one or more organs.

Pathological Classification (pTNM)

- pT, pN, and pM correspond to T, N, and M categories.

- pN0 requires histological examination of adequately sampled hilar and mediastinal lymph nodes.

Key Pearls

Tumour size is based on **invasive component only** in adenocarcinoma.

- **Malignant pleural effusion** = M1a, *not T4*.
- Separate nodules:
  - Same lobe → **T3**
  - Different ipsilateral lobe → **T4**
  - Contralateral lung → **M1a**
- AIS and MIA are staged as **Tis** and **T1mi** respectively.

ASSESS YOURSELF RESPIRATORY SYSTEM

Essay Questions (10 marks each)

1. Describe the grossing technique of a total laryngectomy specimen.
   Discuss orientation, tumour assessment, margins, and sampling strategy.
2. Discuss the grossing and TNM-oriented sampling of a lung resection specimen for carcinoma.
   Include tumour assessment, margins, lymph nodes, and pleural involvement.
3. Describe the pathology of granulomatous diseases of the lung.
   Include tuberculosis, sarcoidosis, and fungal infections with key microscopic features.

Short Answer Questions (5 marks each)

4. Describe the anatomy of the larynx relevant to grossing.
5. Enumerate the bronchopulmonary segments and their surgical importance.
6. Describe the gross features and sampling of pleural decortication specimens.
7. Write a note on usual interstitial pneumonia (UIP) and its key microscopic features.
8. Discuss the gross and microscopic features of bronchiectasis.

Short Notes (3 marks each)

9. Pre-epiglottic space and its significance
10. Paraglottic space
11. Pancoast tumour
12. Hepatization in pneumonia
13. Pleural plaques in asbestos exposure

MCQs

1. **The most common site for aspiration pneumonia is:**

   A. Left upper lobe
   B. Right upper lobe
   C. Right lower lobe
   D. Left lower lobe

2. **Which of the following is a feature of usual interstitial pneumonia (UIP)?**

   A. Uniform fibrosis
   B. Temporal heterogeneity
   C. Absence of fibroblastic foci
   D. Prominent eosinophils

3. **The commonest peripheral lung tumour is:**

   A. Squamous cell carcinoma
   B. Small cell carcinoma
   C. Adenocarcinoma
   D. Large cell carcinoma

Answer Key for MCQs

1. C
2. B
3. C

Chapter 6
CARDIOVASCULAR SYSTEM (CVS)

Chapter outline – I Cardiovascular System Anatomy ; II Cardiovascular System Grossing Techniques; III Cardiovascular System Gross Pearls; IV Cardiovascular System Microscopy; V Cardiovascular System Synoptic Reporting.

I CARDIOVASCULAR SYSTEM ANATOMY

The cardiovascular system consists of the heart, which acts as the central pump, and a network of arteries, veins, and capillaries that traverse the entire body to transport blood. Blood delivers oxygen and nutrients, removes metabolic waste products, and carries immune and other functional cells essential for the maintenance of normal cellular and organ function.

The heart is a muscular organ weighing approximately 250–350 g in adults. It lies posterior to the sternum within the mediastinum, with about two-thirds of its mass to the left of the midline. The apex of the heart rests on the superior surface of the diaphragm.

From outside to inside, the heart wall is composed of three layers:

- Epicardium (outer layer),
- Myocardium (middle muscular layer), and
- Endocardium (inner endothelial-lined layer).

The myocardium forms the bulk of the cardiac tissue and is responsible for contractile function. The heart is enclosed by the pericardium, a fibroserous sac consisting of an outer parietal layer and an inner visceral layer, with a small amount of lubricating fluid between them that permits smooth cardiac movement.

The heart contains four chambers. The right-sided chambers receive deoxygenated blood and pump it into the pulmonary circulation, while the left-sided chambers receive oxygenated blood from the lungs and supply the systemic circulation. The right-sided chambers form the major portion of the anterior surface of the heart.

Each atrium communicates with its corresponding ventricle through an atrioventricular (AV) valve. The mitral (bicuspid) valve separates the left atrium and left ventricle, while the tricuspid valve separates the right atrium and right ventricle. Blood flow through the heart is unidirectional and regulated by the opening and closure of valves in response to pressure gradients.(Fig 6.1)

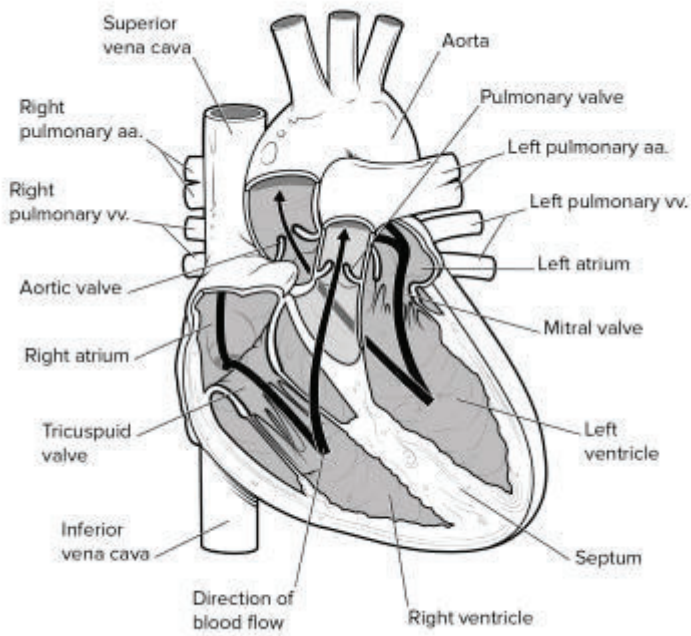


Fig 6.1 Cross section of heart showing the chambers, and blood flow through then.

The heart's conduction system consists of specialized cardiac muscle fibers that coordinate rhythmic contractions. These include:

- the sinoatrial (SA) node, located in the right atrium and acting as the natural pacemaker;
- the atrioventricular (AV) node, which delays impulse conduction between the atria and ventricles; and
- the Purkinje fiber network, which distributes electrical impulses throughout the ventricular myocardium.

The myocardium receives its blood supply through the **right and left coronary arteries**, which arise from the base of the ascending aorta. Deoxygenated blood from the myocardium is drained by the cardiac veins into the **coronary sinus**, which opens into the right atrium.

Systemic Circulation

The systemic circulation begins in the left side of the heart. Oxygenated blood enters the left atrium from the lungs and passes into the left ventricle through the mitral valve. During ventricular contraction, blood is ejected through the **aortic semilunar valve** into the aorta. The aorta branches into arteries, arterioles, and capillaries, allowing exchange of gases and nutrients at the tissue level. Deoxygenated blood returns to the heart through veins and ultimately drains into the right atrium, completing the systemic circuit.

Pulmonary Circulation

The pulmonary circulation serves the function of gas exchange. Deoxygenated blood returns to the right atrium via the superior vena cava, inferior vena cava, and coronary sinus. Blood then

passes into the right ventricle through the tricuspid valve and is pumped through the pulmonary valve into the pulmonary trunk and pulmonary arteries. Gas exchange occurs in the lungs, and oxygenated blood is returned to the left atrium through the pulmonary veins, completing the pulmonary circuit.

Coronary Circulation

The coronary circulation supplies the heart itself (Fig 6.2) . The right and left coronary arteries arise from the ascending aorta and, through their branches—including the anterior and posterior interventricular, marginal, and circumflex arteries—supply the myocardium. Venous blood is collected by the cardiac veins (great, middle, small, and anterior) and drains into the coronary sinus. Myocardial perfusion occurs predominantly during diastole, when the heart muscle is relaxed.

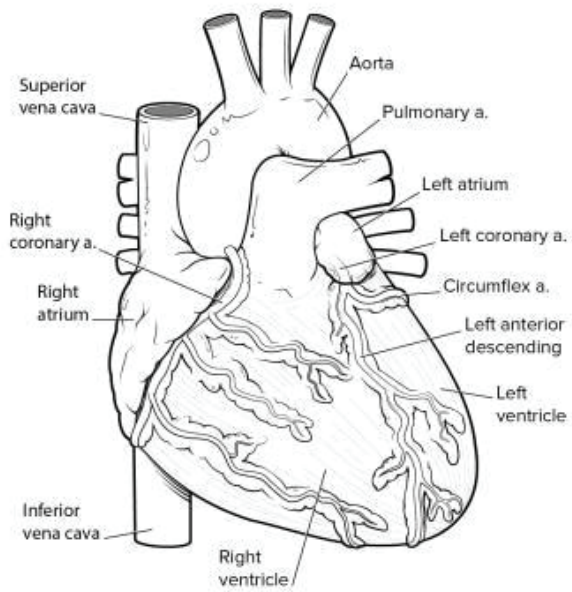


Fig 6.2 shows the coronary system

II. GROSSING TECHNIQUES CARDIOVASCULAR SYSTEM

A. Heart Biopsies

Biopsies of the heart are collectively referred to as endomyocardial biopsies (also termed cardiac or endocardial biopsies, depending on the sampled layer). These are usually obtained via cardiac catheterization, most commonly from the right ventricular septum. The specimen typically consists of multiple small tissue fragments.

Indications for endomyocardial biopsy include:

- Cardiomyopathies
- Myocarditis

- Infiltrative disorders, especially amyloidosis (e.g., transthyretin [ATTR] amyloidosis)
- Drug- or chemotherapy-induced cardiotoxicity
- Assessment of graft rejection following cardiac transplantation- done sequentially as per protocol.

Grossing Guidelines

- Identify the specimen as 'endomyocardial biopsy' and note the number of tissue fragments received.
- Measure each fragment (or the aggregate) in millimeters.
- Submit all tissue for processing, as the specimens are usually scant.
- Avoid over-handling to prevent tissue loss.

B. Valvular Specimens

Valvular heart disease (VHD) results from structural or functional abnormalities of one or more cardiac valves—aortic, mitral, tricuspid, or pulmonary. Valvular lesions may be congenital or acquired.

- Common risk factors for acquired valvular disease include:
- Increasing age
- Male gender
- Tobacco use
- Hypercholesterolemia
- Hypertension
- Type 2 diabetes mellitus

Degenerative calcific aortic valve disease (CAVD) is the most frequent indication for valve replacement. Nearly one-third of elderly individuals show echocardiographic or radiological evidence of aortic valve sclerosis, a subclinical precursor of calcific aortic stenosis.

Grossing of Valvular Specimens

- Identify the valve received (aortic, mitral, tricuspid, or pulmonary).
- Record measurements in three dimensions, including cusp or leaflet thickness.
- Describe gross features such as:
  - Calcification
  - Fibrosis
  - Commissural fusion
  - Vegetations or perforations
- Take representative sections from abnormal areas.
- Calcified valves should be submitted for 'decalcification' prior to processing.

C. Blood Vessels

Specimens from blood vessels may include segments of arteries or veins removed for aneurysms, atherosclerotic plaques, occlusive disease, or inflammatory conditions.

- Identify the vessel and anatomical site.
- Measure length, circumference, and wall thickness.
- Note luminal narrowing, atheroma, thrombosis, calcification, or aneurysmal dilatation.
- Take representative sections, including areas of maximum pathology.

III CARDIOVASCULAR SYSTEM GROSS PEARLS

The surgical pathologist is only occasionally involved in gross examination of cardiovascular specimens, as most cardiac pathology is encountered at autopsy. However, small specimens—such as endomyocardial biopsies, excised valves, and valvular vegetations—may be received, particularly in centers with active cardiothoracic services.

Recognition of the gross morphology of valvular lesions, especially vegetations, is critical, as it often provides immediate diagnostic clues.

Rheumatic Endocarditis (Rheumatic Vegetations)

- Occurs on previously non-diseased valves
- Represents an autoimmune reaction following Group A β-hemolytic streptococcal infection, due to molecular mimicry
- A post-streptococcal phenomenon

Gross features

- Small, warty vegetations
- Firm and non-friable
- Located along the line of closure of the valve cusps
- Most commonly involve:
  - Mitral valve
  - Aortic valve
  - Less commonly, the tricuspid valve

(Classical gross images show bead-like vegetations along the line of valve closure(Fig 6.3.))

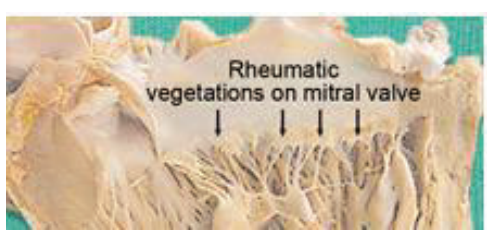


Fig 6.3 – shows rheumatic vegetations

Infective Endocarditis

May occur on **normal or abnormal valves**.

Common organisms

- Staphylococcus aureus – often on normal valves (acute IE)
- Streptococcus viridans – typically on diseased valves (subacute IE)
- Enterococci
- HACEK group organisms

Valves involved

- Aortic and mitral valves most commonly
- Tricuspid valve—especially in intravenous drug users
- Multiple valves may be affected

Gross features

- Large, bulky, friable vegetations (Fig 6.4)
- Often destructive, with ulceration of the valve leaflets
- Ring abscesses may be present, involving adjacent valvular or myocardial tissue
- Vegetations contain abundant microorganisms
- High risk of embolization due to friability

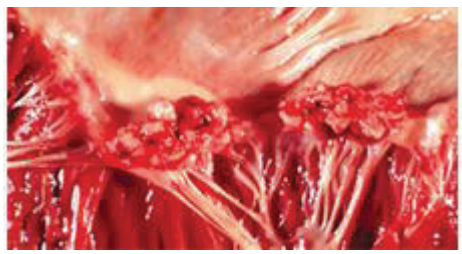


Fig 6.4 : Mitral valve with large bulky vegetations of infective endocarditis

Subacute infective endocarditis tends to show less aggressive destruction, and host factors such as underlying heart disease, immunosuppression, and drug abuse influence disease severity.

Libman–Sacks Endocarditis

- A form of non-infective endocarditis
- Seen in systemic lupus erythematosus (SLE)
- Valves are usually structurally normal

Gross features

- Sterile vegetations

- Small to medium sized
- Occur on either surface of the valve:
  - Ventricular surface of the mitral valve
  - Atrial surface of the mitral valve (Fig 6.5)
  - Ventricular surface of the aortic valve (Fig 6.5)
- Lesions may occur anywhere on the valve, not restricted to the line of closure

(Characteristic finding: vegetations on both sides of the valve leaflet.)

Fig 6.5 : Libman's Endocarditis- numerous small vegetations on the ventricular surface of the aortic valve and on the atrial surface of the mitral valve.

Non-Bacterial Thrombotic Endocarditis (NBTE)

Previously termed 'marantic endocarditis'.

Associations

- Disseminated intravascular coagulation (DIC)
- Hypercoagulable states
- Malignancies, especially mucin-secreting adenocarcinomas
- Severe chronic illness

Gross features

- Occurs on normal valves
- Small, bland, sterile thrombotic vegetations (Fig 6.6)
- Composed of fibrin and platelets
- Any valve may be involved
- Endocardial trauma predisposes

Fig 6.6 : NBTE – Consists of formation of sterile vegetations on cardiac valves and adjacent endocardium.

Although sterile, these vegetations may serve as a nidus for secondary bacterial colonization and are a frequent source of systemic emboli.

Fig 6.7 shows the location of the lesions in various conditions for comparison..

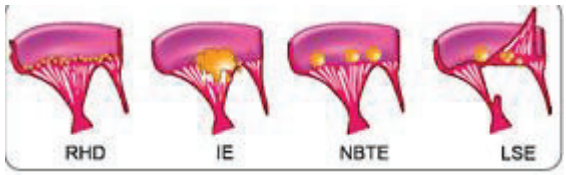


Fig 6.7 : Diagrammatic representation of various types of vegetations.

Vegetations of Cardiac Valves

Feature	Rheumatic (Acute RF)	Infective Endocarditis (Subacute)	Marantic (NBTE)	Libman–Sacks (SLE)
Etiology	Post-streptococcal immune reaction	Low virulence organisms (e.g., viridans streptococci)	Hypercoagulable states (malignancy, cachexia)	Autoimmune (SLE)
Valve involved	Mitral > Aortic	Mitral, Aortic	Mitral, Aortic	Mitral, Tricuspid
Location on valve	Along line of closure	Along line of closure but may extend	Along line of closure	Both sides of leaflet + chordae
Size	Small (1–2 mm)	Moderate, variable	Small	Small–medium
Appearance	Multiple, warty (verrucae)	Friable, irregular	Small, bland	Verrucous, irregular
Attachment	Firmly attached	Loosely attached	Loosely attached	Moderately adherent

Feature	Rheumatic (Acute RF)	Infective Endocarditis (Subacute)	Marantic (NBTE)	Libman–Sacks (SLE)
Composition	Fibrin + platelets	Fibrin + platelets + organisms	Fibrin + platelets (sterile)	Fibrin + immune complexes
Valve damage	Minimal (acute phase)	Destructive (ulceration, perforation)	None	Mild–moderate
Embolization	Rare	Common (septic emboli)	Common (bland emboli)	Possible
Healing outcome	Chronic RHD (fibrosis, calcification)	Scarring or persistent infection	No significant sequelae	Fibrosis, deformity
Key diagnostic clue	Associated with Aschoff bodies	Organisms on stain/culture	Seen in malignancy	Both surfaces involved

Mnemonic for vegetations:

- R → Rheumatic → "Rim" (line of closure)
- I → Infective → "Irregular + Infective organisms"
- M → Marantic → "Malignancy + Minimal reaction"
- L → Libman-Sacks → "Leaflet BOTH sides"

MacCallum's Patch (MacCallum's Triangle)

Seen in chronic rheumatic heart disease.

- Results from regurgitant jets (usually mitral regurgitation) striking the left atrial endocardium
- Leads to subendocardial fibrosis and thickening

Location

- Posterior wall of the left atrium

Significance

- Indicates long-standing rheumatic disease
- There is always a history of, or evidence of, rheumatic fever

Gross Diagnostic Clue

Location, size, friability, and surface involvement of vegetations are often sufficient to suggest the underlying etiology even before microscopy.

Myocardial Infarction (The "Macroscopic" Clues)

1. The 24-Hour Rule (TTC Staining): In the first 12–24 hours, an MI is often invisible to the naked eye. The most vital pearl is the use of *Triphenyl tetrazolium chloride* (TTC); viable myocardium turns deep red (due to lactate dehydrogenase activity), while the infarcted area remains pale/tan, allowing for early identification before permanent structural changes occur.
2. The "Hyperemic Rim" (Days 3–7): By the end of the first week, the gross appearance shows a central yellow-tan softening (myomalacia) surrounded by a distinctive red-purple hyperemic border. This border represents the "battlefront" where granulation tissue and new blood vessels are starting to invade the dead muscle.
3. Thinning & "Puckering" (Chronic): Healed infarcts appear as firm, white-grey fibrous scars. A key gross pearl is that the ventricular wall will be significantly thinner in this area compared to the surrounding muscle, often leading to a "puckered" or concave appearance of the endocardial surface.

IV CARDIOVASCULAR SYSTEM MICROSCOPY

1. Atherosclerosis

Atherosclerosis is the most common and clinically significant form of arteriosclerosis, involving large and medium-sized arteries.

Microscopy

Early lesion (Fatty streak)

- Focal accumulation of lipid-laden foam cells within the intima
- Foam cells are derived from macrophages and smooth muscle cells
- No significant luminal narrowing

Atheromatous (fibrofatty) plaque

- Marked intimal thickening
- Necrotic lipid core containing cholesterol clefts, cellular debris, and foam cells
- Overlying fibrous cap composed of smooth muscle cells and collagen
- Media shows thinning and loss of elastic tissue

Complicated plaque

- Surface ulceration or rupture
- Superimposed thrombosis
- Dystrophic calcification
- May show embolization

Common Sites

- Abdominal aorta (most frequent)
- Coronary arteries
- Popliteal arteries
- Internal carotid arteries (near bifurcation)

Complications

- Progressive luminal narrowing and ischemia
- Plaque rupture with thrombus formation
- Myocardial infarction, stroke, peripheral arterial disease
- Formation of aneurysm

2. Arteriolosclerosis

Involves small arteries and arterioles, commonly associated with hypertension and diabetes mellitus.

Microscopy

- Hyaline arteriolosclerosis:
  - Homogeneous eosinophilic hyaline thickening of vessel wall
  - Luminal narrowing
- Hyperplastic arteriolosclerosis:
  - Concentric, laminated **"**onion-skin**"** thickening
  - Seen in severe or malignant hypertension

3. Monckeberg Medial Calcific Sclerosis

- Characterized by dystrophic calcification of the tunica media (see fig
- Involves medium-sized muscular arteries
- Lumen is usually preserved
- Commonly seen in elderly individuals
- Usually of no clinical significance

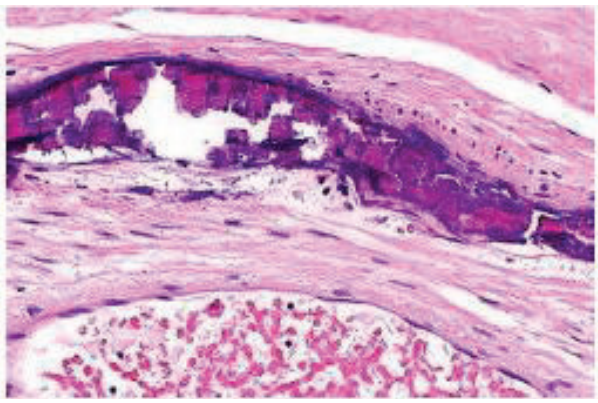


Fig 6.8: shows tunica media of a muscular artery with calcification. H&E x 100

Aneurysms

An aneurysm is a localized abnormal dilatation of a blood vessel or cardiac chamber, resulting from weakening of the vessel wall.

A. Atherosclerotic (True) Aneurysm

- Most commonly involves the abdominal aorta, below the renal arteries
- Caused by atherosclerotic destruction of the media

Microscopy

- Marked thinning of the tunica media
- Loss and fragmentation of elastic fibers
- Prominent intimal atherosclerotic plaques
- Mural thrombus may be present

B. Syphilitic Aneurysm

- Seen in tertiary syphilis
- Predominantly affects the ascending thoracic aorta

Microscopy

- Obliterative endarteritis of the vasa vasorum
- Ischemic injury to the media
- Loss of elastic tissue with replacement fibrosis

C. Aortic Dissection

Aortic dissection occurs when blood enters the aortic wall through an intimal tear and dissects along the media.

Microscopy

- Cystic medial degeneration:
  - Loss of smooth muscle cells
  - Fragmentation of elastic fibers
  - Accumulation of basophilic ground substance
- May occur with or without pre-existing atherosclerosis

Microscopic Pearl

Aneurysm formation reflects medial weakening, most commonly due to atherosclerosis in the abdominal aorta and cystic medial degeneration in the thoracic aorta.

Vasculitis

Vasculitis refers to a heterogeneous group of disorders characterized by inflammation and destruction of blood vessel walls, leading to vascular compromise, tissue ischemia, and organ damage. From a surgical pathology perspective, vasculitis is most often encountered in biopsy specimens, and recognition is largely pattern-based.

1. Giant Cell Arteritis (Temporal Arteritis)

A large-vessel granulomatous vasculitis, predominantly affecting elderly individuals.

Vessels involved

- Temporal artery (most commonly biopsied)
- Other branches of the carotid artery

Microscopy

- Segmental (skip) lesions
- Granulomatous inflammation of the vessel wall
- Multinucleated giant cells adjacent to fragmented internal elastic lamina
- Marked intimal thickening with luminal narrowing
- Chronic inflammatory infiltrate composed of lymphocytes and macrophages

Microscopic pearl

A negative temporal artery biopsy does **not** exclude the diagnosis due to skip lesions.

2. Takayasu Arteritis

A chronic granulomatous vasculitis of large vessels, seen in younger individuals.

Vessels involved

1. Aorta and its major branches

Microscopy

- Granulomatous inflammation involving all layers of the vessel wall
- Destruction and fragmentation of elastic lamina
- Fibrosis of media and adventitia in healed lesions
- Marked luminal narrowing in chronic stages

Microscopic pearl

Healed Takayasu arteritis shows dense fibrosis with loss of elastic tissue, often without active inflammation.

3. Polyarteritis Nodosa (PAN)

A medium-sized muscular artery vasculitis, sparing the pulmonary circulation.

Vessels involved

- Renal, gastrointestinal, hepatic, and coronary arteries

Microscopy

- Segmental transmural necrotizing inflammation
- Fibrinoid necrosis of vessel wall
- Mixed inflammatory infiltrate (neutrophils, lymphocytes, macrophages)
- Lesions at different stages of healing within the same specimen

Microscopic pearl

The coexistence of acute necrosis and healed fibrosis in different vessels is characteristic of PAN.

4. ANCA-Associated Small Vessel Vasculitis

Includes microscopic polyangiitis, granulomatosis with polyangiitis, and related entities.

Vessels involved

- Small arteries, arterioles, capillaries, and venules

Microscopy

- Necrotizing vasculitis with minimal or absent immune complex deposition
- Fibrinoid necrosis of vessel walls
- Prominent neutrophilic infiltrate with nuclear debris (leukocytoclasia)
- Adjacent tissue necrosis may be present

Microscopic pearl

These are pauci-immune vasculitides, with little or no immunoglobulin deposition on immunofluorescence.

Summary Table: Microscopic Patterns in Vasculitis

Type	Vessel Size	Key Microscopic Feature
Giant cell arteritis	Large	Granulomas with giant cells, elastic lamina fragmentation
Takayasu arteritis	Large	Panarteritis with fibrosis
Polyarteritis nodosa	Medium	Transmural necrotizing vasculitis
ANCA-associated	Small	Necrotizing vasculitis with leukocytoclasia

Practical Pearl

Vessel size, depth of wall involvement, and type of inflammation are more diagnostically useful than etiologic classification in biopsy specimens.

Rheumatic Fever – Microscopic Morphology

Rheumatic fever is a post-streptococcal immune-mediated disease that primarily affects the heart. The characteristic cardiac lesion is pancarditis, involving the endocardium, myocardium, and pericardium. From a microscopic standpoint, the most diagnostic feature is the Aschoff body.

Myocardium

Aschoff Bodies

- Pathognomonic lesion of rheumatic myocarditis is
- Seen in the interstitium of the myocardium

Microscopy

- Central area of fibrinoid necrosis
- Surrounding inflammatory infiltrate composed of:
  - Lymphocytes
  - Plasma cells
  - Activated macrophages (Anitschkow cells)

Anitschkow cells

- Enlarged macrophages with abundant cytoplasm
- Characteristic elongated nucleus with central, wavy chromatin
- Referred to as “caterpillar cells”

With healing, Aschoff bodies are replaced by fibrous scars, indicating prior rheumatic activity.

Endocardium and Valves- Acute Rheumatic Valvulitis

- Characterized by verrucae (small sterile vegetations)

Microscopy

- Verrucae composed of:
  - Fibrin
  - Platelets
- Located along the line of valve closure
- Underlying valve shows:
  - Edema
  - Inflammatory cell infiltration
  - Fibrinoid change

Chronic Rheumatic Valvular Disease

Microscopy

- Dense fibrous thickening of valve leaflets
- Commissural fusion
- Shortening and thickening of chordae tendineae
- Dystrophic calcification in long-standing disease

The mitral valve is most commonly affected, followed by the aortic valve.

Pericardium

- Acute fibrinous pericarditis may be seen
- Microscopically shows fibrin deposition with mild inflammatory infiltrate
- Healed lesions may show fibrous thickening.

Myocardial infarction

Microscopic Features (The "Diagnostic" Trio)

- Coagulative Necrosis & "Ghost" Cells: The hallmark of acute MI. Look for myocytes that have lost their nuclei and striations (Fig S but retain their basic cell shape (like an empty house). This is typically accompanied by "contraction band necrosis"—thick, dark eosinophilic transverse bands caused by hypercontraction of the sarcomeres.(Fig 6.9A)
- The Neutrophilic Wave (24–72 Hours): (Fig 6.9 B) In the acute phase, there is a massive influx of polymorphonuclear leukocytes (neutrophils) into the interstitium. This is the

body's primary inflammatory response to tissue death and is the most reliable microscopic marker that the event is between 1 and 3 days old.

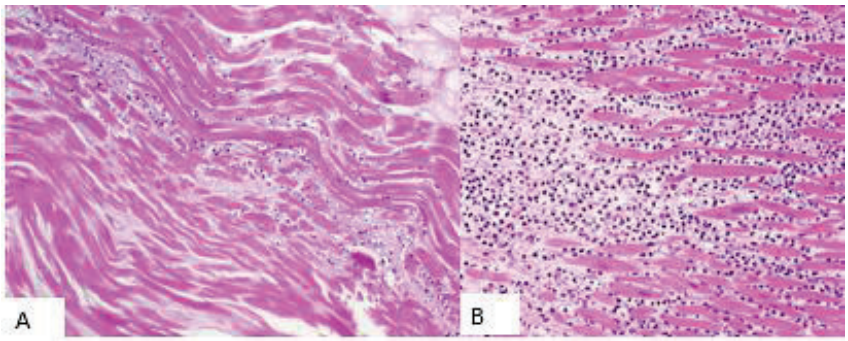


Fig 6.9 A : shows early infarction with wavy cardiac fibre bundles and loss of striations . H&E x 100. B : shows end of 24 hours showing infiltration by numerous neutrophils. : H&E x 400

Granulation Tissue & Hemosiderin (1–3 Weeks): As the infarct heals, we see a "proliferative" look: a rich network of newly formed capillaries (neovascularization), proliferating fibroblasts, and hemosiderin-laden macrophages clearing away the debris. This transition from "dead muscle" to "active repair" is the classic microscopic sign of a subacute MI.

Pro-Tip for the Microscopy Section: Always mention **"Wavy Fibers"** as the earliest microscopic sign (visible within 1–3 hours). These are thin, elongated myocytes stretched by the forceful contraction of adjacent viable muscle—a great "early catch" pearl for the reader.

V CARDIOVASCULAR SYSTEM SYNOPTIC REPORTING

A. Endomyocardial Biopsy

	Data/Options
Specimen type	Endomyocardial biopsy (Right ventricle, septal) / Other
Indication	Cardiomyopathy / Myocarditis / Amyloidosis / Cardiotoxicity / Post-transplant rejection / Other
Number of tissue fragments	___
Size	___ mm (aggregate)
Adequacy	Sufficient / Insufficient
Microscopic findings	Myocyte injury / Fibrosis / Inflammation / Amyloid deposition / Other
Special stains	Congo red, Trichrome, Immunohistochemistry (if performed)
Diagnosis	___
Comments	___

B. Cardiac Valves :

1. Specimen Details

- Type of specimen: Valve replacement / valvectomy
- Valve received: Mitral / Aortic / Tricuspid / Pulmonary
- Number of cusps/leaflets: _____

2. Measurements

- Valve circumference (if intact): _____ cm
- Leaflet thickness: Thin / Thickened
- Chordae tendineae: Normal / Thickened / Fused / Shortened

3. Gross Description

Valve Leaflets

- Surface: Smooth / Rough / Nodular / Ulcerated
- Thickening: Diffuse / Focal
- Calcification: Present / Absent (extent)
- Commissures: Normal / Fused

Vegetations (if present)

- Present / Absent
- Location: Line of closure / Both surfaces / Other
- Size: _____ mm
- Number: Single / Multiple
- Appearance:
  - Warty
  - Friable
  - Nodular
- Attachment: Firm / Loose

4. Associated Findings

- Ulceration: Present / Absent
- Perforation: Present / Absent
- Chordal rupture: Present / Absent
- Thrombus: Present / Absent

5. Microscopy

Valve Tissue

- Fibrosis: Mild / Moderate / Severe
- Hyalinization: Present / Absent
- Calcification: Present / Absent
- Neovascularization: Present / Absent

Inflammation

- Acute / Chronic / Mixed
- Granulomatous (Aschoff bodies): Present / Absent

Vegetation

- Composition:
  - Fibrin + platelets
  - Inflammatory cells
  - Organisms (if present)

Special stains

- Gram stain: Positive / Negative
- PAS / GMS: _____
- AFB: _____

6. Final Diagnosis

- Rheumatic valvular disease
- Infective endocarditis (acute/subacute)
- Non-bacterial thrombotic endocarditis (marantic)
- Libman–Sacks endocarditis
- Degenerative (calcific) valvular disease
- Myocardial infarction : Present / Absent / Site
- Other: _____

7. Comment (if needed)

- Correlate with blood cultures / serology
- Suggest infective etiology if organisms seen
- Mention risk of embolization (if friable vegetations)

8. Cardiac Transplant Rejection

Graded based on inflammatory infiltrate and myocyte injury.

No rejection / Acute cellular rejection (grade ___)

Myocarditis

Lymphocytic infiltrate + myocyte necrosis.

Infiltrative Disease

- Congo red → amyloid
- Granulomas → sarcoidosis

C. Vascular Specimens

	Data/Options
Vessel type	Artery / Vein / Aorta / Other
Site	___
Specimen type	Resection / Aneurysm / Biopsy / Dissection
Measurements	Length / Diameter / Wall thickness
Gross description	Atherosclerosis / Thrombosis / Ulceration / Aneurysmal dilatation / Calcification / Other
Microscopic findings	Arteriosclerosis type / Necrotizing vasculitis / Fibrosis / Medial degeneration / Other
Special stains	Elastic Van Gieson (EVG) / Trichrome / Immunohistochemistry / Others
Diagnosis	___
Comments	___

Note: Include specimen adequacy for small biopsies (endomyocardial, temporal artery).

- Highlight key diagnostic features on microscopy.
- Always comment on presence or absence of active inflammation, fibrosis, or calcification.
- For vascular specimens, mention aneurysm type, vessel size, and presence of thrombus.
- Optional fields allow for special stains or immunohistochemistry as needed.

ASSESS YOURSELF CVS

Essay Questions (10 marks each)

1. Classify and describe vegetations of the heart valves.
   Discuss the gross and microscopic features of rheumatic, infective (subacute), and marantic vegetations, with clinicopathological correlation.
2. Describe the grossing technique of cardiac valves in surgical pathology.
   Include orientation, measurements, identification of lesions, and sampling.
3. Discuss the pathology of rheumatic fever with emphasis on cardiac involvement.
   Describe the microscopic features of rheumatic carditis.
4. Discuss the types of valvular vegetations and their diagnostic significance in surgical pathology."

Short Answer Questions (5 marks each)

- Differentiate between rheumatic, infective, and non-bacterial thrombotic (marantic) endocarditis.
- Describe the microscopic features of rheumatic myocarditis (Aschoff body).
- Write a note on vasculitis involving the cardiovascular system.
- Enumerate the gross features of chronic rheumatic valvular disease.
- Describe the sampling of vegetations in suspected infective endocarditis.

Short Notes (3 marks each)

- Aschoff body
- Verrucae of rheumatic fever
- Libman–Sacks endocarditis
- Marantic (NBTE) vegetations
- Fibrinoid necrosis in vasculitis

MCQs

1. **The characteristic microscopic lesion of rheumatic carditis is:**
   A. Caseating granuloma
   B. Aschoff body
   C. Foreign body giant cell
   D. Langhans giant cell
2. **Marantic endocarditis is most commonly associated with:**
   A. Rheumatic fever
   B. Septicaemia
   C. Malignancy
   D. Tuberculosis

3. **Vegetations in subacute infective endocarditis are typically:**
   A. Small, sterile, along lines of closure
   B. Large, friable, destructive
   C. Nodular, fibrotic, calcified
   D. Verrucous, sterile, on both sides of valve

Answer Key for MCQs

1. 1.B
2. 2.C
3. 3.B

Chapter 7
Breast

Chapter out line – I Breast Anatomy ; II Breast Grossing Techniques ; III Breast Gross Pearls ; IV Breast Microscopy ; V Breast Synoptic Reporting

I BREAST ANATOMY

Each breast is made up of-

Skin & Nipple–Areola Complex

- The nipple contains the lactiferous duct (major) openings.
- These major ducts converge toward the nipple and are important to identify in retroareolar specimens.
- The areola has sebaceous glands and smooth muscle in addition.

Parenchyma (Glandular Tissue)

- Is organized into 15–20 lobes, each drained by a lactiferous duct.
- Lobes subdivide into lobules, made of terminal duct–lobular units (TDLUs). (see also fig 7.)
- TDLUs are the most common site for benign and malignant lesions.

Stroma

- Fibrous stroma supports ducts and lobules. This is divided into inter and intralobular connective tissue.
- Adipose tissue fills the remainder, especially in older women.

Grossing-Relevant points -

- **Quadrants**: Breast is divided into upper outer, upper inner, lower outer, lower inner, plus central (retro-areolar) regions. The Upper outer quadrant is most frequent site of carcinoma.
- **Margins**: Pay attention to superficial (skin), deep (pectoral fascia), and peripheral parenchymal margins.
- **Lymphatics**: Drain mainly to axillary nodes (levels I–III), also to internal mammary nodes.
- **Consistency**: Fatty vs fibrous tissue affects specimen handling.
- **Lesion Location**: Always note quadrant, distance from nipple, and relation to skin or chest wall.

- **Special Areas**: Axillary tail of Spence (part of breast glandular tissue) extends into the axilla .

II BREAST GROSSING TECHNIQUES

Specimens received

Tru cut biopsy

Incisional biopsy

Excision biopsy (lumpectomy) removal of lump with clear margins

Wide local excision

Mastectomy specimens –

- Simple mastectomy: Breast with Skin, Nipple, Areola (NO axillary tail)
- Extended simple mastectomy (modified radical mastectomy) : Simple PLUS axillary tail and pectoralis fascia
- Radical mastectomy: Simple PLUS axillary tail and pectoralis major muscle removed
- Skin-sparing mastectomy**.** The surgeon removes the whole breast, as in a total mastectomy, but leaves the skin. A skin-sparing mastectomy leaves less scarring and makes for a more natural-looking reconstructed breast. But if the tumor is large or near the skin, this procedure may not be an option. Skin-sparing mastectomies require breast reconstruction right after surgery.
- Single (unilateral) mastectomy: only the breast with cancer is removed. A double (bilateral) mastectomy involves the removal of both breasts . A double mastectomy is done if cancer is in both breasts, unilateral cancer but a high risk of developing cancer in the other breast because of a genetic mutation or strong family history of breast cancer

Radical mastectomy is no longer performed and most surgeries done these days are those of extended simple mastectomy ie modified radical mastectomy. This includes simple mastectomy & axillary lymph node clearance.

Tru cut biopsy & incisional biopsy :

1. Measure specimen in 3 dimensions
2. Note colour & texture
3. All for section if less than 4mm
4. If more than 4mm then section the specimen at 3-4 mm thickness and block all in one or more blocks
5. Tissue may be taken for hormone receptor studies from tumour proper if available.

Excision biopsy & wide local excision : (see fig 7.1)

1. Measure in 3 dimensions
2. Section along the greatest dimension
3. Describe the cut surface – size of lesion, colour, consistency; cysts – number, size; distance from the closest resected surgical margin in a suspected neoplasm, borders, presence of haemorrhage, necrosis, fibrosis etc.
4. Paint entire outer surface with India ink in all cases of malignancy/suspected malignancy as shown in the figure.
5. Small specimens – submit in their entirety
6. Larger specimens > 3cm, section thoroughly at 3-4mm thickness & block all grossly visible lesions & suspicious areas (5-6 casettes may be used). Tissue should be submitted for receptor studies in the fresh state.
7. Conventionally all excision biopsies are marked by the surgeon as shown in the figure to have a short suture in the superior margin and a long suture in the lateral margin [superior short (ss) ; lateral long(LL)] in case of tumours.

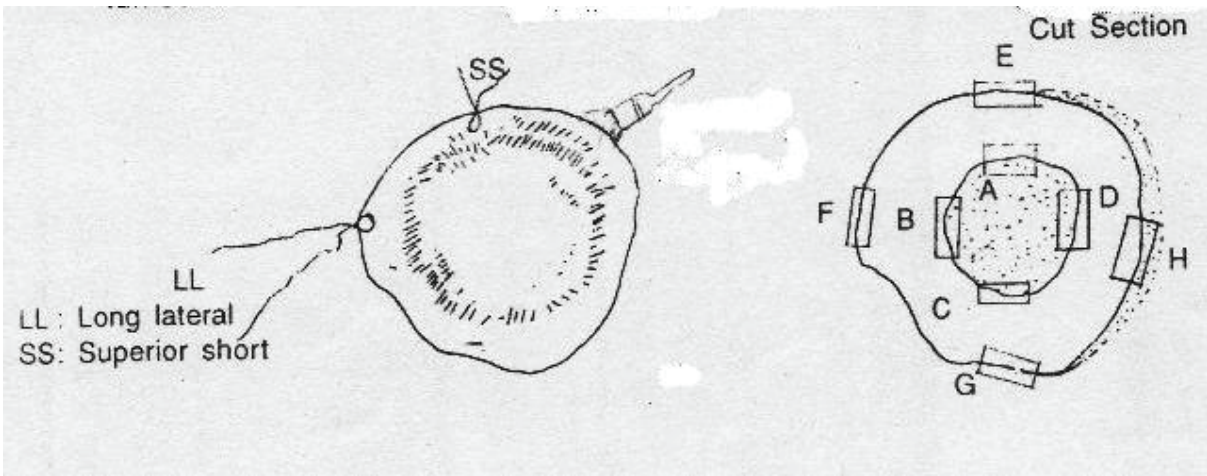


Fig 7. 1: Fig shows grossing of excision biopsy with wide margins

Bits

A to D – four bits may be taken from a central thin slice

E to H – four bits surgical margins in case of suspected carcinoma (each marked separately)

Mastectomy

1. One of the most important aspects of grossing breast specimens is to correlate what you see to what was seen radiographically. Prior to grossing, always check radiology reports
2. Verify that the orientation and 'side of breast' matches the container label. If there are discrepancies with the orientation or laterality, speak to the concerned surgeon before proceeding.

3. Orient the specimen (Fig 7.2) by identifying the upper outer quadrant using the axillary fat as the marker. For this place the specimen with the elliptical portion of skin bearing nipple & areola on the upper aspect facing the dissector in such a way that the axillary tail with fat should point upwards & laterally.

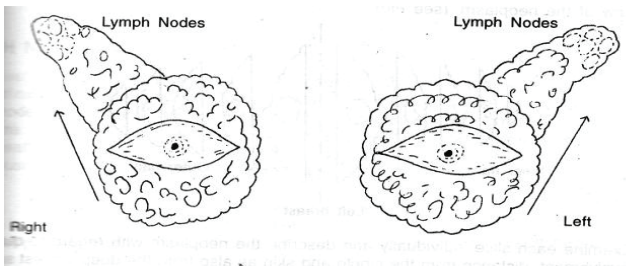


Fig 7.2: Orientation of simple mastectomy specimen.

4. Palpate the specimen & identify in which quadrant the neoplasm is located.
5. Quadrant identification : draw an imaginary vertical line through the nipple to divide the specimen into an outer and inner half. The outer most limit of the outer half may be marked with eosin to indicate at any future date that this half is the outer half. Similarly the skin margin of the upper half may also be marked with eosin . See figure 7.3 below (xxxx : indicates eosinated margins).

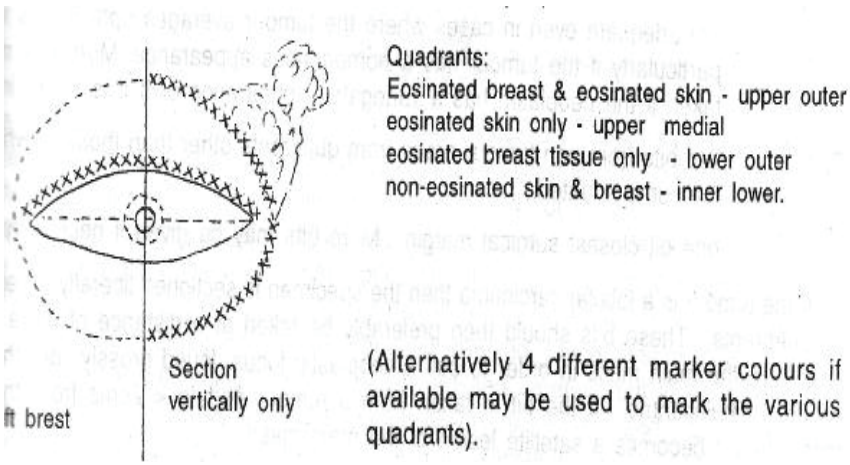


Fig 7. 3: Quadrant identification in mastectomy specimen.

6. Paint the rest of the entire resection (fatty surface) with India ink
7. Separate the axillary lymph nodes from the mastectomy specimen
8. Give the measurement of the specimen in 3 dimensions and of the surface skin in 2 dimensions
9. Describe the nipple & areola- puckered, normal eroded or otherwise; and the skin- scars, peau d' orange appearance , ulceration, etc
10. Section the specimen such that the first section passes through the nipple and areola along the imaginary vertical line drawn on the skin.

11. Make parallel cuts to the first cut at 1 cm distance in both halves.
12. After sectioning place the slices adjacent to one another sequentially to get a proper view of the neoplasm (see figure 7.4)

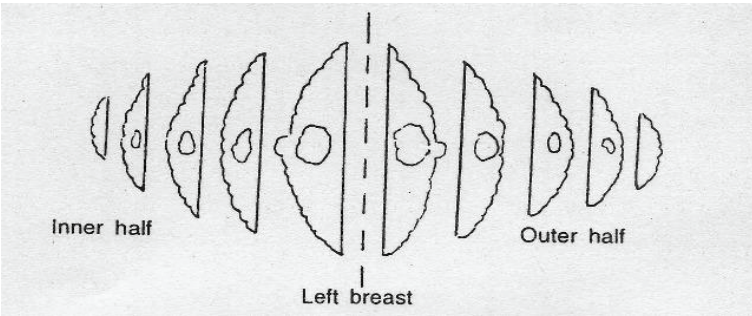


Fig 7. 4 : Arrangement of cut slices after excision

Examine each slice individually and describe the neoplasm with regard to the quadrant involvement, distance from the nipple & skin & deep surgical margin. Size to be taken along i) the greatest dimension & 2) a dimension perpendicular to it. Colour, appearance of previous biopsy site, etc. Comment on the surrounding breast tissue.

13. Identify the closest deep surgical margin and mark it for a bit to be taken.
14. ***If there are multiple lesions, note spatial relation (i.e. lesion 1 is superior and located in …. Quadrant, to lesion 2…), distance between lesions, and total span of each lesion, etc .***

Bits (Fig 7.5)

A – one bit nipple

B, C, D – three bits from the tumour, one or two to include the surrounding breast tissue are adequate even in cases where the tumour averages upto 5 cm in size, particularly if the tumour has a homogenous appearance. More bits may be taken if the neoplasm has a variegated appearance or is larger in size.

E, F – two bits from non tumour areas from quadrants other than those in which the tumour is situated.

G – One/ more bits , closest surgical margin

Others – representative bits from all other surgical margins ie superior, inferior, lateral, medial, deep margin (one from each is adequate).

Extra bits if lesions are more than one.

If the tumour is a lobular carcinoma (known at FNA or previous biopsy) then the specimen is sectioned liberally and bits taken to represent all quadrants in order to exclude multicentric carcinoma which is more common with lobular carcinoma.

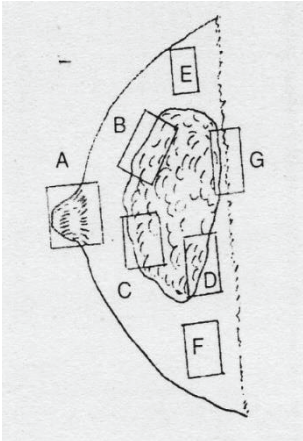


Fig 7. 5 : to show grossing and bits taken in a specimen of the breast

Lymph nodes

The various levels of lymph nodes which are sent with a mastectomy specimen should be marked by the surgeon as belonging to level 1, 2, or even 3. (the pectoralis minor muscle divides the lymph nodes into three levels the involvement of which is of prognostic significance in the staging of carcinoma of the breast. Level 1 lies inferior & lateral to the pectoralis minor, level 2 lies beneath the pectoralis minor and level 3 lies superior & medial to the pectoralis minor muscle Fig 7.6).

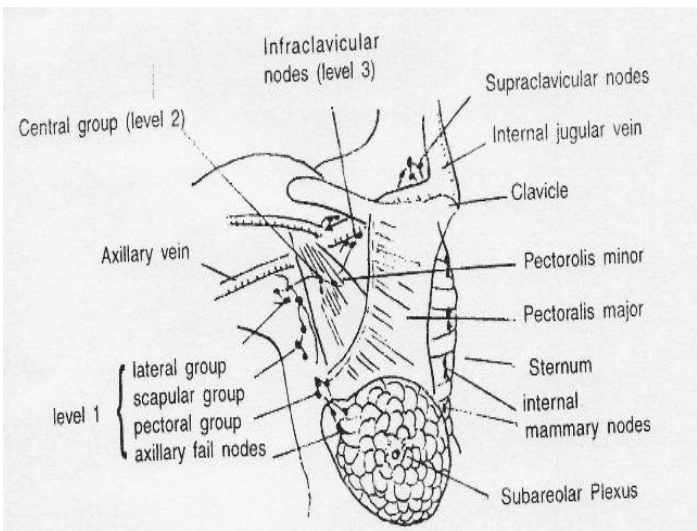


Fig 7. 6 : Shows lymphatic drainage of the breast with axillary group of lymph nodes.

In case they have not been marked separately then the axillary tissue bearing the nodes is separated from the breast tissue after orientation; as outlined above. Section it in the greatest dimension and identify all lymph nodes which stand out as white nodules. Make serial sections subsequently parallel to this through the entire fat at a thickness of 5mm in order not to miss any

nodes. Feel your way as you cut and gross all lymph nodes. If a node is 5mm or less in size, take it entirely; if more then bisect the node & take half for section.

Count all lymph nodes and mention the number grossly involved. In a radical mastectomy specimen about 18-20 nodes are identified on careful examination but in an extended mastectomy the number may be much less as routinely only levels 1 & 2 are removed.

All half lymph nodes remaining in the specimen are to be marked with eosin to indicate that they have been sampled.

III BREAST GROSS PEARLS

1. Depressed nipple : May be congenital (go into the history)
2. Depressed & puckered nipple : Involvement by carcinoma
3. Nipple eczyma : Pagets disease of nipple
4. Peau d' orange appearance of skin : lymphatic involvement in carcinoma breast. Skin appears like the peel of an orange with multiple pits.
5. Cut surface shows bluish multiple cysts : Blue domed cysts ; Fibrocystic disease.
6. Cut section single well circumscribed nodule, white homogenous- fibroadenoma.
   - well circumscribed, white homogenous with slit like spaces : - cellular fibroadenoma & benign phyllodes tumour
7. Well circumscribed mass, firm in consistency. C/S solid & grey white, with cleft like Spaces; - also phyllodes tumour .
8. Areas of necrosis, cystic degeneration & haemorrhage may be seen
   - large size with slit like spaces (cut cabbage appearance)giant fibroadenoma, s phyllodes tumour
9. Diffuse mass with rubbery consistency – fibrocystic disease (mammary dysplasia)
10. Single mass , non rubbery, firm consistency, irregular margins – carcinoma breast.
11. Obviously neoplastic lesions , large in size firm grey white appearance : carcinomas, carcinosarcomas.
12. Tumour with mucoid cut surface – colloid carcinoma
13. Firm lesion with irregular infiltrative margins, cut surface appears concave – radial scar, complex sclerosing lesion, (if the lesion is<1cm it is generally called a radial scar, if > 1cm then complex sclerosing lesion. Infiltrating carcinoma is also a differential.
14. Dilated duct with papillary mass - intraductal papilloma, papillary carcinoma.
15. Multiple papillary fronds in several spaces which are apparently dilated ducts : multiple papillomas, associated carcinoma to be ruled out.

IV BREAST MICROSCOPY

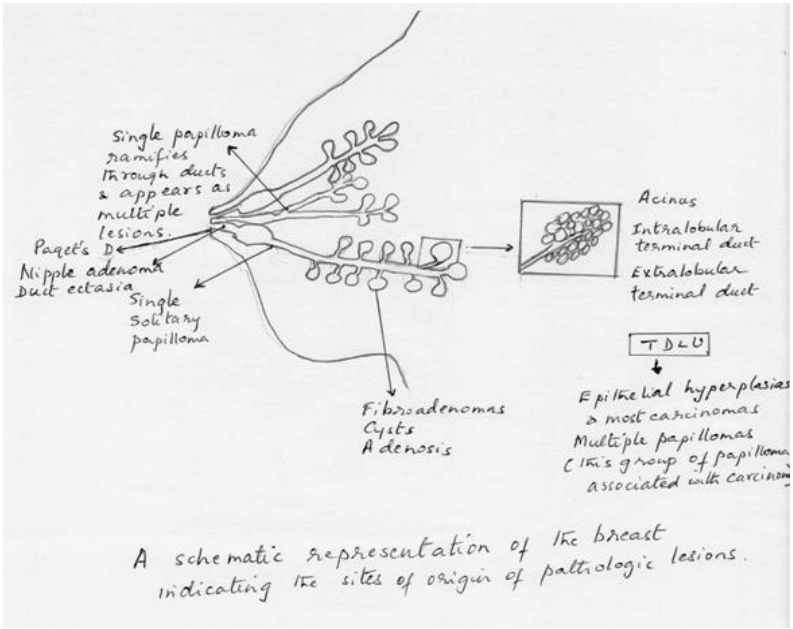


Fig 7.7 : shows sites of origin of lesions in breast

INFLAMMATORY LESIONS OF THE BREAST

Inflammatory lesions of the breast comprise a heterogeneous group of conditions that may be **infectious, autoimmune, obstructive, or idiopathic**. They may mimic carcinoma clinically and radiologically, making recognition important.

1. Acute (Puerperal) Mastitis

- Etiology: Usually seen during lactation. Caused by *Staphylococcus aureus* (most common) or *Streptococcus* spp., entering through cracked nipples.
- Clinical features: Painful, swollen, erythematous breast; fever; may progress to abscess.
- Pathology: Neutrophilic infiltration, possible liquefaction necrosis → abscess formation.
- Management**:** Antibiotics + continued drainage (feeding or pumping).

2. Non-Puerperal / Periductal Mastitis

- Typically seen in **smokers** due to squamous metaplasia of lactiferous ducts (SMOLD).
- Mechanism: Keratin plugging → duct obstruction → rupture → inflammation and abscess.
- Pathology: Mixed inflammation, duct rupture, keratin debris.
- Clinical significance: Recurrent subareolar abscesses, nipple inversion.

3. Mammary Duct Ectasia

- Etiology: Occurs in middle-aged to elderly women; non-infective.

- Pathogenesis: Dilated large ducts with thick, cheesy secretions → periductal inflammation.
- Microscopy: Dilated ducts, foamy histiocytes, plasma cells, lymphocytes; periductal fibrosis.
- Clinical: Periareolar mass, nipple retraction, sticky discharge.
- Mimics: Carcinoma due to fibrosis.

4. Granulomatous Mastitis

A group of disorders characterised by granuloma formation in breast tissue. Categories include:

a. Idiopathic Granulomatous Mastitis (IGM)

- Often in parous women within a few years of pregnancy.
- Pathology: Non-caseating granulomas around lobules; giant cells; microabscesses.
- Etiology: Uncertain; possible autoimmune reaction to milk proteins.
- Note: Must exclude infections.

b. Infective Granulomatous Mastitis

- Tuberculosis: Caseating granulomas, sinus tracts; common in endemic regions.
- Fungal infections: Histoplasmosis, cryptococcosis (rare).

c. Xantho-granulomatous inflammation is an uncommon type of inflammation seen in the breast. It involves infiltration by foamy histiocyte clusters interspersed with a few inflammatory cells. Foamy histiocytes are bland with small pyknotic nuclei.

Xanthogranulomatous mastitis may be associated with fat necrosis, multinucleated giant-cell response, and cholesterol crystals. Duct ectasia with foamy histiocytes are seen and it is suggested that the etiology of xanthogranulomatous mastitis is obstruction and rupture of the ectatic duct., as a result of which foam cell response is noted.

Differentials on a core biopsy include a granular cell tumor and invasive histiocytoid carcinoma or lipid-rich carcinoma. Differentiation is made from these by using cytokeratin and histiocytic markers (alpha1-anti-trypsin and CD 68).

Other possible locations include endometrium, ovary, fallopian tubes, vagina, testis, epididymis, gall bladder, stomach, bone, skin, appendix, urinary , bladder, thyroid and adrenal glands and colon.

The large cells are negative for cytokeratin (CK) and leukocyte common antigen (LCA). CD68 immunostain was positive.

These reactions predominantly occur in the kidney and gallbladder and are generally triggered by abscesses, necrosis, and hemorrhage. They are characterized by eosinophilic, granular, periodic acid-Schiff (PAS)-positive histiocytes in initial stages, followed by foamy macrophages, activated plasma cells, suppurative foci, and hemorrhages

5. Fat Necrosis

- Etiology: Trauma, surgery, radiotherapy.
- Gross: Irregular firm areas; may calcify.
- Microscopy: Necrotic fat cells, foamy macrophages, giant cells; later fibrosis and calcification.
- Clinical significance**:** Mimics carcinoma both clinically and radiologically.

6. Autoimmune / Systemic-Related Mastitis

Includes:

- Sarcoidosis (non-caseating granulomas)
- Wegener's / GPA
- Sclerosing lymphocytic lobulitis (seen in diabetics; lymphocytic infiltration of lobules)
- IgG4-related mastitis (storiform fibrosis, IgG4+ plasma cells)

7. Recurrent Subareolar Abscess

- Often overlaps with periductal mastitis.
- Chronic inflammation → fistula formation.
- Risk factor: Smoking.

8. Lactational Adenoma with Inflammation

- Benign proliferation during pregnancy; may undergo infarction → acute inflammatory reaction.

Summary

- Inflammatory breast lesions may mimic malignant tumours.
- Key entities: Acute mastitis, periductal mastitis, duct ectasia, granulomatous mastitis, fat necrosis.
- Always rule out tuberculosis, especially in endemic regions.
- Smoking is a major risk factor for periductal mastitis.
- Granulomatous mastitis requires exclusion of infectious causes before diagnosing IGM.

BENIGN TUMOURS OF THE BREAST :

1. **Fibroadenoma** : Young women, firm, mobile (called by surgeons as 'mouse in the breast', non-tender mass; Usually <3 cm; well circumscribed and rubbery on C/s; it is a neoplasm which occurs due to prileferation of ducts as well as stroma giving miscroscopically to an intracanalicular and pericanalicular pattern. Type of pattern seen has no significance. If large in size may be giant fibroadenoma.

2. **Phyllodes tumor (benign)** : Usually older women; May recur ; Leaf-like histology on C/S; usually larger than 3 cm. Stromal overgrowth and leaflike cut surface is characteristic. There is always a confusion between fibroadenomas and phyllodes tumours.

   Microscopically shows basic architecture of a fibroadenoma but with a prominent intracanalicular pattern. The increased stromal hypercellularity, differentiates this from a fibroadenoma. Amidst the stroma are seen benign glandular elements. The benign stromal component is fibroblastic with intervening adipose tissue foci.

   Metaplasia of stroma occurs more frequently in phyllodes tumour and besides fat, bone, cartilage and skeletal muscle have all been recorded.

   Grading: benign → malignant depending on stromal cellularity and bizzare nature of cells.

   Malignancy is characterized by marked nuclear atypia, numerous mitoses (away from the ducts) & loss of relationship between glands & stroma. Mitotic rates of > 5/10hpf indicate malignancy and ≥10/10hpf are significant. The stromal component is malignant & there is overgrowth of the stromal element with areas devoid of epithelial components.

   Fibrosarcoma, malignant fibrous histiocytoma or liposarcoma with metaplastic carlilage may be seen. Heterologous elements denote a poor prognosis. An infiltrative margin and necrosis and haemorrhage are other features of malignancy.

   The stromal cells are +ve for CD34 & bcl_2. Incidence of metastasis is seen in 3-12% of cases.

3. **Lactating adenoma :** Shows lactating change in a fibroadenoma. . The breast acini in all lobules are hyperplastic with cells showing secretary change.

OTHER LESIONS

1. **Adenosis :** indicates an increase in the number of glandular components. The lobular proliferation seen in pregnancy is a physiologic form of adenosis.

   Various forms of adenosis exist:

- **Sclerosing adenosis :** Numeric increase in glandular elements accompanied by stromal proliferation, rich in elastic tissue.

  The latter results in compression and distortion of glandular tissue.

  Microcalcification may be seen.

  Generally seen as a microscopic change but may be extensive producing a palpable abnormality.

  Mimicks carcinoma both mammographically and histologically.

  Distinguished from carcinoma by : Low power examination : multiple nodular areas with over all retention of its lobular pattern.; 2 cell lining

- **Tubular adenosis :** Is an unusual benign lesion misinterpreted as carcinoma.

  Micros **:** haphazard proliferation of uniform, elongated, sinuous tubules with bilatered epithelium of luminal epithelium & myoepithelium Lacks circumscription Microcalcification seen
- **Microglandular adenosis** :Uncommon form of adenosis

  Micros : rule of "2 cell type" is broken. Proliferating tubules are lined by a layer of single epithelium.

  Small regular rounded glands without angulation

  Eosinophilic, PAS +ve, diastase resistant material is seen within lumina as also calcium deposits

  Epithelial cells are cuboidal or flattened without apical snouts & cytologically benign

  Staining for basement membrane by the PAS stain is negative but positive by IHC (laminin & collagen IV). Complete reticulin rings can be demonstrated around the tubules by the silver stain.

  It is a benign process.
- **Blunt duct adenosis:**

  The lobular atchitecture is maintained with retention of specialized stroma.

  The acini are more in number, distorted and variably dilated. Some argue that these could be terminal ducts which proliferate without the formation of acini.

  The luminal cells lining these spaces are columnar, with apical snouts.

  Columnar cell change, Columnar cell hyperplasia, and columnar alteration with prominent apical snouts. (CAPSS).

2. PAPILLARY LESIONS

a. Solitary Intraductal Papilloma

Typically central, presents with nipple discharge; rarely palpable.

b. Multiple Papillomas

Peripheral, often associated with ductal hyperplasia and higher risk of carcinoma.

Differences Between Benign Papilloma & Papillary Carcinoma

Feature	Benign Papilloma	Papillary Carcinoma
Fibrovascular cores	Well developed	Delicate or absent
Luminal epithelium	May show hyperplasia	Stratified/tall cells
Myoepithelial cells	Present	Absent or discontinuous
Nuclear features	Benign	Malignant, may be subtle

Feature	Benign Papilloma	Papillary Carcinoma
Apocrine metaplasia	Common	Rare
Adjacent tissue	Usually benign	DCIS often present
Clinical	Nipple discharge	Often palpable mass

3. **RADIAL SCAR :** Syn : complex sclerosing lesion, scleroelastotic scar, indurative mastopathy, non encapsulated sclerosing **lesion**, sclerosing papillary proliferation.

   Mimicks carcinoma mammographically, in gross appearance as well as

   Histologically. (Fig 7.8)

   Generally non palpable, often multiple and frequently bilateral

   Incidental finding or picked up mammographically as suspicious for carcinoma.

   Low power : head of a flower. Periphery composed of epithelial elements with dilatation, adenosis, epithelial hyperplasia and rarely small intraduct papilloma.

   Calcification may be seen

   Most are benign, increased incidence of carcinoma & atypical ductal hyperplasia (varies in literature from 7 to 24%) associated with large lesions.

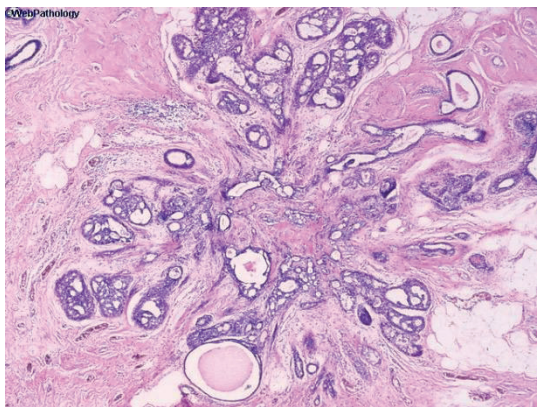


Fig 7.8 : shows radially epithelial components of radial scar . H& E x100

4. ATYPICAL DUCTAL HYPERPLASIA (ADH) AND DUCTAL CARCINOMA IN SITU (DCIS)

- **Atypical Ductal Hyperplasia (ADH)**

  ADH represents a proliferation of ductal epithelial cells that show some but not all the architectural and cytological features of DCIS. Both low-power architectural and high-power cytological features are required for diagnosis.

Diagnostic Criteria (Page et al.)

ADH is characterized by:

1. A relatively uniform population of epithelial cells.
2. Regular, smooth geometric spaces between cells forming micropapillae, tufts, fronds, bridges, solid and cribriform patterns.
3. Evenly spaced, mildly hyperchromatic nuclei.

Features fall short of DCIS when:

- The abnormal proliferation occupies < 2 duct spaces, or
- The aggregate size is < 2 mm, or
- There is partial involvement of a terminal duct–lobular unit (TDLU). ADH is often admixed with usual ductal hyperplasia.

5. DUCTAL CARCINOMA **IN SITU (DCIS)**

DCIS is defined as a **clonal proliferation of malignant epithelial cells confined within the basement membrane**, without stromal invasion.
According to Page et al. and Tavassoli & Norris, features of DCIS should be present in:

- ≥ 2 duct spaces, or
- A minimum extent of 2 mm.

DCIS incidence has increased with mammographic screening, accounting for **15–20%** of screening-detected breast lesions.
Clinically, DCIS may present as microcalcifications, occasionally as a palpable mass (typically comedo type), or as Paget disease / nipple discharge.

Differences Between ADH and DCIS

Feature	ADH	DCIS
Cell morphology	Polymorphous population including myoepithelial cells; "streaming effect"	Monomorphic population of malignant ductal cells
Extent	< 2 duct spaces or < 2 mm total extent	≥ 2 ducts or ≥ 2 mm involvement
Myoepithelial cell layer	Present throughout	Absent or only focally at periphery
Intercellular spaces	Irregular, peripheral	Regular punched-out "cookie-cutter"
Bridges & arcades	Nuclei parallel to bridge axis	Nuclei perpendicular ("Roman arches")

Grading Systems for DCIS

Accurate **nuclear grade** and **necrosis** assessment is central to modern reporting and management.

1. NHSBSP (UK) Nuclear Grading System

- **High grade**: large pleomorphic nuclei (>2.5× normal), irregular contours, prominent nucleoli, frequent mitoses, often central comedo necrosis.
- **Low grade**: small uniform nuclei, fine chromatin, inconspicuous nucleoli; common cribriform/micropapillary patterns.
- **Intermediate grade**: between the above categories.

2. Van Nuys Grading System

Combines **nuclear grade** and **necrosis**:

- High grade = poorly differentiated nuclei
- Non–high grade **with** necrosis
- Non–high grade **without** necrosis

3. Nottingham (UK) DCIS Classification

- **Pure comedo type** (high grade + necrosis)
- **Non-comedo with necrosis**
- **Non-comedo without necrosis**

4. European 1994 Grading

- Well-differentiated
- Intermediately differentiated
- Poorly differentiated

Differences Between High- and Low-Grade DCIS

Feature	High Grade	Low Grade
Proliferation	High	Low
c-erbB2/HER2	Often positive	Negative
p53	Often positive	Negative
ER/PR	Usually negative	Positive
Bcl-2	Negative	Positive

Recent advances & modern concepts on DCIS

A. WHO 5th Edition (2019) Updates

- Stresses **nuclear grade**, **necrosis**, **extent**, **microinvasion**, and **biomarkers** (ER recommended; HER2 optional).
- Recognizes molecular heterogeneity and clonal relationships between DCIS and invasive carcinoma.

B. Surgical Margins (Updated Standard)

The SSO–ASTRO–ASCO Consensus now recommends:

- A 2 mm negative margin is adequate for DCIS treated with breast-conserving surgery + whole-breast radiotherapy.
- Wider margins (>10 mm) do not provide additional benefit.
- When radiotherapy is omitted, margin decisions are individualized.

C. Genomic Tools (Oncotype DX DCIS Score)

- A validated **12-gene assay** provides individualized estimates of recurrence risk.
- Helps guide radiotherapy decisions in selected patients (not universally required).

D. Active Surveillance / De-escalation Trials

Large ongoing trials (COMET, LORIS, LORD, LORETTA) assess whether selected **low-risk DCIS** can be safely managed by:

- **Active surveillance** ± endocrine therapy
  instead of immediate surgery.
  These apply to screen-detected, **small, low/intermediate-grade, ER-positive** lesions.
  Current practice still favours surgical management until long-term results mature.

E. Biomarkers

- **ER testing** is recommended in DCIS to guide endocrine therapy.
- **HER2** is frequently positive in high-grade DCIS; routine testing varies by centre.
- **Ki-67** may support risk assessment, but is not mandatory.

F. Endocrine Therapy

For **ER-positive DCIS**, endocrine therapy (tamoxifen or aromatase inhibitors) reduces:

- Ipsilateral recurrence
- Contralateral new primary events
  Low-dose tamoxifen (5 mg/day) is emerging as a lower-toxicity option.

G. Sentinel Lymph Node Biopsy (SLNB)

Not recommended for:

- **Pure DCIS undergoing breast-conserving surgery**

Indicated for:

- **Mastectomy** (cannot perform SLNB later)
- Suspicion of invasion on imaging/core biopsy
- Very large/extensive DCIS

H.Van Nuys Prognostic Index (VNPI) for DCIS

Feature	1 Point	2 Points	3 Points
Size	≤1.5 cm	1.6–4.0 cm	≥4.1 cm
Margins	≥1 cm	0.1–0.9 cm	<0.1 cm
Histology	Non–high grade, no necrosis	Non–high grade with necrosis	High grade

Score interpretations:

- **3–4** → No benefit from radiotherapy
- **5–7** → Radiotherapy beneficial
- **8–9** → Mastectomy recommended

Margin status is the strongest predictor.

I. Variants of DCIS

Comedo Type

- High-grade nuclei + central necrosis.

Non-Comedo Types

- Small-cell solid DCIS
- Apocrine DCIS
- Neuroendocrine DCIS
- Signet-ring cell DCIS
- Cystic hypersecretory DCIS
- Clinging (flat) DCIS

J. Cancerisation of Lobules (FIG 7.9)

- Extensive DCIS may extend into lobular units ("cancerisation of lobules"). Important: **not** to be misinterpreted as invasive carcinoma (see fig).

Lobular Cancerization (or cancerization of lobules, COL) is the presence in a lobule of ductal carcinoma in situ (DCIS) tumor cells with preservation of the normal lobular pattern.

- It appears to represents a variation in the growth pattern of DCIS, not secondary extension of DCIS into a lobule.

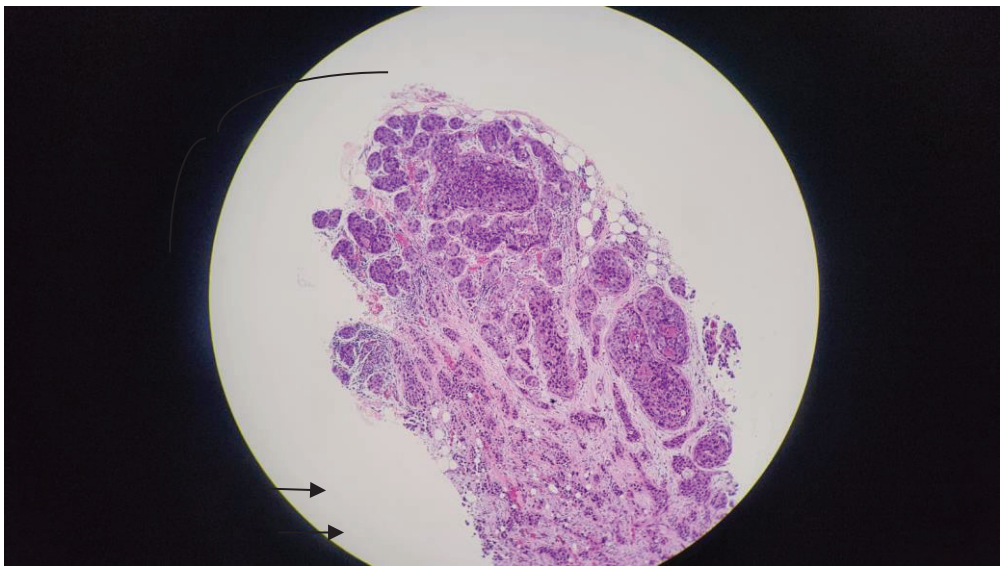


Fig 7.9 . Shows breast core biopsy with the upper part showing cancerisation of breast lobule. Lower part shows an invasice ductal carcinoma. (arrows)

K. Extensive Intraductal Component (EIC)

Defined when **>50%** of the tumour consists of DCIS within or adjacent to invasive carcinoma. Associated with a higher risk of recurrence after breast-conserving therapy.

L. Treatment Overview for DCIS (Current Practice)

Management is **individualized** based on grade, size, margin status, imaging, biomarker profile, and patient preference.

1. Breast-Conserving Surgery (BCS)

- Aim: complete excision with **≥2 mm** margin (if followed by radiotherapy).
- Radiotherapy reduces local recurrence by ~50%.

2. Mastectomy

Indications:

- Very large/multifocal DCIS
- High VNPI score
- Patient preference
- Contraindication to radiotherapy

3. Endocrine Therapy

For **ER-positive DCIS**, either tamoxifen or aromatase inhibitor.

4. Active Surveillance

Possible option **only in clinical trial/registry settings** for selected low-risk cases.

4. Lobular Carcinoma In Situ (LCIS)

A **marker** of increased bilateral breast cancer risk rather than a direct precursor.

5. Myoepithelial Cell Markers

Useful for distinguishing in situ lesions from invasive carcinoma:

- p63
- Smooth muscle myosin heavy chain
- Calponin
- Actin
- CD10
- Type IV collagen, laminin

5.DIAGNOSTIC TECHNIQUES IN CARCINOMA BREAST

a) FNAC : Easy technique

20- or 22- gauge needle

- a high level of diagnostic accuracy
- -10-15% false negative
- rare false positive
- negative FNA cytology results do not exclude malignancy

Indications

- Palpable lesions – done in clinic
- Cystic lesions
- Core biopsy not available
- Impalpable lesions via USS or MMG localisation

Requires a cytopathologist for evaluation – preferably on site to ensure specimen adequate- Diff Quick stain

Results are seen within few hours

b) Core needle biopsy :

- 8 – 18 gauge needles. Large enough to withdraw “chunks” of tissue, with cellular layers undisturbed
- 14G needle on a spring loaded biopsy gun, core sampled under LA; Obtain 4-6 cores

- Pathologist can give more info about the tumor – can see if it is invading surrounding tissue or still within ducts
- Can be used for palpable & non-palpable lumps
- Most often used with image-guidance, and for non-palpable lumps
- Two types:
    - Stereotactic - Ultrasound

Stereotactic core-needle biopsy using a 14-gauge needle is widely accepted to be sensitive (90.5%) and specific (98.3%) in diagnosing breast masses, compared with 62.4% and 86.9%, respectively, for FNA.

Categorisation of results (as per National Health Service Breast Screening Programme)

FNAC :

- C1 Non-Diagnostic
- C2 Benign
- C3 Atypical /Equivocal
- C4 Highly Suspicious
- C5 Malignant

Core Biopsy :

- B1 Unsatisfactory or normal tissue
- B2 Benign representative lesion
- B3 Equivocal
- B4 Malignant, (owing to a technical artifact or the small size of the biopsy)
- B5 Malignant, either in situ or invasive

c) Incision biopsy & open biopsy : in cases of failure of above techniques and clinical strong suspicion.

- **Sentinal lymph node :** is defined as the first lymph node(s) draining the tumour bed. Absence of tumour in the sentinel lymph node accurately predicts a lack of metastasis in the remaining axilla in 95 -100% of cases. . If +ve then the chance that other nodes of that group will show metastasis is one third.
- Biopsy of this has gained popularity in recent times in the evaluation & management of breast carcinoma, in clinical node negative patients
- Methodologies for evaluating a sentinel node include- intra-operative frozen section analysis, multiple sections, serial sectioning and immunoperoxidase staining for tumour with cytokeratin marker.
- Can be done as intra-operative procedure or otherwise

- Most workers accept that if frozen section is negative :
    - 3 step sections stained with H & E
    - one section immunostained for keratin (AE1 / AE3) is adequate to pick up metastasis > 2mm in size.
- Generally diagnosis of metastatic carcinoma is not made based on immunohistochemistry alone
- The College of American Pathologists recommends that sentinel lymph nodes be sectioned as close to 2mm as possible and entirely submitted for histological evaluation, regardless for node size.
- A single microscopic section from each block is then regarded as sufficient and would identify virtually all patients with metastasis > 2mm
- Micrometastais : < 2mm , this is as good as being node negative

6.MICROINVASION : invasive foci 1mm or less in greatest dimension.

7.EARLY BREAST CANCER : These are T1 and T2 cases with lymph nodes negative.

8.MUTIFOCAL CARCINOMA : Two or more foci of carcinoma seen in the same quadrant of the breast .

9.MULTICENTRIC CARCINOMA : disease in multiple quadrants, (foci being situated > than 5cm apart), it is a contraindication to breast conserving therapy.

10. HISTOLOGIC TYPES OF CARCINOMA BREAST

Broadly divided into 2 categories- Not otherwise specified (NOS) type of invasive breast carcinoma is the most common type (50-75%). It is also called as no special type (NST). The other category is the special type – tubular carcinoma, colloid carcinoma, medullary carcinoma, invasive papillary carcinoma, secretary carcinoma, metaplastic carcinoma , etc.

Invasive lobular carcinoma arises from the lobular units whereas the above mentioned types arise from the ductal components.

A carcinoma is placed in a special category only if 90% or more of the tumour shows features of that category. It is called a mixed tumour if 50 -90% of the tumour belongs to the special category and the remaining is of the NST category.

Medullary carcinoma; should be diagnosed only if it shows the complete constellation of all of the following features- well circumscribed tumour border, syncitial sheets of large undifferentiated tumour cells with indistinct cytoplasmic margins, pleomorphic nuclei, pushing

margins, dense lymphoplasmacytic infiltration of the scanty stroma. It is a rare variant of invasive breast carcinoma and is seen in 0.5% of cases.

Tumours which show some of these features are medullary like and the behaviour of these is closer to a ductal carcinoma- NOS type.

Classic invasive lobular carcinoma (4% of breast carcinomas) is diagnosed only when the tumour (90% of the tumour should show these features) exhibits a single file growth pattern, targetoid growth pattern and a monotonous population of small cells with very low grade nuclei and low cell density. Tumours with a diffuse infiltrative growth pattern that do not fullfil the above criteria for classic invasive lobular carcinoma should be reported with their histologic grade with the suffix as "having lobular features".

All infiltrating lobular carcinomas lack E-cadherin protein expression as detected by IHC.

Lobular neoplasia (LN) of the breast is a term which includes includes atypical lobular hyperplasia involving breast lobules (ALH) and lobular carcinoma in situ (LCIS). Distinction between ALH and LCIS is the percentage of the TDLU involved (> 50% in LCIS and < 50% in ALH) with acini that are filled and expanded to qualify as LCIS. Noninvasive, neoplastic proliferation of small, uniform, dyscohesive cells, which originates in the terminal duct lobular unit (TDLU) and fills and distends most of the acini of the involved lobule

A diagnosis of **pure mucinous carcinoma** requires the presence of low-grade nuclei and extracellular mucin in atleast 90% of the tumour. Tumours with less extensive mucin production should be reported primarily by their histologic grade with the suffix "with mucinous features"

Invasive micropapillary carcinoma : An unusual variant of invasive papillary carcinoma in which epithelial pearls without fibrovascular cores are located within clear spaces. In fact the pattern of growth of the papillae is described as being inside out and this feature is best brought out by the EMA stain which shows a reversal of polarity in the papillae.

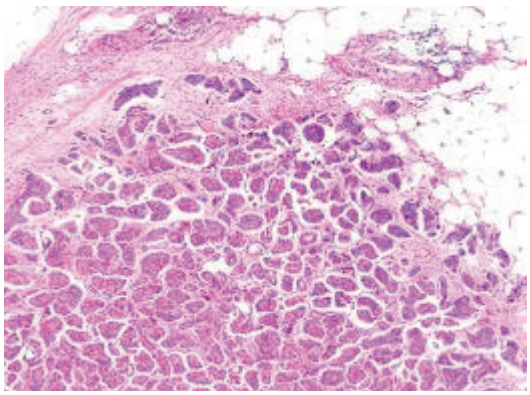


Fig 7.10 Invasive micropapillary carcinoma; note space around papillae **H&E x 100**

Prognosis regarding Special Types of Breast Carcinoma

- Genomic and gene expression studies have shown that special histological types are much more homogenous than the non-specific ductal carcinoma. This emphasizes the importance of morphological assessment made by histopathologists .
- Indeed, some special types are associated with specific genetic alterations that describe both their special developmental pattern and their specific behavior . Eg The most descriptive molecular feature of lobular carcinoma is the loss of E-cadherin.
- Weigelt et al. demonstrated that neuroendocrine and mucinous carcinomas have very similar genetic profiles, which was an expected finding due to the histopathologic observation of these two components within the same tumor.
- Adenoid cystic, medullary, and metaplastic carcinomas were within basal group.

11.CARCINOMA AND GENES : Approximately 10% of cases of breast cancer are associated with germline mutations. Mutations in these are responsible for 5% of all breast cancers

- BRCA 1 (chromosome 17q21) : high susceptibility to breast & ovarian carcinoma; less risk for prostate & colon cancer.
- BRCA 2 (13q12-13) : breast, ovary (< BRCA 1), male breast carcinoma, melanomas & pancreatic tumours.
- Protein products of both genes are localised to the nucleus & believed to be involved in transcription regulation.
- BRCA 1 : regulation of ER receptor activity & co-activation of androgen receptor.
- 1&2 : homologous recombination of DNA repair
- 1&2 : have a close connection with components of G1/S checkpoint that delays the cell cycle to allow for DNA repair.
- Both act as tumour suppressors & it is loss of function that confers the risk of malignancy.
- Lifetime breast cancer risk for female carriers is 60 to 85% and median age at diagnosis is 20 years earlier compared to women without these mutations
- Risk for ovarian cancers is 20 to 40%
- Other cancers : Male breast cancer, colon, prostate & pancreas
- BRCA 1 associated breast cancers are more poorly differentiated, have a syncitial growth pattern with pushing margins, lymphocytic response, do not express ER, PR or overexpress HER-2/neu
- Carcinomas with BRCA 1 mutation carriers are associated with a worse over-all survival.
- Identification of carriers of clinically significant mutations is important as prophylactic mastectomy and /or oophorectomy can reduce the risk of cancer mortality
- A +ve test result may have important health & social implications for family members including future generations

1. Carcinoma of the breast is presently associated with mutations in several genes; some associated with tumour types like E-cadherin gene is associated with lobular carcinoma; TP53 mutation **is** linked to aggressive subtypes eg triple negative

   ESR1 mutations are associated with endocrine resistance

12.MOLECUALR CLASSIFICATION OF BREAST CARCINOMA

The Classification is defined by gene expression profiling and is as follows :

- Luminal A
- Luminal B
- HER 2 enriched
- Triple negative / Basal-like

It guides therapy and is prognosis related. (table)

Molecular subtype	Luminal A	Luminal B	Her2/new	Basal like
Gene expression pattern	Expression of luminal (low molecular weight) cytokeratins, high expression of hormone receptors and related genes	Expression of luminal (low molecular weight) cytokeratins, moderate-low expression of hormone receptors and related genes	High expression of HER2/ne low expression of ER and related genes	High expression of basal epithelial genes and basal cytokeratins, low expression of ER and related genes, low expression of HER2/neu
Clinical and biologic properties	50% of invasive bresat cancer, ER/PR positive, HER2/neu negative	20% of invasive breast cancer ER/PR positive, HER2/neu expression variable, higher proliferation than Luminal A, higher histologic grade than Luminal A	15% of invasive breast cancer, ER/PR negative, HER2/neu positive, high proliferation, diffuse TP53 mutation, high histologic grade and nodal positivity	~15% of invasive breast cancer, most ER/PR/HER2/neu negative (triple negative), high proliferation, diffuse TP53 mutation, BRCA1 dysfunction (germline, sporadic)
Histologic correlation	Tubular carcinoma, Cribrifo carcinoma, Low grade invas ductal carcinoma, NOS, Cla lobular carcinoma	Invasive ductal carcinoma, NO Micropapillary carcinoma	High grade invasive ductal carcinoma, NOS	High grade invasive ductal carcinoma, NOS Metaplastic carcinoma, Medullary carcinoma
Response to treatment and prognosis	Response to endocrine therapy Variable response to chemotherapy Good prognosis	Response to endocrine therapy (tamoxifene and aromatase inhibitors) not as good as Luminal A Variable response to chemotherapy (better than Luminal A) Prognosis not as good as Luminal A	Response to trastuzumab (Herceptin) Response to chemotherapy with antracyclins Usually unfavorable prognosis	No response to endocrine therapy or trastuzumab Sensitive to platinum group chemotherapy and PARP inhibitors Not all, but usually worse prognosis

13.PROGNOSTIC MARKERS IN BREAST CARCINOMA

- The Prognosis depends not only on the histological parameters but also on the molecular characteristics. The markers thus indicating 'high risk' group or poor prognosis in breast cancer are, negative immunostaining of ER, PR, nm23 and; positive staining of c-erb-B2, cathepsin D and MIB-1 antibody. Nm23 and Cathepsin D gene positivity indicates metastatic potential.
- The diametrically apposite pattern of staining of these parameters on the other hand indicates better prognosis.
- **Age :** the younger the patient the worse the prognosis (due to hormones)
- Tumour type, tumour size, histological subtype.

  Tumour size: is one of the most powerful predictors of tumour behaviour. The frequency of nodal metastasis in tumours < 1cm is 10-20% and node negative patients with tumours < 1cm have a 10 year disease free survival rate of 90%.

  Tumour should be measured in atleast 2 dimensions and the single greatest dimension of the invasive tumour is used for determining the stage. For smaller tumours measurements should be taken on the slide. *For tumours with both invasive and insitu components, only the invasive component is included in the tumour measurement for staging purposes.*

  Scarff – Bloom – Richard histological grading system :

  Based on 3 parameters- tubule formation (glandular differentiation) ; nuclear pleomorphism and mitotic counts. A semi-quantitative scoring method has been devised.

  A numeric scoring system on a scale of 1-3 is used to assess each parameter individually.

Tubule formation	
Majority of tumour >75%	1 point
Moderate degree 10-75%	2 points
Little or none <10%	3 points
Nuclear pleomorphism	
Small regular uniform nuclei	1 point
Moderate increase in size and variability	2 points
Marked variation	3 points
Mitotic counts (no of mitoses /10hpfs)(field diameter 0.44 mm- Nikon)	
0-5 mitoses/10hpfs	1 point
6-10/10hpfs	2 points
>11/10hpfs	3 points

The mitotic figure count is reported as the number of mitotic figures found in 10 consecutive hpfs in the most mitotically active part of the tumour. Clearly identifiable mitotic figures should be counted. Apoptotic figures which can be mistaken for mitoses should be ignored.

Points of all the 3 parameters are added and the grades allotted.

Grade 1 is a total points of 3, 4, and 5

Grade 2 is total points of 6 and 7

Grade 3 is total points of 8 and 9.

Grading is carried out on all histologic subtypes of breast carcinoma except medullary carcinoma.

- Lymph node status
- Nottingham prognostic index (NPI) for invasive ductal carcinoma:
  (0.2 x size of tumour in cms) + (histological grade) + lymphnode stage) . Three prognostic groups are identified: Good – with scores of <3.4, moderate with scores of 3.4 to 5.4 and a poor group with scores of > 5.4.

  Lymph node stage **:** Stage 1 : No lymph node metastasis

  Stage 2: Metastasis in 1-3 axillary L nodes

  Stage 3 : Metastasis in 4 or > axillary L nodes

  NPI is Still valid in 2025 though genomic /AI models supplement this.
- Lymphovascular invasion
- Ki-67 proliferarive index (MIB-1 antibody)(PCNA) – on histological sections. MIB-1 antibody : a high MIB-1 labelling index (90%) indicates active proliferation and aggressiveness of tumour. Proliferation markers tumour grade, stage and potential for recurrence and metastasis.
- ER, PR and HER2 status (IHC /FISH) (see below)
- S phase fraction, DNA ploidy, p53, Topoisomerase II alpha (drug- anthracycline response or resistance based on +ve or -ve). Flow cytometric DNA analysis (S phase fraction) – aneuploid tumours and higher S phase fraction are associated with a shorter DFS (disease free survival) and OS (over all survival).
- Cathepsin D, nm23 gene (for metastasis)
- **Axillary lymph nodes:** For many years axillary lymph nodes metastasis was the most important prognostic factor to predict the overall prognosis.
  This in combination with other parameters like age of the patient, tumour size, histological type and tumour grade failed to predict the prognosis accurately in about 25-30% of cases.

  New parameters which are biological or molecular in nature have been discovered. These factors not only predict prognosis but are also able to identify 'high risk' group of patients.

However while grossing uninvolved lymph nodes should be entirely submitted for histologic examination, whereas representative sections for grossly positive nodes are adequate for submittion..

- **Multigene Prognostic panels** : Predicts metastatic potential and prognosis by specific gene signatures of tumors. Doing this identifies distinct subtypes of breast cancer—poor prognosis gene signature.
    1. Oncotype DX (21-gene assay): - Depicts Recurrence Score √
        - Predicts benefit from chemotherapy in HR+/HER-2 –ve ; node – ve or limited node +ve tumours
    2. Mamma Print (70-genesignature) - High vs low genomic risk; Useful in early stage breast cancer, regardless of nodal status
    3. Prosigna (PAM 50) : It has been FDA approved.
    4. Based on 50 gene + 10 year ROR score

Provides intrinsic subtype and +Prog information

4. Endo Predict (12 genes): Combines gene expression + clinical features EP score-chemotherapy benefit in ER+/HER2 –

Identifies patients who may avoid extended endocrine therapy

Breast Cancer Index (BCI)

Prediction of late recurrence In HR+ tumours

5. A mutation in the PIK3CA gene can cause cells to divide and replicate uncontrollably. It contributes to the growth of many cancers, including metastatic breast cancer (MBC).

If the tumor has a PIK3CA mutation, new treatments that specifically target this mutation could be used to treat the cancer cancer.

See Table on molecular classification.

The Food and Drug Administration (FDA) has approved a treatment called alpelisib (Piqray) specifically for people with HR-positive and HER2-negative MBC whose tumors have a PIK3CA mutation. PIK3CA mutation, constitutes one of the most frequently mutated genes (34%) in breast cancer.

PIK3CA serves as the catalytic subunit of PI3Ks, which are phosphoinositide 3-kinases that function as signal transducers in various signaling pathways.

Mutations in PIK3CA usually result in poor prognosis in breast cancer.

A mutation in the PIK3CA gene can cause cells to divide and replicate uncontrollably. It contributes to the growth of many cancers, including metastatic breast cancer (MBC).

If the tumor has a PIK3CA mutation, new treatments that specifically target this mutation could be used to treat the cancer.

Predict Online Tool

- This is an online prognostic model developed by UK NHS and Cambridge University designed for use in early invasive breast cancer.
- The parameters used are Age, tumour size, grade, nodal status, ER status, HER 2 status, KI-67 and treatment details(Surgery, chemo, hormones, transtuzumad)
- Predicts 5-,10-, 15- overall survival, breast cancer survival and treatment benefit estimates. Compares estimates with each treatment like chemo, hormone and transtusumad etc aiding decision making.
- Patient friendly, more accessible than Oncotype and prosigna; used in UK NICE guidelines foe treatment decisions.
- **Prognostic Factors in India**
- IHC 4 Testing : ER,PR, HER2, Ki-67.
- The scores of IHC4 combines semi-quantitative values from these 4 markers into an algorithm that estimates :

  Risk of distant recurrence

  Benefit from endocrine therapy

- Particularly useful in ER=ve,HER2 _ve early breast cancer.
- IHC4+clinical (IHC4 +C) : combines IHC4 results with clinical parameters (age, size, grade and node status)for enhanced prediction-comparable to Oncotype DX in some validation studies(transATAC trial)
- **Liquid Biopsy in Prognosis**
- Circulating tumour cells (CTCs)
- Circulating tumour DNA (ct DNA)
- Applications - Monitoring minimal residual disease
  - Predicting relapse
  - Non –invasive prognostic tool
- **Tumour Environment and Immune Prognosis**
- Tumour-infiltrating lymphocytes (TILs):
  High TILs=better prognosis in TNBC and HER2 +
- PD-L1 expression : (Programmed death Ligand -1)
  Predictive marker for immunotherapy in TNBC (tumour evades immune evasion)
- Immune gene signatures – under evaluation in trials

HORMONE RECEPTOR STATUS: Estrogen and progesterone receptors are members of family of nuclear receptors.

They are normally expressed in many tissues of the body including: reproductive system, mammary gland, brain, bone, liver etc

ER and PR receptor studies are done in many malignancies like ovarian carcinomas, prostatic, esophageal carcinoma and in many non neoplastic conditions.

Estrogen receptor (ER) and Progesterone receptor (PR) determinations are established procedures in the routine management of patients with breast cancer., primarily as predictive factors for response to therapeutic and adjuvant hormonal therapy. It predicts responses to hormonal therapy like tamoxifen, selective estrogen receptor modulators and aromatase inhibitors. Positive ER and PR receptor indicates good response to hormonal therapy and good prognosis.

- *Er & PR status in newly diagnosed breast cancer is – a cornerstone in planning ; to select patients for hormone therapy; useful to predict risk of progressive disease*
- *60% of ER +ve women are PR +ve (75% of these respond)*
- *ER-ve & PR +ve (uncommon) : intermediate response*
- *Topic of debate : ER, PR status in DCIS. If both invasive and DCIS are present ER & PR scores of invasive component is reported.*

IHC has become the preferred method for determining the estrogen and progesterone receptor status of breast cancer though ligand binding assays could also be used.

ER β determination is more specific as an indicator of response to hormonal therapy

Method and criteria :

Hormone receptors should be routinely performed on all primary breast carcinomas.

When both the primary tumour and nodal metastasis are available, analysis is preferably performed on the primary tumour. If only lymph node tissue is available then the latter can be used for receptor analysis as there is a good correlation between ER/PR staining of a primary breast tumour and its lymph node metastasis. ER/PR staining of a metastatic tumour of unknown primary can help establish or exclude the possibility of a primary of breast origin in addition to staining of Gross Cystic Disease Protein-15 (GCDFP-15)

Immunohistochemistry is preferred over ligand binding assays for smaller tumours. The advantage of immunohistochemistry is that the positivity obtained can be directly visualized with morphology. Microwave / pressure cooker antigen retrieval systems may be used for optimal results.

The name of the primary antiboby used (6F11, H222, H226, D547, D75, ID5 etc), method, paraffin vs frozen sections, the name of the reagent kit used and fixative used, should be recorded as a diagnostic comment.

Preferably the surgeons should be asked to send the fresh tissue containing the tumour to the laboratory as soon as it is removed. The specimen is then sampled immediately and fixed in adequate amount of fixative (10% neutral buffered formalin is preferred). Tissue cassettes are immersed in a fixative for 18-24 hrs. Prolonged fixation for upto 72 hrs (as occurs during week-end receipt of samples) also does not affect the staining of ER/PR of the fixed tissue sample. If

tumours are not going to be sampled immediately , it is advisable to slice the specimen thinly to allow proper exposure of tumour cells to a fixative(this avoids cytoplasmic positivity).

A tissue control (with positive cancer and benign adjacent epithelium) is recommended.

Scorings systems :

Immunohistochemical Evaluation of ER and PR in Breast Carcinoma:

1. Modified McCarty's H-Score (Histoscore)

- Entire section scanned at low power
- Brown nuclear staining in tumour cells is considered positive
- Percentage of tumour cells showing weak, moderate, or strong intensity is estimated

Scoring:

H-score =
(% weak × 1) + (% moderate × 2) + (% strong × 3)

Score range: 0–300

Interpretation (descriptive):

- 0–100 : Weak expression
- 101–200 : Moderate expression
- 201–300 : Strong expression

Note: H-score is semi-quantitative and mainly used for comparison and research; **positivity is not defined by a fixed H-score cut-off**.

1. **Quick Score (QS)**
   Assesses:

1. **Proportion of positive cells**
2. **Intensity of staining**

Proportion Score:

% Positive Cells	Score
0	**0**
1–25	**1**
26–50	**2**
51–75	**3**
76–100	**4**

Intensity Score:

Intensity	Score
None	0
Mild	1
Moderate	2
Strong	3

Total score = Proportion + Intensity (Max = 7)
Score ≥3 is considered positive

3. Allred Score (Most Widely Used System)

Combines:

- **Proportion score: 0–5**
- **Intensity score: 0–3**
  (Proportion score: A score of 0 is assigned if no cells are stained, and a score of 1 represents less than 1% positivity. The numerical scale increases progressively: 2 covers 1−10% of positive cells, 3 corresponds to 11−33%, and 4 is assigned if 34−66% are positive. The maximum Proportion Score of 5 is given when 67% or more of the cells exhibit positive staining)

Total score: 0–8

Total Score	Interpretation
0–2	Negative
3–8	Positive

4. ASCO–CAP RECOMMENDATIONS (Current Standard)

- **ER or PR is POSITIVE if ≥1% of tumour cell nuclei show staining**
- Tumours with **1–10% positivity** should be reported as
  "ER (or PR) low positive"
- Intensity and scoring system used should be mentioned in the report

RESPONSE TO HORMONAL THERAPY

- **ER+/PR+** : ~70–80% response
- **ER+/PR−** : ~40–50% response
- **ER−/PR+** : Uncommon; low and inconsistent response

- **ER−/PR−** : Minimal response

ER status is the most important predictive marker; PR provides additional prognostic information.

STAINING ON CORE BIOPSIES

- Minimum **100 invasive tumour cells** should be evaluated
- **Excellent concordance** between ER status on core biopsy and excision specimens
- **PR expression is more heterogeneous**, leading to slightly lower concordance

MODEL HISTOPATHOLOGY REPORTING TEMPLATE

(ER / PR Immunohistochemistry)

- **Antibody clone: _____**
- **Specimen:** Core biopsy / Excision
- **Percentage of positive tumour nuclei:** ____ %
- **Staining intensity:** Weak / Moderate / Strong
- **Scoring system used:** Allred / H-score / Quick score

Final Interpretation:

- ER: Positive / Negative / Low positive
- PR: Positive / Negative / Low positive

Summary :

- ≥1% nuclear staining = positive (ASCO–CAP)
- Allred score is the most commonly used system
- ER is predictive; PR is supportive
- H-score is descriptive, not decisive

15.HER2-NEU (C-ERB B2) : gene amplification which usually results in over expression of the encoded transmembrane protein p185 occurs in one third of breast cancers.. The 2 most common assay systems use fluorescence in situ hybridization (FISH) and IHC. Numerous commercial reagents are available. Clinical use of anti-c erb B2 drug Herceptin (transtuzumab) requires c-erb B2 testing on tumours from potentially eligible patients before therapy.

- Amplification & overexpression of HER-2 / neu (C-erbB-2) gene & protein has been seen in 10-34% of invasive breast cancers.
- Associated with adverse prognosis in both node negative & node positive disease
- It's chief application lies in it's prediction of response to therapy with transtuzumab (Herceptin) and pertuzumab

The interpretation of the IHC staining is done as follows:

More than 10% of cells show weak staining of the cell membrane 1+

More than 10% of cells show moderate staining of the cell membrane 2+

More than 10% of cells show a continuous strong staining of the cell membrane 3+

It is recommended that all 2+ results need to be confirmed by the FISH technique

A V BREAST SYNOPTIC REPORTING

EXCISION /MASTECTOMY

Side Left □ Right □

Specimen type

Excision biopsy □

Wide local excision □

Simple mastectomy □

Simple mastectomy with axillary clearance □

Radical mastectomy □

Re-excision □

Other □ Specify …….

Gross

Specimen weight ..... g

Ellipse of skin ..... × ..... cm

Nipple Normal □ Indrawn □ Not assessable □

Fibrofatty tissue ..... × ..... × ..... cm

Lesion measures ..... × ..... × ..... cm

Site OUQ □ OLQ □ IUQ □ ILQ □ Retroareolar □ Not known □

Distance to superior margin ……cm

Distance to inferior margin …….cm

Distance to lateral margin ……cm

Distance to medial margin ……..cm

Distance to deep margin …….cm

Macroscopic distance to nearest resection margin ……

Axillary Lymph nodes present Yes □ No □ No sampled …….

Other nodes present No □ Yes □ Site …….

Total number ..... Number positive .....

Histology :

Part A : Invasive carcinoma present Yes □ No □

No special type (ductal NST) □

Pure special type (90% purity, specify components present below) □

Mixed tumour type (50–90% special type component,

specify components present below) □

Other malignant tumour (please specify) ……

Specify type component(s) present for pure special type and mixed tumour types:

Tubular/cribriform □ Lobular □ Mucinous □ Medullary like □ Ductal /no special type

Other (please specify) □ .....

Histological Grade 1 □ 2 □ 3 □ N/A

Vascular invasion Not seen □ Present□ Possible □

Associated DCIS None □ Minimal (< 1 mm beyond) □ Extensive □

DCIS grade Low □ Intermediate □ High □

In situ lobular neoplasia present Yes □ No □

Paget's disease present Yes □ No □

Tumour extent Localised □ Multifocal (within 5cm) □ Multicentric (beyond 5cm and in different quandrants) □

Invasive tumour reaches margin Yes □ No □ Not assessable □

Closest relevant margin(s) to invasive tumour .....margin; …… mm distant

Axillary nodes Total number examined..... Number positive .....

For single node positivity, specify Metastasis (> 2 mm) □

Micrometastasis □ (= 2 mm to > 0.2 mm)

Isolated tumour cells □ (= 0.2 mm)

Other nodes Site …… Total number examined ..... Number positive .....

Site …… Total number examined ..... Number positive .....

TNM stage : pT 1 □ 2 □ 3 □ N/A □

pN 1 (1-3 nodes) □ 2 (3-10)□ 3 (>10)□ N/A □

M 1 □ 2 □ 3 □ Mx □ N/A □

Nottingham prognostic index (0.2 x size of tumour + histological grade + lymphnode stage : Good □ Moderate □ Poor □

Oestrogen receptor status Positive □ Negative □

Quickscore ……/8

H Score ..... /300

Progesterone receptor status Positive □ Negative □

Quickscore ……/8

H Score ..... /300

Additional comments ……..

SNOMED

Breast T04 M.....; T04 M.....

Lymph node T08 Node negative M00100 □

Node positive M81406 □

Pathologist's signature ……..Date ....

Part B :Synoptic reporting Final pathology : DCIS

Pure DCIS size ..... mm in maximum extent

DCIS grade

Low □ Intermediate □ High□

DCIS architecture

Solid □ Cribriform □ Micropapillary □ Papillary □

DCIS necrosis Yes □ No □

LCIS present Yes □ No □

Microinvasion (< 1 mm) Yes □ No □

Paget's disease Yes □ No □ Not assessable □

Excision

DCIS reaches margin Yes □ No □

Closest relevant margin(s) to DCIS..... ..... mm distant

Comments…….

Lymph nodes sampled Yes □ No □

Number of axillary nodes sampled .....

Number of axillary nodes containing tumour .....

Details of other nodes

Additional comments

SNOMED T04 – M85002 T04 ..... T08000 – M 001

Pathologist's signature and date ....

Part C : Final diagnosis benign lesion

Normal breast tissue □

Radial scar/complex sclerosing lesion □

Periduct mastitis/duct ectasia □

Fibroadenoma □

Fibrocystic change □

Multiple papillomata □

Solitary papillomata □

Surgical biopsy cavity □

Columnar cell change □

Other benign lesion □

Benign lesion size (mm) .....

Epithelial hyperplasia : Not present □

Present without atypia □

Atypical ductal hyperplasia □

Atypical lobular hyperplasia □

Present with atypia, both ductal and lobular □

Comments

SNOMED T04 M.....; T04 M.....

Consultant pathologist's signature and date .....
.....

Part C: Separate axillary staging procedure (clearance, sampling or sentinel node)

Axillary nodes present: No □ Yes □

Total number ..... Number positive .....

For single node positivity, specify Metastasis (> 2 mm) □

Micrometastasis (= 2 mm to > 0.2 mm) □

Isolated tumour cells (=0.2 mm) □

Other nodes present No □ Yes □

Total number ..... Number positive .....

Site of other nodes .....

Comments

SNOMED T08 □

Node negative M00100 □

Node positive M81406 □

Pathologist's signature and date .....

WIDE BORE NEEDLE BIOPSY FORM (WBN)

Side : Right □ Left □

Quadrant UOQ □ LOQ □ UIQ □ LIQ □ AXL □

Number of cores .....

Specimen type

WBN □ Vacuum assisted excision biopsy □

Vacuum assisted diagnostic biopsy □

Calcification present on specimen x-ray? Yes □ No □ Radiograph not seen □

Histological opinion

B1 Unsatisfactory/normal tissue only □

B2 Benign □

B3 Uncertain malignant potential □

B4 Suspicious □

B5 Malignant □

In situ □

Invasive □

not assessable □

Histological calcification Absent □ Benign □ Malignant □ Both □

OPTIONAL FURTHER INFORMATION

Benign lesion

Complex sclerosing lesion/radial scar □ Fibroadenoma □ Multiple papilloma □

Periductal mastitis/duct ectasia □ Fibrocystic change □ Solitary papilloma □

Sclerosing adenosis □ Solitary cyst □ Columnar cell change □ Other (please specify)

Epithelial proliferation

Not present □ Present without atypia □ Present with atypia (ductal) □ Present with atypia (lobular) □

Malignant lesion

In situ carcinoma □ Not present □ Ductal □ Lobular □

DCIS grade High □ Intermediate □ Low □ Not assessable □

Invasive carcinoma

Not present □

Size invasive tumour.....mm (largest dimension, if available)

Type No special type (ductal NST) □

Pure special type (90% purity specify components present below): □

Mixed tumour type (50–90% special type component, specify components present below): □

Other malignant tumour □ (please specify) .....

Specify type component(s) present for pure special type and mixed tumour types:

Lobular □ Mucinous □ Medullary/atypical medullary □

Ductal/no special type □ Other □ (please specify) .....

Invasive grade 1 □ 2 □ 3 □ Not assessable □

Oestrogen receptor status : *(ER / PR Immunohistochemistry)*

- Antibody clone: _____
- Specimen: Core biopsy / Excision
- Percentage of positive tumour nuclei: ____ %
- Staining intensity**:** Weak / Moderate / Strong

  Negative . □

  Moderate . □

  Strong Positive . □

  Not performed □
- **Scoring system used:** Allred / H-score / Quick score

Final Interpretation:

- ER: Positive / Negative / Low positive
- PR: Positive / Negative / Low positive

Pathologist's signature …… Date……. Snomed code

TNM classification on breast :

Tumor (T)

The T category in the TNM staging system describes the size and location of the tumor:

TX: The primary tumor cannot be assessed.

T0: There is no sign of a primary tumor in the breast.

Tis: There is carcinoma in situ in the breast.

- **Tis (DCIS):** Abnormal cells have been found in the breast ducts and have not spread past the layer of tissue where they began.
- **Tis (Paget disease):** A rare form of early, noninvasive cancer where abnormal cells are found in the skin of the nipple and have not spread beyond where they first formed. No tumor or DCIS is found in the breast tissue under the nipple.

T1: The tumor is 20 millimeters or smaller. There are four subtypes of a T1 tumor depending on the size of the tumor:

- **T1mi:** The tumor is 1 millimeter or smaller.

- **T1a:** The tumor is larger than 1 millimeter but not larger than 5 millimeters.
- **T1b:** The tumor is larger than 5 millimeters but not larger than 10 millimeters.
- **T1c:** The tumor is larger than 10 millimeters but not larger than 20 millimeters.

T2: The tumor is larger than 20 millimeters but not larger than 50 millimeters.

T3: The tumor is larger than 50 millimeters.

T4: The tumor is described as one of the following:

- **T4a:** The tumor has grown into the chest wall.
- **T4b:** The tumor has grown into the skin. An ulcer has formed on the surface of the skin of the breast, small tumor nodules have formed in the same breast as the primary tumor, and/or the skin of the breast is swollen.
- **T4c:** The tumor has grown into the chest wall and the skin.
- **T4d:** One-third or more of the skin on the breast is red and swollen (called peau d'orange). T4d includes inflammatory breast cancer.

Lymph node (N)

This section describes pathological staging of lymph nodes. Pathological staging is done when the lymph nodes are removed by surgery and studied under a microscope by a pathologist. When the lymph nodes are checked using mammography or ultrasound, it is called clinical staging. Clinical staging of lymph nodes is not described here.

The N category describes the location of the lymph nodes where cancer has spread and the size of the cancer in the lymph nodes:

NX: The lymph nodes cannot be assessed.

N0: There is no sign of cancer in the lymph nodes, or there may be tiny clusters of cancer cells not larger than 0.2 millimeters in the lymph nodes.

N1: Cancer is described as one of the following:

- **N1mi:** Cancer has spread to the axillary (armpit area) lymph nodes and is larger than 0.2 millimeters but not larger than 2 millimeters.
- **N1a:** Cancer has spread to one to three axillary lymph nodes and the cancer in at least one of the lymph nodes is larger than 2 millimeters.
- **N1b:** Cancer has spread to lymph nodes near the breastbone on the same side of the body as the primary tumor. The cancer is larger than 0.2 millimeters and is found by sentinel lymph node biopsy. Cancer is not found in the axillary lymph nodes.
- **N1c:** Cancer has spread to one to three axillary lymph nodes, and the cancer in at least one of the lymph nodes is larger than 2 millimeters. Cancer is also found by sentinel lymph node biopsy in the lymph nodes near the breastbone on the same side of the body as the primary tumor.

N2: Cancer is described as one of the following:

- **N2a:** Cancer has spread to four to nine axillary lymph nodes, and the cancer in at least one of the lymph nodes is larger than 2 millimeters.
- **N2b:** Cancer has spread to lymph nodes near the breastbone, and the cancer is found by imaging tests. Cancer is not found in the axillary lymph nodes by sentinel lymph node biopsy or lymph node dissection.

N3: Cancer is described as one of the following:

- **N3a:** Cancer has spread to 10 or more axillary lymph nodes. The cancer in at least one of the lymph nodes is larger than 2 millimeters, or the cancer has spread to lymph nodes below the collarbone.
- **N3b:** Cancer is described as one of the following:
    - Cancer has spread to one to nine axillary lymph nodes, and the cancer in at least one of the lymph nodes is larger than 2 millimeters. Cancer has also spread to lymph nodes near the breastbone, and the cancer is found by imaging tests.
    - Cancer has spread to four to nine axillary lymph nodes, and the cancer in at least one of the lymph nodes is larger than 2 millimeters. Cancer has also spread to lymph nodes near the breastbone on the same side of the body as the primary tumor. The cancer in these lymph nodes is larger than 0.2 millimeters and is found by sentinel lymph node biopsy.
- **N3c:** Cancer has spread to lymph nodes above the collarbone on the same side of the body as the primary tumor.

Metastasis (M)

The M category describes the spread of cancer to other parts of the body.

M0: There is no sign that cancer has spread to other parts of the body.

M1: Cancer has spread to other parts of the body, most often to the bones, lungs, liver, or brain. If cancer has spread to distant lymph nodes, the cancer in the lymph nodes is larger than 0.2 millimeters. The cancer is called metastatic breast cancer.

Note : 1 -microinvasion is the invasion of cancer cells beyond the basement membrane into the adjacent tissues with no focus more than 0.1 cm in greatest dimension. When there are multiple foci of microinvasion the size of only the largest focus is used to classify the microinvasion (Do not use the sum of all the individual foci) The presence of multiple foci of microinvasion should be noted, as it is with multiple larger invasive carcinomas.

2.Inflammatory carcinoma of the breast is characterized by diffuse brawny induration of the skin with an erisepheloid edge, usuallu with no underlying mass. If the skin biopsy is negative and there is no localized measurable primary cancer the T category is pTX when pathologically staging a clinical inflammatory carcinoma (Td4). Dimpling of the skin, nipple retraction or other

skin changes except those in T4b and T4d may occur in T1, T2, or T3 without affecting the classification.

N – Regional lymph nodes

NX – Regional lymph nodes cannot be assessed (eg previously removed)

N0 No regional lymph node metastasis

N1 Metastasis to movable ipsilateral axillary node(s)

N2 Metastasis to ipsilateral axillary node(s) fixed or matted or in clinically apparent ipsilateral internal mammary nodes in the absence of clinically evident axillary lymph node matastasis.

N2a Metastasis to ipsilateral axillary node(s) fixed to one another or to other structures

N2b Metastasis in clinically apparent ipsilateral internal mammary nodes and in the absence of clinically evident axillary lymph node matastasis.

N3 Metastasis to ipsilateral infraclavicular lymph nodes with or without axillary lymph node involvement , or in clinically apparent ipsilateral internal mammary nodes & in the presence of clinically evident axillary lymph node metastasis; or metastasis in ipsilateral supraclaclavicular lymph node (s) with or without axillary or internal mammary lymph node involvement .

N3a - Metastasis to ipsilateral infraclavicular lymph nodes

N3b- Metastasis in ipsilateral internal mammary nodes & and axillary nodes

N3c - Metastasis in ipsilateral supraclaclavicular lymph node (s)

(Clinically apparent is defined as detected by imaging studies or by clinical examination or by grossly visible pathologically)

Pathologic (pN)

pNX Regional lymph nodes cannot be assessed (eg previously removed or not removed for pathologic study)

pN0 No regional lymph node metastasis , no additional examination for isolated tumour cells(ITC)

pN0(i -) : No regional lymph node metastasis histologically , negative IHC

pN0(i+) : No regional lymph node metastasis histologically , +ve IHC; no IHC cluster greater than 0.2mm

pN0(mol-) : No regional lymph node metastasis histologically , negative molecular finding (RT-PCR)

pN0(mol+) : No regional lymph node metastasis histologically , +ve molecular finding (RT-PCR)

pN1 : Metastasis in 1-3 axillary lymph nodes & /or in internal mammary nodes with microscope disease detected by sentinel lymph node dissection but not clinically apparent

pN1Mi : Micrometastasis (greater than 0.2mm, none greater than 2mm)

pN1a : Metastasis in 1-3 axillary lymph nodes

pN1b : Metastasis in internal mammary nodes with microscope disease detected by sentinel lymph node dissection but not clinically apparent

pN1c : Metastasis is in 1-3 axillary lymph nodes & in internal mammary nodes with microscope disease detected by sentinel lymph node dissection but not clinically apparent (if associated with greater than 3 +ve axillary nodes the internal mammary nodes are classified as pN3b to reflect increased tumour burden)

PN2 : Metastasis in 4-9 axillary lymph nodes or in clinically apparent internal mammary nodes in the absence of axillary lymph node metastasis.

pN2a : Metastasis in 4-9 axillary lymph nodes (atleast one tumour deposit >2.00mm)

pN2b : Metastasis in clinically apparent internal mammary lymph nodes in the absence of axillary lymph node metastasis.

pN3 : Metastasis in 10 or more axillary lymph nodes or infraclavicular lymph nodes or in clinical apparent ipsilateral internal mammary nodes in the presence of one or more positive axillary lymph nodes or in > 3 axillary lymph nodes with clinically negative microscopic metastasis in internal mammary lymph nodes ; or ipsilateral supraclaclavicular lymph nodes.

pN3a : Metastasis in 10 or more axillary lymph nodes (atleast one tumour deposit >2.00mm), or metastasis to the infraclavicular lymph nodes.

pN3b : Metastasis in clinically apparent ipsilateral internal mammary lymph nodes in the presence of one or > +ve axillary lymph nodes; or in > than 3 axillary lymph nodes and in internal mammary lymph nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent.

pN3c : Metastasis in ipsilateral supraclaclavicular lymph nodes.

Note : Isolated tumour cells (ITC) are defined as single tumour cells oe small cell clusters not greater than 0.2mm usually detected only by immunohistochemical (IHC) or molecular methods but which may be verified by H&E stains. ITCs do not usually show evidence of malignant activity eg proliferation or stromal reaction.

Classification is based on axillary lymph node dissection with or without sentinel lymph node dissection without subsequent axillary lymph node is designated (sn) for sentinel nodeeg pN0 (i+)(sn).

M- Distant metastasis

MX Distant metastasis cannot be assessed

M0 No distant metastasis

M1 Distant metastasis

The pT categories correspond to the T categories.

Note : when classifying pT the tumour size is a measurement of the invasive component . If there is a large in situ component (eg 4 cm) and a small invasive component (eg 0.5 cm) the tumour is coded pT1a.

For bilateral disease each primay is staged separately.

ASSESS YOURSELF BREAST

Essay-Type Questions (10 marks each)

1. Discuss the classification, pathogenesis and morphology of benign epithelial lesions of the breast. Add notes on their clinical significance.
2. Describe fibroadenoma and phyllodes tumour in detail. Compare and contrast their clinical, gross and microscopic features.
3. Enumerate inflammatory lesions of the breast. Describe in detail acute mastitis, granulomatous mastitis and mammary duct ectasia.
4. Enumerate the steps involved in the grossing of a simple mastectomy specimen with axillary clearance

Short Paragraph Questions (5 marks each)

1. Apocrine metaplasia — definition, morphology and clinical relevance.
2. Usual ductal hyperplasia (UDH) — microscopic features and risk of carcinoma.
3. Duct ectasia — pathogenesis and microscopic features.
4. Grossing of breast lumpectomy

Point-Type Questions (3 marks each)

1. Three features of complex fibroadenoma.
2. Types of granulomatous mastitis.
3. Key microscopic features of sclerosing adenosis.
4. Two differences between UDH and ADH (Atypical Ductal Hyperplasia).

Multiple Choice Questions (MCQs)

1. **Sclerosing adenosis is characterised by:**
   A. Loss of myoepithelial cells
   B. Proliferation of small ductules with stromal fibrosis

C. Sheets of atypical epithelial cells
D. Presence of necrosis

2. **A biphasic tumour with leaf-like architecture is:**
   A. Fibroadenoma
   B. Phyllodes tumour
   C. Lactational adenoma
   D. Sclerosing papilloma
3. **The hallmark of duct ectasia is:**
   A. Caseating granulomas
   B. Dilated ducts with lipid-laden macrophages and periductal fibrosis
   C. Rapidly enlarging mass
   D. Microcalcifications
4. **Painful, erythematous breast during lactation is most commonly due to:**
   A. Mammary duct ectasia
   B. Fat necrosis
   C. Acute mastitis (Staph aureus)
   D. Sarcoidosis

Answer Key for MCQs

1. B
2. B
3. B
4. C

Chapter 8
Lymphoreticular System

Chapter outline –A Lymph nodes; B Spleen; C Thymus

A I Lymph Nodes Anatomy ; A II lymph Nodes Grossing Techniques ; A III Lymph nodes Gross Pearls ; A IV Lymph nodes Microscopy ;A V Lymph nodes Synoptic reporting

B I Spleen Anatomy; B II Spleen Grossing Techniques; B III Spleen Gross Pearls; B IV Spleen Microscopy; B V Spleen Synoptic Reporting

C I Thymus Anatomy; C II Thymus Grossing Techniques ; C III Thymus Gross Pearls ; C IV Thymus Microscopy; C V Thymus Synoptic Reporting.

A I LYMPH NODES ANATOMY

Lymph Nodes are small rounded structures (Fig 8.0) which lodge lymph fluid within. They have-

- **Capsule & Hilum**: Fibrous capsule with trabeculae; hilum is entry/exit point for vessels (See fig below)
- **Cortex**: Contains lymphoid follicles (primary and secondary with germinal centers).
- **Paracortex**: T-cell zone, important in reactive changes.
- **Medulla**: Medullary cords and sinuses, continuous with efferent lymphatics.
- **Grossing relevance**: Nodes are usually small, ovoid, tan-gray and have great significance when enlarged in malignancy.

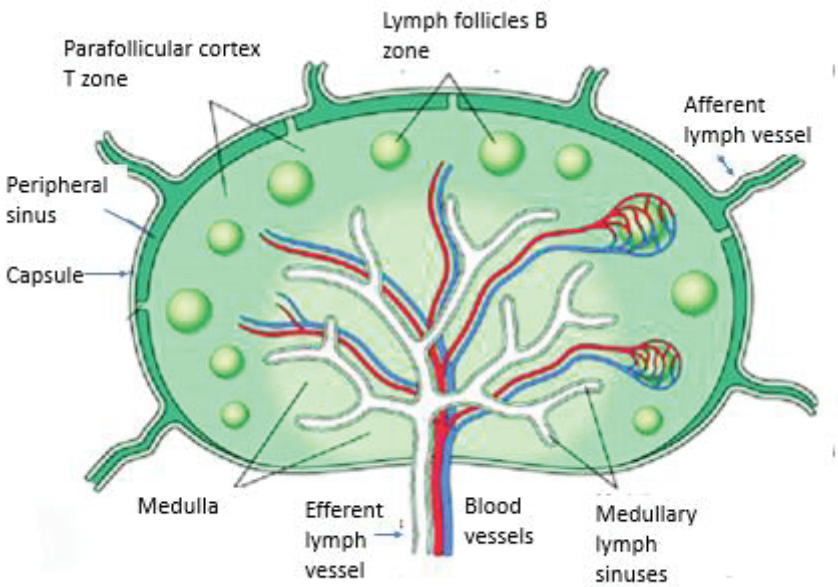


Fig 8.0-shows diagrammatic representation of the structure of a lymph node node

A II Lymph Nodes Grossing Techniques

- **Weigh/Measure**: Record size (length, breadth, thickness).
- **Count**: In dissections, enumerate all nodes retrieved.
- **Capsule**: Note integrity, thickening, or extracapsular spread.
- **Cut Surface**: Describe color (tan, gray, white), homogeneity, necrosis, or nodularity.
- **Hilum**: Identify fatty hilum; note obliteration.
- **Lymph nodes** may be received: Singly or as part of a 'regional lymph node dissection '/ clearance

A. SINGLE LYMPH NODE

1. Measure the lymph node in three dimensions and record the external appearance (capsule smooth / congested / matted).
2. State the fixative in which the specimen is received (usually 10% neutral buffered formalin).
3. Fresh lymph node – suspected infection
   - Bisect using a sterile blade.
   - Submit a portion for microbiological culture and sensitivity, including mycobacterial and fungal cultures where clinically indicated.
4. Suspected hematolymphoid neoplasm – Imprints
   - Bisect the fresh node.
   - Prepare 4 imprint smears by gently touching the cut surface on clean glass slides.
   - Fix:
     - 2 slides in alcohol (for H&E / PAP)
     - 2 air-dried slides (for Wright / Giemsa)
5. Tissue triage (only if fresh tissue is received):
   - Flow cytometry:
     Submit a small piece (≤0.5 cm) in appropriate transport medium.
   - Cytogenetics / molecular studies (if required):
     Fresh tissue may be submitted as per laboratory protocol.

     Note: In most laboratories today, immunohistochemistry is routinely performed on formalin-fixed paraffin-embedded tissue, and fresh tissue is not mandatory.
6. Sectioning
   - If the node measures ≤5 mm → submit entirely without bisecting.
   - If >5 mm → bisect through the hilum and describe the cut surface, noting:
     - Homogeneous / nodular appearance
     - Areas of necrosis, hemorrhage
     - Loss of corticomedullary distinction

BITS

- Nodes ≤5 mm: submit entirely.
- Nodes >5 mm: submit at least one complete cut surface.

B. LYMPH NODE DISSECTION FOR MALIGNANCY (Regional lymph node clearance)

Includes:

- Axillary nodes (mastectomy)
- Radical / modified neck dissections (Fig 8.1)
- Nodes with gastrointestinal resections
- Inguinal and pelvic dissections

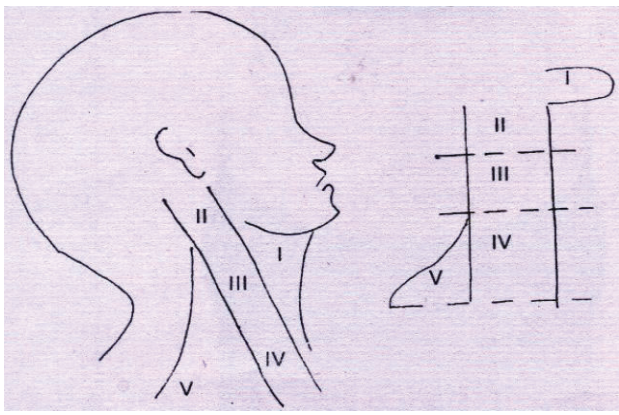


Fig 8.1 shows lymph node levels in the neck as seen in radical node dissection.

Procedure

1. Identify and separate nodal levels if designated by the surgeon.
   Each level must be grossed and submitted separately.
2. Dissect lymph nodes from fat:
   - Best done after overnight fixation.
   - Fat-clearing solutions may be used where required (as per lab policy).
3. Count all lymph nodes and record:
   - Total number
   - Size of largest and smallest node
4. Sectioning
   - Nodes >5 mm: bisect and describe cut surface.
   - Nodes ≤5 mm: submit entirely.

BITS

- **All lymph nodes must be submitted.**

- For large nodes, submit one representative section, and mark the remaining tissue with eosin/ink to avoid double counting during regrossing**.**

NECK DISSECTION SPECIMENS

Types of specimens received

1. **Radical Neck Dissection**
   - Lymph nodes
   - Sternocleidomastoid muscle
   - Internal jugular vein
   - Spinal accessory nerve
   - Submandibular salivary gland
   - ± Tail of parotid gland
2. **Modified Radical Neck Dissection (Fig 8.2)**
   - One or more non-lymphatic structures preserved

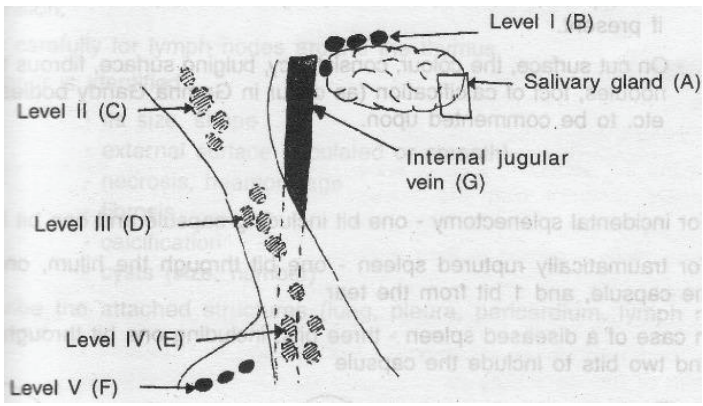


Fig 8.2 shows various levels and bits (A to F) to be taken from them.

3. **Extended Radical Neck Dissection**
   - Includes additional nodal groups (retropharyngeal, paratracheal, mediastinal, etc.)
4. **Selective / Regional Neck Dissection**
   - Only selected nodal levels removed

Procedure

i. Orient the specimen
   - Preferably by the surgeon; otherwise by the pathologist.
   - Identify nodal levels (I–V).

ii. Dissect lymph nodes level-wise.

iii. Separate non-nodal structures (salivary gland, muscle, vein).

iv. Examine salivary gland
   - External and cut surface

- Tumour infiltration, if any

v. Section lymph nodes
- 5 mm: bisect
- ≤5 mm: submit entirely

vi. Examine internal jugular vein
- Look specifically for tumor invasion or thrombosis

BITS

- A: Submandibular salivary gland
- B: Level I nodes
- AC: Level II nodes
- D: Level III nodes
- E: Level IV nodes
- F: Level V nodes
- G: Internal jugular vein
- H: Thyroid gland (if present)

(More blocks may be submitted from any level if indicated.)

A III LYMPH NODES GROSS PEARLS

- **Enlarged lymph node (s), single or multiple, usually 2–3 cm**
  → Reactive lymphoid hyperplasia
- **Enlarged, matted lymph nodes**
  → Tuberculosis (also consider metastatic carcinoma in appropriate setting)
- **Markedly enlarged lymph nodes**
  → Lymphomas
  → Castleman disease
  → Rosai–Dorfman disease
  → HIV / AIDS-related lymphadenopathy
  → Other histiocytic disorders

Cut Surface Appearance

- **Homogeneous, pink / tan cut surface**
  → Castleman disease
  → Rosai–Dorfman disease
- **"Fish flesh" appearance**
  → Hodgkin lymphoma
  → Non-Hodgkin lymphoma

- **Caseous necrosis**
  → Tuberculosis
- **Hemorrhagic areas**
  → Infarction
  → High-grade lymphoma (occasionally)
- **Solid foci different from nodal parenchyma**
  → Metastatic carcinoma
- **Black foci within node**
  → Metastatic malignant melanoma
- **Black linear pigment at periphery / subcapsular region**
  → Dermatopathic lymphadenitis
- **Cystic lymph node**
  → Necrotic metastatic carcinoma
  → Papillary carcinoma (thyroid) metastasis
- **Minute punctate foci of necrosis**
  → Cat scratch disease
  → Lymphogranuloma venereum

A IV LYMPH NODES MICROSCOPY

Microscopy of Non-Neoplastic Lymph Node Diseases

1. Tuberculosis

- Granulomas:
  - Presence of well-formed granulomas composed of epithelioid cells and Langhans giant cells.(see fig 8. 3 -diagrammatic fig and section of lymph node with granulomatous inflammation on the right.)
  - **Necrosis:** Caseous necrosis is commonly seen in the center of granulomas.

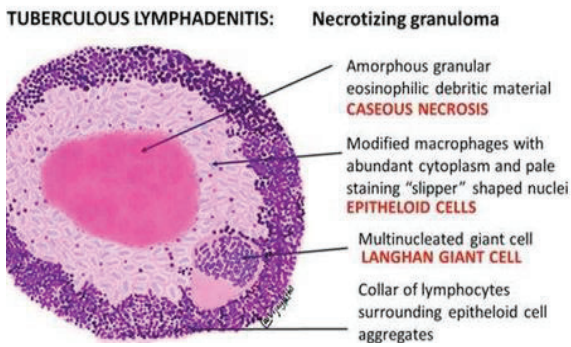


Fig 8.3

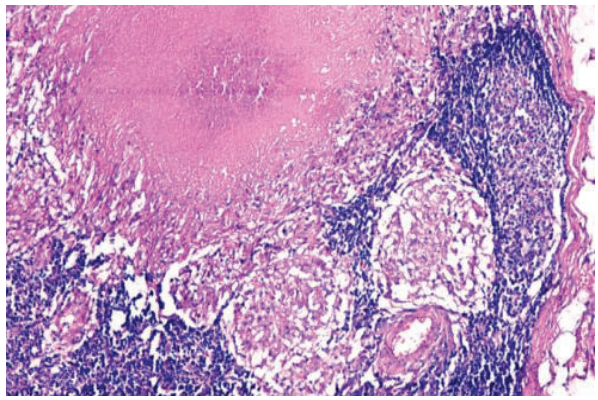


Fig 8.4 H&E x 100

Fig 8.3 and Fig 8.4 shows features of tuberculous inflammation -section above and below shows caseous necrosis at the center of the granuloma.

- Additional Features:
  - Staining: Acid-fast bacilli may be identified with Ziehl-Neelsen stain.

2. Sarcoidosis

- Granulomas:
  - Non-caseating, confluent granulomas, often without central necrosis.9fig 8.5)

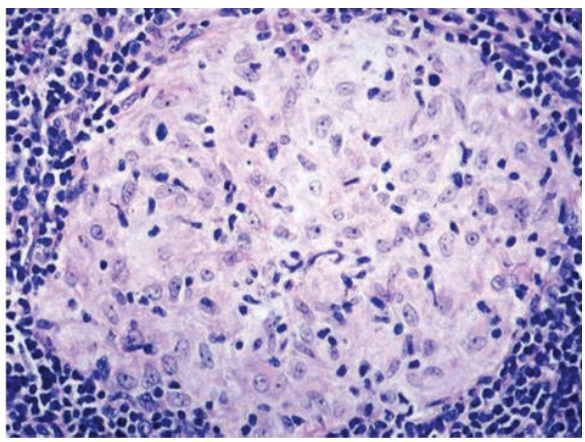


Fig 8.5 shows non necrotic granuloma of sarcoidosis

  - Giant Cells: Schaumann bodies and asteroid bodies (fig8.6) may be present within the granulomas.

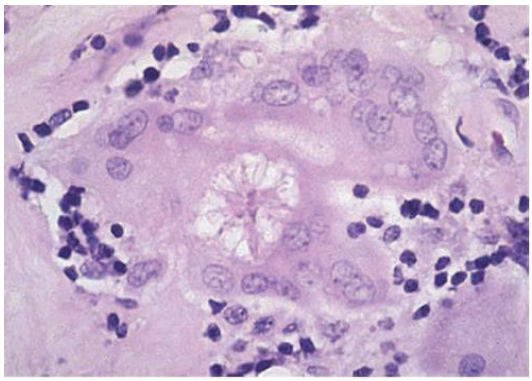


Fig 8.6 : shows asteroid body in the giant cell in a sarcoid granuloma

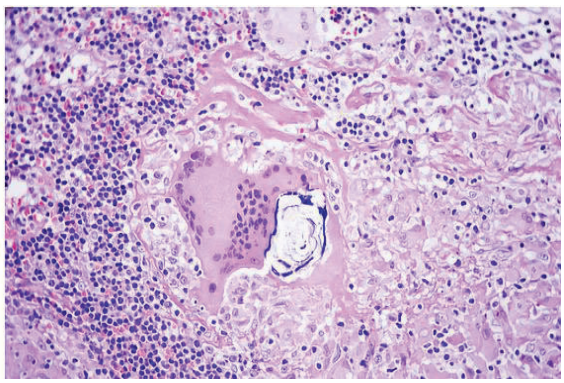


Fig 8.7 shows laminated calcium of Shaumann body within a giant cell in sarcoidosis. Distribution**:**

- Granulomas are typically found in a diffuse pattern, often involving multiple organs.

3. Kikuchi-Fujimoto Disease (Kikuchi's Lymphadenitis)

- Necrosis:
  - Focal, well-circumscribed paracortical necrosis with abundant karyorrhectic debris.
- Cell Types:
  - Plasmacytoid monocytes (activated T cells) are prominent.
  - Minimal neutrophils and plasma cells.
- Additional Features:
  - Often seen in young women, with a history of a painless cervical lymphadenopathy.

4. Toxoplasmosis

- Architecture:
  - Preserved lymph node architecture with follicular hyperplasia.
- Granulomas:
  - Small granulomas within hyperplastic follicles.(fig 8.8)
  - Monocytoid B-cell hyperplasia is commonly observed.

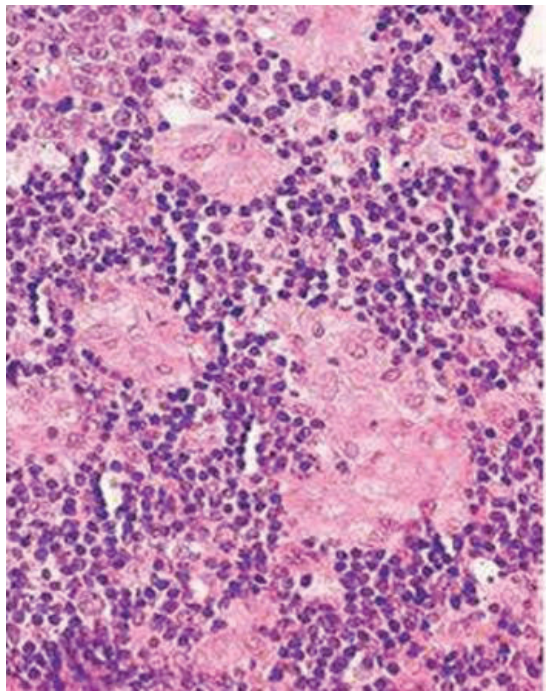


Fig 8.8 : small granulomas of toxoplasmosis.H & E x 100

- Additional Features:
  - Confirmed by serologic studies (IgM immunofluorescence).

5. Fungal Granulomas – Granulomatous inflammation due to fungal infections like caaspergillus (fig 8.9), histoplasmas (fig 8.10) etc

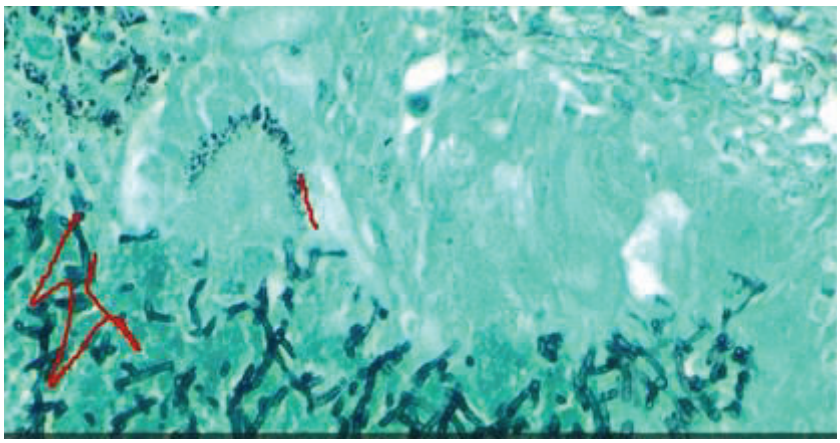


Fig 8.9 Shows a fugal granuloma of aspergillus in the lymph node stained by the GMS stain

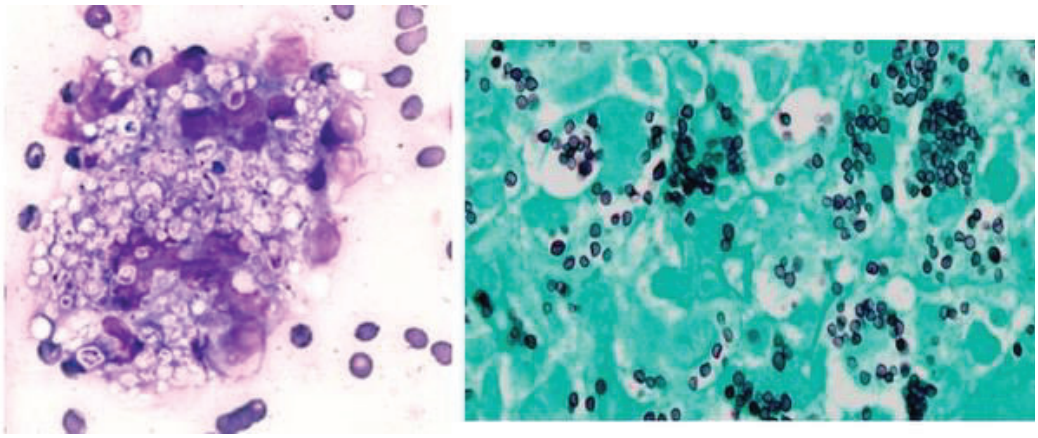


Fig 8. 10 Show a high power view of a macrophage with histoplasma organisms within, the right side shows these organisms stained by the GMS stain.

6. Dermatopathic Lymphadenitis

- Architecture:
  - Preserved with pale widening of the paracortical zone.
- Cell Types:
  - Histiocytes containing phagocytosed melanin or fat.
  - Plasma cell infiltrates and follicular hyperplasia.

7. Sinus Hyperplasia

- Architecture:
  - Dilated sinuses filled with histiocytes.
- Cell Types:
  - Histiocytes positive for S-100 protein.
  - Common in conditions like infection drainage or certain clonal proliferative disorders.

1. **Infections leading to lymphomas : i)** EBV is seen in 100% of endemic Burkitt's lymphoma and in 40% of sporadic and HIV associated cases.

   ii) HHV8/ Kaposi's sarcoma herpes virus (KSHV) in primary effusion lymphoma and lymphoma occurring in multicentric Castleman's disease in HIV infected patients.

   iii) Hepatitis C virus in lymphoplasmacytic lymphoma associated with type II cryoglobulinemia and lymphoma of the liver and salivary glands

   iv) H pylori in Maltomas of GIT.

   v) Borrelia Burgdorferi in the pathogenesis of cutaneous MALT lymphomas.

   vi) HIV (AIDS associated) lymphomas : Diffuse large B-cell lymphomas (plasmablastic lymphomas & primary effusion lymphomas); Burkitt's lymphomas; Hodgkin's lymphoma (classical -mixed cell& lymphocyte depletion types).

8.Secondaries in lymphnodes are a more common cause of lymphadenopathy than primary lymphomas. Metastasis in lymphnodes occurs initially in the subcapsular sinus from which it progresses into the parenchyma.

9.Castleman's disease : morphological distinct form of lymph node hyperplasia . Microscopically two major categoryies are seen :

a) **Hyaline vascular type** or the angiofollicular type :

i) large follicles scattered in a mass of lymphoid tissue.

ii) Follicles are abnormal with central vascular proliferation & hyalinasation of their germinal centers. They are an example of regressively transformed germinal centers.

iii) Large vesicular follicular dendritic cells are seen in the center which are CD21 and CD35 +ve.

iv) An onion – skin pattern is noted due to the concentric layering of the lymphocytes at the periphery corresponding to the mantle zone.

v) interfollicular stroma is prominent with numerous hyperplastic venules, and plasma cells, eosinophils, and immunoblasts. DD : Hassel's corpuscles of thymus & splenic white pulp.

b) **Plasma cell type** :

i) Diffuse plasma cell proliferation in the interfollicular tissue with numerous Russell bodies (due to secretion of interleukin-6)

ii) hyaline vascular changes are inconspicuous

iii) deposition in the center of the follicles of an acidophilic material that contains fibrin & immune complexes.

Clinically Castleman's disease can present as a "solitary mass" or as a "multicentric form". The former occurs commonly in the mediastinum but may be seen in the axilla, lung, mesentry, broad ligament, retroperitoneum, nasopharynx and soft tissue of extremities. The mass may measure upto 15 cm and 90% are of the hyaline vascular type. The other 10% are of the plasma cell type & associated with hypergammaglobulinemia & hypoalbuminemia.

The multicentric form is generally always of the .plasma cell type with generalized lymphadenopathy and splenomegaly. Prognosis over time is poor.

Neoplasms associated with Castleman's disease are : Kaposi's sarcoma, Follicular lymphoma, large cell lymphoma, plasmacytomas, Hodgkin's disease and angiomatous hamartomas. Angiogenic factors secreted by the lymphoid cells result in the vascular proliferation.

10.Stellate abscesses : occur in the following conditions :

i) Cat scratch disease –The primary lesion is a red papule at the site of inoculation 7- 12 days following contact followed by regional lymph node enlargement

- Axillary and cervical lymph nodes are commonly enlarged.
- At microscopy stellate abscesses (fig 8.11) are seen with necrosis and neutrophils, surrounded by palisades of histiocytes. Sinuses are filled with monocytoid cells. Follicular hyperplasia is observed.
- Etiology : cocco-bacillary extracellular bacterium identified by the Warthin- Starry stain.

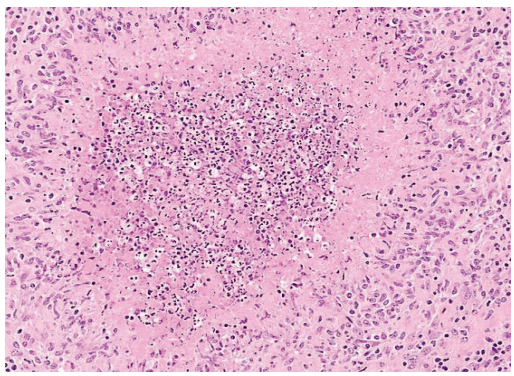


Fig 8.11 shows an abcess with numerous neutrophils in cat-scratch disease.

ii) Lymphogranuloma Venereum (LGV) (fig 8.12)– Sexually transmitted disease caused by the Chlamydia group of organisms serotypes L1, L2, L3.

- initial lesion is small (2-3mm), painless genital vesicle or ulcer and heals in a few days.
- Inguinal lymphadenopathy is prominent
- Microscopy : Early – tiny necrotic foci infiltrated by neutrophils, they enlarge & coalesce to form stellate abscesses which is the characteristic feature of the disease. Later stage – epitheliod cells , scattered Langhan's giant cells & fibroblasts are seen to line the walls.
- Confluence of the abscesses results in cutaneous sinus tract formation.
- LGV should be confirmed by Frei's test (a delayed hypersensitivity skin test using a chlamydia antigen)

iii) Tularemia – bacterial disease caused by Francisella tularensis (a virulent pathogen)due to handling of rabbits.

- Axillary, cervical & inguinal regions are involved.
- Microscopically early stages show widespread (stellate) necrosis , later followed by a granulomatous inflammation.

iv) Atypical mycobacteria – stellate or geographic necrosis. Acid fast stain shows more elongated and coarsely beaded bacilli as compared to the typical mycobacteria.

LYMPHOMAS

General Principles

- The **WHO classification of lymphoid neoplasms** is currently followed and is based on:
  - Morphology
  - Immunophenotype
  - Genetic abnormalities
  - Clinical features
- It includes:
  - Hodgkin lymphomas
  - Non-Hodgkin lymphomas
  - Precursor lymphoid neoplasms
  - Mature B-cell, T-cell and NK-cell neoplasms
  - Certain histiocytic and dendritic cell tumours
- **All lymphoid neoplasms are monoclonal**, arising from a single transformed lymphoid cell.
- During normal B- and T-cell development, **antigen receptor gene rearrangement** occurs:
  - Immunoglobulin genes in B cells
  - T-cell receptor genes in T cells
- Since gene rearrangement precedes malignant transformation, all tumour cells share identical receptor gene configuration, which helps differentiate:
  - Monoclonal neoplasms from
  - Polyclonal reactive lymphoid proliferations

Overview of Commonly Encountered Lymphomas

- Most common B-cell lymphomas:
  - Diffuse large B-cell lymphoma (DLBCL)
  - Small lymphocytic lymphoma / CLL
  - Follicular lymphoma
- Common Hodgkin lymphomas:
  - Nodular sclerosis type
  - Mixed cellularity type

- Less common:
  - Burkitt lymphoma
  - Mantle cell lymphoma
  - Marginal zone lymphomas
  - Nodular lymphocyte-predominant Hodgkin lymphoma
  - Peripheral T-cell lymphomas
- Incidence varies with **geography, race, immune status and viral infections**.

B-CELL LYMPHOMAS – CONCEPTUAL FRAMEWORK

Most B-cell lymphomas resemble **normal stages of B-cell differentiation** (fig 8.12), but WHO classification is **not purely "cell-of-origin" based** and requires clinical correlation.

Understanding normal B-cell maturation helps in interpreting morphology and immunophenotype.

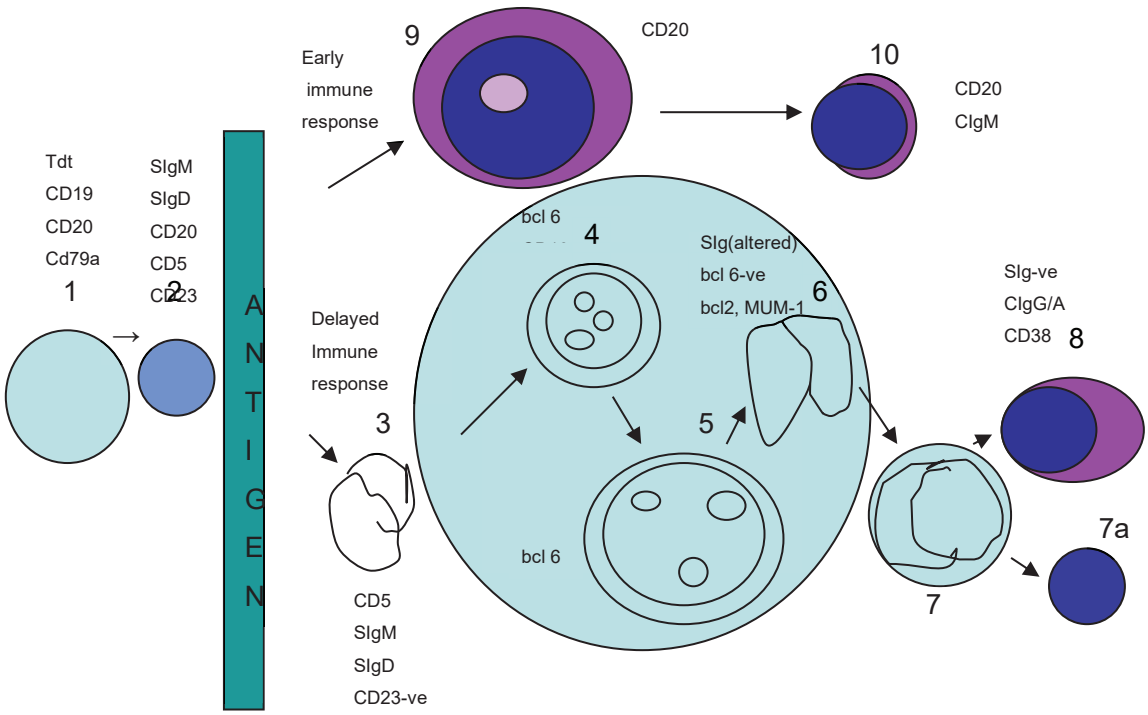


Fig 8.12 Diagrammatic representation of B cell maturation with some important markers

1. Precursor B-cell (Lymphoblast)

Neoplasm

- B-lymphoblastic leukemia / lymphoma

Microscopy

- Sheets of small to medium lymphoblasts

- Scant cytoplasm, fine chromatin
- Inconspicuous nucleoli
- Frequent mitoses
- Starry-sky pattern may be seen

Immunophenotype

- TdT positive
- CD19, CD79a, CD22 positive
- CD10 positive (CALLA) in many cases
- CD20 negative or weak
- Surface immunoglobulin negative

Clinical

- Common in children
- Often presents with leukemia
- Lymphoma preferred term when marrow blasts <20%

2. Naïve B-cell (Mantle Zone / Primary Follicle)

Neoplasms

- Small lymphocytic lymphoma / CLL
- Prolymphocytic leukemia (subset)

Microscopy

- Diffuse sheets of small lymphocytes
- Clumped (“soccer ball”) chromatin
- Scant cytoplasm
- Proliferation centers (pseudofollicles)

Immunophenotype

- CD19, CD20 (dim), CD79a
- CD5 positive
- CD23 positive
- Low-density surface IgM and IgD
- Cyclin D1 negative

Clinical

- Indolent course

- Bone marrow and blood involvement common

3. Mantle Cell (Antigen-exposed Naïve B Cell)

Neoplasm

- Mantle cell lymphoma

Microscopy

- Monomorphic small to medium lymphoid cells
- Irregular / cleaved nuclei
- Diffuse, nodular or mantle zone growth pattern
- Blastoid variant may occur

Immunophenotype

- CD19, CD20, CD79a positive
- CD5 positive
- CD23 negative
- Cyclin D1 positive (hallmark)
- BCL2 positive, BCL6 negative

Genetics

- t(11;14) → Cyclin D1 overexpression

Clinical

- Widespread disease
- Poor prognosis

4–6. Germinal Center B-cells

(Centroblasts → Centrocytes)

Follicular Lymphoma

Definition & Epidemiology

- Neoplasm of follicular center cells (centrocytes and centroblasts)
- ~30% of NHL
- Older adults
- Rare below 20 years
- Usually nodal; cutaneous variant common in skin
- 25–35% transform to DLBCL

Morphology

- Closely packed, uniform follicles
- Loss of mantle zones
- Decreased tingible body macrophages
- Interfollicular tumour infiltration with progression

Cell Morphology

- **Centrocytes:**
  - Small to medium
  - Cleaved nuclei
  - Inconspicuous nucleoli
- **Centroblasts:**
  - Large cells
  - Vesicular nuclei
  - Peripheral nucleoli
- Follicular dendritic cells: CD21, CD23 positive

Growth Pattern

- Follicular (>75%)
- Follicular and diffuse (25–75%)
- Minimally follicular (<25%)

Grading (WHO)

- Grade 1: 0–5 centroblasts/HPF
- Grade 2: 6–15 centroblasts/HPF
- Grade 3:
  - 3a: centroblasts with centrocytes
  - 3b: solid sheets of centroblasts

Genetics & IHC

- **t(14;18) BCL2** in 70–95%
- CD19, CD20, CD22, CD79a positive
- CD10 positive (~60%)
- BCL2 positive (except cutaneous variant)
- BCL6 nuclear positivity

Diffuse Large B-Cell Lymphoma (DLBCL)

Definition

Diffuse large B-cell lymphoma is a neoplasm of large B lymphoid cells growing in a diffuse pattern, in which tumour cells are more than twice the size of a normal lymphocyte or larger than a macrophage nucleus**.**

Epidemiology & Clinical Features

- Most common non-Hodgkin lymphoma
- Constitutes **30–40% of adult NHL** in Western countries
- Broad age range; may occur in children
- Nodal involvement (~60%)
- Extranodal disease (~40%):
    - Skin, CNS, bone, testis, soft tissue, lung, kidney, liver, spleen
- May arise:
    - De novo
    - By transformation of indolent lymphomas:
        - CLL/SLL
        - Follicular lymphoma
        - Marginal zone lymphoma
        - Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL)

Patients typically present with a rapidly enlarging mass, nodal or extranodal. Although disease may appear localized, dissemination is common on staging.

- DLBCL arising in immunodeficiency states is more often EBV-positive than sporadic cases.

Morphology

- Diffuse effacement of nodal architecture
- Sheets of large atypical lymphoid cells
- High mitotic activity and apoptosis
- Necrosis may be present

Morphologic Variants of DLBCL

1. Centroblastic (Most Common)

- Medium to large cells
- Round to oval vesicular nuclei
- Fine chromatin

- **2–4 peripheral or membrane-bound nucleoli**
- Moderate basophilic to amphophilic cytoplasm
- Polymorphism and multilobated nuclei may be seen

2. Immunoblastic Variant

- 90% immunoblasts
- Single, centrally placed prominent nucleolus
- Abundant basophilic cytoplasm
- Centroblasts <10%
- Plasmacytoid differentiation may be present
  DD: Plasmablastic myeloma (extramedullary)

3. T-Cell / Histiocyte-Rich DLBCL

- <10% neoplastic B cells
- Background rich in:
  - Reactive T cells
  - Histiocytes
- Large tumour cells resemble:
  - L&H cells
  - Centroblasts
  - Immunoblasts
  - Reed–Sternberg–like cells
- Diffuse growth pattern helps distinguish from NLPHL

4. Anaplastic Variant

- Very large pleomorphic tumour cells
- Round, oval or polygonal shapes
- Bizarre nuclei; RS-like cells
- Cohesive growth pattern, mimicking carcinoma
- Often CD30 positive

5. Unusual Morphologic Patterns

- Myxoid or fibrillary matrix
- Pseudo-rosettes
- Spindle cells
- Signet-ring cells
- Microvillous projections

- Intercellular junctions

Immunophenotype

- Pan B-cell markers:
  - CD19, CD20, CD22, CD79a (may lack one or more)
- Cytoplasmic Ig: IgM > IgG > IgA
- CD10 positive in ~40%
- CD5 positive in ~10% (Cyclin D1 negative → excludes mantle cell lymphoma)
- CD23 variably expressed
- Anaplastic variant: CD30 positive
- Ki-67 proliferation index: high (often >90%)
- T-cell receptor genes: no rearrangement

Molecular Genetics

- Clonal rearrangement of Ig heavy and light chains
- t(14;18) (BCL2) in ~20–30% (usually transformed FL)
- BCL6 (3q27) abnormalities in up to 30%
- Two major molecular subtypes (cell-of-origin):
  1. Germinal center B-cell–like (GCB)
  2. Activated B-cell–like (ABC)

Cell of Origin

- Peripheral B cell of:
  - Germinal center origin (better prognosis)
  - Post-germinal center origin

Prognosis

- Aggressive but potentially curable
- Outcome predicted by International Prognostic Index (IPI)
- Adverse factors:
  - High Ki-67
  - BCL2 overexpression
  - p53 abnormalities
- **BCL6 rearrangement → better prognosis**
- GCB subtype has superior survival

SPECIAL SUBTYPES OF DLBCL

Mediastinal (Thymic) Large B-Cell Lymphoma

- Arises from thymic B cells
- Young adults; female predominance
- Presents as anterior mediastinal mass
- May cause SVC syndrome
- Morphology:
    - Large cells with pale cytoplasm
    - Compartmentalizing fibrosis
    - Residual thymic tissue
    - Reactive lymphocytes and eosinophils
- IHC:
    - CD19, CD20 positive
    - CD5 and CD10 negative
    - CD30 frequently positive
- May resemble Hodgkin lymphoma

Microscopy

- Diffuse growth pattern
- Large lymphoid cells
- Vesicular nuclei
- Prominent nucleoli
- High mitotic rate

Immunophenotype

- Pan B-cell markers positive
- Variable BCL2, BCL6

Clinical

- Most common NHL
- Aggressive but potentially curable

7. Marginal Zone / Memory B-cell

Microscopy

- Heterogeneous infiltrate
- Centrocyte-like cells

- Monocytoid B cells
- Lymphoepithelial lesions
- Reactive follicles colonized

Immunophenotype

- CD19, CD20, CD79a positive
- CD5, CD10, CD23 negative
- Cyclin D1 negative

Clinical

- Extranodal sites (stomach, salivary gland, thyroid)
- Indolent
- Often associated with chronic inflammation

8–10 Plasma Cell / Plasmacytoid Differentiation

Lymphoplasmacytic Lymphoma / Waldenström Macroglobulinemia

Microscopy

- Small lymphocytes
- Plasmacytoid lymphocytes
- Plasma cells

Immunophenotype

- Cytoplasmic IgM positive
- CD19, CD20 positive
- CD5, CD10, CD23 negative

Clinical

- IgM paraproteinemia
- Hyper viscosity syndrome

KEY PEARLS IN MICROSCOPY

- Architecture (nodular vs diffuse) is critical
- Cytology + Immunophenotype defines subtype
- Cyclin D1 positivity = Mantle cell lymphoma
- BCL2 positivity in follicles = Follicular lymphoma
- TdT positivity = precursor lymphoid neoplasm
- Lymphoepithelial lesions = MALT lymphoma

Other lymphomas : 1.Intravascular Large B-Cell Lymphoma

- Rare extranodal DLBCL
- Tumour cells confined to **small blood vessels**
- Common sites:
  - CNS
  - Skin
  - Lung
  - Adrenals
- Due to lack of adhesion molecules (CD29, CD54)
- IHC: CD19, CD20, CD22, CD79a positive; CD5 variable

2.Primary Effusion Lymphoma (PEL)

- Presents as serous effusions without tumour mass
- Associated with HHV-8 (KSHV)
- Often in immunodeficiency
- Pleural, pericardial, peritoneal cavities
- Poor response to therapy
- Median survival <6 months
- IHC:
  - CD45 positive
  - CD19, CD20, CD79a negative
  - Surface and cytoplasmic Ig negative
  - Plasma cell markers: CD30, CD38, CD138
  - Aberrant CD3 expression may occur
- Associated with multicentric Castleman disease

3.Plasmablastic Lymphoma

- Variant of DLBCL
- Commonly oral cavity
- Strong association with HIV
- EBV positive in ~60%
- IHC:
  - Plasma cell markers (VS38c, CD138)
  - CD20 often negative

4.Burkitt lymphoma

- Highly aggressive lymphoma

- Endemic, sporadic, immunodeficiency-associated
- **Starry-sky pattern**
- Ki-67 ~100% (diagnostic)
- CD10, CD20, CD79a positive
- MYC translocation: t(8;14), t(2;8), t(8;22)
- BCL2 negative; BCL6 positive
- Cell of origin: germinal center B blast

3.Hairy cell leukemia

- Post-germinal center B cell
- CD103, CD11c, CD25 strong positive
- TRAP positive
- CD5, CD23 negative

PLASMA CELL NEOPLASMS (Brief Note)

Plasmacytoma

- Localized plasma cell tumour
- Bone or soft tissue
- Risk of progression to myeloma (bone > soft tissue)

Multiple Myeloma

- Plasma cell infiltrate
- Bone destruction
- Monoclonal protein
- Diagnostic criteria: Durie–Salmon

COMPARATIVE TABLE – COMMON B-CELL LYMPHOMAS (REVISION AID)

Feature	DLBCL	Follicular Lymphoma	Burkitt Lymphoma	Mantle Cell Lymphoma	CLL / SLL
Clinical behaviour	Aggressive, potentially curable	Indolent, relapsing	Highly aggressive	Aggressive	Indolent
Age group	Adults (wide range)	Older adults	Children & young adults	Older males	Elderly
Architecture	Diffuse	Follicular	Diffuse	Diffuse / nodular	Diffuse
Cell size	Large	Small cleaved + large	Medium, monomorphic	Small–medium	Small mature
Nucleoli	Prominent	Inconspicuous	Multiple small	Inconspicuous	Inconspicuous
Starry sky	±	–	**Prominent**	–	–
Ki-67	High (>40–90%)	Low–moderate	**~100%**	Moderate	Low
CD20	+	+	+	+	+ (dim)
CD10	± (40%)	+	+	–	–
BCL2	±	+	–	+	+
BCL6	+	+	+	–	–
CD5	± (10%)	–	–	+	+
CD23	±	–	–	–	+
Cyclin D1	–	–	–	+	–
Key genetics	BCL6, ±BCL2	t(14;18) BCL2	MYC translocation	t(11;14) CCND1	del 13q, trisomy 12

Exam Pearls:

- *CD5 + CD23 = think of CLL*
- *CD5 + Cyclin D1 = think of Mantle cell*
- *CD10 + BCL2 in follicles = Follicular lymphoma*
- *Ki-67 ~100% = Burkitt*

ONE-PAGE IHC ALGORITHM – LYMPHOMA DIAGNOSIS

STEP 1: Is it Lymphoid?

- **CD45 (LCA)**
  - Positive → Lymphoma
  - Negative → Consider carcinoma / sarcoma / melanoma

STEP 2: B-cell or T-cell?

Marker	Interpretation	
CD20 / CD79a / PAX5	B-cell lineage	
CD3 (surface or cytoplasmic)	T-cell lineage	

→ If B-cell, proceed to Step 3

→ If T-cell, defer to T-cell lymphoma section

STEP 3: Low-grade vs High-grade B-cell

Feature	Low-grade	High-grade
Cell size	Small	Large
Ki-67	Low	High
Architecture	Nodular / diffuse	Diffuse
Mitoses	Few	Numerous

STEP 4: Germinal Center Markers

- CD10
- BCL6

Pattern	Suggests
CD10 + / BCL6 +	Follicular lymphoma, Burkitt, GC-type DLBCL
CD10 – / BCL6 –	CLL, Mantle cell, Marginal zone

STEP 5: CD5-Positive B-Cell Lymphomas

CD5	CD23	Cyclin D1	Diagnosis
+	+	–	CLL / SLL
+	–	+	Mantle cell lymphoma
+	–	–	CD5+ DLBCL (exclude MCL)

STEP 6: Proliferation Index (Ki-67)

Ki-67	Diagnosis
~100%	Burkitt lymphoma
High (40–90%)	DLBCL
Low	Indolent lymphomas

STEP 7: Special Situations

- CD30 + → Anaplastic DLBCL, Hodgkin mimic
- CD138 + / CD20 – → Plasmablastic lymphoma
- CD20 – / CD45 + / HHV-8 + → Primary effusion lymphoma

FINAL DIAGNOSTIC INTEGRATION IS A MUST :

Diagnosis = Morphology + IHC + Clinical setting

T-CELL AND NK-CELL LYMPHOMAS

General principles

- T-cell lymphomas are less common, more heterogeneous and biologically aggressive than B-cell lymphomas.
- Classification is broadly into:
  1. Precursor T-cell neoplasms
  2. Mature (peripheral) T-cell lymphomas
  3. NK-cell neoplasms
- **T- cell maturation stages : (**Algorithm 8.13 below **)**

  The stages in the maturation and development of T cells are not as well developed as those in B cell.

  The blast cell committed to a T cell lineage is called the T lymphoblast. It is released from the bone marrow and the various stages of it's maturation are outlined below:

T-lymphoblast (prothymocyte) Acute lymphoblastic
(CD7+ve, Tdt+ve, cyt CD3+ve) leukaemia/lymphoma

↓

(in the thymus) Early thymocyte Acute lymphoblastic
(CD7+ve, Tdt+ve, cyt CD3+ve leukaemia/lymphoma
CD5+ve & or CD2+ve)

↓

(in thymus) Cortical thymocyte (CD7+ve, Tdt+ve, cyt CD3+ve CD5+ve, CD1a +ve) Acute lymphoblastic leukaemia/lymphoma

↓

↓

↓

medullary thymocyte (mature T cell)

(CD7+ve, CD5+ve, cytCD3+ve, CD1a+ve membrane CD3+ve) CD 3 comes to the surface

↓

enters circulation as mature T cell (mature T cell neoplasms)

Algorithm 8.13 (above)

- **Mature T cell (8.14)**

|

90-95% have TCR ά & β chains chains (At first TCR **β** gene rearrangement, later TCR ά gene rearrangement occurs

Location is paracortex of lymph nodes, malpigian corpuscle- splenic pulp

5-10% have TCR for γ & δ

Location is GIT epithelium, respiratory epithelia, splenic red pulp

↓

|

↓

CD4 CD8

At 1st it is double negative then becomes Double positive

+

CD3 molecule

CD4-ve, CD8-ve (primitive type of immune response)

(composed of

γδε polypeptide

chains)

+

2 chains of ζ

(see fig below)

Besides CD3, CD4 &CD3 mols

these cells can also have CD2, CD28

and integrin molecules

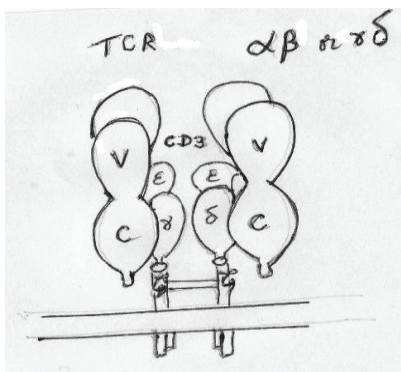


Fig 8.14 : shows alpha and beta gene chains of T cells

CD4 cells : (60% of the TCR άβcells). Function with the help of antigen presenting cells. Related to MHC class II. Based on their cytokine secretion they are divided into 2 groups.

TH1 cells secrete IL-2 and interferon gamma. Function via cell presentation by macrophages.

TH2 cells secrete IL-4, IL-5, IL-13. Help B cells in their function.

CD8 cells : (30% of the TCR άβcells) : have no subsets. Function by direct killing action. They are granzyme +ve, perforin +ve, TIA-1 (T cell intracellular antigen-1) +ve. They are associated with MHC class I.

NK cells have only ε chain of CD3 molecule , they are related to cytotoxic T cells. They are CD2, CD7, CD8, CD56 & CD57+ve. Also perforin, granzymeB and TIA-1+ve .

The mature T cell gives rise to all the T cell lymphomas/laekaemias- these neoplasms show varying positivity for various antigens and molecules outlined above. The precursor T lymphoblast (prothymocyte to cortical thymocyte) gives rise to the acute lymphoblastic leukaemia/lymphoma

I. PRECURSOR T-CELL NEOPLASM

T-lymphoblastic leukaemia / lymphoma (T-LBL)

- Arises from immature thymic T-cell precursors
- Common in children and adolescents
- Classically presents with anterior mediastinal mass
- Bone marrow blasts <25% → lymphoma; ≥25% → leukaemia

Microscopy

- Sheets of small to medium-sized lymphoblasts
- Scant cytoplasm, fine chromatin, inconspicuous nucleoli
- Frequent mitoses; starry-sky pattern may be present

Immunophenotype

- TdT +
- Cytoplasmic CD3 + (most specific T-cell marker)
- CD7 (earliest marker), CD2, CD5, CD1a variably +
- CD99 (MIC2) often +
- May aberrantly express myeloid markers (CD13, CD33)

II. MATURE (PERIPHERAL) T-CELL LYMPHOMAS

Peripheral T-cell lymphoma, NOS (PTCL-NOS)

- Diagnosis of exclusion
- Occurs in older adults
- Presents with generalized lymphadenopathy and extranodal disease

Microscopy

- Diffuse effacement
- Medium to large atypical lymphoid cells
- Marked pleomorphism, frequent mitoses

IHC

- CD3+, CD5+, CD7 often lost
- CD4 or CD8 variable
- High Ki-67

Angioimmunoblastic T-cell lymphoma (AITL)

- Systemic disease with immune dysregulation
- Associated with rash, hepatosplenomegaly, polyclonal hyper gamma globulinaemia

Microscopy (triad)

1. Polymorphous infiltrate (T cells, plasma cells, eosinophils, B immunoblasts)
2. Prominent high endothelial venules
3. Expanded follicular dendritic cell meshwork

Cell of origin

- T-follicular helper (TFH) cell

IHC

- CD3+, CD4+
- TFH markers: CD10, BCL6, PD-1, CXCL13
- CD21 highlights expanded FDC network

Adult T-cell leukaemia / lymphoma (ATLL)

- Caused by **HTLV-1**
- Endemic areas: Japan, Caribbean
- Hypercalcaemia common

Microscopy

- Highly pleomorphic cells
- Characteristic **"**flower cells**"** in blood

IHC

- CD3+, CD4+, CD25+
- CD7 often lost
- ALK negative

Prognosis

- Poor (acute and lymphomatous forms)

Anaplastic large cell lymphoma (ALCL)

ALK-positive ALCL

- Younger patients
- Better prognosis

ALK-negative ALCL

- Older adults
- More aggressive

Microscopy

- Large pleomorphic cells
- Hallmark cells with kidney-shaped or horseshoe nuclei
- Sinusoidal growth pattern common

IHC

- CD30 strong and diffuse
- ALK ±
- EMA +
- Often CD3 negative
- Cytotoxic markers positive

III. EXTRANODAL T-CELL / NK-CELL LYMPHOMAS

Extranodal NK/T-cell lymphoma, nasal type

- Strongly associated with **EBV**
- Angiocentric and angiodestructive growth

Microscopy

- Coagulative necrosis
- Vessel destruction
- Polymorphous infiltrate

IHC

- CD2+, cytoplasmic CD3ε+
- CD56+
- Surface CD3 −
- Granzyme B, TIA-1, perforin +
- EBV positive (EBER)

Enteropathy-associated T-cell lymphoma (EATL)

- Associated with celiac disease
- Jejunum and ileum commonly involved

Microscopy

- Medium to large pleomorphic cells
- Necrosis and inflammation

IHC

- CD3+, CD7+
- CD5−, CD4−
- CD8 variable
- CD103 +

Hepatosplenic T-cell lymphoma

- Young males
- Marked hepatosplenomegaly

Cell of origin

- γδ T-cells

IHC

- CD3+
- CD4−, CD8−
- Cytotoxic markers +

Subcutaneous panniculitis-like T-cell lymphoma

- Lobular panniculitis pattern
- Cytotoxic αβ or γδ T-cell origin

IV. CUTANEOUS T-CELL LYMPHOMAS

Mycosis fungoides

- Epidermotropic small to medium T cells
- Cerebriform nuclei
- Pautrier microabscesses

IHC

- CD3+, CD4+, CD5+
- CD8−

Sézary syndrome

- Leukemic variant of MF

- Erythroderma + circulating cerebriform cells

HODGKIN'S LYMPHOMA

1. Reed–Sternberg (RS) Cell and Variants (High-yield Table)

Cell type	Morphology	Seen in
Classic RS cell	Large (20–50 μ), binucleate/bilobed, thick nuclear membrane, vesicular chromatin, large eosinophilic nucleolus with halo ("owl-eye")	All subtypes of classical HL
Mononuclear variant (Hodgkin cell)	Single nucleus with RS-like nucleolus	All subtypes (not diagnostic alone)
Lacunar cell	Multilobated nucleus, cytoplasmic retraction → pericellular lacuna (formalin fixation artefact)	Nodular sclerosis CHL
Pleomorphic / anaplastic RS	Giant, bizarre, hyperchromatic nuclei	Lymphocyte depletion CHL
Mummified (necrobiotic)	Shrunken, pyknotic, apoptotic-appearing nucleus	All subtypes
Popcorn cell (L&H cell)	Multilobated "popcorn" nucleus, small nucleoli	NLPHL only

Minimum diagnostic requirement: A bilobed nucleus with at least one prominent eosinophilic nucleolus.

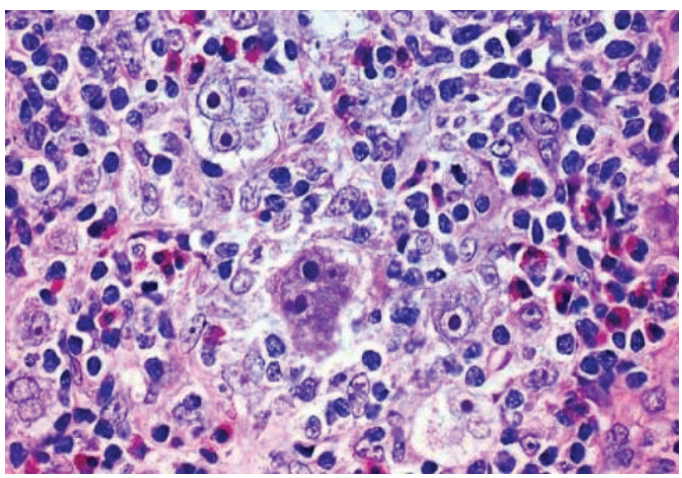


Fig 8.15 : Hodgkin's Lymphoma with binucleate R-S cells H7E x 400

2. Immunophenotype of Reed–Sternberg Cell

Marker	Expression
CD30	+ in ~90% (membranous + Golgi)
CD15	+ in 75–85% (paranuclear/diffuse cytoplasmic)
PAX5	Weak +
CD20	Weak/variable (10–20%)
CD45 (LCA)	Negative
EMA	Negative
EBV (LMP1 / EBER)	+ in many cases (esp. MC & LD types)

3. Classification (WHO-aligned)

A. Classical Hodgkin's Lymphoma (CHL) (Fig 8.15)

1. Nodular sclerosis
2. Mixed cellularity
3. Lymphocyte-rich
4. Lymphocyte depletion

B. Nodular Lymphocyte Predominance HL (NLPHL)

4. Classical Hodgkin's Lymphoma – Subtypes

Subtype	Key morphology	Background	EBV
Nodular sclerosis	Lacunar RS cells, broad collagen bands, nodular pattern	Lymphocytes, eosinophils	±
Mixed cellularity	Numerous classic RS cells	Lymphocytes, eosinophils, plasma cells	+ common
Lymphocyte-rich	Scattered RS cells	Predominantly small lymphocytes	Rare
Lymphocyte depletion	Numerous pleomorphic RS cells, necrosis	Sparse lymphocytes	+ common

Most common subtype overall: Nodular sclerosis
Common in developing countries / HIV: Mixed cellularity

5. Nodular Lymphocyte Predominance HL (NLPHL)

Morphology

- Nodular growth pattern
- Absence of classic RS cells
- Presence of L&H (popcorn) cells
- Background: small B lymphocytes
- PTGC may be present

Immunophenotype (Diagnostic)

- CD20, CD79a, PAX5: +
- CD30, CD15: −
- EMA: + (≈50%)
- EBV: −

Cell of origin: Germinal center B cell
Clinical course: Indolent, excellent prognosis

6. CHL vs NLPHL – Comparative Table

Feature	CHL	NLPHL
Frequency	~95%	~5%
Architecture	Diffuse / nodular	Nodular
Tumour cell	Classic RS	L&H (popcorn)
CD30	+	−
CD15	+	−
CD20	Weak / variable	Strong +
EBV	Often +	−
PTGC	−	+
Prognosis	Variable	Excellent

7. Diagnostic Algorithm

Step 1: Large atypical cells present?
↓
Step 2: CD30 positive?

- **Yes** → Think CHL or ALCL
- **No** → Consider NLPHL or non-HL

↓

Step 3: CD15 positive, CD45 negative, PAX5 weak?

- **Yes → CHL**

↓

Step 4: Nodular architecture + CD20 strong + CD30 negative?

- **Yes → NLPHL**

A V LYMPH NODES SYNOPTIC REPORTING

1. Lymph Nodes Synoptic Reporting

A. Reactive/Benign Lymph Nodes

- Specimen: Lymph node (site: _____)
- **Architecture:**
  - ☐ Preserved
  - ☐ Follicular hyperplasia
  - ☐ Sinus histiocytosis
- **Background:**
  - ☐ Polymorphous cell population (lymphocytes, histiocytes)
  - ☐ No significant atypia
- **Final Diagnosis:**
  - ☐ Reactive lymphoid hyperplasia
- **Comments:**
  - Correlate clinically for infection or autoimmune etiology.

B. Granulomatous Lymphadenitis

- **Specimen:** Lymph node (site: _____)
- **Architecture:**
  - ☐ Preserved
  - ☐ Granulomas present
- **Granuloma Type:**
  - ☐ Caseating (e.g., tuberculosis)
  - ☐ Non-caseating (e.g., sarcoidosis)
- **Background:**
  - ☐ Epithelioid histiocytes
  - ☐ Langhans giant cells

- **Final Diagnosis:**
  - ☐ Granulomatous lymphadenitis (Specify etiology: _____)
- **Comments:**
  - Consider further tests (e.g., AFB staining, special stains).

Detailed reports for specific conditions:

1. Tuberculosis

- **Specimen:** Lymph node (site: _____)
- **Architecture:**
  - ☐ Granulomas present
  - ☐ Caseous necrosis
- **Granuloma Features:**
  - ☐ Epithelioid cells
  - ☐ Langhans giant cells
- **Final Diagnosis:**
  - ☐ Tuberculous lymphadenitis
- **Comments:**
  - Consider AFB staining and molecular tests for confirmation.

2. Sarcoidosis

- **Specimen:** Lymph node (site: _____)
- **Architecture:**
  - ☐ Non-caseating granulomas
  - ☐ Confluent granulomas
- **Granuloma Features:**
  - ☐ Schaumann bodies
  - ☐ Asteroid bodies
- **Final Diagnosis:**
  - ☐ Sarcoid lymphadenitis
- **Comments:**
  - Evaluate for systemic involvement (lungs, skin, liver).

3. Fungal Infections (e.g., Histoplasmosis)

- **Specimen:** Lymph node (site: _____)
- **Architecture:**

- ☐ Granulomas present
- ☐ Fungal organisms (special stains positive)

- **Granuloma Features:**
  - ☐ Epithelioid cells
  - ☐ Possible yeast forms on PAS/GMS staining
- **Final Diagnosis:**
  - ☐ Fungal lymphadenitis (Specify organism: _____)
- **Comments:**
  - Confirm with culture and further molecular testing.

4. Kikuchi-Fujimoto Disease (Kikuchi's Lymphadenitis)

- **Specimen:** Lymph node (site: _____)
- **Architecture:**
  - ☐ Paracortical necrosis
  - ☐ Abundant karyorrhectic debris
- **Microscopic Features:**
  - ☐ Plasmacytoid monocytes
  - ☐ Minimal neutrophils and plasma cells
- **Final Diagnosis:**
  - ☐ Kikuchi-Fujimoto disease
- **Comments:**
  - Clinical correlation important; typically self-limiting.

5. Toxoplasmosis

- **Specimen:** Lymph node (site: _____)
- **Architecture:**
  - ☐ Preserved architecture
  - ☐ Follicular hyperplasia
- **Granuloma Features:**
  - ☐ Small granulomas in hyperplastic follicles
  - ☐ Monocytoid B-cell hyperplasia
- **Final Diagnosis:**
  - ☐ Toxoplasmic lymphadenitis

- **Comments:**

- Confirm with serology (IgM immunofluorescence).

6. Dermatopathic Lymphadenitis

- **Specimen:** Lymph node (site: _____)
- **Architecture:**
  - ☐ Preserved architecture
  - ☐ Pale widening of paracortical zone
- **Microscopic Features:**
  - ☐ Histiocytes with melanin or fat
  - ☐ Plasma cell infiltrate
- **Final Diagnosis:**
  - ☐ Dermatopathic lymphadenitis
- **Comments:**
  - Often associated with chronic dermatitis (e.g., psoriasis, mycosis fungoides).

7. Sinus Hyperplasia

- **Specimen:** Lymph node (site: _____)
- **Architecture:**
  - ☐ Dilated sinuses
  - ☐ Histiocytes present
- **Microscopic Features:**
  - ☐ S-100 positive histiocytes

C. Lymphoma

- **Specimen:** Lymph node (site: _____)
- **Architecture:**
  - ☐ Effaced architecture
  - ☐ Monomorphic cell population
- **Tumour Cells:**
  - ☐ Reed–Sternberg cells (if CHL)
  - ☐ Large B/T cells (if non-Hodgkin lymphoma)
- **Immunohistochemistry:**
  - CHL: CD30+, CD15+, PAX5 weak
  - B-cell lymphoma: CD20+, CD79a+
  - T-cell lymphoma**:** CD3+, CD5+

- **Final Diagnosis:**
  - ☐ Hodgkin's lymphoma (Subtype: _____)
  - ☐ B-cell lymphoma (Subtype: _____)
  - ☐ T-cell lymphoma (Subtype: _____)
- **Comments:**
  - Correlate with clinical staging.

Synoptic detailed reporting on Lymphomas Report: Hodgkin's Lymphoma (Lymph Node Biopsy)

Specimen

- ☐ Lymph node (site: _____)
- ☐ Excision / Core biopsy

Architecture

- ☐ Preserved
- ☐ Partially effaced
- ☐ Completely effaced
- Pattern: ☐ Nodular ☐ Diffuse

Tumour Cells

- Reed–Sternberg cells: ☐ Present ☐ Frequent ☐ Scattered
- Variant identified: ☐ Classic ☐ Lacunar ☐ Pleomorphic ☐ Mononuclear

Background Infiltrate

- ☐ Lymphocytes
- ☐ Eosinophils
- ☐ Plasma cells
- ☐ Fibrosis / sclerosis
- ☐ Necrosis

Immunohistochemistry

- CD30: ☐ + ☐ −
- CD15: ☐ + ☐ −
- CD45: ☐ + ☐ −
- CD20: ☐ + ☐ −

- PAX5: ☐ Weak + ☐ −
- EBV (LMP1 / EBER): ☐ + ☐ − ☐ Not done

Final Diagnosis

- ☐ Classical Hodgkin's Lymphoma
  Subtype: _____

Comment (optional)

- Features are consistent with classical Hodgkin's lymphoma. Clinical and radiologic staging correlation advised.

Separate Synoptic Report for : NLPHL

Final Diagnosis

- Nodular Lymphocyte Predominance Hodgkin's Lymphoma

Supporting Features

- ☐ Nodular architecture
- ☐ L&H (popcorn) cells
- ☐ CD20 / PAX5 positive
- ☐ CD30 / CD15 negative

Common Pitfalls

CHL vs ALCL → CD45 −, PAX5 weak in CHL

- NLPHL vs Follicular lymphoma → L&H cells, PTGC, CD30 −
- Lymphocyte-rich CHL vs NLPHL → Immunophenotype decides

T and B-Cell Lymphomas Synoptic Reporting

A. B-cell Lymphomas

- **Specimen:** Lymph node (site: _____)
- **Architecture:**
  - ☐ Effaced architecture
  - ☐ Monomorphic B-cell population
- **Immunohistochemistry:**
  - CD20: +
  - CD79a: +
  - BCL6, CD10, BCL2: (as subtype dictates)

- **Final Diagnosis:**
  - ☐ Diffuse large B-cell lymphoma (DLBCL)
  - ☐ Follicular lymphoma (Grade: _____)
  - ☐ Mantle cell lymphoma (MCL)
- **Comments:**
  - Correlate with clinical staging and molecular findings.

B. T-cell Lymphomas

- **Specimen:** Lymph node (site: _____)
- **Architecture:**
  - ☐ Effaced architecture
  - ☐ Monomorphic T-cell population
- **Immunohistochemistry:**
  - CD3: +
  - CD5, CD7: (as subtype dictates)
  - CD30, ALK: (for ALCL)
- **Final Diagnosis:**
  - ☐ Peripheral T-cell lymphoma (NOS)
  - ☐ Anaplastic large cell lymphoma (ALCL)
  - ☐ T-cell lymphoblastic lymphoma (T-LBL)
- **Comments:**
  - Correlate with clinical staging and molecular findings.
  - Stage of lymphoma

D. Metastatic Disease in Lymphnode

- **Specimen:** Lymph node (site: _____)
- **Architecture:**
  - ☐ Effaced by metastatic cells
  - ☐ Disruption of normal lymph node architecture
- **Tumour Cells:**
  - ☐ Adenocarcinoma cells
  - ☐ Squamous cell carcinoma cells
  - ☐ Other (Specify: _____)
- **Immunohistochemistry:**
  - Cytokeratin: + (if epithelial origin)

- TTF-1, PSA, etc.: (as appropriate)

- **Final Diagnosis:**
  - ☐ Metastatic carcinoma (Primary site: _____)
- **Comments:**
  - Correlate with known primary malignancy.

Pathologist's signature……. Date ……..

The Lugano Classification (2014)

The Lugano system is the most recent refinement of lymphoma staging, building on the Ann Arbor system:

Stage	Definition
Stage I	Involvement of a single lymph node region or a single extralymphatic site (IE).
Stage II	Two or more lymph node regions on the same side of the diaphragm, or localized involvement of an extralymphatic site with regional nodes (IIE).
Stage III	Lymph node involvement on both sides of the diaphragm, possibly with spleen involvement.
Stage IV	Diffuse or disseminated involvement of one or more extralymphatic organs (e.g., bone marrow, liver, lung).

Modifiers:

- **A/B**: Absence/presence of systemic symptoms (fever, night sweats, weight loss).
- **X**: Bulky disease (mass >10 cm or mediastinal mass >1/3 chest diameter).
- **E**: Extranodal involvement.

Key Updates in Lugano vs Ann Arbor

- **PET-CT is central** for staging and response assessment.
- **Bulky disease** is defined more precisely.
- **Bone marrow biopsy** is no longer routine if PET-CT is available.
- **Response criteria** (complete metabolic response, partial response, progressive disease) are integrated into staging.

Clinical Implications

- For **Hodgkin lymphoma**, staging guides whether combined chemo-radiotherapy or chemotherapy alone is used.
- For **Non-Hodgkin lymphoma**, staging helps determine intensity of therapy but is less prognostic than histology and molecular subtype.

- TNM staging remains irrelevant; instead, biological markers (e.g., cell of origin, genetic mutations) are increasingly important for prognosis.

B I SPLEEN ANATOMY

- **Weight/Size**: (Normal ~150 g); record actual.
- **Capsule**: Smooth vs thickened, ruptured, or adherent.
- **Cut Surface**: Red pulp (dark red) and white pulp (gray-white nodules).
- **Lesions**: Infarcts, congestion, nodules, or focal masses.
- **Hilum**: Note vessels, adhesions, or hilar masses.

B II SPLEEN GROSSING TECHNIQUES

- Weight and measurements
- Record parenchymal pattern
- Note nodularity, necrosis, infarcts, or cysts.

Indications for splenectomy

- Hemolytic anemias / thrombocytopenia
- Trauma
- Incidental (e.g., gastrectomy)
- Portal hypertension
- Staging for lymphomas

Splenectomy – Procedure

1. Weigh and measure the spleen.
2. Sectioning
    - First cut along the greatest dimension through the hilum.
    - Serially slice at 1 cm intervals.
3. Examination
    - Inspect each slice carefully for focal lesions.
    - Dab excess blood with moist gauze (do not wash).
    - Always measure, weigh, and describe capsule, parenchyma, and hilum
4. Identify
    - Hilar lymph nodes
    - Accessory spleens

5. Special procedures
    - Culture in inflammatory conditions (if required)

- Imprint smears in hematologic disorders
- In sickle cell disease, sample from interior and fix immediately.

6. Describe
    - Capsule (thickening, rupture, adhesions)
    - Cut surface (color, consistency, bulging)
    - Fibrous trabeculae
    - Nodules, infarcts, calcification (Gamna–Gandy bodies)

BITS (Fig 8.16)

- Incidental splenectomy:
    - One capsular section
    - One hilar section
- Ruptured spleen:
    - Capsule
    - Hilum
    - Tear
- Diseased spleen:
    - Hilum
    - Two capsular sections

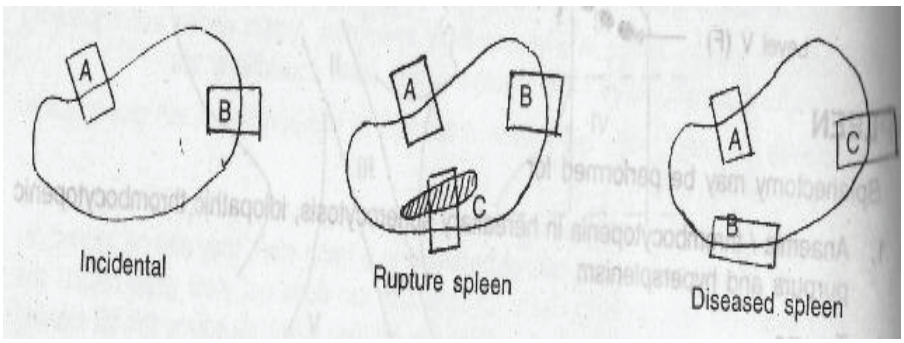


Fig8.16 showing bits being taken for various conditions in the spleen

Splenectomy for Lymphoma Staging

- Grossly normal spleen: 8 random blocks
- Enlarged but unremarkable spleen: up to 10 blocks
- Grossly involved spleen: Representative blocks from lesions

Always submit:

- Hilar lymph nodes
- Liver biopsy
- Bone marrow biopsy (if sent)

B III SPLEEN GROSS PEARLS

- **Nodules / masses in spleen**
  → Lymphoma
  → Mastocytosis
- **Micronodules in spleen**
  → Lymphoma (especially small lymphocytic lymphoma)
  → *Sago spleen* in amyloidosis
- **Splenomegaly**
  - Typhoid fever
  - Tropical splenomegaly (often massive)
  - Langerhans cell histiocytosis
  - Hairy cell leukemia (classically massive)
  - *Lardaceous spleen* in amyloidosis
- Dark red, moderately firm, enlarged spleen with **hemorrhagic areas**
  → Myelofibrosis
- **Haemorrhagic lesions of spleen**
  → Hemangioma
  → Angiomatosis
  → Littoral cell angioma
  → Angiosarcoma

B IV SPLEEN MICROSCOPY

Microscopy of the Spleen

- **Thrombocytopenia-related changes**
  - Splenomegaly is common in thrombocytopenia, with a soft, congested spleen.
  - Congested red pulp with increased phagocytosis of platelets by splenic macrophages.
  - Expansion of splenic cords due to heightened reticuloendothelial activity.
  - Sometimes associated with follicular hyperplasia in the white pulp.

- **Splenic infarcts**

- Infarcts appear as pale, wedge-shaped, firm areas beneath the capsule and show coagulative necrosis at microscopy with preserved outlines early, progressing to fibrosis and scarring
- Chronic insults may leave fibrotic scars or calcified nodules
- Early lesions show pale eosinophilic necrotic tissue with preserved architecture.
- Later, infarcts become fibrotic and may calcify, leaving firm, scarred areas.
- Surrounding tissue often shows reactive hyperemia and inflammatory infiltrates.

- **General background changes**
  - In chronic conditions, the spleen may show fibrosis of trabeculae and capsule.
  - Hemosiderin-laden macrophages can be present due to repeated red cell breakdown.
  - In severe or repeated insults, architectural distortion with loss of normal white/red pulp distinction.
- **Correlation**
  - The pale wedge-shaped infarcts seen grossly correspond to areas of coagulative necrosis microscopically.
  - Splenomegaly grossly reflects the microscopic congestion and hyperplasia of the red pulp.
  - Fibrotic scars grossly match the healed infarcts with collagen deposition microscopically.

Clinical Relevance – Spleen

- **Thrombocytopenia**: Splenic sequestration of platelets contributes to low counts, which can manifest clinically as easy bruising, mucosal bleeding, or petechiae. Recognition of splenic involvement helps explain persistent thrombocytopenia despite adequate marrow production.
- **Splenic infarcts**: Often present with left upper quadrant pain and tenderness. They may complicate hematologic disorders (e.g., sickle cell disease, myeloproliferative states) or embolic phenomena. Chronic infarcts can lead to scarring and functional hyposplenism, predisposing to infections by encapsulated organisms.
- **Integration with lymphomas**: While lymphomas primarily involve lymph nodes, splenic pathology adds to systemic disease burden and can influence prognosis and therapeutic decisions. Thrombocytopenia-related changes and infarcts in the spleen not only contribute to systemic picture of hematologic disease but also highlight the spleen's role as both a diagnostic and clinically significant organ in hematopathology."

B V SPLEEN SYNOPTIC REPORTING

Specimen : Splenectomy / Partial splenectomy / Wedge biopsy

Indication for Splenectomy (if provided)

- Hypersplenism
- Hematologic disorder (e.g. ITP, hemolytic anemia, leukemia/lymphoma)
- Splenic mass / Nodules
- Trauma
- Other (specify)

Gross Examination

- **Weight:** ____ g (Normal adult: ~150–200 g)
- **Size:** ____ × ____ × ____ cm
- **Capsule:** Intact / Thickened / Ruptured
- **Cut Surface:**
  - Congested
  - Firm / Soft
  - Nodules present (number, size, appearance)
  - Infarcts / Hemorrhage
  - Focal lesions (specify)

Microscopic Findings

- **Architecture:** Preserved / Distorted
- **Red Pulp:**
  - Congestion
  - Expansion by histiocytes / fibrosis
  - Infiltration by hematolymphoid cells
- **White Pulp:**
  - Hyperplasia
  - Atrophy
  - Replacement by neoplastic cells
- **Specific Findings (if present):**
  - Granulomas
  - Amyloid deposition
  - Extramedullary hematopoiesis
  - Vascular lesions
  - Neoplastic infiltration

Ancillary Studies (if performed)

- Special stains (e.g. Congo red)

- Immunohistochemistry
- Flow cytometry
- Molecular studies

Final Diagnosis

- Reactive / Congestive splenomegaly
- Specific hematologic disorder (specify)
- Neoplastic involvement (primary / secondary)
- Other (specify)

Comment (optional)

- Correlation with clinical and hematologic findings advised.

Signature …….

C I THYMUS ANATOMY

- **Location**: Anterior mediastinum, bilobed organ, usually in the pre-vascular compartment.
- **Capsule & Lobules**: Thin capsule divides into lobules with cortex (lymphocyte-rich) and medulla (lighter, Hassall's corpuscles). Unifocal or multifocal.
- **Age-related changes**: Prominent in children, involutes with age, replaced by fat.
    - In young patients: soft, pink-gray tissue.
    - In adults: fatty tissue with residual islands of thymic tissue.

C II THYMUS GROSSING TECHNIQUES

Specimens received

- Needle biopsy
- Thymectomy

Needle Biopsy

- Measure and submit **entirely**.

Thymectomy Specimen

1. Record weight and dimensions.
2. Serial sectioning
    - 1 cm thick slices
    - First cut along greatest dimension.
3. Diffuse enlargement, lobulated, soft
   → Thymic hyperplasia

Look for perithymic lymph nodes, and presence of nodules, cysts, or masses (Thymoma) .

1. If neoplasm present, record
    - Size, shape
    - Capsular integrity
    - Necrosis, hemorrhage
    - Fibrosis, calcification
    - Cystic change
    - Thymoma; Necrosis rare in low-grade tumors, may be present in high-grade types
2. Firm, infiltrative mass with invasion of adjacent structures
   → Thymic carcinoma
3. Cystic changes
   → Thymic cyst or Cystic thymoma
4. Examine attached structures
    - Lung, pleura, pericardium, lymph nodes
5. Bits – shown in fig 8. 17.

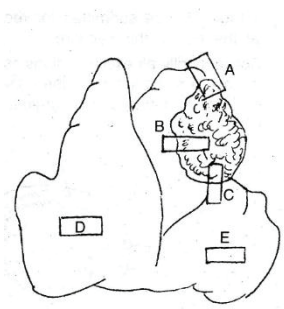


Fig 8.17 Shows grossing of thymic tumour.

- Tumor with capsule – 3 blocks
- Uninvolved thymus – 2 blocks
- Other attached structures – as indicated

C IV THYMUS MICROSCOPY

THYMOMA

Thymomas are primary epithelial neoplasms of the thymus, characterized by variable admixture of neoplastic thymic epithelial cells and non-neoplastic immature T lymphocytes. They show a wide spectrum of morphology, biological behaviour, and clinical associations, particularly myasthenia gravis.

CLASSIFICATION OF THYMOMAS

I. Historical Classifications

1. Müller–Hermelink Classification Based on resemblance to normal thymic architecture:

1. Medullary thymoma
2. Mixed thymoma
3. Predominantly cortical (organoid) thymoma
4. Cortical thymoma
5. Well-differentiated thymic carcinoma

This classification emphasized histogenetic origin but had limited reproducibility.

2. Lattes and Bernatz Classification (1962)

Based on relative proportions of epithelial cells and lymphocytes:

1. Predominantly spindle cell thymoma
2. Predominantly lymphocytic thymoma
3. Predominantly epithelial thymoma
4. Predominantly mixed thymoma

The above are useful historically, but lacked correlation with prognosis.

II. WHO Classification of Thymic Epithelial Tumours

The **WHO classification (1999 onwards, refined in later editions**) integrates morphology with clinical behavior and now remains the standard classification.

Thymomas are categorized based on the shape of neoplastic epithelial cells, nuclear features, and relative lymphocyte content.

WHO CLASSIFICATION OF THYMOMAS

1. Thymomas

Type A Thymoma

(Spindle cell / Medullary thymoma)

- Composed of spindle or oval epithelial cells
- No or minimal nuclear atypia
- Scant or absent lymphocytes
- Architectural patterns:
    - Fascicular or storiform
    - Rosette-like formations (without lumen)
    - Gland-like spaces

- Generally resembles a mesenchymal tumor
- Rare findings: focal pleomorphism, mitoses, necrosis
- Excellent prognosis

Type AB Thymoma

(Mixed thymoma)

- **Most common subtype**
- Mixture of:
  - Type A–like spindle cell areas
  - Lymphocyte-rich areas resembling Type B thymoma
- Transition between areas may be sharp or indistinct
- Type A component may be scant
- Prognosis similar to Type A

Type B Thymomas

Characterized by increasing epithelial prominence and decreasing resemblance to normal thymus.

Type B1 Thymoma

(Lymphocyte-rich / Predominantly cortical / Organoid thymoma)

- Closely resembles normal functional thymus
- Prominent cortex-like areas with scattered epithelial cells
- Medullary-like areas may be present
- Lymphocytes predominate
- Epithelial cells are inconspicuous
- Excellent prognosis

Type B2 Thymoma

(Cortical thymoma)

- Increased number of plump epithelial cells
- Epithelial cells:
  - Vesicular nuclei
  - Distinct nucleoli
  - Oval to polygonal shape
- Dense background of lymphocytes (less than B1)
- Perivascular spaces are prominent and characteristic
- Intermediate prognosis

Type B3 Thymoma

(Epithelial / Atypical / Squamoid thymoma; Well-differentiated thymic carcinoma)

- Predominantly epithelial cells in sheets
- Mild to moderate cytologic atypia
- Sparse lymphocytes
- Squamoid appearance common
- Represents the borderline between thymoma and thymic carcinoma
- Less favorable prognosis among thymomas

2. Thymic Carcinoma

(Formerly Type C thymoma)

- Exhibits overt cytologic malignancy
- Lacks thymic organoid features
- Histology resembles carcinomas of other organs
- Common subtypes:
  - Squamous cell carcinoma
  - Lymphoepithelioma-like carcinoma
  - Sarcomatoid carcinoma
  - Spindle cell carcinoma
- Aggressive clinical course

3. Borderline Thymic Epithelial Tumours

- Tumours with features intermediate between **thymoma and thymic carcinoma**
- Difficult to classify unequivocally
- Require correlation with invasion and clinical behavior

C V THYMOMA SYNOPTIC REPORTING

Thymoma

- **Specimen:** Thymic tissue (site: _____)
- **Gross Findings:**
  - ☐ Encapsulated
  - ☐ Lobulated
  - ☐ Cystic areas present (if any)

- **Microscopy:**
  - Type A: Spindle epithelial cells, few lymphocytes
  - Type AB: Mixed areas of type A and lymphocyte-rich zones
  - Type B1: Resembles normal thymic cortex
  - Type B2: Cortical thymoma with abundant lymphocytes
  - Type B3: Predominantly epithelial cells, mild atypia
- **Immunohistochemistry:**
  - Epithelial cells: Cytokeratin, p63+
  - Thymocytes: TdT+, CD1a+, CD3+
- **Final Diagnosis:**
  - ☐ Thymoma (Type: _____)
- **Comments:**
  - Correlate with clinical symptoms (e.g., myasthenia gravis).

Signature…… Date……..

CLINICAL STAGING OF THYMOMA

Modified Masaoka Staging System (1981, 1994)

Stage	Description
Stage I	Completely encapsulated tumor; no capsular invasion (T1)
Stage II	Microscopic transcapsular invasion **or** macroscopic invasion into thymus or mediastinal fat, or adherence to pleura/pericardium without penetration (T2)
Stage III	Macroscopic invasion into adjacent organs (lung, pericardium, great vessels) (T3)
Stage Iva	Pleural or pericardial dissemination
Stage IVb	Lymphatic or hematogenous metastasis (T4)

Stage I thymomas are considered **benign**, while all higher stages are regarded as **malignant thymomas**.

TNM LYMPH NODE STAGING (THYMOMA)

- **N0**: No lymph node metastasis
- **N1**: Intrathoracic lymph nodes (excluding anterior mediastinal)
- **N2**: Anterior mediastinal lymph nodes
- **N3**: Extrathoracic lymph nodes

METASTASIS (M FACTOR)

- **M0**: No hematogenous metastasis
- **M1**: Hematogenous metastasis present

Exam & Reporting Pearls

- Histologic subtype does not always predict behavior—stage is the most important prognostic factor
- Thymomas may show benign cytology but malignant behavior
- Always correlate WHO type + Masaoka stage in reports

ASSESS YOURSELF LYMPHORETICULAR SYSTEM

A Long answer questions (10 marks each)

1. How would you gross right sided cervical lymph node resection?
2. How does the thymus contribute to T-cell maturation?
3. How are non-Hodgkins Lymphomas Classified?

B. Short essay type questions (5 marks each)

4. Classification of Hodgkin's Lymphomas
5. Briefly describe 'Plasma cell lymphomas'
6. Gamna -Gandy bodies

C. Short answer questions (3 marks each)

7. Types of Reed Sternberg Cells
8. Causes of lymphadenopathy
9. B cell function

MCQs

1.The nodular lymphocytic predominant Hodgkin's arises from :

A. B lymphocyte
B. T lymphocyte
C. Reticulum cell
D. Dendritic cell …….

2.All of the following are types of Reed Sternberg cells except :

A. Lacunar type of cell
B. Classic R S cell
C. Popcorn cell

D. Multinucleate giant cell ……

3.The location of Ghon's focus is at :

A. Apex of the left lung
B. Upper part of the lower lobe and lower part of the upper lobe
C. Hilar region
D. Apex of right lung ……

Answer Key for MCQs

1. 1.A

2. 2.D

3. 3.B

Chapter 9
Urinary System

Chapter out line -A Kidneys ; B Ureters; C Urinary bladder ;D urethra

A I Kidneys Anatomy ; A II Kidneys Grossing Techniques; A III Kidneys Gross Pearls; A IV Kidneys Microscopy; A V Kidneys Synoptic Reporting.

B I Pelvis & ureters (Upper Urinary Tract) Anatomy ; B II Pelvis & ureters Grossing Techniques; B III Pelvis & ureters Gross Pearls ; B IV Pelvis & ureters Microscopy; B V Pelvis & ureters Synoptic Reporting

C I Urinary Bladder Anatomy; C II Urinary Bladder Grossing Techniques; C III Urinary Bladder Gross Pearls; C IV Urinary Bladder Microscopy; C V Urinary Bladder Synoptic Reporting

D Urethra

A I KIDNEYS ANATOMY

Salient Anatomical Features

The kidneys are paired, retroperitoneal organs situated on either side of the vertebral column, extending approximately from T12 to L3 vertebral levels, with the right kidney lying slightly lower due to the liver.

Each kidney measures approximately 10–12 cm in length, 5–7 cm in width, and 3–4 cm in thickness, and is enclosed by a fibrous capsule, perirenal fat, and Gerota's fascia.

On cut section, the kidney is divided into:

Cortex – outer granular zone containing glomeruli and cortical tubules

Medulla – inner region composed of pyramids

Renal pyramids – whose apices form papillae opening into minor calyces

Columns of Bertin – cortical extensions between pyramids

The renal sinus contains the renal pelvis, calyces, vessels, and fat, and is an important site for tumour spread.

The functional unit of the kidney is the **nephron**, consisting of:

Glomerulus

Proximal tubule

Loop of Henle

Distal tubule

Collecting duct

Each kidney contains approximately **1 million nephrons**.

Normal Glomerulus

Anastomosing network of capillaries invested by 2 layers of epithelium. The parietal epithelium lines the Bowman's space and the visceral epithelium forms an integral part of the capillary wall.

The glomerular capillary wall is the filtering membrane and consists of –
a) A thin layer of fenestrated endothelial cells with fenestrations measuring 75–100 nm in diameter.
b) The glomerular basement membrane (GBM): a central dense layer with thinner electron-lucent layers (lamina rara interna and externa), composed mainly of type IV collagen; thickness ~250–350 nm in adults.
c) Visceral epithelial cells (podocytes) with interdigitating foot processes forming filtration slits (20–30 nm).

Mesangial cells: Provide structural support, and are contractile., They also have phagocytic and proliferative capabilities in laying down matrix and collagen and secreting biologically active mediators (cytokines) of inflammation. The basement membrane like mesangial matrix forms a network through which mesangial cells are scattered.

Juxtaglomerular apparatus: Located at the vascular pole and composed of: – 1) specialized cells of the distal convoluted tubule called macula densa with reversed polarity 2) terminal portion of the afferent arteriole and initial portion of the efferent arteriole 3) lacis cells or Goormaghtigh cells –modified smooth muscle cells which are continuous with the mesangial cells of the glomerulus.

Vasculature : Main renal artery → segmental arteries → interlobar arteries → arcuate arteries → interlobular arteries → afferent arterioles → glomerular capillary tuft → efferent arteriole →

- Cortical peritubular capillaries
- Medullary vasa recta

Venous drainage parallels arterial supply → interlobular → arcuate → interlobar veins → renal vein.

A II KIDNEYS GROSSING TECHNIQUES

Types of specimens received

Renal needle biopsy
Nephrectomy

Partial nephrectomy
Simple nephrectomy

Radical nephrectomy
Nephro-ureterectomy

A. RENAL NEEDLE BIOPSY

Receipt and triage

Renal biopsy is usually performed percutaneously under ultrasound guidance.

Specimens should ideally be received **fresh** and triaged into:

Light microscopy (LM)

Immunofluorescence (IF)

Electron microscopy (EM)

Adequacy should be assessed at grossing:

Presence of renal cortex must be confirmed.

A core may be examined under low power (10×) to identify glomeruli.

Adequate native kidney biopsy usually contains ≥10–15 glomeruli for LM and ≥1 glomerulus for IF.

EM is **selective** and not mandatory in all cases; it is reserved for suspected glomerular basement membrane or podocyte disorders.

Description

Number of cores/fragments

Colour

Length of each core

Homogeneity

Processing

Entire tissue should be submitted.

Recommended sections:

2 sections: Haematoxylin & Eosin

2 sections: Periodic acid–Schiff (PAS)

2 sections: Periodic acid–methenamine silver (PASM / Jones silver)

1 section: Masson's trichrome

Special stains as indicated:

Congo red for amyloidosis

Utility

Renal biopsy is useful in diagnosing primary renal disease, assessing renal involvement in systemic disorders, monitoring transplant dysfunction, and establishing diagnoses such as amyloidosis.

B. NEPHRECTOMY SPECIMENS

Types

Partial nephrectomy – localized non-neoplastic lesions or small renal tumours

Simple nephrectomy – chronic pyelonephritis, end-stage kidney disease

Radical nephrectomy – renal tumours

Nephro-ureterectomy – upper tract urothelial carcinoma

I. NON-TUMOURAL NEPHRECTOMY

Gross examination

Inspect the intact specimen; identify renal artery, vein and ureteric stump.

Determine laterality using hilar structures (anterior to posterior: vein, artery, pelvis/ureter).

Overnight fixation is ideal; formalin may be injected through the ureter.

Record:

- Weight (with fat if adherent)
- Measurements: pole-to-pole length, width, thickness
- Length of ureter and renal vessels
- Pelvis: intrarenal or extrarenal

Bisect kidney longitudinally to expose cortex, medulla, pelvis and calyces.

Description

Capsule: thickness, adherence, scars

Cortex and medulla: cortical thickness (normal ~8 mm–1.2 cm), colour, corticomedullary differentiation

Cysts: size, location, contents

Pelvis and calyces: dilatation, haemorrhage, calculi

Ureter: length, diameter, strictures (especially PUJ)

Renal vessels: thrombosis, stenosis

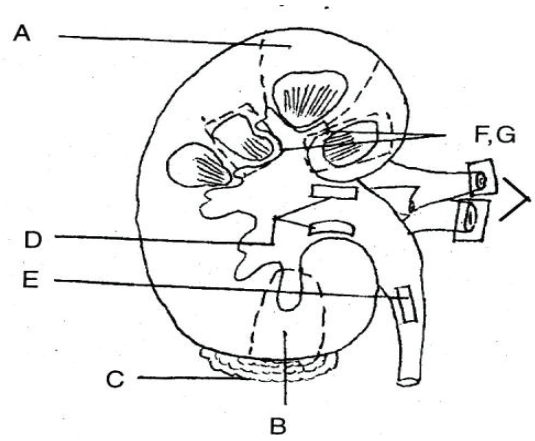


Fig 9.1: grossing for non tumoural conditions

Sampling (Fig 9.1)

A, B: Cortex, medulla and pyramid with capsule (upper and lower poles)

C: Perinephric fat (if adherent)

D, E: Pelvis

F: Ureter

G, H: Tips of pyramids (for papillary necrosis when indicated)

Renal vessels: cross-sections in transplant/allograft kidneys – to rule out fungal infection as a cause of rejection.

II. TUMOURAL NEPHRECTOMY

Gross examination

Open kidney longitudinally from outer to inner aspect; open pelvis and ureter.

Identify renal vessels and surgical margins.

Description

Weight and dimensions of kidney

Tumour:

Location and size (maximum dimension)

Appearance: colour, consistency, necrosis, haemorrhage

Relationship to capsule (intact/breached)

Renal sinus invasion

Perinephric fat involvement

Involvement of calyces, pelvis

Renal vein or segmental vein invasion

Uninvolved kidney: cortex and medulla

Pelvis: dilatation, stones, tumour spread

Lymph nodes: number, size, cut surface

Adrenal gland: involved or uninvolved

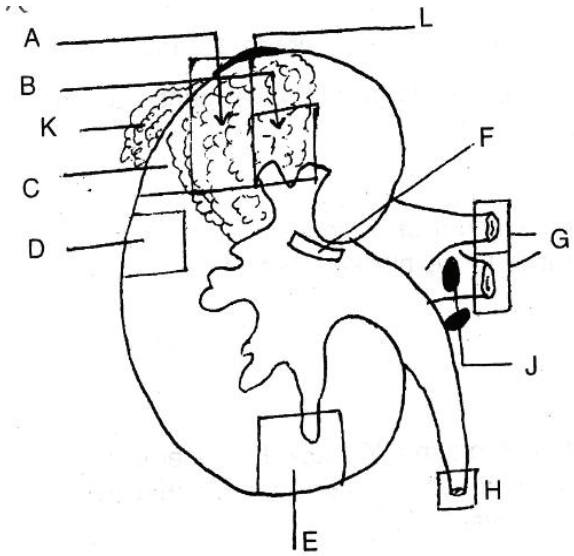


Fig 9.2 to show grossing in kidney tumour .

Sampling (Fig 9.2)

A, B, C: Tumour (one with adjacent kidney; one from periphery; one from centre)

Additional tumour–sinus and tumour–perinephric fat interface blocks

Wilms' tumour: one block per cm of tumour diameter

D, E: Uninvolved kidney (upper and lower poles)

F: Pelvis

G: Renal artery and vein margins

H: Ureteric margin

I: Perihilar lymph nodes (entirely submitted)

J: Perinephric fat

K: Capsule if adherent

L: Adrenal gland (even if grossly normal)

A III KIDNEYS GROSS PEARLS

Capsule and Surface Changes

Capsule adherent: Seen in chronic pyelonephritis and over healed infarcts.

Flea-bitten kidney: Multiple petechial haemorrhages on the cortical surface. Classically described in subacute bacterial endocarditis (now rare); commonly seen in malignant hypertension, crescentic glomerulonephritis, severe acute glomerulonephritis, and any condition producing marked haematuria.

Scarring Patterns

V-shaped cortical scars: Vascular scars due to renal infarcts and hypertensive renal disease.

U-shaped scars: Broad-based depressed scars, characteristically at the upper and lower poles in reflux nephropathy; may also be seen in chronic pyelonephritis due to causes other than reflux.

Kidney Size and Consistency

Large, oedematous kidney: Acute pyelonephritis.

Large kidney with yellowish discoloration; greasy bulging cut surface: Fatty change.

Small Contracted Kidney

Benign nephrosclerosis: Bilateral, symmetric contraction; largest among the small contracted kidneys (≈110–130 g); fine granular surface; V-shaped scars may be present.

Chronic glomerulonephritis: Bilateral, symmetric contraction (≈90–100 g); coarse granular surface.

Chronic pyelonephritis: Smallest among the contracted kidneys (≈80–90 g); often unilateral; asymmetric contraction with irregular scarring.

Cut Surface Clues

Pale cortex sharply demarcated from medulla: Cortical necrosis.

CYSTIC LESIONS – GROSS PATTERNS

Autosomal dominant polycystic kidney disease (ADPKD) (Fig 9.3 A & B)

Bilateral massively enlarged kidneys (often 2–4 kg each)

Bosselated external surface

Cut surface: cysts of varying sizes involving cortex and medulla

(Corresponds to Potter type III)

Fig 9.3 A Cysts : Potter typr III Surface view

Fig 9.3 B Potter type III – cut surface ; cysts

of varying sizes

Autosomal recessive polycystic kidney disease (ARPKD) (Fig 9.4)

Bilateral enlarged kidneys with smooth external surface

Cut surface: elongated cylindrical cysts arranged perpendicular to the surface (radiating appearance)

(Potter type I)

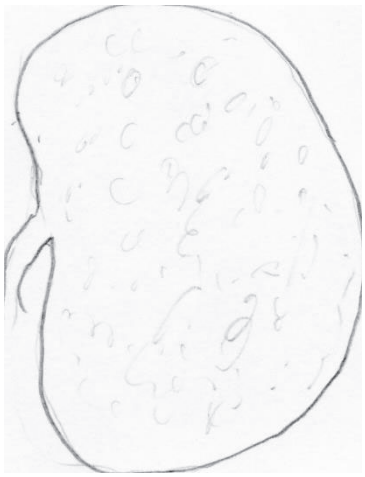


Fig 9.4 A Potter type I : Surface view enlarged kidney

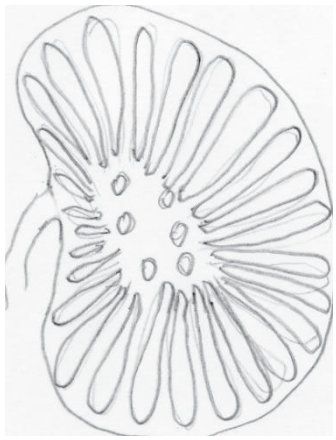


Fig 9.4 B Potter type I : Cut surface shows radial cysts.

Simple renal cysts

Occasional cysts, usually cortical; kidney otherwise normal.

Medullary sponge kidney (Fig 9.5)

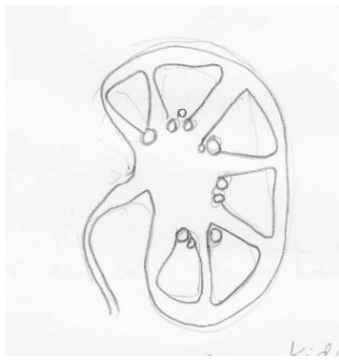


Fig 9.5 Medullary sponge kidney

Cysts confined to tips at the medulla

Cysts confined to medullary and papillary collecting ducts.

Nephronophthisis / medullary cystic disease complex (FIG 9.6)

Normal-sized or small contracted kidneys

Multiple small cysts (1–15 mm) located at the corticomedullary junction.

Fig 9.6 : Fig shows : Variably sized cysts in the corticomedullary junction

Renal cystic dysplasia

Reniform mass replaced by cysts of varying sizes; unilateral or bilateral; kidney may be enlarged, normal or contracted.

Multicystic dysplastic kidney: Usually unilateral, enlarged kidney with irregular cysts; typically not associated with other congenital anomalies (Potter type II).(Fig 9.7)

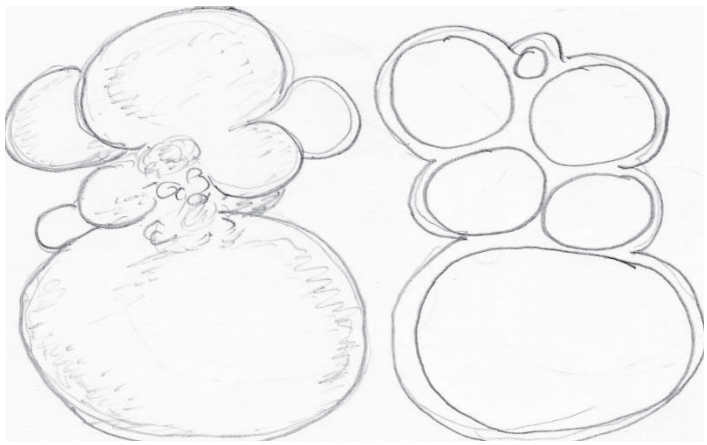


Fig 9.7 shows : Surface view and c/s of Potter type II kidneys

Diffuse cystic dysplasia (Fig 9.8 A & B) Bilateral, symmetric involvement with maintained reniform shape; commonly associated with other congenital abnormalities.

Fig 9.8 A Surface view

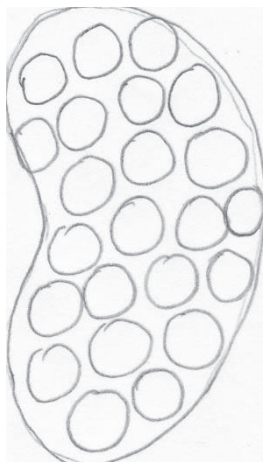


Fig 9.8 B Cut surface

Dilatation and Parasitic Lesions

Unilateral dilated kidney with dilated pelvis and calyces: Advanced hydronephrosis.

Cyst with thick laminated membrane: Hydatid cyst**.**

Tumours – Gross Diagnostic Clues

Thick-walled cyst with variegated cut surface and necrosis: Cystic change in renal cell carcinoma.

Small cortical nodule ≤5 mm: Papillary adenoma.

Polar renal mass with variegated appearance; yellow solid-cystic areas and haemorrhage: Renal cell carcinoma.

Large, well-circumscribed tumour; homogeneous pale pink cut surface; soft or cystic: Wilms' tumour.

Medullary-based tumour: Collecting duct carcinoma or renal medullary carcinoma.

Mahogany brown/tan, well-circumscribed tumour with central stellate scar: Oncocytoma (in contrast to the bright yellow colour of RCC).

Other tumours with central scars: focal nodular hyperplasia of liver; complex scars of breast.

Yellow, fatty or lipoma-like tumour: Angiomyolipoma.

GROSS DIAGNOSIS AT A GLANCE (KIDNEY)

Gross Appearance	Most Likely Diagnosis
Capsule adherent, irregular surface	Chronic pyelonephritis; healed infarcts
Flea-bitten cortical surface	Malignant hypertension, crescentic GN, severe acute GN, marked haematuria

V-shaped cortical scars	Renal infarcts; hypertensive nephrosclerosis
U-shaped scars at upper & lower poles	Reflux nephropathy (classical); chronic pyelonephritis
Large, oedematous kidney	Acute pyelonephritis
Large yellow kidney; greasy cut surface	Fatty change
Small contracted kidney, fine granularity, bilateral symmetric (110–130 g)	Benign nephrosclerosis
Small contracted kidney, coarse granularity, bilateral symmetric (90–100 g)	Chronic glomerulonephritis
Smallest contracted kidney, unilateral/asymmetric, irregular scars	Chronic pyelonephritis
Pale cortex sharply demarcated from medulla	Cortical necrosis
Bilateral massive kidneys, bosselated surface, cysts of varying size	ADPKD
Bilateral enlarged kidneys, smooth surface, radiating cylindrical cysts	ARPKD
Occasional cortical cysts, otherwise normal kidney	Simple renal cysts
Medullary and papillary cysts only	Medullary sponge kidney
Small/normal kidney with small corticomedullary cysts	Nephronophthisis / medullary cystic disease
Reniform mass replaced by irregular cysts, unilateral	Multicystic dysplastic kidney
Bilateral cystic kidneys with maintained reniform shape	Diffuse cystic dysplasia
Unilateral dilated kidney with dilated pelvis and calyces	Advanced hydronephrosis
Thick laminated cyst wall	Hydatid cyst
Thick-walled cyst with necrosis, variegated cut surface	Cystic RCC
Small cortical nodule ≤5 mm	Papillary adenoma
Variegated yellow, solid-cystic polar mass with haemorrhage	Renal cell carcinoma

Homogeneous pale pink, soft/cystic tumour	Wilms' tumour
Medullary-based tumour	Collecting duct carcinoma; renal medullary carcinoma
Mahogany brown/tan tumour with central scar	Oncocytoma
Yellow, fatty/lipoma-like tumour	Angiomyolipoma

A III KIDNEYS MICROSCOPY

RENAL BIOPSY

Indications

Indications for renal biopsy are not uniform and may vary among nephrologists; however, the following are **widely accepted indications**:

- Idiopathic nephrotic syndrome in adults, prior to initiation of glucocorticoid therapy
- Non-nephrotic proteinuria (≈1–2 g/day) of unknown cause associated with abnormal urinary sediment
- Proteinuria with glomerular haematuria, after excluding urinary tract infection and neoplasia
- Acute kidney injury of unknown cause, once obstruction has been ruled out
- Suspected rapidly progressive glomerulonephritis (RPGN) or acute nephritic syndrome
- Persistent haematuria (>6 RBCs/hpf) with proteinuria and elevated serum creatinine
- Suspected renal vasculitis, especially ANCA-associated vasculitis (e.g. granulomatosis with polyangiitis), where aggressive immunosuppression is considered
- Renal allograft dysfunction, to evaluate rejection, acute tubular injury, drug-induced interstitial nephritis, infarction, or recurrence of the original disease

Additional / Selective Indications

- Persistent isolated glomerular haematuria without proteinuria
- Proteinuria >1 g/day with otherwise normal urinary sediment
- Suspected inherited glomerular disorders, such as Alport syndrome or Fabry disease
- Diabetic patients with an atypical clinical course, including:
    - Rapid unexplained deterioration of renal function
    - Sudden onset of nephritic syndrome
    - Early azotaemia in the absence of diabetic retinopathy

Situations Where Renal Biopsy is Generally Not Useful

Malignant hypertension

Polycystic kidney disease

Hepatorenal syndrome

Acute pyelonephritis

Advanced chronic kidney disease with bilaterally shrunken kidneys

Typical long-standing diabetic nephropathy

Clinically silent lupus nephritis (controversial)

Contraindications for renal needle biopsy

Absolute

Uncorrectable severe bleeding diathesis

Patient refusal

Relative

Severe thrombocytopenia (<50,000/mm³), usually correctable

Solitary native kidney (renal allograft is an exception)

Renal artery aneurysm

Active pyelonephritis or perinephric abscess

Hydronephrosis

Uncontrolled severe hypertension

High-risk situations (not absolute contraindications)

Moderate thrombocytopenia (50,000–100,000/mm³)

Moderate hypertension

Blood urea nitrogen >100 mg/dL

Microscopic Evaluation – Techniques

Types of Microscopy

1. Light Microscopy (LM)
Thin sections stained with:

Haematoxylin and Eosin (H&E)

Periodic acid–Schiff (PAS)

Jones methenamine silver stain (PASM)

Masson's trichrome

LM permits assessment of glomerular cellularity, sclerosis, crescent formation, basement membrane alterations, tubulointerstitial changes and vascular lesions.

2.Immunofluorescence Microscopy (IF)

Fluorochrome-labelled antibodies are used to localize immunoreactants (IgG, IgA, IgM, C3, C1q and light chains) within glomeruli, tubules or vessels, providing crucial information on immune-complex and complement-mediated renal diseases.

3.Electron Microscopy (EM)

Ultrastructural examination is used **selectively** to demonstrate:

Location of immune-complex deposits

Glomerular basement membrane abnormalities

Podocyte foot process effacement and epithelial injury

EM is particularly valuable in minimal change disease, hereditary nephropathies and early immune-complex glomerulonephritis.

4. Terms used in the Evaluation of Glomerular Diseases

Diffuse : > than 50% glomeruli affected

Focal : < 50% of glomeruli affected

Global : >than 50% of glomerular lobules to entire glomerulus is involved

Segmental : < than 50% of segments (glomerular lobules) involved

Peripheral mesangial area : mesangium at the periphery of the glomerulus with 2-3 mesangial nuclei.

Mesangial hypercellularity : more than 3 mononuclear cells or nuclei in atleast one mesangial area in a section 3 μ in thickness

Crecentic glomerulo-nephritis : > 50% of glomeruli show crescents.

Extracapillary proliferation or cellular crescent : Extracapillary cell proliferation of two or more than 2 cell layers of parietal and visceral epithelial cells occupying one fourth or more of the glomerular capsular circumference.

Endocapillary proliferation : Proliferative glomerulonephritis with a complex mixture of different cell types including mesangial cells, endothelial cells and infiltrating leukocytes-neutrophils monocytes, macrophages and lymphocytes.

5. Clinical Patterns of Glomerular Diseases

Nephrotic Syndrome :

Characterised by heavy proteinuria (>3.5g/24 hours) , consequent hypoalbuminemia (serum albumin <3.5g/dl, generalized oedema, hyperlipidemia and hyperlipiduria.

Protein “re-absorption droplets” in the proximal tubular epithelial cells

Lipid within tubular epithelial cells and peritubular macrophages : appears in urine as free fat or oval fat bodies.

The structural /charge barrier of the GBM is lost . Effacement of epithelial cell foot processes and swelling of podocytes.

Patients may develop renal vein thrombosis due to loss of coagulation factors (proteinuria). Patients are vulnerable to infections with staphylococci and pneunococci.

Nephrotic syndrome in children : protein excretion of more than 40mg/m^2/hour body surface area in an overnight collection of urine (as compared to adults of > 3.5g/1.73m^2/day body surface area.

Nephritic syndrome :

Characterised by haematuria, variable proteinuria (non-nephrotic), diminished GFR, with some degree of oliguria, azotemia (↑ BUN and creatine) and hypertension.

Caused by diseases that evoke an inflammatory proliferative response within the glomeruli

Proliferation may involve endothelial, mesangial or epithelial cells. Exudation of neutrophils may be present.

Uraemia : refers to clinical manifestations of azotemia

6. Classification of Glomerular Diseases

Primary Glomerular Diseases : Disease process is confined to the kidney and does not involve other organs.

Those which cause nephritic syndrome: Post -infectious glomerulonephritis
Rapidly proliferative glomerulo-nephritis
IgA nephropathy/ Henoch Schonlein purpura
Membrano-proliferative glomerulo-nephritis
(20% of cases)

Those which cause nephrotic syndrome: Minimal change disease
Focal segmental glomerulosclerosis
Membranous glomerulonephritis
Membrano-proliferative glomerulo-nephritis
(50% of cases)

Secondary diseases : Renal involvement is part of a systemic disease.

Those which cause nephritic syndrome : Systemic lupus erythematosus
Vasculitis

Those which cause nephrotic syndrome : Diabetes mellitus
Amyloidosis

ACUTE POST-INFECTIOUS GLOMERULONEPHRITIS

(Classically post-streptococcal)

Definition

An immune-mediated glomerulonephritis occurring after infection with a variety of organisms, most classically **group A β-haemolytic streptococci**.

Clinical Features

Age group: Predominantly children, 6–10 years; less common but more severe in adults.

Antecedent infection:

Usually group A β-haemolytic streptococci (>90%)

Nephritogenic strains: types 12, 4, 1

Latent period:

Develops 1–4 weeks after streptococcal infection

Respiratory infections predominate in temperate climates

Skin infections (impetigo) more common in tropical regions

Clinical presentation:

Abrupt onset of haematuria, proteinuria, periorbital oedema, hypertension and renal insufficiency

Serum complement levels are low, particularly C3

Anti-streptolysin-O (ASO) titres elevated (less reliable after skin infections)

90% show ↓ serum C3 and ↓ total haemolytic complement (CH50)

C4 is reduced in <10% of cases

C3 returns to normal within 6–8 weeks, helping distinguish it from MPGN

Other causative organisms:

Staphylococcus (second most common)

Gram-negative bacteria

Viruses and parasites (less common)

Pathogenesis

Primarily immune-complex mediated

Immune complexes may be:

Circulating and trapped in glomeruli, or

Formed in situ due to planted streptococcal antigens

Streptococcal antigens implicated include:

Endostreptosin

Streptokinase

Streptococcal pyrogenic exotoxin B (SpeB)

Streptococcal enzymes may alter GBM components, rendering them antigenic

Complement activation (mainly alternative pathway) plays a key role

Pathology - Light Microscopy

Diffuse global endocapillary hypercellularity (Fig 9.9)

Partial obliteration of capillary lumina

Prominent neutrophilic infiltration (exudative GN) (Fig 9.9)

Mild interstitial oedema with mononuclear infiltrate

Crescents occur only in severe cases

On Masson's trichrome, large subepithelial deposits appear as fuchsinophilic red granules

Resolving phase: Residual mesangial hypercellularity

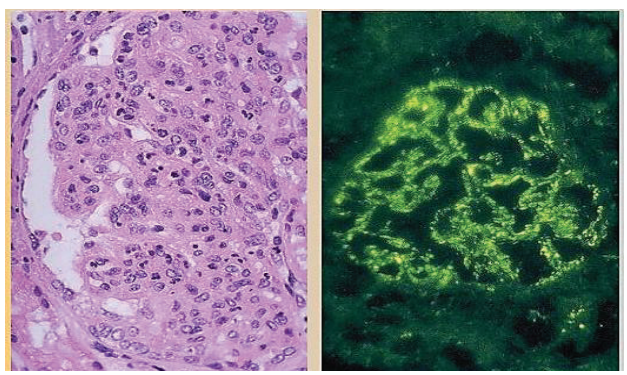


Fig 9.9 shows hypercellurity of glomerulus by H&E stain x 400. Right side shows granular deposits along capillary wall by IF

Immunofluorescence

Coarsely granular ("lumpy-bumpy") deposits of IgG and C3 (FIG 9.9)

Located along capillary walls and mesangium

IgM may be present

Electron Microscopy

Large subepithelial "hump-shaped" electron-dense deposits (diagnostic hallmark)

Deposits may also be mesangial or subendothelial

Prognosis

Prognosis depends on age and whether disease is epidemic or sporadic

Children:

Complete recovery in most cases

Epidemic cases have best outcome

Children progressing to RPGN or CKD: ~2–3%

Adults:

15–50% may progress to chronic renal insufficiency

RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS (RPGN)

(Crescentic glomerulonephritis)

Definition

A clinicopathologic syndrome characterized by rapid loss of renal function over weeks to months, with crescent formation in ≥50% of glomeruli (WHO criterion).

Clinical Features

Rapidly progressive renal failure with oliguria

Proteinuria, haematuria, azotaemia and anaemia are constant

Nephrotic syndrome is uncommon

Untreated cases progress to death from renal failure within weeks to months

Classification

Type I – Anti-GBM antibody–mediated RPGN

Caused by in situ binding of anti-GBM antibodies

Often associated with **Goodpasture syndrome** (pulmonary haemorrhage + GN)

Type II – Immune-complex RPGN

Occurs in:

Post-infectious GN

Lupus nephritis

IgA nephropathy / Henoch–Schönlein purpura

MPGN

Type III – Pauci-immune RPGN

Little or no immune deposits on IF

Most common form

80–90% are **ANCA-positive**

Associated with small-vessel vasculitis:

Granulomatosis with polyangiitis

Microscopic polyangiitis

Eosinophilic granulomatosis with polyangiitis

Age association

Pauci-immune RPGN: more common in older patients

Immune-complex RPGN: more common in children and young adults

Type I: Anti-GBM Glomerulonephritis

Bimodal age distribution:

Young adults (18–35 years)

Older adults (50–60 years)

Most common presentation: Goodpasture syndrome

Pulmonary haemorrhage often precedes renal disease

Severe renal failure at presentation is common

Treatment: plasmapheresis + immunosuppression

Pathogenesis of Crescent Formation

Breach in glomerular capillary wall → leakage of plasma proteins into Bowman's space

Fibrin polymerisation acts as a potent stimulus for crescent formation

Activated epithelial cells, macrophages and monocytes proliferate

Cytokines, growth factors and coagulation mediators amplify injury

Crescents consist predominantly of **parietal epithelial cells**, with variable macrophages

Anti-GBM and ANCA-associated RPGN show more macrophage-rich, disruptive crescents

EM demonstrates breaks in the GBM allowing inflammatory mediators to enter Bowman's space

Pathology- Light Microscopy

Crescent formation and fibrinoid necrosis are diagnostic hallmarks (Fig 9.10)

Crescents may be:

Cellular → fibrocellular → fibrous

Anti-GBM and ANCA-associated RPGN show:

Necrotizing lesions

Disruption of Bowman's capsule

Periglomerular inflammation

Immune-complex RPGN shows:

More orderly crescents

Intact Bowman's capsule

Tubules: mild tubular atrophy

Interstitium: variable inflammation and fibrosis

Arterioles may show fibrinoid necrosis

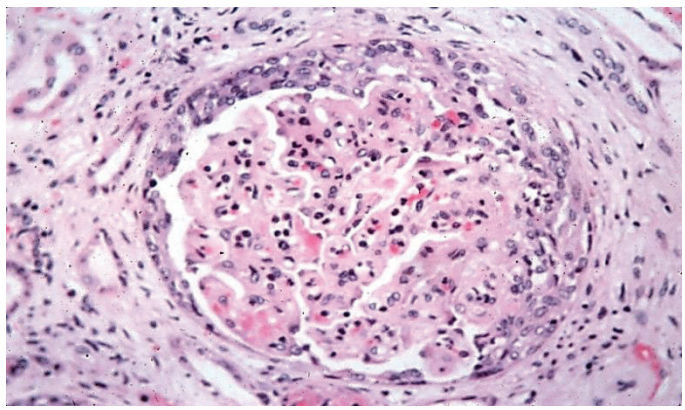


Fig 9.10 shows an epithelial crescent around a glomerulus. H & E x 400

Immunofluorescence – patterns

Type I: Linear IgG ± C3 along GBM

Type II: Granular immune-complex deposits

Type III: Minimal or absent staining (pauci-immune)

Electron Microscopy

Type I: Subendothelial lucent widening

Type II: Immune-complex deposits

Type III: Usually no deposits

GBM breaks often seen near crescents

IGA NEPHROPATHY / HENOCH–SCHÖNLEIN PURPURA (IGA VASCULITIS)

IgA nephropathy is the **most common primary glomerulonephritis worldwide**.

Clinical Features – IgA Nephropathy

Patients typically present with recurrent episodes of gross or microscopic haematuria, often accompanied by variable proteinuria.

More common in children and young adults, with a male predominance.

Episodes frequently follow upper respiratory tract, gastrointestinal or urinary tract infections ("synpharyngitic haematuria").

Most cases are idiopathic, but IgA nephropathy may be associated with:

Chronic liver disease

Ankylosing spondylitis

Psoriasis, Reiter's syndrome

Coeliac disease

Inflammatory bowel disease

Dermatitis herpetiformis

Serum IgA levels are elevated in approximately 30–50% of patients.

Serum complement levels are usually normal, helping to distinguish IgA nephropathy from post-infectious glomerulonephritis.

Henoch–Schönlein Purpura (IgA Vasculitis)

Henoch–Schönlein purpura is a **systemic small-vessel vasculitis** characterized by **IgA-**dominant immune complex deposition involving capillaries, venules and arterioles.

Clinical Features

Classical clinical tetrad:

Palpable purpura (skin rash)

Abdominal pain ± gastrointestinal bleeding

Non-migratory arthralgia

Renal involvement
(All components may not be present simultaneously.)

Renal manifestations include microscopic or gross haematuria and proteinuria; nephrotic syndrome may occur.

A small proportion, especially adults, develop rapidly progressive glomerulonephritis.

Occurs most commonly in children aged 3–8 years, but adults may also be affected.

Onset often follows an upper respiratory tract infection.

The presence of systemic manifestations distinguishes Henoch–Schönlein purpura from primary IgA nephropathy.

Pathogenesis (Current Concept)

IgA nephropathy is now considered a multi-hit disease:

Production of galactose-deficient IgA1 (Gd-IgA1).

Formation of autoantibodies against Gd-IgA1.

Circulating immune complexes deposit predominantly in the mesangium.

Mesangial activation leads to complement activation (alternative and lectin pathways), cytokine release, and progressive glomerular injury.

Henoch–Schönlein purpura shares the same pathogenic mechanism, differing mainly in its systemic vascular involvement.

Pathology- *(Similar in IgA nephropathy and Henoch–Schönlein purpura)*

Light Microscopy

The histological picture is variable and includes:

Normal glomeruli (early disease)

Focal or diffuse mesangial proliferative glomerulonephritis (most common)

Endocapillary proliferative glomerulonephritis

Crescentic glomerulonephritis (especially in severe cases)

Segmental or global glomerulosclerosis in advanced disease
(Type I MPGN pattern is rare)

Immunofluorescence

Dominant or co-dominant mesangial IgA deposition

Usually accompanied by **C3**

IgG and/or IgM may be present but with lesser intensity

Electron Microscopy

Electron-dense deposits in the mesangium (characteristic)

Subendothelial and subepithelial deposits may occasionally be seen

Podocyte foot process effacement correlates with proteinuria

Oxford Classification (MEST-C) – Prognostic Significance of IgA Nephropathy

Renal biopsy findings are graded using the **Oxford classification**, which predicts outcome:

M – Mesangial hypercellularity

E – Endocapillary hypercellularity

S – Segmental glomerulosclerosis

T – Tubular atrophy / interstitial fibrosis

C – Crescents

(T and S lesions are strongest predictors of progression.)

Prognosis

IgA nephropathy generally shows **slow progression**.

Approximately 20–40% of patients progress to end-stage renal disease over 20–25 years.

Poor prognostic factors include:

- Persistent proteinuria
- Hypertension
- Reduced renal function at presentation
- Extensive tubular atrophy/interstitial fibrosis
- Crescent formation

Most children with Henoch–Schönlein purpura recover completely; however, adults and those with severe renal involvement may develop chronic renal failure.

Recurrence in renal allografts occurs in up to 50**%** of cases, though graft loss is less common.

MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS (MPGN)

(Mesangiocapillary glomerulonephritis)

Definition

Membranoproliferative glomerulonephritis is a **pattern of glomerular injury** characterized by:

Mesangial cell proliferation and matrix expansion

Thickening of glomerular capillary walls

Alteration of the glomerular basement membrane (GBM), often with **double-contour ("tram-track") formation**

Contemporary concept: MPGN is no longer regarded as a single disease entity but as a **histologic pattern** resulting from different pathogenic mechanisms.

Clinical Features

Majority of patients present between **5–30 years** of age.

Clinical presentation:

Nephrotic syndrome – ~50%

Asymptomatic proteinuria – ~30%

Acute nephritic syndrome – ~20%

Hypocomplementemia, especially low C3, is common due to complement activation.

Course is typically chronic and progressive.

Pathogenetic Classification (Current)

1. Immune Complex–Mediated MPGN

Caused by deposition of circulating or in situ–formed immune complexes

Activates the **classical complement pathway**

Associated with infections, autoimmune diseases, and monoclonal gammopathies

2. Complement-Mediated MPGN (C3 Glomerulopathy)

Due to dysregulation of the alternative complement pathway

Dominant C3 deposition with minimal or absent immunoglobulins

Includes:

Dense deposit disease (DDD) – former MPGN Type II

C3 glomerulonephritis (C3GN)

Traditional Morphologic Classification (For Examination Purposes)

MPGN Type I (Classic Type)

Clinical

Most common type

Presents with nephrotic, nephritic, or mixed features

Complement Profile

C3 decreased in ~80% (may be normal)

C1q, C4, C2 may be reduced

C3 nephritic factor (C3NeF) present in <50%

Light Microscopy

Diffuse glomerular hypercellularity

Lobular accentuation (Fig 9.11)

Mesangial expansion ± nodularity

Double-contour ("tram-track") GBM due to mesangial interposition (Fig 9.11)

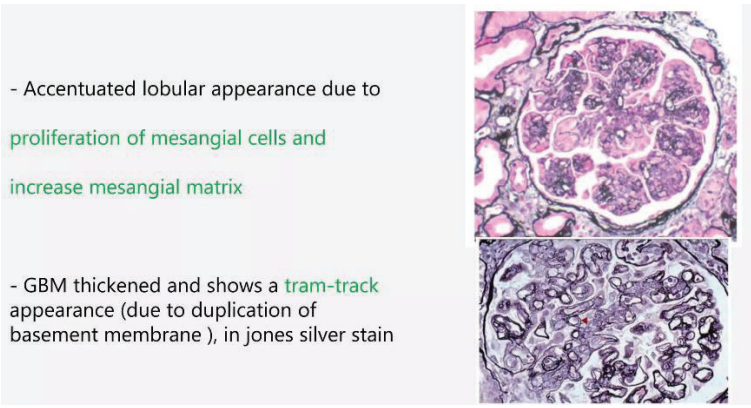


Fig 9.11 MPGN type 1 (slide share .net)

Immunofluorescence

Granular C3 along capillary walls and mesangium

IgG commonly present; C1q and C4 often detected

Properdin positive

Electron Microscopy

Predominantly subendothelial deposits

Mesangial interposition into GBM

Occasional mesangial and subepithelial deposits

Associated Conditions (Secondary MPGN)

Hepatitis C and B

Cryoglobulinemia

SLE

Chronic liver disease

Sickle cell disease

Schistosomiasis

Malignancies

MPGN Type II – Dense Deposit Disease - *(Now classified under C3 glomerulopathy)*

Clinical

Children and young adults

Often nephritic presentation with haematuria

More aggressive course

Complement Profile

Persistently low C3 in nearly 100%

C1q and C4 normal (classical pathway not activated)

C3NeF frequently present

Factor B and properdin reduced

Light Microscopy

Variable hypercellularity

Ribbon-like thickening of GBM

PAS-positive, silver-negative deposits

Tram-tracks usually poorly developed

Immunofluorescence

Strong C3 positivity in GBM (“railroad track” pattern)

Mesangial C3 rings

Immunoglobulins absent or minimal

Electron Microscopy

Pathognomonic dense, ribbon-like electron-dense material within the lamina densa of GBM

Associated Condition

Partial lipodystrophy

Prognosis

Worse than Type I

High risk of progression to end-stage renal disease

Frequent recurrence after transplantation

MPGN Type III

Clinical

Similar to Type I

Complement Profile

C3 decreased in ~50%

C1q and C4 normal

C3NeF usually absent

Light Microscopy

Similar to Type I

More diffuse capillary wall thickening

Double contours with **additional spikes** on silver stain

Immunofluorescence

Fine granular C3 along GBM and mesangium

Properdin parallels C3 deposition

Electron Microscopy

Subendothelial and subepithelial deposits

Deposits may communicate through defects in lamina densa

Strife and Anders type

"Washed-out" deposits may be seen

Burkholder type

Prognosis

Similar to Type I

Associated Conditions

Sjögren syndrome

Cirrhosis

HIV infection

Malignant melanoma

Prognosis (Overall)

MPGN is typically chronic and slowly progressive

Many patients eventually progress to end-stage renal disease

Prognosis is worse in:

Dense deposit disease

Presence of crescents

Persistent hypocomplementemia

RECENT CONCEPTS – MPGN

MPGN is a pattern of injury, not a single disease

Dense deposit disease is now classified as C3 glomerulopathy

Classification should integrate LM, IF, and EM findings

Dominant C3 deposition with minimal immunoglobulin suggests alternative pathway dysregulation

Recognition of C3 glomerulopathy has therapeutic implications (complement-targeted therapy)

SYSTEMIC LUPUS ERYTHEMATOSUS – RENAL INVOLVEMENT (LUPUS NEPHRITIS)

Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disease characterized by the production of autoantibodies against nuclear and cytoplasmic antigens, leading to immune complex–mediated tissue injury. Renal involvement, termed lupus nephritis (LN), is one of the most important determinants of morbidity and mortality.

Clinical Features

Renal disease may occur at any stage of SLE and may precede systemic manifestations. Clinical presentation ranges from asymptomatic haematuria and proteinuria to nephrotic syndrome,

rapidly progressive glomerulonephritis, or chronic kidney disease. Renal biopsy is essential for diagnosis, classification, assessment of activity and chronicity, and therapeutic decision-making.

PATHOGENESIS

Renal injury in lupus nephritis is primarily mediated by immune complex deposition, occurring through:

Deposition of circulating immune complexes

In situ formation following binding of autoantibodies (notably anti–dsDNA) to planted antigens such as nucleosomes

Subsequent activation of complement (classical and alternative pathways) **and Fc receptor–**mediated inflammation leads to glomerular, tubulointerstitial, and vascular injury.

Recent pathogenetic concepts include:

Type I interferon (IFN-α) pathway activation

NETosis, contributing to autoantigen exposure

Direct podocyte injury, particularly in membranous LN

ANCA-associated mechanisms in a subset of patients

Prothrombotic injury related to antiphospholipid antibodies

ISN/RPS CLASSIFICATION OF LUPUS NEPHRITIS *(2003; revised recommendations 2018) is the current classification in use.*

Class I – Minimal mesangial LN
Normal LM; mesangial immune deposits on IF/EM

Class II – Mesangial proliferative LN
Mesangial hypercellularity and expansion with mesangial immune deposits

Class III – Focal LN
<50% glomeruli involved; segmental or global endocapillary/extracapillary lesions, subendothelial deposits, necrosis, crescents

Class IV – Diffuse LN
≥50% glomeruli involved; diffuse endocapillary/extracapillary proliferation, wire-loop lesions, necrosis, crescents
(2018 revision: IV-S and IV-G subdivisions removed)

Class V – Membranous LN
Diffuse subepithelial immune deposits with or without mesangial proliferation; may occur alone or with Class III/IV

Class VI – Advanced sclerosing LN
≥90% globally sclerosed glomeruli; no residual activity

Prognosis as per the Class

The prognosis of lupus nephritis depends upon the histological class, severity of activity and chronicity indices, and response to therapy. Diffuse proliferative lupus nephritis (Class IV) carries the worst prognosis, while mesangial (Class I and II) and membranous lupus nephritis (Class V) generally have a more favourable outcome. Clinical predictors of poor prognosis in Class IV nephritis include high serum creatinine at presentation, anemia, hypertension, and delayed response to immunosuppressive therapy. With current immunosuppressive regimens, long-term renal survival has improved significantly, particularly in patients diagnosed early and treated adequately.

WHO classification was put forth in 1974 and modified in 1982 which is as follows; but is rarely used presently and is now of historical importance only.

Modified WHO classification of Lupus Nephritis 1982

Class I Normal glomeruli

Nil by all techniques

Normal by LM but deposits seen by IF & /or (EM Full house)

Class II Purely mesangial alterations (mesangiopathy)

mesangial widening & /or moderate mesangial alterations

moderate hypercellularity

Class III Focal segmental glomerulonephritis (associated with mild or mod mesangial alterations)

with active necrotizing lesions

with active & sclerosing lesions

with sclerosing lesions

Class IV Diffuse glomerulonephritis (severe mesangial, endocapillary, or mesangiocapillary proliferation &/or extensive subendothelial deposits). Mesangial deposits are present invariably & subepithelial deposits often, & may be numerous

without segmental lesions

with active necrotizing lesions

with active & sclerosing lesions

with sclerosing lesions

Class V membranous glomerulonephritis

a pure membranous glomerulonephritis

b associated with lesions of category II (a or b)

c. associated with lesions of category III (a , b or c)

d. associated with lesions of category IIV (a , b, c, or d)

Class VI Advanced sclerosing glomerulonephritis

ACTIVITY AND CHRONICITY INDICES in lupus nephritis (Austin et al.)

Activity Index (0–24)
Includes endocapillary hypercellularity, karyorrhexis, fibrinoid necrosis, crescents, subendothelial deposits, interstitial inflammation

Chronicity Index (0–12)
Includes glomerulosclerosis, fibrous crescents, tubular atrophy, interstitial fibrosis

Index of Activity (0-24):

Endocapillary hypercellularity	(0-3+)
Leukocyte infiltration	(0-3+)
Subendothelial hyaline deposits	(0-3+)
Fibrinoid necrosis/ karyorrhexis	(0-3+) x 2
Cellular crescents	(0-3+) x 2
Interstitial inflammation	(0-3+)

Index of Chronicity (0-12) :

Glomerular sclerosis	(0-3+)
Fibrous crescents	(0-3)
Tubular atropy	(0-3+)
Interstitial fibrosis	(0-3+)

Chronicity index and tubulointerstitial fibrosis are the strongest predictors of long-term outcome

VASCULAR LESIONS IN LUPUS NEPHRITIS :

Arteriosclerosis and arteriolosclerosis

Uncomplicated vascular immune deposits

Non inflammatory necrotizing vasculopathy (so called lupus vasculopathy)

Thrombotic microangiopathy

Associated with HUS/TTP syndromes

Associated with antiphospholipid antibodies

Associated with scleroderma/ mixed connective tissue disease

Necrotizing vasculitis (PAN type)

IMMUNOFLUORESCENCE

"**Full house**" staining (IgG, IgA, IgM, C3, C1q) is characteristic

Complement activation predominantly via classical pathway

Fibrin-related antigens highlight necrosis and crescents

SPECIAL ENTITIES

Lupus Podocytopathy

Nephrotic syndrome

Minimal change or FSGS pattern on LM

No immune deposits on IF

Steroid responsive

ANCA-positive Lupus Nephritis

Necrotizing crescentic GN

Overlap of immune complex and pauci-immune injury

REPORTING RECOMMENDATIONS (in lupus nephritis)

A renal biopsy report should include:

ISN/RPS class (single or combined)

Activity and chronicity indices

Percentage of glomeruli with crescents and necrosis

Tubulointerstitial inflammation, fibrosis, tubular atrophy

Vascular lesions

IF and EM findings

Comparison with prior biopsies where available

PROGNOSIS LUPUS NEPHRITIS

Overall renal survival has significantly improved

10-year renal survival now exceeds **80–90%** in many cohorts

Poor prognostic factors:

High chronicity index

Extensive tubulointerstitial fibrosis

Persistent activity despite therapy

Antiphospholipid-associated vascular lesions

RECENT ADVANCES IN LUPUS NEPHRITIS

1. ISN/RPS 2018 REVISIONS – CONCEPTUAL SHIFT

Retains six-class framework

Eliminates Class IV-S and IV-G subdivision

Mandates reporting of:

Activity index

Chronicity index

Tubulointerstitial inflammation and fibrosis

Recognizes that class alone does not predict outcome

2. IMPORTANCE OF TUBULOINTERSTITIAL DISEASE

Tubulointerstitial inflammation and fibrosis are now recognized as:

Independent predictors of renal outcome

Stronger predictors than glomerular class alone

Interstitial inflammation even without active glomerular lesions is prognostically significant

3. LUPUS PODOCYTOPATHY (NEW ENTITY)

Presents with nephrotic syndrome

LM shows:

Minimal change disease or FSGS-like pattern

IF: No immune deposits

EM: Diffuse foot process effacement

Often steroid responsive

Considered distinct from classical immune complex–mediated LN

4. ANCA-POSITIVE LUPUS NEPHRITIS

Subset of patients show:

Necrotizing crescentic GN

Pauci-immune features superimposed on immune complex disease

Represents an overlap syndrome

Associated with more aggressive disease

5. UPDATED PATHOGENETIC INSIGHTS

Nucleosomes act as planted antigens

Type I interferon (IFN-α) signature central to immune dysregulation

NETosis contributes to autoantigen exposure and immune complex formation

Complement dysregulation involves both classical and alternative pathways

6. ROLE OF ELECTRON MICROSCOPY

Detects early membranous change

Identifies organized vs non-organized deposits

Assesses podocyte injury

Essential in suspected lupus podocytopathy

7. IMPROVED PROGNOSIS IN THE MODERN ERA

With current immunosuppressive regimens:

10-year renal survival **>80–90%**

Prognosis correlates best with:

Chronicity index

Extent of interstitial fibrosis

Response to there

COMPARATIVE TABLE IN THE EVOLUTION OF CLASSIFICATION OF LUPUS NEPHRITIS

Feature	WHO 1974 / 1982	ISN/RPS 2003	ISN/RPS 2018 (Current Concept)
Basis of classification	LM predominant	LM + IF + EM	Integrated morphology + indices
Number of classes	I–VI	I–VI	I–VI (unchanged)
Subdivision of Class IV	Multiple subtypes	IV-S and IV-G	**Removed**
Emphasis on immune deposits	Limited	Strong	Strong

Activity & chronicity indices	Adjunct	Recommended	**Mandatory to report**
Tubulointerstitial disease	Secondary	Mentioned	**Major prognostic factor**
Combined classes (III+V / IV+V)	Complex	Defined	Retained
Prognostic stratification	Class-based	Class + activity	**Chronicity + fibrosis dominant**
New entities included	None	None	**Lupus podocytopathy, ANCA overlap**

TAKE-HOME MESSAGE

WHO classification → historical

ISN/RPS → current standard

Activity = potentially reversible

Chronicity = irreversible damage

Tubulointerstitial fibrosis predicts outcome better than class

Lupus podocytopathy = nephrotic syndrome + no deposits

CAP-STYLE RENAL BIOPSY REPORTING CHECKLIST

(Lupus Nephritis – ISN/RPS aligned)

Patient / Specimen Details

Specimen type: ☐ Native kidney ☐ Transplant kidney

Biopsy adequacy:

Total glomeruli: _____

Globally sclerosed glomeruli: _____

Adequate cortex present: ☐ Yes ☐ No

LIGHT MICROSCOPY

Glomerular findings:

Endocapillary hypercellularity ☐ Absent ☐ Present

Mesangial hypercellularity ☐ Absent ☐ Present

Subendothelial deposits / wire loops ☐ Absent ☐ Present

Necrosis / karyorrhexis ☐ Absent ☐ Present

Crescents:

Cellular _____ %

Fibrocellular _____ %

Fibrous _____ %

Segmental sclerosis ☐ Absent ☐ Present

Global sclerosis _____ %

Tubulointerstitial findings:

Interstitial inflammation ☐ None ☐ Mild ☐ Moderate ☐ Severe

Tubular atrophy ☐ None ☐ Mild ☐ Moderate ☐ Severe

Interstitial fibrosis ☐ None ☐ Mild ☐ Moderate ☐ Severe

Vascular findings:

Immune deposits ☐ Yes ☐ No

Thrombotic microangiopathy ☐ Yes ☐ No

Lupus vasculopathy ☐ Yes ☐ No

Necrotizing vasculitis ☐ Yes ☐ No

Arteriosclerosis / arteriolosclerosis ☐ Mild ☐ Moderate ☐ Severe

IMMUNOFLUORESCENCE

Immunoglobulins:

IgG ☐

IgA ☐

IgM ☐

Complement:

C3 ☐

C1q ☐

C4 ☐

Pattern: ☐ Full house ☐ Non–full house

Distribution:

Mesangial ☐

Subendothelial ☐

Subepithelial ☐

Vascular ☐

ELECTRON MICROSCOPY

Immune deposits:

Mesangial ☐

Subendothelial ☐

Subepithelial ☐

Podocyte foot process effacement ☐ Present ☐ Absent

Basement membrane changes ☐ Present ☐ Absent

DIAGNOSIS (MANDATORY)

ISN/RPS Class:

☐ I ☐ II ☐ III ☐ IV ☐ V ☐ VI

Combined class (if applicable): ☐ III+V ☐ IV+V

Activity Index (0–24): _____

Chronicity Index (0–12): _____

Percentage of glomeruli with:

Necrosis: _____ %

Crescents: _____ %

FINAL INTERPRETIVE COMMENT

Dominant pattern of injury

Prognostic indicators (chronicity, interstitial fibrosis)

Comparison with prior biopsy (if available)

REVISION – SUMMARY OF LUPUS NEPHRITIS

Definition

Immune complex–mediated glomerulonephritis occurring in systemic lupus erythematosus, diagnosed and classified by renal biopsy.

Pathogenesis (Key Points)

Anti-dsDNA antibodies + nucleosomes → immune complex deposition

Classical + alternative complement activation

Type I interferon pathway

NETosis

Podocyte injury (membranous LN, lupus podocytopathy)

ISN/RPS CLASSIFICATION (CURRENT)

Class I: Minimal mesangial

Class II: Mesangial proliferative

Class III: Focal LN (<50% glomeruli)

Class IV: Diffuse LN (≥50% glomeruli)

Class V: Membranous LN

Class VI: Advanced sclerosing LN (≥90% sclerosis)

ACTIVITY vs CHRONICITY

Activity index: Reversible lesions
(necrosis, crescents, endocapillary proliferation)

Chronicity index: Irreversible damage
(glomerulosclerosis, fibrosis, tubular atrophy)

Chronicity predicts outcome better than class

IMMUNOFLUORESCENCE

"Full house" staining: IgG, IgA, IgM, C3, C1q

C1q dominance → classical pathway

Fibrin in necrosis and crescents

SPECIAL ENTITIES

Lupus podocytopathy

Nephrotic syndrome

MCD / FSGS pattern

No immune deposits

ANCA-positive LN

Necrotizing crescentic GN

Overlap immune + pauci-immune injury

PROGNOSIS

10-year renal survival: **>80–90%**

Poor prognostic factors:

High chronicity index

Interstitial fibrosis

Persistent activity

Vascular lesions (APS/TMA)

Antiphospholipid syndrome in lupus nephritis

Lupus anticoagulant / Antiphospholipid syndrome (APS):
Antiphospholipid syndrome is characterized by antibodies directed against phospholipid-binding plasma proteins, particularly β2-glycoprotein I, resulting in a hypercoagulable state. The antibodies include lupus anticoagulant, anticardiolipin antibodies, and anti-β2-glycoprotein I antibodies. APS has varied clinical manifestations with multisystem involvement and may be seen in association with systemic lupus **e**rythematosu**s** or as a primary disorder, including in pregnant women.

Laboratory diagnosis is established by the demonstration of lupus anticoagulant by coagulation assays and/or detection of anticardiolipin and anti-β2-glycoprotein I antibodies by ELISA. Renal involvement is common and is termed antiphospholipid antibody–associated nephropathy, characterized by acute and chronic vascular lesions, ranging from glomerular capillary and arteriolar microthrombosis to thrombosis of the main renal artery or vein, resulting in cortical necrosis and renal infarction.

Points needing correction

Antibody specificity

APS antibodies are *directed mainly against phospholipid-binding proteins* (especially β2-glycoprotein I), not phospholipids alone.

Laboratory diagnosis

ELISA detects anticardiolipin and anti-β2-glycoprotein I antibodies

Lupus anticoagulant is detected by coagulation assays, not ELISA.

Renal pathology

The preferred term now is antiphospholipid antibody–associated nephropathy, characterized by:

Acute thrombotic microangiopathy

Chronic vascular lesions (fibrous intimal hyperplasia, recanalized thrombi)

Minor language edits needed for flow and punctuation.

HEREDITARY NEPHRITIS (ALPORT SYNDROME)

Definition

Hereditary nephritis (Alport syndrome) is an inherited disorder characterized by progressive renal disease associated with sensorineural deafness and ocular abnormalities, caused by defects in type IV collagen of basement membranes.

Clinical Features

Renal disease is associated with sensorineural hearing loss and ocular abnormalities, including anterior lenticonus (characteristic), posterior subcapsular cataracts, corneal dystrophy, and retinal flecks.

Males are more commonly and more severely affected, especially in X-linked disease, and are more likely to progress to renal failure.

Persistent microscopic or gross hematuria is the earliest manifestation; RBC casts may be present.

Symptoms usually begin in childhood or adolescence (5–20 years). Progressive renal insufficiency leading to ESRD commonly develops in affected males between 20–40 years.

Inheritance is genetically heterogeneous:

X-linked (most common)

Autosomal recessive

Rarely autosomal dominant

Pathogenesis

The disease results from defective synthesis of the glomerular basement membrane (GBM) due to mutations in genes encoding type IV collagen α chains.

In X-linked Alport syndrome, mutations involve the COL4A5 gene encoding the α5 chain of type IV collagen.

Autosomal forms involve COL4A3 or COL4A4 genes.

Defective collagen leads to structural instability and progressive damage of glomerular, tubular, cochlear, and ocular basement membranes.

Pathology

Light Microscopy (LM)

Glomeruli are **variably affected**.

Early stages may show minimal changes; later stages show:

- Focal segmental glomerulosclerosis
- Increased mesangial matrix
- Tubular atrophy and interstitial fibrosis
- Vascular narrowing

Foam cells (lipid-laden tubular epithelial cells) may be present in the interstitium.

Immunofluorescence (IF)

Negative for immune complex deposition.

Absence of α3, α4, or α5 chains of type IV collagen in the GBM on special collagen staining helps differentiate from anti-GBM disease (Goodpasture syndrome).

Electron Microscopy (EM)

Characteristic and diagnostic

GBM shows:

- Irregular thinning and thickening
- Splitting and lamellation of the lamina densa producing a “basket-weave” or “moth-eaten” appearance

Similar changes are seen in the tubular basement membrane.

Recent Advances / Current Concepts

Molecular genetic testing is now the gold standard for definitive diagnosis and family screening.

Early ACE inhibitor or ARB therapy significantly delays progression to ESRD.

Recognition of autosomal dominant Alport syndrome as more common than previously thought.

Alport syndrome is now viewed as a spectrum disorder rather than a single phenotype.

MINIMAL CHANGE DISEASE (MCD, LIPOID NEPHROSIS)

Definition

Minimal change disease is a glomerular disease characterized by nephrotic syndrome with minimal or no abnormalities on light microscopy, but with diffuse podocyte foot process effacement on electron microscopy.

Clinical Features

Most common cause of nephrotic syndrome in children, accounting for 70–90% of cases.

Peak age: **2–6 years**

Usually presents with:

Heavy proteinuria

Hypoalbuminemia

Edema

Blood pressure is usually normal, and renal function is preserved at presentation.

Hematuria and hypertension are uncommon.

Etiology and Pathogenesis

The exact cause is unknown, but strong evidence supports an immune-mediated podocytopathy.

Supporting features include:
a) Frequent onset following respiratory infections or immunizations
b) Dramatic response to corticosteroids
c) Association with atopic disorders (eczema, asthma, allergic rhinitis)
d) Increased frequency of HLA haplotypes associated with atopy
e) Increased association with Hodgkin lymphoma, suggesting T-cell dysfunction

Activated T cells are believed to release a circulating permeability factor (cytokine-like mediator) that causes podocyte injury and foot process effacement.

Proteinuria is selective, mainly involving albumin.

Tubular epithelial cells reabsorb filtered lipoproteins, leading to lipid-laden tubular cells, hence the term *lipoid nephrosis*.

Secondary Minimal Change Disease

Secondary MCD may be seen in association with:

NSAIDs

Rarely lithium therapy

Malignancies (especially Hodgkin lymphoma)

Withdrawal of the offending drug usually results in remission within weeks.

Pathology

Light Microscopy (LM)

Glomeruli appear normal.

Mild mesangial prominence may be seen.

Proximal tubules show lipid and protein droplets due to reabsorption.

Immunofluorescence (IF)

Negative for immunoglobulins and complement.

Electron Microscopy (EM)

Diffuse and uniform effacement of podocyte foot processes

Microvillous transformation of podocytes

Changes are completely reversible with remission.

Prognosis

Excellent prognosis in both children and adults.

Most patients show **complete remission within 4–8 weeks** of corticosteroid therapy.

Relapses are common, especially in children, but:

There is **no progression to chronic kidney disease** in typical cases.

Histologic Variants of Minimal Change Disease

1. Minimal Change Disease with Diffuse Mesangial Hypercellularity

Definition:
Presence of **>4 mesangial cells per mesangial area** in ≥80% of glomeruli.

Clinical Features

Accounts for ~3% of pediatric idiopathic nephrotic syndrome.

Patients more likely to have:

Hematuria

Hypertension

Steroid dependence

Pathology

LM: Mesangial hypercellularity without capillary lumen occlusion

IF: Usually negative; occasional **IgM and C3 mesangial positivity**

EM: Diffuse podocyte foot process effacement

2. IgM Nephropathy

Characterized by diffuse and global mesangial IgM deposition (≥2+) on IF.

LM shows normal glomeruli or mild mesangial hypercellularity.

EM shows podocyte foot process effacement.

Considered by many as a variant or overlap with MCD/FSGS spectrum.

3. Minimal Change Disease with IgA Nephropathy

Patients present with nephrotic syndrome.

IF shows mesangial IgA deposits.

EM demonstrates diffuse foot process effacement typical of MCD.

No capillary wall immune deposits.

LM shows little or no mesangial hypercellularity.

FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FSGS)

Definition

Focal segmental glomerulosclerosis is a glomerular disease characterized by nephrotic-range proteinuria, focal and segmental sclerosis of glomeruli, and diffuse podocyte foot process effacement, in the absence of glomerular immune complex deposition.

Minimal change disease (MCD) and FSGS are considered part of a **podocytopathy spectrum**, with MCD representing the milder, steroid-responsive end and FSGS representing a more severe, often steroid-resistant form that frequently progresses to end-stage renal disease (ESRD).

Clinical Features

Accounts for approximately 10% of nephrotic syndrome in children and 10–20% in adults.

Many patients present with hypertension and/or microscopic haematuria.

Most cases are idiopathic (primary) FSGS, many of which progress to renal failure over 5–10 years.

Genetic forms are increasingly recognized:

Mutations in genes encoding podocyte proteins such as NPHS2 (podocin), NPHS1 (nephrin), ACTN4, TRPC6, and INF2.

Secondary FSGS occurs in association with:

Reduced nephron mass (reflux nephropathy, obesity, unilateral renal agenesis)

HIV infection (collapsing FSGS)

Heroin abuse

Sickle cell disease

Long-standing hypertension

Pathology

The lesion often begins in juxtamedullary glomeruli and may be missed on early biopsy. Repeat biopsy is indicated in patients diagnosed as MCD who fail to respond to corticosteroids.

Light Microscopy (LM)

Segmental sclerosis and hyalinosis involving a portion of the glomerular tuft, with sparing of other segments and glomeruli.

Hyalinosis represents plasma protein insudation beneath the GBM:

PAS positive

Trichrome red

Non-argyrophilic

Some glomeruli may be globally sclerosed.

Foam cells may be present in affected segments.

Tubulointerstitial changes:

Tubular atrophy

Interstitial fibrosis

Lymphocyte and plasma cell infiltration

Cystic dilatation of tubules and Bowman's space with proteinaceous material

Variants (Columbia Classification)

Collapsing variant (common in HIV-associated nephropathy):

Collapse and wrinkling of GBM

Podocyte hypertrophy and hyperplasia

Cellular variant:

Endocapillary hypercellularity

Foam cells, karyorrhexis

Podocyte hypertrophy and hyperplasia

Tip lesion, perihilar variant, and NOS variant (for classification completeness)

Immunofluorescence (IF)

Usually negative or shows nonspecific trapping of IgM and C3 in sclerotic segments.

Electron Microscopy (EM)

Diffuse effacement of podocyte foot processes

Folding and thickening of GBM

Capillary loop collapse in advanced lesions

Electron-dense material within sclerotic segments (plasma protein trapping)

Tubuloreticular inclusions in endothelial cells are seen in HIV-associated FSGS, not in idiopathic or heroin-related disease.

Prognosis

Approximately 50% of patients show progressive decline in renal function within 10 years.

Around 20–30% progress rapidly to ESRD within 3–5 years.

FSGS has a high recurrence rate after renal transplantation (25–50%), suggesting a circulating permeability factor.

Differentiation from MCD in children is critical due to marked differences in response to therapy and outcome.

Note: Up to 10% of glomeruli may be globally sclerosed by age 40 due to aging and should not be misinterpreted as FSGS.

Recent Advances / Current Concepts

FSGS is now regarded as a heterogeneous group of podocyte disorders, not a single disease.

Recognition of primary (circulating factor–mediated) vs secondary (adaptive) vs genetic FSGS.

Identification of suPAR and other circulating permeability factors (still under investigation).

Genetic testing is recommended in steroid-resistant nephrotic syndrome, especially in children.

Primary FSGS recurs post-transplant, while genetic and secondary forms usually do not.

MEMBRANOUS GLOMERULONEPHRITIS

(Membranous Nephropathy)

Definition

Membranous nephropathy is a glomerular disease characterized by subepithelial immune complex deposition along the GBM, leading to diffuse thickening of the capillary walls and nephrotic syndrome.

Clinical Features

Most common cause of nephrotic syndrome in adults, accounting for **30–40%** of cases.

Occurs predominantly in middle-aged and older adults.

Can be:

Primary (idiopathic) – majority

Secondary – associated with systemic diseases, infections, drugs, or malignancies

Pathogenesis

The disease is caused by immune complex deposition on the epithelial (subepithelial) side of the GBM.

Primary membranous nephropathy is now established as an autoimmune disease.

Genetic susceptibility (HLA alleles) influences antibody formation.

Known Antigens

Primary (idiopathic):

PLA2R (M-type phospholipase A2 receptor) – most common

THSD7A (minor subset)

Secondary membranous nephropathy:

Tumor antigens (lung, colon, breast, melanoma)

HBsAg

DNA–anti-DNA complexes in SLE

Drugs: gold, penicillamine, captopril

Infections: malaria, syphilis, schistosomiasis

Immune complexes are largely formed **in situ** due to binding of antibodies to planted or intrinsic podocyte antigens.

Complement Activation

Complement activation via the lectin and alternative pathways.

C3 levels are usually normal.

Formation of C5b–C9 membrane attack complex (MAC) damages podocytes, increasing glomerular permeability and causing proteinuria.

Urinary excretion of C5b–C9 correlates with disease activity.

Pathology -Light Microscopy (LM)

Glomeruli are normocellular or mildly enlarged.

Capillary walls appear normal early, then become diffusely thickened.

Silver stain shows characteristic “spikes” projecting from the GBM.

Late stages show glomerulosclerosis and hyalinization.

Immunofluorescence (IF)

Granular deposition of IgG and C3 along the GBM.

In secondary forms, mesangial deposits and hypercellularity may be present.

Electron Microscopy (EM)

Subepithelial electron-dense deposits along the GBM.

GBM forms spike-like projections around the deposits.

With progression, deposits are incorporated into the GBM.

Late stages show resorption of deposits, leaving lucent GBM defects.

Prognosis

Approximately:

- 25% undergo spontaneous remission
- 25% have persistent proteinuria with stable renal function
- 50% progress to renal failure over 10–15 years

Progression is variable and difficult to predict at diagnosis.

Poor prognostic factors include:

- Persistent heavy proteinuria
- Male sex
- Elevated serum creatinine at presentation

Recent Advances / Current Concepts

Discovery of PLA2R and THSD7A antibodies has revolutionized diagnosis.

Serum anti-PLA2R levels correlate with disease activity and prognosis.

Distinction between primary and secondary membranous nephropathy is now immunologically based.

Targeted immunotherapy (e.g., rituximab) increasingly used.

COMPARATIVE FEATURES OF MINIMAL CHANGE DISEASE, FOCAL SEGMENTAL GLOMERULOSCLEROSIS AND MEMBRANOUS NEPHROPATHY

Feature	Minimal Change Disease (MCD)	Focal Segmental Glomerulosclerosis (FSGS)	Membranous Nephropathy (MN)
Typical age group	Mainly children (peak 2–6 years); also adults	Children and adults	Adults (middle-aged and elderly)
Frequency as cause of nephrotic syndrome	Most common in children (70–90%)	10% children, 10–20% adults	Most common in adults (30–40%)
Nature of proteinuria	Heavy, **selective** (albumin)	Heavy, usually **non-selective**	Heavy, non-selective
Hematuria / Hypertension	Rare	Common	Occasional
Etiology / Pathogenesis	Immune-mediated podocyte injury due to circulating permeability factor; T-cell dysfunction	Podocyte injury due to circulating factor, genetic mutations, or adaptive responses; part of podocytopathy spectrum	Immune complex–mediated disease with subepithelial deposition; autoimmune (PLA2R/THSD7A)
Primary vs secondary forms	Mostly primary; secondary with NSAIDs, Hodgkin lymphoma	Primary, secondary (HIV, reflux, obesity, heroin), and genetic forms	Primary (idiopathic) and secondary (SLE, infections, drugs, malignancy)

Light microscopy (LM)	Glomeruli appear normal or show minimal mesangial prominence	Focal and segmental sclerosis and hyalinosis; foam cells; tubulointerstitial fibrosis	Diffuse thickening of capillary walls; spikes on silver stain
Immunofluorescence (IF)	Negative	Negative or nonspecific IgM and C3 trapping in sclerotic segments	Granular IgG and C3 along GBM
Electron microscopy (EM)	Diffuse, uniform foot process effacement	Diffuse foot process effacement with GBM folding and segmental collapse	Subepithelial electron-dense deposits with GBM spike formation
Complement levels	Normal	Normal	Usually normal
Response to steroids	Excellent	Poor to variable	Variable
Progression to ESRD	Rare	Common	Occurs in ~50% over 10–15 years
Recurrence after renal transplant	Rare	Common (25–50%)	Occasional
Prognosis	Excellent; relapses common but renal function preserved	Guarded; many progress to ESRD	Variable; spontaneous remission in ~25%

Key Pearls

MCD → normal LM, negative IF, diffuse foot process effacement, steroid responsive

FSGS → focal & segmental sclerosis, IgM/C3 trapping, poor steroid response, recurrent post-transplant

Membranous nephropathy → subepithelial immune deposits, spikes on silver stain, PLA2R antibodies

CONGENITAL NEPHROTIC SYNDROME (CNS)

Definition

Congenital nephrotic syndrome refers to the development of nephrotic syndrome at birth or within the first 3 months of life. It is caused by a heterogeneous group of disorders, most commonly genetic defects affecting podocyte structure and function.

The two classical genetic forms unique to this age group are:

Congenital nephrotic syndrome of the Finnish type (CNF)

Diffuse mesangial sclerosis (DMS)

Congenital Nephrotic Syndrome of the Finnish Type (CNF)

Etiology and Genetics

Autosomal recessive disorder caused by mutations in the **NPHS1 gene**, encoding **nephrin**, a key component of the slit diaphragm.

High carrier frequency in Finland (~1 in 200), hence the name.

Clinical Features

Affected fetuses are often premature and may have postural deformities (contractures of knees and elbows).

Markedly increased placental weight with a high placental-to-fetal weight ratio is a characteristic prenatal clue.

Infants are small for gestational age and often have edema at birth; massive ascites may cause respiratory distress.

Massive proteinuria (often 1–6 g/day or more).

Progressive disease with early development of end-stage renal disease, usually in early childhood.

Pathology -Light Microscopy (LM)

Cystic dilatation of proximal tubules, initially in the inner cortex and corticomedullary junction.

Tubular epithelial cells contain protein resorption droplets (PAS positive, trichrome red) and lipid droplets.

Glomeruli may be:

Normal initially

Microglomeruli may be present

Mild mesangial expansion

With progression, FSGS and global glomerulosclerosis develop.

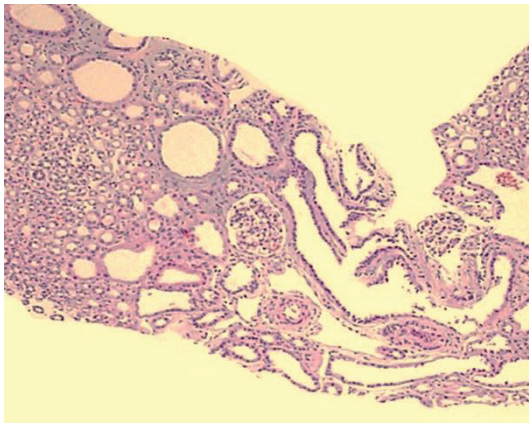


Fig 9.12 shows dilated tubules in a case of congenital nephrotic syndrome of the Finnish type H & E x 100

Prognosis

Poor, without renal replacement therapy.

Most patients progress to ESRD in early childhood.

Recent Advances / Current Concepts

CNS of Finnish type is now classified as a podocytopathy.

Genetic diagnosis allows prenatal screening and family counseling.

Early nephrectomy with dialysis followed by renal transplantation has improved survival.

Disease does not recur in the transplanted kidney.

Diffuse Mesangial Sclerosis (DMS)

Definition

Diffuse mesangial sclerosis is a severe form of congenital nephrotic syndrome characterized by diffuse and global mesangial matrix expansion, leading to rapid renal failure.

Clinical Features

Onset is usually later than CNF, typically between 3–9 months of age.

Unlike CNF, DMS is not associated with placentomegaly, prematurity, or low birth weight.

Hypertension is common, and renal failure progresses rapidly within 1–3 years.

Mostly renal-limited, but may be associated with syndromic abnormalities.

The association of Diffuse mesangial sclerosis, Wilms tumor, and male pseudohermaphroditism constitutes **Denys–Drash syndrome**.

Genetics

Most commonly caused by mutations in the WT1 gene.

Can be sporadic or familial.

Pathology - Light Microscopy (LM)

Diffuse and global increase in mesangial matrix, often with mesangial hypercellularity.

Progressive FSGS or global glomerulosclerosis.

Podocyte capping over sclerotic tufts may be seen.

Tubulointerstitial changes:

Tubular ectasia and microcyst formation

Hyaline casts

Protein resorption droplets

Prognosis

Very poor, with rapid progression to ESRD.

Recent Advances / Current Concepts

DMS is now recognized as a developmental podocytopathy.

WT1 mutation testing is crucial due to the risk of Wilms tumor.

Early bilateral nephrectomy and transplantation improve outcomes.

Disease does not recur post-transplant.

DIABETIC NEPHROPATHY *(Diabetic Kidney Disease)*

Definition

Diabetic nephropathy is a progressive microvascular complication of diabetes mellitus, characterized by proteinuria, declining renal function, and characteristic glomerular, tubular, and vascular lesions.

Clinical Features

Usually develops after **≥10 years of diabetes**, earlier in poorly controlled disease.

Microalbuminuria is the earliest detectable abnormality, progressing to overt proteinuria.

Edema is often the first clinical manifestation.

Diabetic retinopathy is commonly present.

Hypertension is frequent and accelerates progression.

Pathogenesis

Diabetic nephropathy results from a combination of metabolic and hemodynamic mechanisms:

Metabolic Factors

Non-enzymatic glycation of basement membrane proteins → advanced glycation end-products (AGEs).

Increased synthesis of type IV collagen, fibronectin, and laminin.

Activation of TGF-β, PKC, and oxidative stress pathways.

Hemodynamic Factors

Early glomerular hyperfiltration due to afferent arteriolar dilation.

Increased intraglomerular pressure leads to:

- Mesangial expansion
- Glomerulosclerosis

Progressive loss of podocytes contributes to proteinuria.

Pathology - Light Microscopy (LM)

Glomerulomegaly.

Diffuse diabetic glomerulosclerosis:

- Diffuse GBM thickening
- Increased mesangial matrix

Nodular glomerulosclerosis (Kimmelstiel–Wilson nodules):

- Rounded, acellular mesangial nodules
- Laminated appearance on silver stain

Exudative lesions:

- Capsular drops
- Fibrin caps

Hyaline arteriolosclerosis involving both afferent and efferent arterioles (characteristic).

Armanni–Ebstein lesion: glycogen accumulation in tubular epithelium.

Thickening of tubular basement membranes.

Immunofluorescence (IF)

Diffuse linear staining for IgG and albumin along GBM and TBM (non-immune trapping).

Electron Microscopy (EM)

Uniform and diffuse thickening of the GBM (up to 6–10 times normal).

Mesangial expansion with increased matrix.

Prognosis

Approximately **30–40% of type 1** and **15–20% of type 2 diabetics** develop diabetic nephropathy.

Because type 2 diabetes is more prevalent, it accounts for the majority of ESRD cases worldwide.

Tight glycemic control, blood pressure control, and RAAS blockade significantly delay progression.

Recent Advances / Current Concepts

Term "Diabetic kidney disease (DKD)" increasingly used.

Microalbuminuria screening is standard for early detection.

SGLT2 inhibitors and GLP-1 receptor agonists provide renoprotection beyond glycemic control.

Podocyte loss is now recognized as a key driver of progression.

Regression of early diabetic nephropathy is possible with aggressive therapy.

AMYLOIDOSIS (RENAL)

Definition

Amyloidosis is defined as extracellular deposition of misfolded proteins arranged in a characteristic β-pleated sheet conformation, resulting in:

Congo red positivity on light microscopy with apple-green birefringence under polarized light

Formation of non-branching, randomly oriented fibrils measuring 7–10 nm on electron microscopy.

Clinical Features

Renal involvement is most common in:

AA (secondary) amyloidosis, associated with:

Chronic inflammatory and infectious diseases

Rheumatoid arthritis

Chronic osteomyelitis

- Inflammatory bowel disease
- Malignancies
- Familial Mediterranean fever (FMF) and other autoinflammatory syndromes (AA type)

AL (primary) amyloidosis

- Commonly associated with plasma cell dyscrasias
- Occurs predominantly in λ light chain–restricted myeloma
- Also seen in other B-cell lymphoproliferative disorders

Clinical presentation

- Proteinuria (often massive)
- Nephrotic syndrome
- Progressive renal insufficiency
- Renal failure may be rapid or slowly progressive
- Renal involvement is a major determinant of prognosis and mortality

Pathology

1. Distribution of amyloid deposits

- Glomeruli (mesangium → capillary walls)
- Blood vessels (arteries and arterioles)
- Interstitium
- Tubular basement membranes

Deposits are amorphous and weakly acidophilic, less dense than sclerosis or mesangial matrix.

2. Light Microscopy

- Amyloid deposits in mesangium and capillary walls
- Positive staining with:
  - Congo red
  - Thioflavin T
  - Crystal violet
- **Apple-green birefringence** under polarized light
- Non-argyrophilic (negative on silver stains)

Potassium permanganate test:

AA amyloid: loss of Congo red staining

AL amyloid: staining retained

(Note: now largely of historical interest)

3. Immunofluorescence

Typically negative in AA amyloidosis

AL amyloidosis may show monoclonal κ or λ light-chain staining

Routine IF has limited sensitivity

4. Electron Microscopy

Non-branching fibrils

Diameter: 7–10 nm

First appear in mesangium → extend to capillary walls and vessels

Recent Advances in Renal Amyloidosis

Proteomic typing using laser microdissection with mass spectrometry is now the gold standard for amyloid typing

Recognition of newer renal amyloidoses:

- ALECT2 amyloidosis (common in elderly, often renal-limited)
- ATTR amyloidosis (hereditary and wild-type)

SAP scintigraphy useful for assessing systemic amyloid burden (limited renal resolution)

Congo red remains diagnostic, but typing is essential for therapy

Prognosis and Therapy

Prognosis depends on:

- Amyloid type
- Degree of renal involvement
- Response to treatment

AL amyloidosis:

- Treated with plasma-cell–directed therapy (bortezomib-based regimens)

AA amyloidosis:

- Control of underlying inflammatory disease
- **Colchicine** effective in FMF

Renal transplantation possible in selected cases after disease control

THROMBOTIC MICROANGIOPATHY (TMA)

Definition

Thrombotic microangiopathy is a pathologic term describing a pattern of microvascular injury characterized by:

Endothelial damage

Platelet-rich thrombi

Microangiopathic hemolytic anemia

Thrombocytopenia

Organ ischemia (kidney predominant)

Conditions Associated with TMA

Hemolytic uremic syndrome (HUS)

Thrombotic thrombocytopenic purpura (TTP)

Disseminated intravascular coagulation

Malignant hypertension

Scleroderma renal crisis

Preeclampsia/eclampsia

Antiphospholipid antibody syndrome

Drug-induced TMA (calcineurin inhibitors, chemotherapy)

Transplant-associated TMA

Relationship Between HUS and TTP

Morphologic renal lesions are virtually identical

Differences are primarily clinical and pathogenetic

Currently regarded as distinct entities within the TMA spectrum:

TTP: ADAMTS13 deficiency, CNS-predominant

HUS: renal-predominant

Hemolytic Uremic Syndrome (HUS)

Clinical Forms

1. Typical (Diarrhea-positive, D+) HUS

Seen mainly in children

Associated with Shiga toxin–producing E. coli (STEC)

Presents with:

- Bloody diarrhea
- Hemorrhagic colitis
- Acute kidney injury

Renal TMA predominantly glomerular

Generally better prognosis

2. Atypical (Diarrhea-negative, D–) HUS

Occurs in children and adults

Accounts for 5–10% of cases

Caused by dysregulation of the alternative complement pathway

- Mutations in CFH, CFI, MCP, C3, factor B

Characterized by:

- Severe hypertension
- Heavy proteinuria
- Progressive renal failure

Renal arterial involvement is prominent

Poor prognosis without treatment

Transplant-Associated HUS

May be:

- Recurrent (genetic aHUS)
- De novo (calcineurin inhibitors, antibody-mediated rejection)

Differentiation from rejection is difficult

Presence of MAHA and thrombocytopenia supports TMA

Pathology of Renal TMA

Gross

- Enlarged swollen kidneys
- Patchy or diffuse cortical necrosis
- Petechial hemorrhages

Light Microscopy

Glomeruli

- Endothelial swelling with subendothelial expansion
- Double contours (“tram-tracking”)
- Occluded capillary lumina (“bloodless glomeruli”)
- Platelet-fibrin thrombi
- Mesangiolysis and capillary ectasia
- Late stages show sclerosis and ischemic changes

Vessels

- Endothelial swelling
- Fibrinoid necrosis
- Mucoid intimal hyperplasia
- Concentric “onion-skin” lesions in chronic disease

Comparative Table — Typical HUS vs Atypical HUS vs TTP

Feature	Typical HUS (STEC-HUS)	Atypical HUS (aHUS)	TTP (Thrombotic Thrombocytopenic Purpura)
Etiology	Shiga toxin-producing *E. coli* (STEC) infection	Complement dysregulation (genetic/acquired)	Severe ADAMTS13 deficiency (autoantibodies or rare genetic)
Pathogenesis	Endothelial injury via Shiga toxin → complement activation and TMA	Uncontrolled activation of alternative complement pathway → endothelial damage and TMA	Accumulation of ultra-large vWF multimers → platelet aggregation and microthrombosis
Typical Preceding Event	Gastroenteritis with bloody diarrhea	None specific (may be triggered by infection, pregnancy, drugs)	None specific
ADAMTS13 Activity	Normal or modestly reduced	Normal	**Severe deficiency (<10%)**
Complement Abnormalities	Not primary	Common (mutations in factor H, MCP/CD46, factor I, C3, factor B)	Not typical
Age Distribution	Mainly children	Children and adults	Mostly adults
Prominent Organ Involvement	**Kidneys predominantly**	Kidneys (may have multi-organ involvement)	**CNS predominantly**
Renal Dysfunction	Severe AKI common	Severe AKI frequent	Mild to moderate
Neurologic Symptoms	Less common	May occur	Prominent (confusion, seizures)
Platelet Count	Low	Low	Very low
Hemolytic Anemia	Present	Present	Present

Treatment	Supportive; limited specific therapy	**Complement blockade** (eculizumab/ravulizumab)	**Plasma exchange + immunosuppression**
Prognosis	Often good with supportive care	Variable; risk of ESRD if untreated	

NEPHROSCLEROSIS

Nephrosclerosis refers to chronic ischemic renal injury resulting from **long-standing** hypertension and aging, characterised by progressive vascular, glomerular, tubular, and interstitial sclerosis.

A. Benign (Hypertensive) Nephrosclerosis

Benign nephrosclerosis is renal injury caused by chronic mild to moderate hypertension. Similar morphologic changes may be seen with advanced aging even in the absence of overt hypertension.

Pathogenesis

Chronic endothelial injury leads to arteriolar hyalinosis and arteriosclerosis, with:

Plasma protein insudation

Increased deposition of extracellular matrix

Smooth muscle cell migration and proliferation

Hyaline arteriolosclerosis results from leakage of plasma constituents (proteins, lipids) into the arteriolar wall.

Progressive luminal narrowing causes chronic ischemia, leading to:

Glomerular sclerosis

Tubular atrophy

Interstitial fibrosis

Glomerular sclerosis involves excess production of type IV collagen (GBM) and type I/III collagen (mesangium/interstitium).

Pathology

Light Microscopy

Focal and global glomerulosclerosis

Arteriolar hyalinosis (especially afferent arterioles)

Fibroelastic intimal thickening of arteries

Contracted, wrinkled sclerosed glomeruli often retracted toward the vascular pole

Tubular atrophy and interstitial fibrosis

Important distinction:
In diabetes mellitus, hyaline arteriolosclerosis involves both afferent and efferent arterioles, unlike benign nephrosclerosis.

Special stains

PAS: strongly positive

Silver stain: negative

Trichrome: fuchsinophilic

Immunofluorescence

Usually negative

Nonspecific trapping of IgM and C3 may be seen in sclerotic areas

Electron Microscopy

Thickened, wrinkled GBM

Increased mesangial matrix

B. Malignant Nephrosclerosis (Hypertensive Emergency)

Represents a form of **thrombotic microangiopathy** due to abrupt, severe hypertension.

Gross

Petechial cortical hemorrhages ("**flea-bitten kidney**")

Possible cortical infarction

Microscopy

Fibrinoid necrosis of afferent arterioles and small arteries

Hyperplastic arteriolosclerosis ("**onion-skin**" intimal thickening)

Glomerular ischemic collapse

Patchy cortical necrosis

PYELONEPHRITIS

Inflammation of the renal parenchyma and pelvis caused by **bacterial infection**. May be **acute or chronic**.

A. Acute Pyelonephritis

Pathogenesis

- Ascending infection from the lower urinary tract (most common)
- Hematogenous spread (rare; e.g. infective endocarditis)

Causative organisms

- *Escherichia coli* (most common)
- *Proteus mirabilis*
- *Klebsiella*, *Enterococcus*

Predisposing factors

- Urinary tract obstruction
- Vesico-ureteric reflux
- Instrumentation
- Diabetes mellitus
- Pregnancy

Gross

- Enlarged, edematous kidneys
- Yellow-white cortical microabscesses
- Patchy suppuration

Microscopy

- Neutrophilic infiltration of tubules (tubulitis)
- Interstitial neutrophils and microabscesses

Tubular necrosis in severe cases

Complications

- Papillary necrosis
- Pyonephrosis
- Sepsis

B. Chronic Pyelonephritis

Results from **recurrent infection**, usually associated with:

Chronic obstruction

Vesicoureteric reflux (reflux nephropathy)

Pathogenesis (Reflux nephropathy)

Incompetent UV junction → vesicoureteric reflux

Intrarenal reflux occurs at compound papillae, especially at poles

Leads to focal polar scarring

Gross

Asymmetrically shrunken kidneys

Adherent capsule

Irregular **U-shaped cortical scars** overlying distorted calyces

Polar predominance

Microscopy

Interstitial fibrosis with lymphocytes and plasma cells

Tubular atrophy and dilation

Thyroidisation of tubules

Periglomerular fibrosis

Secondary glomerulosclerosis

Arteriolosclerosis

END-STAGE RENAL DISEASE (ESRD)

ESRD is defined as irreversible renal failure requiring dialysis or transplantation to sustain life, typically persisting beyond 90 days.

Etiology

Common causes include:

Diabetic nephropathy

Hypertensive nephrosclerosis

Chronic glomerulonephritis

Chronic pyelonephritis / reflux nephropathy

Gross

Kidneys are bilaterally shrunken (late stages)

Cortical thinning

Exception: diabetic nephropathy and amyloidosis may have enlarged kidneys initially

Microscopy

Global glomerulosclerosis

Tubular atrophy and cystic dilation

Extensive interstitial fibrosis

Thickened, hyalinised blood vessels

Marked loss of nephron architecture

Oxalate crystal deposition may be seen in advanced uremia

Clinicopathologic Correlation

Histologic features become nonspecific in ESRD

Original disease may no longer be identifiable

RENAL NEOPLASMS

Renal neoplasms comprise a heterogeneous group of epithelial, mesenchymal, and embryonal tumours. The majority arise from renal tubular epithelium and are collectively termed renal cell carcinoma (RCC).

Renal Cell Carcinoma – Current Classification (WHO-based)

Tumour Type	Approx. Frequency
Clear cell RCC	65–70%
Papillary RCC	10–15%
Chromophobe RCC	5–7%
Collecting duct carcinoma	<1–2%
Other rare RCC subtypes (MiT family, FH-deficient, SDH-deficient, etc.)	Rare
Renal oncocytoma (benign)	~5%

Histologic, Immunohistochemical & Molecular Features of Major RCCs

1. Clear Cell Renal Cell Carcinoma

Histopathology

Alveolar, acinar, tubular, or cystic patterns

Cells with optically clear cytoplasm (lipid and glycogen) (Fig 9.13)

Delicate, branching vascular network ("chicken-wire")

Low N:C ratio

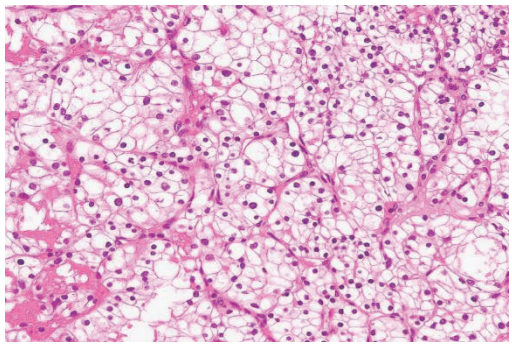


Fig 9.13 Clear neoplastic cells arranged in a tubular fashion H & E x 400

Genetics / Molecular

Chromosome 3p deletion

Inactivation of VHL gene

↑ HIF → ↑ VEGF, PDGF (basis for targeted therapy)

Immunohistochemistry

CD10: positive (membranous)

CAIX: strong diffuse positivity

PAX8: positive

CK7: negative or focal

Vimentin: positive

2. Papillary Renal Cell Carcinoma

Histopathology

Papillary or tubulopapillary architecture

Foamy macrophages within papillary cores

Psammoma bodies may be present

Type 1: basophilic cells, low-grade

Type 2: eosinophilic cells, higher-grade

Genetics

Trisomy of chromosomes 7 and 17

Loss of Y chromosome (males)

MET alterations (especially type 1)

Immunohistochemistry

CK7: positive

AMACR: positive

PAX8: positive

CD10: variable

3. Chromophobe Renal Cell Carcinoma

Histopathology

Solid sheets and nests

Pale or eosinophilic cytoplasm

Prominent cell borders ("plant cell" appearance)

Perinuclear halos

Diffuse colloidal iron positivity

Genetics

Multiple chromosome losses: **1, 2, 6, 10, 13, 17, 21**

Immunohistochemistry

CK7: diffuse strong positivity

CD117 (KIT): positive

CD10: negative

Vimentin: negative

Prognosis

Generally better than clear cell RCC

4. Collecting Duct Carcinoma (Bellini Duct Carcinoma)

Histopathology

Medullary location

Tubular, glandular or papillary growth

Hobnail cells

Prominent desmoplastic stroma

Genetics

Complex chromosomal abnormalities

Immunohistochemistry

CK7, CK19: positive

PAX8: positive

CD10: negative or focal

Prognosis

Highly aggressive

5. Renal Oncocytoma (Benign)

Histopathology

Well-circumscribed

Solid nests of uniform oncocytic cells

Abundant granular eosinophilic cytoplasm

Central scar may be present

Genetics

Loss of chromosomes 1 and Y

Mitochondrial DNA alterations

Immunohistochemistry

CD117: positive

CK7: negative or focal

Vimentin: negative

Mitochondrial marker: diffuse positivity

Recent Advances in Renal Cell Carcinoma: Emerging Molecularly Defined Entities

1. MiT Family Translocation Renal Cell Carcinoma

MiT family translocation renal cell carcinomas are characterized by gene fusions involving transcription factors of the microphthalmia (MiT) family, most commonly TFE3 and TFEB.

These tumours occur predominantly in children and young adults, but are increasingly recognized in adults.

Pathogenesis and Genetics

- Chromosomal translocations involving Xp11.2 (TFE3) or 6p21 (TFEB)
- Result in overexpression of MiT transcription factors, leading to dysregulated cell proliferation

Morphology

- Papillary, nested, or alveolar architecture
- Clear to eosinophilic cytoplasm
- Frequent psammoma bodies
- May closely mimic clear cell or papillary RCC

Immunohistochemistry

- Nuclear TFE3 or TFEB positivity
- PAX8 positive
- Variable CK7
- Often positive for Melan-A and HMB45 (melanocytic markers)

Clinical Behaviour

- Indolent in many paediatric cases
- Adults may show aggressive behaviour with late metastasis

2. FH-Deficient Renal Cell Carcinoma

FH-deficient RCC is an aggressive renal carcinoma associated with hereditary leiomyomatosis and renal cell carcinoma (HLRCC) syndrome, caused by mutations in the fumarate hydratase (FH) gene.

Pathogenesis and Genetics

- Germline or somatic inactivation of FH
- Accumulation of fumarate leads to oncogenic metabolic reprogramming (“oncometabolite” effect)

Morphology

- Variable architecture: papillary, tubulocystic, or solid
- Characteristic large eosinophilic nucleoli with perinucleolar halos

High-grade cytology common

Immunohistochemistry

Loss of FH expression

Positive 2-succino-cysteine (2SC) staining

PAX8 positive, CK7 variable

Clinical Behaviour

Highly aggressive

Early metastasis even with small primary tumours

Important to recognize due to genetic counselling implications

3. SDH-Deficient Renal Cell Carcinoma

SDH-deficient RCC is a rare renal tumour associated with germline mutations in succinate dehydrogenase (SDH) genes, particularly SDHB, and occurs in the setting of hereditary paraganglioma–pheochromocytoma syndromes.

Pathogenesis and Genetics

Inactivation of SDHA, SDHB, SDHC, or SDHD

Leads to mitochondrial dysfunction and altered cellular metabolism

Morphology

Solid, nested or tubular growth

Tumour cells have eosinophilic granular cytoplasm with cytoplasmic vacuoles

Often well circumscribed

Immunohistochemistry

Loss of SDHB expression (screening marker)

PAX8 positive

CK7 negative

Clinical Behaviour

Generally indolent

Rare cases may behave aggressively

Recognition prompts family screening

Exam-Oriented Summary Box

Entity	Key Molecular Feature	Hallmark IHC
MiT translocation RCC	TFE3 / TFEB fusion	Nuclear TFE3 / TFEB
FH-deficient RCC	FH mutation (HLRCC)	FH loss, 2SC +
SDH-deficient RCC	SDH gene mutation	SDHB loss

Comparative Table: Major Types of Renal Cell Carcinoma :

Feature	Clear Cell RCC	Papillary RCC	Chromophobe RCC	Collecting Duct RCC	MiT Translocation RCC	FH-Deficient RCC	SDH-Deficient RCC
Relative frequency	~70–75%	~10–15%	~5%	<1–2%	Rare	Rare	Very rare
Typical age	Adults (6–7th decade)	Adults	Adults	Adults	Children & young adults	Young adults	Young to middle-aged
Location	Cortex	Cortex	Cortex	Medulla	Cortex	Cortex	Cortex
Architecture	Alveolar, solid, tubular, cystic	Papillary, tubulopapillary	Solid sheets, trabeculae	Tubular, glandular	Papillary, nested, alveolar	Papillary, tubulocystic, solid	Solid, nested, tubular
Cytoplasm	Clear (lipid & glycogen)	Basophilic (Type 1) or eosinophilic (Type 2)	Pale or eosinophilic, granular	Eosinophilic	Clear to eosinophilic	Eosinophilic	Eosinophilic with vacuoles
Nuclear features	Low–moderate grade	Type 2 shows higher grade	Irregular nuclei, perinuclear halos	High-grade	Variable	Prominent nucleoli with perinucleolar halo	Uniform nuclei
Special histologic features	Rich sinusoidal vasculature	Foamy macrophages, psammoma bodies	Distinct cell borders, plant-cell appearance	Desmoplastic stroma, hobnail cells	Psammoma bodies	High-grade cytology	Well circumscribed
Cytogenetics / Molecular	3p loss, VHL alteration	Trisomy 7, 17; loss of Y	Loss of 1,2,6,10,13, 17	Multiple chromosomal losses	TFE3 / TFEB gene fusion	FH gene mutation (HLRCC)	SDH gene mutation (esp. SDHB)
CD10	Positive	Positive	Negative	±	Variable	Variable	Negative
CK7	Negative	Positive	Positive	Positive	Variable	Variable	Negative

Vimentin	Positive	Positive	Negative	Positive	Variable	Variable	Negative
Special IHC marker	CAIX positive	AMACR positive	KIT (CD117) positive	Ulex europaeus agglutinin	Nuclear TFE3 / TFEB	FH loss, 2SC positive	Loss of SDHB
Melanocytic markers	Negative	Negative	Negative	Negative	HMB45 / Melan-A positive	Negative	Negative
Clinical behaviour	Variable	Type 2 more aggressive	Usually indolent	Highly aggressive	Variable (worse in adults)	Highly aggressive	Usually indolent
Syndromic association	VHL syndrome	–	Birt-Hogg-Dubé	–	–	HLRCC	Paraganglioma – pheochromocytoma
Prognosis	Stage-dependent	Type-dependent	Good	Poor	Variable	Poor	Generally good

OTHER RENAL TUMOURS

Angiomyolipoma (AML)

Benign mesenchymal tumour (PEComa family)

Composed of fat, smooth muscle, and thick-walled blood vessels

25–50% associated with tuberous sclerosis complex

May be sporadic

Microscopy

Variable proportions of components

Epithelioid variant may mimic RCC

HMB45 and Melan-A positive

Clinical importance

Risk of hemorrhage

Rare malignant transformation (epithelioid AML)

Cystic Nephroma

Benign cystic renal tumour

More common in adult females

Multiloculated cystic mass with fibrous capsule

Septa lack clear cells and skeletal muscle

Importance

Differentiation from cystic RCC and cystic Wilms tumour

Diagnostic Immunohistochemistry Algorithm for Renal Tumours

Step 1: Confirm renal epithelial origin

PAX8 positive → Renal epithelial tumour

PAX8 negative → consider metastatic tumour or mesenchymal neoplasm

Step 2: Clear cell vs non–clear cell

CAIX

Diffuse strong positivity → Clear Cell RCC

Negative / focal → proceed to CK7

Step 3: CK7 expression

CK7 diffuse positive

Consider **Papillary RCC** or **Chromophobe RCC**

CK7 negative or focal

Consider **Clear cell RCC** or **Oncocytoma**

Step 4: CD117 and CD10

Marker pattern	Likely diagnosis
CD117 + / CK7 diffuse + / CD10 –	**Chromophobe RCC**
CD117 + / CK7 – or focal	**Oncocytoma**
CD10 + / CK7 +	**Papillary RCC**
CD10 + / CAIX +	**Clear Cell RCC**

Step 5: Additional markers (problem-solving)

AMACR → Papillary RCC

Vimentin → Clear cell RCC

Colloidal iron (diffuse) → Chromophobe RCC

HMB45 / Melan-A → Angiomyolipoma (epithelioid)

CYSTS OF THE KIDNEY

Classification of Cystic Diseases of the Kidney

(Modified and updated from American Academy of Paediatrics; WHO and contemporary genetic insights added)

I. Genetic (Hereditary) Cystic Diseases

Autosomal Recessive Polycystic Kidney Disease (ARPKD)
(Infantile polycystic kidney disease; Potter type I)

Autosomal Dominant Polycystic Kidney Disease (ADPKD)
(Adult polycystic kidney disease; Potter type III)

Nephronophthisis–Medullary Cystic Disease Complex

- Juvenile nephronophthisis (autosomal recessive)
- Autosomal dominant tubulointerstitial kidney disease (ADTKD; previously medullary cystic disease)

Congenital nephrosis (Finnish type) *(NPHS1 mutation)*

Familial hypoplastic / glomerulocystic kidney disease

Renal cysts associated with multisystem genetic syndromes

- Tuberous sclerosis complex
- Von Hippel–Lindau disease
- Meckel–Gruber syndrome
- Joubert syndrome (ciliopathies)

II. Non-Genetic (Sporadic / Developmental / Acquired)

Multicystic dysplastic kidney (MCDK) *(Potter type II)*

Simple renal cysts

Medullary sponge kidney

Sporadic glomerulocystic kidney disease

Acquired renal cystic disease (dialysis-associated)

Calyceal diverticulum (pyelogenic cyst)

Segmental or unilateral ADPKD-like disease

Obstructive cystic disease due to in-utero urinary tract obstruction *(Potter type IV)*

III. Neoplastic Cystic Lesions

Cystic nephroma

Cystic partially differentiated nephroblastoma

Multilocular cystic renal neoplasm of low malignant potential

Cystic change in RCC, Wilms tumour, mesoblastic nephroma, clear cell sarcoma

IV. Miscellaneous

Cystic hamartoma of renal pelvis

Endometriosis

Teratoma

Detail on Renal Cystic Dysplasia

Renal cystic dysplasia results from abnormal differentiation of the metanephric blastema, with arrest of nephron development and persistence of primitive ducts/tubules embedded in undifferentiated mesenchyme, often with heterologous tissue.

Patterns of Renal Dysplasia

Multicystic dysplastic kidney (MCDK) – unilateral, non-genetic (Potter type II)

Aplastic dysplasia – rudimentary dysplastic kidney

Obstructive dysplasia – due to congenital urinary tract obstruction (Potter type IV)

Diffuse dysplasia – genetic, bilateral, syndromic

Key Features

Most common cystic renal disease in the newborn

Often detected as an abdominal mass

Frequently associated with urinary tract anomalies

Gross

Reniform mass replaced by cysts of varying sizes

Unilateral (MCDK) or bilateral (diffuse dysplasia)

Microscopy

Cysts lined by cuboidal epithelium

Immature mesenchyme

Primitive tubules and glomeruli

Heterologous tissue: cartilage, adipose, fibromuscular tissue

Glomerular cysts may be present

Prognosis

Bilateral disease often fatal

Unilateral disease compatible with normal life

Progressive renal insufficiency if bilateral or associated with obstruction

Autosomal Dominant Polycystic Kidney Disease (ADPKD)

(Potter type III)

Prevalence: 1:400–1:1000

Accounts for ~10% of ESRD

Mutations:

PKD1 (85%) – chromosome 16p13.3

PKD2 – chromosome 4q21

Pathogenesis: abnormal ciliary signalling → tubular epithelial proliferation and fluid secretion

Gross

Bilaterally enlarged kidneys (2–4 kg)

Cysts throughout cortex and medulla

Cysts filled with serous or hemorrhagic fluid

Microscopy

Cysts arise from all nephron segments

Variable epithelial lining

Papillary epithelial proliferations may be seen

RCC risk slightly increased

Extra-renal Manifestations

Hepatic cysts (most common)

Pancreatic, splenic cysts

Berry aneurysms

Mitral valve prolapse

Prognosis

Progressive renal failure

Accelerated decline once renal insufficiency develops

Autosomal Recessive Polycystic Kidney Disease (ARPKD)

(Potter type I)

Rare, autosomal recessive

Gene: PKHD1 (fibrocystin)

Associated with congenital hepatic fibrosis

Gross

Bilateral enlarged kidneys

Smooth external surface

Radially arranged cylindrical cysts → spongiform appearance

Microscopy

Uniform dilation of collecting ducts

Cuboidal epithelial lining

Clinical

Perinatal/neonatal forms often fatal

Survivors develop portal hypertension and hepatic fibrosis

Cystic Diseases of the Renal Medulla

Medullary Sponge Kidney

Benign developmental anomaly

Cystic dilatation of collecting ducts

Renal function preserved

Complications: stones, hematuria, infection

Nephronophthisis–Medullary Cystic Disease Complex

Feature	Nephronophthisis	ADTKD
Inheritance	Autosomal recessive	Autosomal dominant
Age	Childhood	Adulthood

Genes	NPHP genes	UMOD, MUC1
Kidney size	Normal to small	Small
Outcome	Early ESRD	Late ESRD

Microscopy

Tubular basement membrane thickening and reduplication

"Puff-pastry" appearance

Tubular atrophy and interstitial fibrosis

Corticomedullary cysts

ALLOGRAFT REJECTION

Acute Renal Allograft Rejection

Acute renal allograft rejection is an immune-mediated injury to the transplanted kidney that usually occurs within the first few weeks after transplantation, though it may develop at any time, particularly when immunosuppression is inadequate. It is broadly classified into acute T-cell–mediated (cellular) rejection and acute antibody-mediated (humoral) rejection.

1. Acute T-cell–mediated (Cellular) Rejection

This is the commonest form of acute rejection and results from recipient T-lymphocyte recognition of donor alloantigens.

Microscopy:

The characteristic histological features include a dense interstitial mononuclear inflammatory infiltrate, composed predominantly of lymphocytes, associated with interstitial edema. A defining feature is tubulitis, seen as infiltration of lymphocytes into the tubular epithelium with damage to tubular cells. In more severe cases, vascular involvement (endothelialitis**)** is present, characterized by lymphocytes beneath the endothelium of arteries with intimal inflammation.

2. Acute Antibody-mediated (Humoral) Rejection

This form is caused by circulating donor-specific antibodies directed against antigens on graft endothelial cells.

Microscopy:

Histological findings reflect microvascular injury and include glomerulitis (inflammatory cells within glomerular capillaries) and peritubular capillaritis. Capillary thrombosis, fibrinoid necrosis, and acute tubular injury may be seen. Deposition of complement split product **C4d** in peritubular capillaries is a supportive diagnostic feature.

Clinical Significance

Acute rejection typically presents with acute graft dysfunction, manifested by rising serum creatinine and reduced urine output. Importantly, acute rejection is potentially reversible if recognized early and treated promptly with intensified immunosuppression.

Key points

Hallmark lesion of acute cellular rejection: **tubulitis**

Vascular involvement indicates **severity**

Antibody-mediated rejection shows **microvascular injury**

Acute rejection is **potentially reversible**

Comparison : RENAL ALLOGRAFT REJECTION – ACUTE vs CHRONIC

Feature	Acute Rejection	Chronic Rejection
Time of onset	Days to weeks (may occur later)	Months to years
Immune mechanism	Predominantly T-cell mediated (± antibodies)	Chronic immune-mediated injury
Interstitium	Dense mononuclear cell infiltration, edema	Interstitial fibrosis
Tubules	Prominent **tubulitis**	Tubular atrophy
Blood vessels	Endothelialitis (intimal lymphocytes)	Fibrous intimal thickening, luminal narrowing
Glomeruli	Usually normal or mild changes	Transplant glomerulopathy (GBM duplication)
Peritubular capillaries	May show capillaritis	Loss of capillaries, chronic injury
Reversibility	Potentially reversible with treatment	Irreversible, progressive
Clinical significance	Acute graft dysfunction	Progressive graft failure

A V KIDNEY SYNOPTIC REPORTING

Kidney Biopsy – Native Kidney

CLINICAL & SPECIMEN DETAILS

Side: Right / Left

Clinical indication: _____

Type of biopsy: Needle / Wedge / Other

Number of cores: ____

Adequacy: Adequate / Inadequate

No of Glomeruli (LM): ____

No of Arteries: ____

Cortex present Yes / No C

Tissue submitted for:

Immunofluorescence: Yes / No

Electron microscopy: Yes / No

B. LIGHT MICROSCOPY

1. Glomeruli

Hypercellularity:

None / Focal / Diffuse

Mesangial: Mild / Moderate / Severe

Endocapillary: Mild / Moderate / Severe

Segmental sclerosis: Yes / No

Globally sclerosed glomeruli: ___ / ___ glomeruli

Crescents:

Present / Absent

Type: Cellular / Fibrocellular / Fibrous

Percentage of glomeruli: ___ %

Basement membrane changes:

Thickening: Focal / Diffuse

Spikes (JMS): Present / Absent

Double contours (tram-tracks): Present / Absent

Mesangial matrix expansion:

Absent / Segmental / Global

Other glomerular lesions:

Hyalinosis: Present / Absent

Mesangiolysis: Present / Absent

Fibrin thrombi: Present / Absent

Fibrinoid necrosis: Present / Absent

Kimmelstiel–Wilson nodules: Present / Absent

Amyloid: Present / Absent

2. Tubules

Tubular injury: Present / Absent

Tubular atrophy:

None / Mild (<25%) / Moderate (26–50%) / Severe (>50%)

Casts: Present / Absent

Protein reabsorption droplets: Present / Absent

Lipid droplets: Present / Absent

Calcification: Present / Absent

3. Interstitium

Inflammation:

None / Mild / Moderate / Severe

Predominant cells:

Lymphocytes / Plasma cells / Neutrophils

Interstitial fibrosis:

None / Mild (<25%) / Moderate (26–50%) / Severe (>50%)

Oedema Present ---- Absent ----

4. Blood Vessels

Arteriosclerosis:

None / Mild / Moderate / Severe

Arteriolosclerosis (hyaline):

None / Mild / Moderate / Severe

Vasculitis / fibrinoid necrosis: Present / Absent

Thrombi: Present / Absent

Amyloid: Present / Absent

C. IMMUNOFLUORESCENCE

(Grade intensity: 0 / 1+ / 2+ / 3+)

Antibody	Mesangial	Capillary wall
IgG		
IgA		
IgM		
C3		
C1q		
Kappa		
Lambda		
Fibrinogen C4d (if transplant): ____		

Pattern: Granular / Linear / Full house / Pauci-immune

D. ELECTRON MICROSCOPY (if available)

GBM thickness: Normal / Increased / Irregular

Electron-dense deposits:

Mesangial / Subendothelial / Subepithelial

Foot process effacement extent :

Focal / Diffuse

Extent ….%

E. SPECIAL SCORING (WHEN APPLICABLE)

Lupus Nephritis (ISN/RPS)

Class: I / II / III / IV / V / VI

Activity index: ___ / 24

Chronicity index: ___ / 12

F. DIAGNOSIS (IMPRESSION)

Primary diagnosis:

Secondary changes:

Clinicopathological correlation is advised

Example -
"Diffuse lupus nephritis, ISN/RPS Class IV (A/C), with moderate activity and mild chronicity. Clinicopathological correlation advised."

G. COMMENTS (Concise interpretative comment)

Signature: _____ **Date: _____**

"Model completed renal biopsy reports" (For Understanding Reporting Style)"

These examples illustrate structured reporting based on the above synoptic format.

1: LUPUS NEPHRITIS

Specimen details

Adequate renal biopsy with 2 cores containing 18 glomeruli, of which 1 is globally sclerosed. Two arteries are identified.
Material submitted for immunofluorescence and electron microscopy.

Light microscopy

Most glomeruli show diffuse endocapillary hypercellularity with leukocyte infiltration. Wire-loop lesions are present.
Cellular crescents are identified in 6 of 18 glomeruli (≈33%).
Fibrinoid necrosis is present in occasional capillary loops.
Basement membranes show focal thickening.

Tubules show focal acute tubular injury.
Interstitium shows moderate inflammatory infiltrate with mild interstitial fibrosis (~20%).
Blood vessels show mild arteriosclerosis; no vasculitis is seen.

Immunofluorescence

Full-house granular immune complex deposition involving mesangium and capillary walls:

IgG: **3**+

IgA: **2**+

IgM: **2**+

C3: **3**+

C1q: **2**+

Electron microscopy

Numerous **subendothelial and mesangial electron-dense deposits** are present, with focal podocyte foot process effacement.

Diagnosis (Impression)

Diffuse lupus nephritis, ISN/RPS Class IV (A/C).
Activity index: 12 / 24
Chronicity index: 3 / 12

MODEL REPORT 2: PRIMARY MEMBRANOUS NEPHROPATHY (MGN)

Specimen details

Adequate renal biopsy with 15 glomeruli, none globally sclerosed. One artery identified.
Material submitted for immunofluorescence and electron microscopy.

Light microscopy

Glomeruli show diffuse uniform thickening of capillary walls without significant hypercellularity.
Spikes are highlighted on silver stain.
No crescents, necrosis, or endocapillary proliferation are seen.

Tubules and interstitium are unremarkable.
Blood vessels are within normal limits.

Immunofluorescence

Granular capillary wall deposits:

IgG: 3+

C3: 2+

IgA and IgM: negative

(PLA2R positive, if tested)

Electron microscopy

Numerous subepithelial electron-dense deposits with intervening basement membrane projections (spikes).
Diffuse podocyte foot process effacement is present.

Diagnosis (Impression)

Membranous nephropathy (primary), Stage II.

MODEL REPORT 3: IgA NEPHROPATHY

Specimen details

Adequate renal biopsy with 20 glomeruli, including 2 globally sclerosed. Two arteries identified.
Material submitted for immunofluorescence.

Light microscopy

Glomeruli show mesangial hypercellularity and mesangial matrix expansion.
No endocapillary hypercellularity, necrosis, or crescents are identified.

Tubules show mild tubular atrophy (<25% of cortex).
Interstitium shows mild interstitial fibrosis.
Blood vessels show mild arteriosclerosis.

Immunofluorescence

Dominant mesangial IgA deposition (3+) with co-dominant C3 (2+).
IgG and IgM are weak/negative.

Diagnosis (Impression)

IgA nephropathy.
Oxford classification: M1 E0 S0 T1.

Model report 4 : **SAMPLE TRANSPLANT BIOPSY REPORT (BANFF 2022)**

SAMPLE: ACUTE T CELL–MEDIATED REJECTION (TCMR) – GRADE IA

Specimen details:
Adequate biopsy with 2 cores containing 12 glomeruli and 2 arteries. IF and IHC performed.

Banff scores:

Interstitial inflammation (i): i2

Tubulitis (t): t2

Glomerulitis (g): g0

Peritubular capillaritis (ptc): ptc0

Arteritis (v): v0

Interstitial fibrosis (ci): ci0

Tubular atrophy (ct): ct0

Chronic glomerulopathy (cg): cg0

Chronic vascular changes (cv): cv0

Arteriolar hyalinosis (ah): ah0

Immunohistochemistry:
C4d staining in peritubular capillaries is negative.

Diagnosis (Impression):
Acute T cell–mediated rejection (TCMR), Banff Grade IA.

Signature: _____ **Date:** _____

Adult Renal Tumour Resection – Synoptic Histopathology Report

SPECIMEN

Type of resection:

☐ Radical (total) nephrectomy

☐ Partial nephrectomy

☐ Portion of kidney

☐ Other (specify): _____

Side:

☐ Right

☐ Left

☐ Not specified

GROSS FINDINGS

Size of kidney (with tumour): _____ × _____ × _____ cm

Tumour location:

☐ Upper pole

☐ Lower pole

☐ Mid zone

☐ Renal sinus / medulla

☐ Other: _____

☐ Not specified

Tumour focality:

☐ Unifocal

☐ Multifocal

Tumour size (largest dimension if multifocal): _____ × _____ × _____ cm

☐ Cannot be determined

Adrenal gland included:

☐ Yes

☐ No

Macroscopic extent of tumour:

☐ Confined to kidney

☐ Infiltrates renal capsule

☐ Infiltrates perirenal fat

☐ Infiltrates renal sinus fat

☐ Invades Gerota's fascia

☐ Invades adrenal gland

☐ Tumour thrombus in renal vein / branches

Cut surface:

☐ Variegated

☐ Homogeneous

☐ Cystic

☐ Hemorrhage / necrosis present

MICROSCOPY

Tumour present:

☐ Yes

☐ No

Nature of tumour:

☐ Benign

☐ Malignant

Histological type:

☐ Renal cell carcinoma (RCC)

☐ Clear cell (conventional)

☐ Papillary

☐ Chromophobe

☐ Collecting duct carcinoma

☐ Medullary carcinoma

☐ **Clear cell papillary RCC**

☐ Oncocytoma

☐ Papillary adenoma

☐ Angiomyolipoma

☐ Other mesenchymal tumour (specify): _____

☐ Other (specify): _____

Tumour necrosis: ☐ Present ☐ Absent

Sarcomatoid change: ☐ Present ☐ Absent

Rhabdoid features: ☐ Present ☐ Absent

HISTOLOGIC GRADE (WHO/ISUP preferred)

☐ Gx – Cannot be assessed

☐ Grade 1

☐ Grade 2

☐ Grade 3

☐ Grade 4

(Fuhrman grading may be mentioned in brackets for legacy correlation, if required.)

PATHOLOGIC EXTENT (MICROSCOPIC)

Tumour confined to kidney: ☐ Yes ☐ No

Renal capsule invasion: ☐ Present ☐ Absent

Perirenal fat invasion: ☐ Present ☐ Absent

Renal sinus fat invasion: ☐ Present ☐ Absent

Renal vein / segmental vein invasion: ☐ Present ☐ Absent

Adrenal invasion: ☐ Present ☐ Absent

Gerota's fascia invasion: ☐ Present ☐ Absent

MARGINS

Parenchymal margin (partial nephrectomy): ☐ Free ☐ Involved

Renal sinus soft tissue margin: ☐ Free ☐ Involved

Renal vein margin: ☐ Free ☐ Involved

Ureteric margin: ☐ Free ☐ Involved

Perinephric fat / Gerota's fascia margin: ☐ Free ☐ Involved

LYMPHOVASCULAR INVASION

☐ Present

☐ Absent

LYMPH NODES

Number examined: _____

Number involved: _____

DISTANT METASTASIS

☐ Cannot be assessed

☐ Present (specify site): _____

PATHOLOGIC STAGING (AJCC – Renal Cell Carcinoma) (Stage to be incorporated in report)

Primary Tumour (pT):

pTX: Cannot be assessed

pT0: No evidence of primary tumour

pT1: Tumour ≤ 7 cm, limited to kidney

pT1a: ≤ 4 cm

pT1b: > 4 cm but ≤ 7 cm

pT2: Tumour > 7 cm, limited to kidney

pT3: Extends into major veins or perinephric tissues but not beyond Gerota's fascia

pT3a: Invades renal vein, segmental branches, renal sinus fat, or perirenal fat

pT3b: Extends into vena cava below diaphragm

pT3c: Extends into vena cava above diaphragm or invades its wall

pT4: Invades beyond Gerota's fascia

Regional Lymph Nodes (pN):

pNX: Cannot be assessed

pN0: No regional lymph node metastasis

pN1: Metastasis in regional lymph node(s)

Distant Metastasis (pM):

pMX: Cannot be assessed

pM1: Distant metastasis present

Final integrated report (example format):

Procedure: _____

Side: _____

Tumour type: _____

Histologic grade (WHO/ISUP): _____

Tumour size: _____

Tumour extent: _____

Margins: _____

Lymphovascular invasion: ☐ Present ☐ Absent

Lymph nodes: ____ / ____ involved

Pathologic stage (pTNM): _____

Signature …… Date ……..

Pathological tumour stages illustrated diagrammatically : (Fig 9.9)

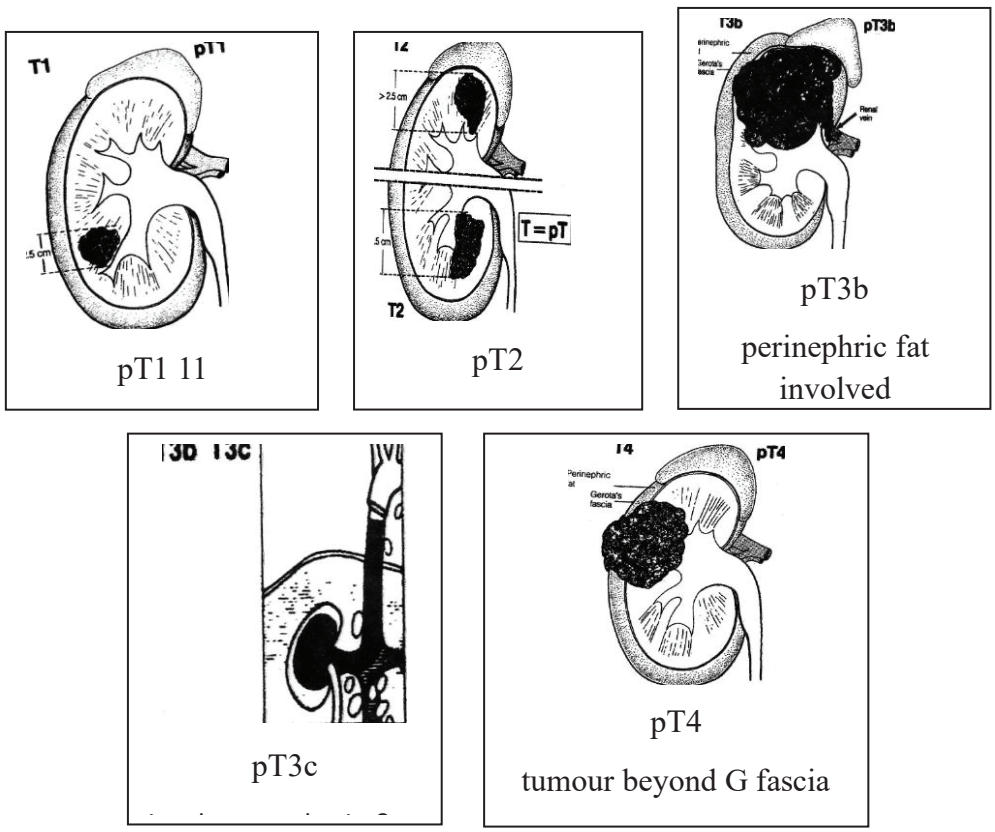


Fig 9.9 -shows diagram with various stages of RCC

"EXPANDED DETAILED SYNOPTIC REPORT (FOR POSTGRADUATE USE)

Adult Renal Cell Carcinoma – Comprehensive Synoptic

Procedure and side

Tumour focality and exact size

Histological subtype (WHO classification)

Histologic grade (WHO/ISUP ± Fuhrman)

Microscopic tumour extent:

- Renal capsule
- Renal sinus fat
- Perirenal fat
- Renal vein / segmental branches
- Adrenal gland

Gerota's fascia

Margin status (parenchymal, renal sinus, vascular, ureteric)

Lymphovascular invasion

Regional lymph node status (number examined / involved)

Distant metastasis (if known)

Final AJCC pTNM stage

B I RENAL PELVIS & URETER ANATOMY

The renal pelvis and ureter are lined by **urothelium (transitional epithelium)**.
The wall consists of:

Mucosa (urothelium with lamina propria)

Muscularis (inner longitudinal and outer circular layers; less well defined in renal pelvis)

Adventitia

There is **no well-defined muscularis mucosae**. The renal pelvis is closely related to the renal sinus fat, which is important in assessing tumour spread.

B II PELVIS & URETER GROSSING TECHNIQUES

GROSSING OF URETER

Indications

Congenital anomalies (megaureter, strictures, diverticula)

Trauma

Neoplasia

Pelvi-ureteric junction (PUJ) specimens

Endoscopic biopsies

Long segment ureter (segmental ureteric resection)

Measure length and diameter.

Open longitudinally and examine mucosa.

Sampling:

Two blocks if grossly unremarkable

In strictures: 2–3 longitudinal blocks including stricture

In tumours: generous sampling including proximal and distal margins

PUJ specimen

Measure length and open longitudinally.

Examine for narrowing, wall thickening or tumour.

Sampling:

- Longitudinal sections from representative areas
- Transverse sections only if lumen cannot be probed

Nephroureterectomy (with bladder cuff) – most common for malignancy

Grossing Techniques

Identify and orient kidney, renal pelvis, ureter, and bladder cuff

Measure:

- Length of ureter
- Size and location of tumour (pelvis vs ureter; upper/mid/lower ureter)

Open ureter longitudinally to expose mucosa

Note:

- Papillary vs infiltrative growth
- Multifocal lesions

Sections to be taken:

Tumour (multiple, including deepest point of invasion)

Tumour with adjacent mucosa

Renal sinus fat (for pelvic tumours)

Ureteric margin and bladder cuff margin

Non-tumorous mucosa

Key Points -

Renal sinus invasion is critical for tumour staging

Ureteric and bladder cuff margins must always be assessed”

All identified lymph nodes should be entirely submitted

A NEPHRO-URETERECTOMY may be done for upper tract urothelial carcinoma . Grossing should be done as for a neoplasm

B III PELVIS & URETER GROSS PEARLS

Gross Pearls

Dilated ureter- megaureter

Strictures- suggest congenital anomalies

Always look for **multifocality** of tumour (field change effect of urothelium)

Small papillary lesions may be easily missed—careful mucosal inspection is essential

Assess carefully for renal sinus involvement in pelvic tumours"

Check for calculi especially in areas known for calculi formation and in history of pain abdomen.

Staghorn calculus -Takes the shape of the pelvis and calyses.

B IV PELVIS & URETER MICROSCOPY

Microscopy

Urothelial carcinoma is the most common tumour

- Low-grade
- High-grade

Growth patterns:

- Papillary
- Flat/infiltrative

Assess for:

Depth of invasion:

- Lamina propria
- Muscular wall
- Periureteric/renal sinus fat

Lymphovascular invasion

Tumour necrosis

Associated carcinoma in situ

Variants : Squamous, glandular, micropapillary, etc.

B V PELVIS & URETER SYNOPTIC REPORTING

Procedure: ☐ Nephroureterectomy ☐ Segmental resection ☐ Biopsy

Tumour site: ☐ Renal pelvis ☐ Ureter (upper/mid/lower)

Max Tumour size: _____ cm

Tumour configuration: ☐ Papillary ☐ Flat/Infiltrative

Focality: ☐ Unifocal ☐ Multifocal

Histologic type: ☐ Urothelial carcinoma ☐ Variant: _____

Histologic grade: ☐ Low grade ☐ High grade

Depth of invasion:

☐ Non-invasive

☐ Lamina propria invasion

☐ Muscularis invasion

☐ Periureteric fat / Renal sinus fat invasion

☐ Adjacent organ invasion

Associated Carcinoma in situ: ☐ Present ☐ Absent

Lymphovascular invasion: ☐ Present ☐ Absent

Margins:

Ureteric margin: ☐ Free ☐ Involved

Bladder cuff margin: ☐ Free ☐ Involved

Lymph nodes:

Number examined: ___

Number involved: ___

Pathological staging based on above findings: pT:…….;pN:……;pM:…….

UROTHELIAL CARCINOMA OF RENAL PELVIS / UPPER TRACT Synoptic report

SPECIMEN

Type of resection:

☐ Nephroureterectomy with bladder cuff

☐ Nephrectomy (specify): _____

Side:

☐ Right

☐ Left

GROSS FINDINGS

Kidney size: _____ × _____ × _____ cm

Tumour site:

☐ Renal pelvis

☐ Pelvicalyceal system

☐ Upper ureter

Tumour configuration:

☐ Papillary

☐ Flat / infiltrative

Tumour size: _____ cm (greatest dimension)

Multifocality:

☐ Present

☐ Absent

Associated hydronephrosis:

☐ Present

☐ Absent

MICROSCOPY

Histological type:

☐ Urothelial carcinoma

Tumour grade (WHO):

☐ Low grade

☐ High grade

PATHOLOGIC EXTENT

Non-invasive papillary carcinoma: ☐ Yes ☐ No

Carcinoma in situ: ☐ Present ☐ Absent

Depth of invasion:

☐ Lamina propria

☐ Muscularis

☐ Peripelvic / periureteric fat

☐ Renal parenchyma

MARGINS

Renal parenchymal margin: ☐ Free ☐ Involved

Ureteric margin: ☐ Free ☐ Involved

Bladder cuff margin (if present): ☐ Free ☐ Involved

LYMPHOVASCULAR INVASION

☐ Present

☐ Absent

LYMPH NODES

Number examined: _____

Number involved: _____

PATHOLOGIC STAGING (AJCC – UPPER TRACT UROTHELIAL CARCINOMA) (incorporate in report)

Primary Tumour (pT):

pTX: Cannot be assessed

pT0: No evidence of primary tumour

pTa: Non-invasive papillary carcinoma

pTis: Carcinoma in situ

pT1: Invades subepithelial connective tissue

pT2: Invades muscularis

pT3: Invades renal parenchyma or periureteric fat

pT4: Invades adjacent organs or through kidney into perinephric fat

Regional Lymph Nodes (pN):

pNX: Cannot be assessed

pN0: No regional lymph node metastasis

pN1: Regional lymph node metastasis

Distant Metastasis (pM):

pMX: Cannot be assessed

pM1: Distant metastasis present

Pathological staging based on gross and microscopic findings

pT:…….;pN:……;pM:……. Signature ……..

RCC vs UROTHELIAL CARCINOMA (RENAL PELVIS)–REVISION AID

Feature	Renal Cell Carcinoma (RCC)	Urothelial Carcinoma (Renal Pelvis / Upper Tract)
Tissue of origin	Renal tubular epithelium	Urothelial lining of pelvis/ureter
Common specimen	Radical / partial nephrectomy	Nephroureterectomy with bladder cuff
Gross appearance	Solid, yellow, variegated	Papillary or flat infiltrative lesion
Multifocality	Uncommon	Common
Key grading system	WHO/ISUP (± Fuhrman)	WHO (Low vs High grade)
Key pathologic focus	Tumour size and extent	Depth of invasion
Important invasion sites	Renal sinus fat, perirenal fat, renal vein, Gerota's fascia	Lamina propria, muscularis, renal parenchyma, periureteric fat
Margin of greatest importance	Renal vein / parenchymal margin	Ureteric and bladder cuff margins
Carcinoma in situ	Not applicable	Important associated lesion
Lymphovascular invasion	Prognostically significant	Prognostically significant
TNM staging basis	Tumour size and spread beyond kidney	Depth of invasion (pTa–pT4)
Common exam pitfall	Confusing renal sinus fat with perirenal fat	Missing CIS or ureteric margin status

C I URINARY BLADDER – ANATOMY

The urinary bladder is a hollow muscular organ located in the pelvis, lined by urothelium (transitional epithelium) and specialized for storage and controlled evacuation of urine.

Gross Anatomy

The bladder is divided into:

Apex – anterior, continuous with the urachus

Body – main distensible portion

Fundus (base) – posterior wall

Neck – inferior portion leading to urethra

A distinct triangular area on the posterior wall, the **trigone**, is formed by the two ureteric orifices and the internal urethral orifice. This region is smooth and **lacks mucosal folds**.

The bladder wall consists of:

Mucosa

Lamina propria

Muscularis propria (detrusor muscle) – thick, interlacing smooth muscle bundles

Adventitia/Serosa (serosa present on superior surface)

Microscopy (Relevant Features)

1. Urothelium

Stratified epithelium with **3–7 layers**

Composed of:

- Basal cells
- Intermediate cells
- Superficial **umbrella cells** (often binucleated)

Shows **distensibility** without loss of integrity

2. Lamina Propria

Loose connective tissue with:

- Blood vessels
- Nerves
- Inflammatory cells

May show **muscularis mucosae (variable and discontinuous)**

⚠ Important: can be mistaken for muscularis propria in superficial biopsies

3. Muscularis Propria (Detrusor Muscle)

Thick bundles of smooth muscle arranged in **interlacing layers**

Critical landmark for:

Distinguishing non-invasive vs invasive carcinoma

Tumour staging (pT2 and beyond)

4. Adventitia / Serosa

Adventitia in most parts

Serosa (peritoneal covering) over the superior surface

C II URINARY BLADER –GROSSING TECHNIQUES

The types of specimens received are : Cystoscopic biopsies and Cystectomies.

Cystoscopic biopsies

Punch biopsies with mucosa for cystitis.

Measure along three dimensions and block all tissues; mucosa should be on one aspect.

Transurethral resection specimens for grade I carcinoma.

Weigh if bits are small. For large bits give measurements in addition to weight.

Bits

On a routine, if the specimen is ≤ 10g, block all to a maximum of 5 blocks. Apart from this for every 10g, one additional block. If muscle is identified then it is to be blocked separately.

Cystectomy

Fixation

Either open with a scissors in a Y-shaped manner or through the junction of the antero-posterior wall and fix it flat on a cork board either overnight or for a minimum period of 5 hours. (fig 9.14) Alternatively,

Fill it with formalin through the urethra. Fix overnight, open it at the junction of the antero-posterior wall as shown in the figure.

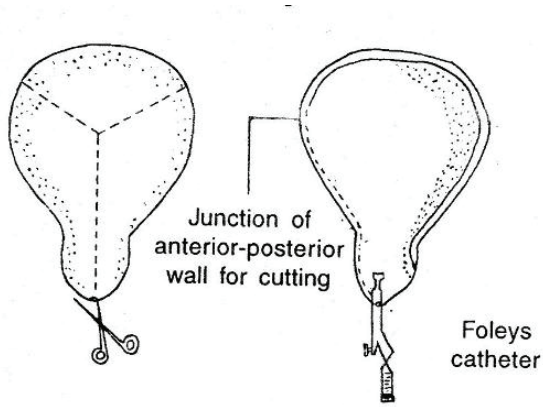


Fig 9.14 : Method of opening the urinary bladder

Important points-

Tumours commonly arise in the **posterolateral wall and trigone**

The **relationship to ureteric orifices** must be noted

Depth of invasion (lamina propria vs muscularis propria) is **critical for staging**

The bladder wall may appear trabeculated in long-standing obstruction

Identify and sample:

Tumour with deepest point of invasion

Adjacent mucosa

Base of tumour

Any suspicious areas (flat lesions/CIS)

Procedure

Size of the bladder- three dimensions (antero-posterior, superior-inferior and right to left)

Length of the ureter and urethra (if measureable)

Tumour characteristics- size, location, extent of invasion, shape and appearance of the neoplastic mucosa.

Appearance of the non-neoplastic mucosa

Thickness of the bladder wall at the tumour and away from the tumour .

Seminal vesicles to examined, measured and sectioned.

Section through the prostate if present. Record involvement of these organs grossly.

Bits (Fig 9. 15)

A,B,C – tumour three bits- to include the full thickness of the wall.

D,E - Trigone - two bits- to include uretero-vesicle junctions

F – One bit anterior wall

G - One bit posterior wall

H – One bit Dome

J – One bit any other abnormal area

K,L – ureter – surgical margins – separate blocks

M – One bit urethral surgical margin

N – Regional lymph nodes; more bits if necessary .

Additional bits in males to include seminal vesicles and prostate.

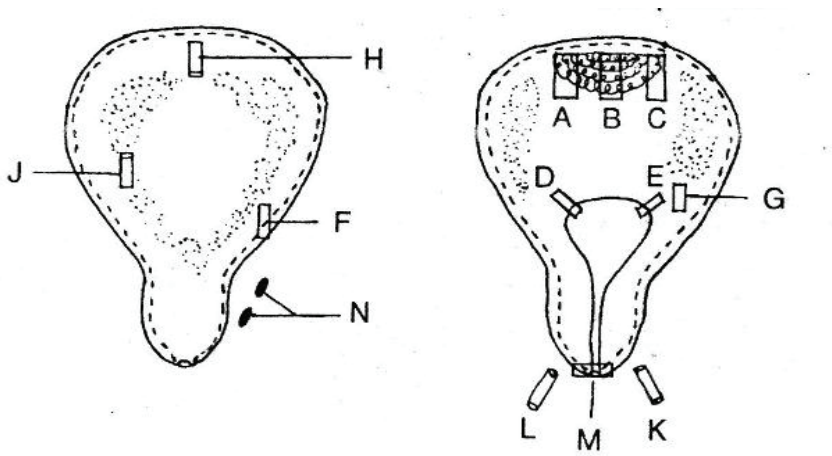


Fig 9.15 : Bits taken in cystectomy specimen in urinary bladder

C III & IV URINARY BLADDER GROSS & MICROSCOPY

Red, hyperemic mucosa

→ Acute cystitis, erosive cystitis

Single or multiple delicate papillary lesions (pedunculated or clustered)

→ Papilloma, well-differentiated papillary urothelial carcinoma, urothelial neoplasm of low malignant potential (PUNLMP), polypoid cystitis and fibroepithelial polyp.

Solitary smooth or lobulated polypoid lesion

→ Inverted papilloma, urothelial carcinoma, von Brunn's cell nests, malakoplakia
(Malakoplakia lesions are yellowish with central umbilication)

Confluent papillary tissue with cauliflower-like appearance ± satellite lesions

→ Papillary urothelial (transitional cell) carcinoma

Bulky polypoid or nodular tumours with full-thickness wall involvement

→ Poorly differentiated carcinoma

(sarcomatoid, squamous cell carcinoma, adenocarcinoma, high-grade urothelial carcinoma)

Cluster of oedematous, smooth-surfaced polypoid fronds

→ Sarcoma botryoides / hamartoma (especially in children)

Black nodular mass

→ Malignant melanoma

Dark blue cystic lesion

→ Hemangioma

Black, hemorrhagic mucosa

→ Hemorrhagic cystitis (e.g. cyclophosphamide toxicity)

Microscopy :

1. Cystitis – Important Variants

Interstitial cystitis (Hunner's cystitis)

Eosinophilic cystitis

Emphysematous cystitis

Chronic tuberculous cystitis

2. Malakoplakia

Multiple nodular thickenings of mucosa and submucosa (often trigone)

Associated with **immunodeficiency**

Microscopy: Sheets of histiocytes with granular eosinophilic cytoplasm

Pathognomonic **Michaelis–Gutmann bodies**

- Basophilic, PAS positive
- Stain for calcium and iron
- Represent calcified phagolysosomes

Electron microscopy may show intracellular bacteria

3. Von Brunn's Cell Nests

Common reactive change due to **invagination of urothelium into lamina propria**

May appear detached within lamina propria

Cystitis cystica: cystic dilatation of nests

Cystitis glandularis: glandular metaplasia with mucin secretion

4.UROTHELIAL (TRANSITIONAL CELL) CARCINOMA

Etiology and Epidemiology

Accounts for **~90% of bladder cancers**

Risk factors:

- Cigarette smoking
- Aromatic amines (benzidine, β-naphthylamine, aniline dyes)
- Schistosoma haematobium (especially squamous carcinoma)

Common after **50 years**, males > females

Most common symptom: **painless hematuria**

Gross

Flat lesions (CIS) – mucosal erythema, often inconspicuous

Infiltrative tumours – indurated, ulcerated masses

Common sites:

- Posterolateral wall
- Trigone

Important to note:

- Size and number of tumours
- Multifocality
- Appearance of base (broad-based vs pedunculated)

Microscopy

. Non-invasive Papillary Urothelial Carcinoma

Papillary fronds with fibrovascular cores

Lined by urothelium showing:

- Loss of polarity
- Nuclear enlargement and hyperchromasia
- Increased mitotic activity

Grading:

Low grade

- Mild to moderate atypia
- Preserved architecture

High grade

- Marked atypia
- Loss of cohesion and polarity
- Frequent mitoses

Carcinoma in Situ (CIS)

- Flat, high-grade lesion
- Marked cytologic atypia:
  - Pleomorphic, hyperchromatic nuclei
  - Loss of maturation
- Intact basement membrane
- Often multifocal

Clinically important as a **precursor to invasive carcinoma**

Invasive Urothelial Carcinoma

- Tumour infiltrates beyond basement membrane into:
  - Lamina propria (pT1)
  - Muscularis propria (pT2)
  - Perivesical fat (pT3)
- Histology:
  - Irregular nests, cords, or single cells
  - Desmoplastic stromal response
  - Possible necrosis

Variants (brief mention)

- Squamous differentiation
- Glandular differentiation
- Micropapillary

Nested variant

(These may have prognostic significance)

Key Points for Reporting

Histologic grade (low vs high)

Depth of invasion (most critical parameter)

Presence of:

Muscularis propria in biopsy (must be commented upon)

Carcinoma in situ

Lymphovascular invasion

Variant histology

Always state whether **muscularis propria is present** in TURBT specimens

Distinguish:

Muscularis mucosae vs muscularis propria

CIS may be **missed grossly**—requires careful microscopy

Multifocality is common

Rule to remember:

Low-grade papillary tumours → recur but rarely invade

High-grade tumours → invade and metastasize

Evaluation of Bladder Biopsies

Always comment on:

Layers present (mucosa, lamina propria, muscularis propria)

Surface epithelium (intact / ulcerated)

Tumour type and growth pattern (papillary vs nodular)

Grade and **depth of invasion (especially muscle)**

Lymphovascular invasion

Adjacent mucosa (dysplasia / CIS)

Immunohistochemistry

Urothelial carcinoma:

CK7+, CK20+

CK8/18 positive at tumour–stroma interface

Other Bladder Carcinomas

Squamous cell carcinoma: 2–7% (common in schistosomiasis areas)

Adenocarcinoma: rare (≤2.5%) – rule out secondary extension

Small cell neuroendocrine carcinoma

Sarcomatoid carcinoma.

C V URINARY BLADDER SYNOPTIC REPORT

SPECIMEN

Type of specimen:

☐ Transurethral resection of bladder tumour (TURBT)

☐ Partial cystectomy

☐ Radical cystectomy

Side / site:

☐ Trigone

☐ Posterior wall

☐ Lateral wall

☐ Dome

☐ Multiple sites (specify): _____

GROSS FINDINGS

Tumour configuration:

☐ Papillary

☐ Flat

☐ Ulcerative / infiltrative

Tumour size: _____ cm (largest dimension)

Tumour focality:

☐ Unifocal

☐ Multifocal

MICROSCOPY

Histological type:

☐ Urothelial carcinoma

☐ Variant histology (specify): _____

Tumour grade (WHO):

☐ Low grade

☐ High grade

Carcinoma in situ:

☐ Present

☐ Absent

DEPTH OF INVASION

☐ Non-invasive papillary carcinoma (pTa)

☐ Lamina propria invasion (pT1)

☐ Muscularis propria present: ☐ Yes ☐ No

☐ Muscularis propria invasion (pT2)

☐ Perivesical fat invasion (pT3)

☐ Adjacent organ invasion (pT4)

LYMPHOVASCULAR INVASION

☐ Present

☐ Absent

MARGINS (CYSTECTOMY SPECIMENS)

Urethral margin: ☐ Free ☐ Involved

Ureteric margin(s): ☐ Free ☐ Involved

Soft tissue margin: ☐ Free ☐ Involved

LYMPH NODES

Number examined: _____

Number involved: _____

PATHOLOGIC STAGING (AJCC – URINARY BLADDER) (to be incorporated in report)

Primary Tumour (pT):

pTX: Cannot be assessed

pT0: No evidence of primary tumour

pTa: Non-invasive papillary carcinoma

pTis: Carcinoma in situ

pT1: Invades subepithelial connective tissue

pT2: Invades muscularis propria

- pT2a: Inner half
- pT2b: Outer half

pT3: Invades perivesical tissue

- pT3a: Microscopic
- pT3b: Macroscopic

pT4: Invades adjacent organs

Regional Lymph Nodes (pN):

pNX: Cannot be assessed

pN0: No regional lymph node metastasis

pN1–pN3: Regional lymph node metastasis

Distant Metastasis (pM):

pMX: Cannot be assessed

pM1: Distant metastasis present

Checklist - DETAILED SYNOPTIC (FOR POSTGRADUATE USE)

Specimen type and tumour site

Tumour configuration, size, and focality

Histologic grade and variant histology

Presence of carcinoma in situ

Adequacy and invasion of muscularis propria

Lymphovascular invasion

Margin status (urethral and ureteric emphasized)

Regional lymph node status

Final AJCC pTNM stage

PEARLS / COMMON PITFALLS

Always comment on **presence of muscularis propria** in TURBT

pT1 disease requires **adequate muscle sampling** to exclude pT2

CIS may be present **away from the main tumour**

Variant histology upgrades risk and management

D - URETHRA

The most common urethral mass is the caruncle . Lesions usually occur on the inferior aspect of the urethral meatus in post menopausal female. They are red tender and bleed on touch.possibly due to increased vascularity of the granulation tissue that compose them. Rarely the stroma may show atypical cells which are reactive in nature. They are invariably benign.

Fibro-epithelial polyps occur in the urethra but are less common than in the ureter.

Condyloma acuminatum of the urethra occurs in association with genital and perineal condylomata and generally the distal urethra is involved.

A “prostatic type polyp” occurs in the prostatic urethra in young men and is composed of papillae covered by cuboidal to low columnar cells similar to those lining the prostatic acini as confirmed by immunohistochemistry. Prostatic glands may be present in the stroma.The differential diagnosis is a prostatic adenocarcinoma particularly if the lesion is seen in older individuals.

Urethral carcinomas are more common in women than in men and majority (60-70%) are squamous cell carcinomas. Next in frequency are the trasitional cell and then adenocarcinomas.

ASSESS YOURSELF URINARY STSTEM

Essay Questions (10 marks)

Discuss the pathology of urothelial carcinoma of the urinary bladder, including classification, grading, and staging.

Describe the microscopic features and staging of invasive carcinoma of the urinary bladder.

Write an essay on carcinoma in situ (CIS) of the urinary bladder, including its significance and diagnosis.

Short Essay Questions (5 marks)

Differences between low-grade and high-grade urothelial carcinoma.

Muscularis mucosae vs muscularis propria – importance in bladder biopsies.

List the vascular complications of lupus nepritis

Short Answer / Point Questions (3 marks)

Enumerate the common sites of bladder tumours.

List the variants of urothelial carcinoma.

Multiple Choice Questions (MCQs)

The most important prognostic factor in carcinoma of the urinary bladder is:
a) Tumour size
b) Histologic subtype
c) Depth of invasion
d) Presence of necrosis

Carcinoma in situ of the urinary bladder is:
a) Papillary lesion
b) Flat high-grade lesion
c) Benign condition
d) Always visible grossly

Which of the following is NOT a variant of urothelial carcinoma?
a) Micropapillary
b) Nested
c) Squamous
d) Serous

Answer Key for MCQs

1. b
2. C
3. d

Chapter 10
Liver And Gall Bladder

Chapter outline – A Liver ; B Gall bladder .

A I Liver Anatomy;; A II Liver Grossing Techniques; A III Liver Gross Pearls; A IV Liver Microscopy; A V Liver Synoptic Reporting.

B I Gall Bladder Anatomy B II Gall Bladder Grossing Techniques ; BIII Gall Bladder Gross Pearls ;B IV Gall Bladder Microscopy; B V Gall Bladder - Synoptic Reporting

A I LIVER ANATOMY

A. Liver

Location & External Anatomy

Largest solid organ, located in the right hypochondrium and epigastrium.

Covered by Glisson capsule, except at the bare area.

Divided anatomically into right and left lobes; functionally into right and left hemilivers based on vascular inflow, biliary drainage, and hepatic venous outflow.

Microscopic Architecture

Classical hepatic lobule: Hexagonal unit centered on a central vein, with portal tracts at the periphery.

Portal lobule: Triangular unit emphasizing bile secretion, centered on a portal tract.

Hepatic acinus (Rappaport): Diamond-shaped functional unit highlighting metabolic zonation (Zone 1 periportal, Zone 2 midzonal, Zone 3 centrilobular).

Hepatocytes

Polygonal cells arranged in one- to two-cell-thick plates.

Abundant eosinophilic granular cytoplasm; centrally placed round nucleus, often binucleate.

Perform metabolic, synthetic, detoxification, and excretory functions.

Sinusoids

Vascular channels between hepatocyte plates.

Lined by fenestrated endothelial cells lacking a basement membrane.

Contain Kupffer cells (resident macrophages).

Space of Disse

Perisinusoidal space between hepatocytes and sinusoidal endothelium.

Contains plasma, microvilli of hepatocytes, and hepatic stellate (Ito) cells, which store vitamin A and play a key role in fibrosis.

Portal Tracts (Portal Triads)

Composed of a branch of portal vein, hepatic artery, and bile duct, embedded in fibrous connective tissue.

Additional components may include lymphatics and nerves.

Biliary System (Intrahepatic)

Bile canaliculi formed by adjoining hepatocyte membranes.

Canaliculi drain into canals of Hering, then into interlobular bile ducts within portal tracts.

A II LIVER GROSSING TECHNIQUES

Types of Specimens

Needle biopsy

Wedge biopsy

Hepatic resections

- Segmental resections
- Right or left hepatectomy
- Trisectionectomy (right anterior + posterior segments with medial segment of left lobe)
- Total hepatectomy (explant following liver transplantation)

Needle Biopsy

Measure the biopsy in two dimensions and record the number of cores.

State the fixative used. Routine biopsies are fixed in formalin. Biopsies for suspected storage or metabolic disorders may be received in alcohol, which preserves water-soluble carbohydrates and mucosubstances.

Record the colour of the biopsy, as it may provide diagnostic clues:

- Green: cholestasis
- Yellow: fatty change
- Black: Dubin–Johnson syndrome

Submit entirely for histology.

Wedge Biopsy

Measure the specimen in three dimensions.

Describe colour, capsular surface, and cut surface.

If the wedge is ≥5 mm in thickness, bisect prior to processing.

Submit **entirely** for histology.

Hepatic Resections for Hepatic Parenchymal Tumours

Measure the specimen in three dimensions and record the weight.

Ink all surgical margins with India ink and allow to dry.

Identify and separate the gall bladder with cystic duct, if present.

Section the liver in the frontal plane, preferably through the hilum if present. (Fig 10.1) Make serial slices at 1 cm intervals parallel to the initial cut.

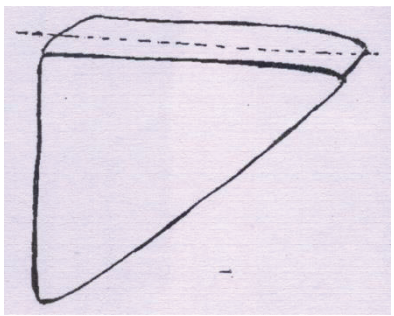


Fig 10.1 Sectioning of liver

Identify the tumour and record:

- Maximum tumour dimensions
- Distance from the closest surgical margin
- Relationship to the capsule and hilar vessels, with measured distances

Describe the tumour with respect to:

- Colour and consistency
- Margins (well circumscribed or infiltrative)
- Presence of bile staining
- Central necrosis or umbilication
- Satellite nodules, if any

Describe the adjacent liver parenchyma, noting features such as congestion, biliary obstruction, fibrosis, or cirrhosis.

Open the gall bladder, measure its length and wall thickness, and gross it as per gall bladder protocol.

Dissect hilar lymph nodes; record number and size of the smallest and largest nodes. Nodes >5 mm are bisected; smaller nodes are submitted entirely.

Sampling (Fig 10.2)

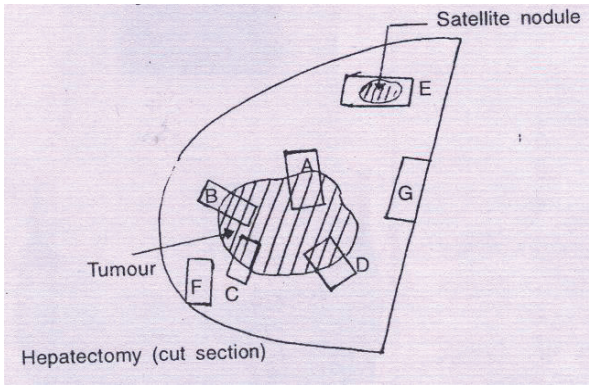


Fig 10.2 : Sampling of liver in hepatic tumours

A–E: Three to four tumour sections depending on size; sample variegated areas and satellite nodules (up to a maximum of five blocks).

F: One section of uninvolved liver.

G: Section from the closest surgical margin (may be included with tumour if very close).

Additional sections:

Hilar lymph nodes and blood vessels, if present

Gall bladder (three sections)

Hepatic Resections for Tumours of Major Bile Ducts

Tumours may be intrahepatic or extrahepatic. Purely intrahepatic cholangiocarcinomas are grossed as hepatic masses. When origin from major bile ducts is evident, proceed as follows:

Measure the specimen in three dimensions and record the weight.

Identify bile duct and vascular surgical margins and submit them en face.

Palpate bile ducts for areas of induration or thickening.

Separate the gall bladder.

Open bile ducts longitudinally with scissors and extend the section into the liver parenchyma.

Measure the tumour and its distance from the bile duct surgical margin.

Describe the tumour, noting:

Colour and consistency

Presence of papillary growth

Adjacent bile duct mucosa and any stenosis

Extension into surrounding liver parenchyma

Presence of gallstones within ducts

Section the tumour along the long axis of the bile duct.

Identify and sample hilar lymph nodes, recording number and size.

Gross the gall bladder as per standard protocol.

Sampling (Fig 10.3)

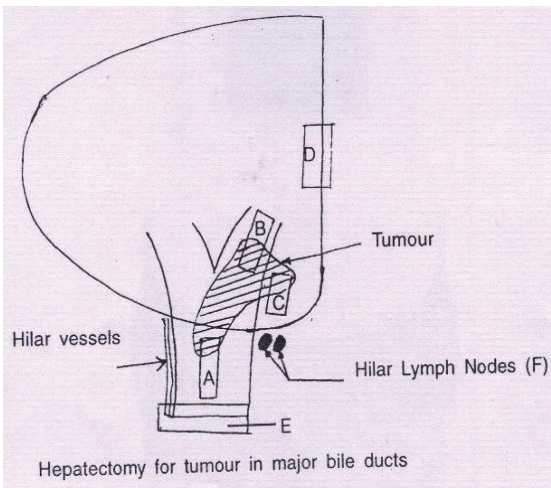


Fig 10.3 : Liver sampling in tumour of the major bile ducts .

A, B: Tumour with bile duct (two sections).

C: Surrounding hepatic parenchyma.

D: Uninvolved liver.

E: Bile duct surgical margin and hilar vessels.

F: Hilar lymph nodes.

Additional: Two to three sections of gall bladder.

A III LIVER GROSS PEARLS

1) Gross appearance of liver biopsies

Gross examination of core needle biopsies of the liver is of limited diagnostic value. However, useful information may be obtained from wedge biopsies and examination of the whole liver.

Gross clues from wedge biopsy:

Pale, greasy appearance; tissue floats in fixative – *Hepatic steatosis*

Deep red to bluish-red coloration – *Passive hepatic congestion*

Increased fragility with nodular surface – *Cirrhosis*

Whitish nodules – *Metastatic carcinoma*

Green discoloration – *Cholestasis*

Black or dark brown liver – *Dubin–Johnson syndrome*

Brown granular pigmentation – *Iron overload (hemochromatosis / hemosiderosis)*

Slate-gray discoloration – *Malarial pigment deposition*

2) Cirrhosis – Gross appearance

Cirrhosis is characterized grossly by diffuse fibrosis with regenerative nodules, leading to distortion of normal hepatic architecture.

WHO emphasizes that micronodular (<3mm nodules) and macronodular patterns often coexist and evolve over time.

Predominantly micronodular cirrhosis is classically seen in:

Alcohol-associated liver disease (ALD) – early stage

Metabolic dysfunction–associated steatohepatitis (MASH) *(formerly NASH)*

Chronic cholestatic liver disease (primary biliary cholangitis, secondary biliary cirrhosis)

Glycogen storage disease type IV

Hereditary hemochromatosis (early stage)

Post–jejunoileal bypass–associated liver disease

Galactosemia

Congestive hepatopathy (cardiac cirrhosis)

Predominantly macronodular cirrhosis is classically seen in:

Chronic viral hepatitis (HBV, HCV)

Wilson disease

Hereditary tyrosinemia

α_1-antitrypsin deficiency

Chronic drug- or toxin-induced liver injury

Alcohol-associated cirrhosis may show early micronodular, later progressing to mixed or macronodular cirrhosis.

3) Budd–Chiari syndrome

The liver is enlarged, congested, and dark red, particularly in the acute phase.
Chronic cases may show **fibrosis and secondary cirrhosis.**

4) Cystic lesions of the liver

Hydatid cyst

Caused by Echinococcus granulosus

Typically **a unilocular, well-defined, fluid-filled cyst**

Enlarges at approximately 1–5 cm per year

Located subcapsular or intraparenchymal

Shows **a thick, white, laminated cyst wall**

Echinococcus multilocularis produces **multilocular (alveolar) echinococcosis**, which is **rare but locally aggressive**.

5) Liver abscess

Amoebic liver abscess

Most commonly involves the right lobe

Usually solitary

Grossly shows yellowish necrotic material

Later forms thick, brownish, blood-stained material ("anchovy sauce" appearance)

Contents are typically sterile on bacterial culture

Multiplicity of lesions:

Seen in ~50% of pyogenic liver abscesses

Seen in ~25% of amoebic liver abscesses

6) Gross differences between hepatocellular carcinoma and metastatic carcinoma

Feature	Hepatocellular carcinoma (HCC)	Metastatic carcinoma
Number of lesions	Usually solitary; may have satellite nodules	Usually multiple, relatively uniform
Texture	Similar to surrounding liver	Different from surrounding liver
Umbilication	Absent	Present (especially subcapsular nodules)

Background liver	Cirrhosis or chronic liver disease usually present	Usually non-cirrhotic
Microscopy	Trabecular, pseudoglandular, or solid patterns	Glandular or squamous patterns

A IV LIVER MICROSCOPY

BIOPSY MATERIAL

Material for diagnosis of liver disease may be obtained by:

Fine-needle aspiration (FNA):

Useful mainly for space-occupying lesions, particularly when image-guided. Limited value for diffuse parenchymal disease.

Core (needle) biopsy:

Preferred for diffuse parenchymal liver diseases.

May be performed as:

Percutaneous blind biopsy

Image-guided biopsy for focal lesions

Wedge biopsy:

Obtained during laparotomy; provides larger tissue sample but may overestimate portal fibrosis.

Fixation

Tissue should be immediately fixed in 10% neutral buffered formalin.

In suspected glycogen storage disorders, two cores are recommended:

One in formalin

One in alcohol (to preserve glycogen)

Liver biopsy interpretation

Adequacy of liver biopsy

Minimum length: ~2–2.5 cm

Portal tracts:

≥ 10–11 portal tracts recommended for optimal assessment

≥ 6 portal tracts acceptable for routine diagnosis

Adequacy is critical for assessment of:

Chronic hepatitis

Fibrosis staging

Biliary disease

Routine stains for liver biopsy (Mandatory stains)

Step sections should be taken.

i) Hematoxylin & Eosin (H&E)

At least '3 step levels'

ii) Reticulin stain

Demonstrates type III collagen

Highlights:

Reticulin framework

Lobular collapse

Regenerative nodules

iii) Periodic acid–Schiff (PAS)

For glycogen

With diastase digestion (PAS-D) to identify:

α_1-antitrypsin globules (diastase-resistant, magenta)

iv) Masson's trichrome

Demonstrates type I collagen

Highlights:

Portal fibrosis

Bridging fibrosis

Pericellular ("chicken-wire") fibrosis

These four stains are essential for interpretation of any liver biopsy.

Special stains (as indicated)

v) Van Gieson stain

Alternative to trichrome

Type I collagen – pink/red

Bile – green

vi) Perls' Prussian blue

Iron (hemosiderin) – blue

vii) Orcein (Shikata)

HBsAg (cytoplasmic positivity)

Copper-associated protein (CAP) – brown-black granules

Elastic fibers – black

viii) Rhodanine / Rubeanic acid

Copper – red / green respectively

ix) Fouchet stain

Bile pigment – emerald green

x) Frozen sections for fat

Oil Red O – red

Sudan III / IV – orange-red

Sudan Black B / Osmium tetroxide – black

xi) Microorganisms

GMS, AFB, PAS, Gram stains

xii) Giemsa stain

Leishman–Donovan bodies

Toxoplasma

xiii) Immunohistochemistry

HBsAg

HBcAg

(Expanded viral and metabolic panels as required)

Alcohol-associated liver disease (ALD) *(WHO-preferred term)*

General features

Histology ranges from steatosis → steatohepatitis → cirrhosis

Changes predominantly involve Zone 3 (centrilobular region)

Microscopy

Steatosis:

Predominantly macrovesicular

Microvesicular steatosis may be seen in acute binge drinking

Ballooning degeneration

Mallory–Denk bodies

Aggregates of cytokeratin intermediate filaments

Prominent in zone 3

Inflammation:

Lobular and portal

Neutrophil-predominant (alcoholic hepatitis)

Necrosis:

Spotty or confluent

Fibrosis:

Begins as pericellular/perisinusoidal fibrosis around central veins

Radiates outward ("chicken-wire" pattern)

Progresses to micronodular cirrhosis

Late lesions:

Centrilobular sclerosis

Sclerosing hyaline necrosis

Differential diagnosis

Viral hepatitis

Chronic biliary disease

A correlation between clinical history, Mallory–Denk bodies, and centrilobular fibrosis supports the diagnosis.

Metabolic dysfunction–associated steatohepatitis (MASH)

Steatotic liver disease (SLD) was chosen as an overarching term by AASLD to encompass the various aetiologies of steatosis.- June 2023

The term steatohepatitis was felt to be an important pathophysiological concept that should be retained.

Nonalcoholic fatty liver disease (NAFLD) will now be metabolic dysfunction-associated steatotic liver disease (MASLD). MASLD encompasses patients who have hepatic steatosis and have at least one of five cardiometabolic risk factors.

A new category, outside pure MASLD, termed MetALD (pronunciation: Met A-L-D) was selected to describe those with MASLD who consume greater amounts of alcohol per week (140 g/week and 210 g/week for females and males respectively).

Metabolic dysfunction-associated steatohepatitis (MASH) is the replacement term for NASH. Those with no metabolic parameters and no known cause have cryptogenic SLD.

Background

MASH represents the inflammatory stage of MASLD

Hepatic manifestation of metabolic syndrome

Requires ≥ 3 of the following:

- Central obesity
- Type 2 diabetes / hyperglycemia
- Hypertriglyceridemia
- Low HDL cholesterol
- Hypertension

Microscopy of MASH

Changes predominantly in Zone 3

Hallmark triad:

- Steatosis (macrovesicular, variable size)
- Hepatocellular injury (ballooning)
- Lobular inflammation

Mallory–Denk bodies:

- Present but often poorly formed and irregular

Inflammation:

- Lobular, predominantly lymphocytic

Fibrosis:

- Perisinusoidal/pericellular
- Begins in zone 3 and progresses outward

Other features:

- Glycogenated nuclei (common)

Fibrosis staging (Brunt system)

Stage 1: Zone 3 perisinusoidal fibrosis

Stage 2: Stage 1 + periportal fibrosis

Stage 3: Bridging fibrosis

Stage 4**:** Cirrhosis

Features favoring ALD over MASH

Sclerosing hyaline necrosis

Veno-occlusive lesions

Ductular reaction

Cholangiolitis

Bilirubinostasis

Histologic distinction between ALD and MASH may be difficult; clinical correlation is essential.

VIRAL HEPATITIS

Etiology

Caused by hepatotropic viruses:

HAV, HBV, HCV, HDV, HEV

Histologic differentiation of etiology is limited, except for certain characteristic features.

Acute viral hepatitis – microscopy

Hepatocellular injury:

- Ballooning degeneration
- Acidophil (Councilman) bodies
- Spotty necrosis

Inflammation:

- Portal and lobular
- Lymphocytes ± neutrophils, plasma cells

Massive hepatic necrosis:

- More common in HAV and HBV
- Rare in HCV

HBV: prominent ballooning degeneration

HCV: sinusoidal lymphocytes

HAV: prominent plasma cells

Severe injury**:**

Collapse of reticulin framework

Ductular (progenitor cell) reaction

Chronic viral hepatitis

Defined as hepatitis with ongoing necroinflammation > 6 months.
Most commonly due to HBV and HCV.

Microscopy

Portal lymphocytic infiltrates

Interface hepatitis (piecemeal necrosis)

Lobular necroinflammation

HBsAg:

"Ground-glass hepatocytes"

HBcAg:

"Sanded nuclei" (IHC marker of activity)

HCV:

Lymphoid aggregates in portal tracts (≈50%)

Strong association with hepatocellular carcinoma

Premalignant lesions:

Dysplastic foci

Dysplastic nodules

Terms such as chronic persistent and chronic active hepatitis are obsolete and no longer used.

Scoring systems for chronic hepatitis

Ishak (modified Knodell) HAI – most widely used

Scheuer / Batts–Ludwig system – simpler

METAVIR – commonly used in Europe

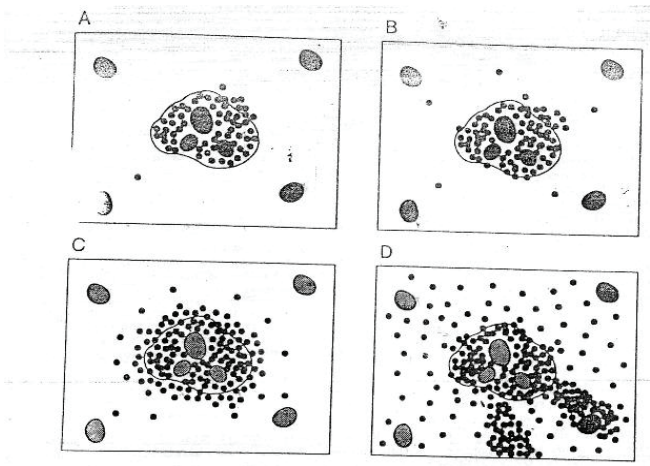


Fig 10.4

Activity panel: In these four panels A to D (of the figure 10.4) the increasing severity of portal inflammation, interface hepatitis, and lobular necro-inflammatory lesions in chronic hepatitis are shown (Batts & Ludwig, 1995)

Ishak's modification for Hepatic activity index (HAI) for scoring of necro-inflammatory activity in chronic hepatitis

Maximum score for grading is 18

(A) Periportal or periseptal interface hepatitis (piecemeal necrosis)	
Absent	0
Mild (focal, few portal areas)	1
Mild/moderate (focal, most portal areas)	2
Moderate (continuous around <50% of tracts or septa)	3
Severe (continuous around >50% of tracts or septa)	4
(*B*) *Confluent necrosis*	
Absent	0
Focal confluent necrosis	1
Zone 3 necrosis in some areas	2
Zone 3 necrosis in most areas	3
Zone 3 necrosis+occasional portal-central (P-C) bridging	4
Zone 3 necrosis+multiple P-C bridging	5
Panacinar or multiacinar necrosis	6

(*C*) *Focal (spotty) lytic necrosis, apoptosis and focal inflammation*	
Absent	0
One focus or less per ×10 objective	1
Two to four foci per ×10 objective	2
Five to ten foci per ×10 objective	3
More than ten foci per ×10 objective	4
(*D*) *Portal inflammation*	
Absent	0
Mild, some or all portal areas	1
Moderate, some or all portal areas	2
Moderate/marked, all portal areas	3
Marked, all portal areas	4

Modified Histological Activity index staging :

Maximum score 6

Change	Score
No fibrosis	0
Fibrous expansion of some portal areas, With or without short fibrous septa	1
Fibrous expansion of most portal areas, With or without short fibrous septa	2
Fibrous expansion of most portal areas, With occasional portal to portal (P-P) bridging	3
Fibrous expansion of portal areas, with marked bridging P-P as well as portal –central (P-C)	4
Marked bridging (P-P and /or P-C) with occasional nodules (incomplete cirrhosis)	5
Cirrhosis, probable or definite	6

ii) Scheurer system:

Necro-inflammatory activity :	Portal periportal activity/	Lobular activity
Grade 0 :	No or minimal inflammation/ none	
Grade 1:	Portal inflammation/ lobular inflammation (no necrosis)	
Grade 2:	Mild piecemeal necrosis/ focal hepatocellular necrosis	
Grade 3 :	Moderate piecemeal necrosis/ severe focal cell damage	
Grade 4:	Severe piecemeal necrosis / bridging necrosis	

(Focal necrosis : not more than one focus of necrosis /lobule.

Severe necrosis: > 2 foci of necrosis in each lobule

Mild piecemeal necrosis : small foci around occasional portal tract

Moderate piecemeal necrosis : diffuse necrosis around some portal tracts/ or focal necrosis around most portal tracts.

Severe piecemeal necrosis : diffuse necrosis around most portal tracts

Bridging necrosis : 2 or > bridges:- P-P, P-C, C-C

Scheurer's Staging :

0 : no fibrosis

I : enlarged fibrotic portal tracts

II : periportal or portal to portal fibrosis

III : bridging fibrosis with architectural distortion but no obvious cirrhosis

IV : cirrhosis.

CIRRHOSIS

Definition: Diffuse liver disease with parenchymal nodules of regenerating hepatocytes separated by irregular, branching, and anastomosing fibrous septa.

Nodular patterns:

Micronodular: nodules < 3 mm

Macronodular: nodules > 3 mm

Progression of fibrosis**:**

Portal expansion

Septal fibrosis

Complete cirrhotic remodelling

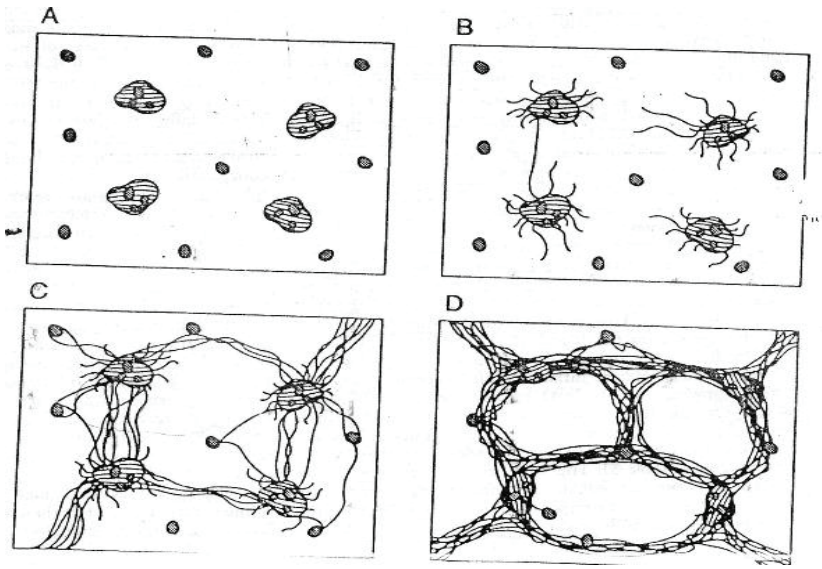


Fig 10.5

Fibrosis Staging : The above panels (Fig 10.5) graphically portray the progression of fibrosis from portal expansion (A), through septal fibrosis (B&C), to complete cirrhotic remodelling (D). Distinction between C & D is difficult at needle biopsy interpretation. (Batts & Ludwig, 1995)

Needle biopsies may not reliably distinguish late septal fibrosis from early cirrhosis.
See gross morphology section for macronodular vs micronodular patterns. Fig 10.6 shows a H & E section of a liver showing evidence of cirrhosis.

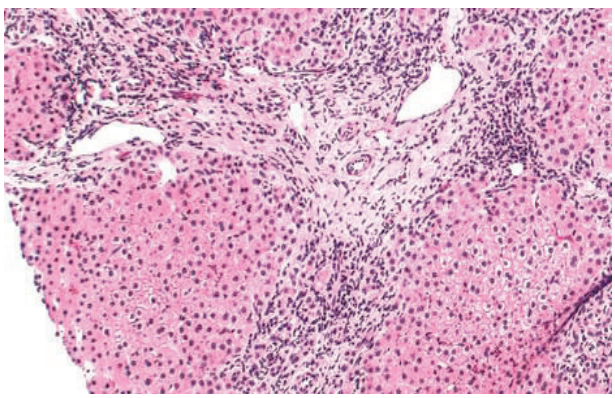


Fig 10.6 Shows liver tissue with 3 pseudo-lobules in a fibrous stroma showing distorted architecture with several inflammatory cells. H&E x 100

AUTOIMMUNE HEPATITIS (AIH)

Epidemiology: Predominantly affects women; correct diagnosis is therapeutically important.

Serological types:

Type I: ANA + ASMA (anti-smooth muscle antibodies) – most common

Type II: Anti-LKM1 (liver/kidney microsomal)

Type III: Anti–soluble liver antigen (SLA/LP)

Histology

Acute phase:

High necroinflammatory activity in untreated patients

Interface hepatitis (piecemeal necrosis)

Hepatocyte ballooning

Bridging/confluent necrosis

Lymphoplasmacytic infiltrate, often with hepatic rosettes and multinucleate giant hepatocytes

Note: Only 'acute HAV' shows abundant plasma cells; rule out serologically in suspected AIH.

Chronic phase:

Portal inflammation, interface hepatitis

Lobular inflammation and hepatocyte regeneration

Histology may resemble chronic viral hepatitis

METABOLIC LIVER DISEASES (OTHER THAN STEATOHEPATITIS)

Hereditary Haemochromatosis (HH)

Hereditary haemochromatosis is a genetic disorder of iron homeostasis characterized by inappropriately increased intestinal iron absorption, leading to progressive iron overload. It shows male predominance and is usually inherited in an autosomal recessive manner.

The most common form (Type 1 HH) is due to a missense mutation (C282Y) in the HFE gene.

Pathogenesis involves impaired hepcidin regulation → increased iron absorption at the enterocyte level.

Histologic distribution of iron

Primary (hereditary) haemochromatosis:
Iron deposition predominantly in hepatocytes (periportal → panlobular).

Secondary haemosiderosis:
Iron initially in Kupffer cells, later extending to hepatocytes.

Causes of secondary haemosiderosis include:

Chronic transfusional iron overload

Haemolytic anaemias

Porphyria cutanea tarda

In advanced cirrhosis, distinction between primary and secondary forms may become difficult.

Hepatic Iron Index (HII)

(Used mainly in non-cirrhotic livers)

[Iron concentration (mg iron per gram dry weight of liver) / 55.846 (atomic weight of iron)] / patient's age = HII in mmol iron/gram liver/year.

HII > 2 → diagnostic of hereditary haemochromatosis. A HII greater than 1.9 in a non-cirrhotic liver is considered strongly suggestive of HH. The theoretical basis for the HII is that iron accumulation increases steadily with age in patients with HH resulting in a correlation between age and hepatic iron content in patients with HH but not in patients who have secondary iron overload .

Not required if:

No iron present

Massive iron overload

Established cirrhosis

Reference values

Normal hepatic iron: < 1,000 µg/g dry wt

HH usually: > 10,000 µg/g

Cirrhosis risk: ~22,000 µg/g

Systemic involvement

Iron deposition also occurs in:

Heart (cardiomyopathy)

Pancreas (“bronze diabetes”)

Testes (hypogonadism)

Wilson Disease (Hepatolenticular Degeneration)

Wilson disease is an autosomal recessive disorder of copper metabolism caused by mutations in the ATP7B gene on chromosome **13**, leading to defective biliary copper excretion and reduced ceruloplasmin synthesis.

Typically presents in the 2nd–4th decades

Liver histology is highly variable and requires clinicopathologic correlation

Histologic features

Early:

- Steatosis
- Hepatocyte ballooning
- Glycogenated nuclei
- Acidophil bodies (periportal)

Progressive disease:

- Portal inflammation (sparse lymphocytes)
- Copper-associated protein deposition
- Cholestasis
- Lipofuscin accumulation (may be striking)

Advanced:

- Chronic hepatitis–like pattern
- Fulminant hepatic failure (may mimic viral hepatitis)
- Fibrosis and cirrhosis

Differential diagnosis of increased hepatic copper

- Chronic cholestasis
- Indian childhood cirrhosis
- Primary biliary cholangitis

Diagnostic criteria (screening)

- Kayser–Fleischer rings (slit lamp)
- Serum ceruloplasmin < 20 mg/dL (85–95%)
- 24-hour urinary copper > 100 µg
- Hepatic copper > 250 µg/g dry wt (gold standard)

REYE SYNDROME

Reye syndrome is a rare but potentially fatal pediatric disorder characterized by:

- Acute encephalopathy
- Hepatic dysfunction

It is associated with mitochondrial dysfunction, particularly affecting fatty acid β-oxidation, and may be precipitated by aspirin (acetylsalicylic acid) use during viral illness.

Liver histology

Diffuse panlobular microvesicular steatosis

Cytoplasmic pallor

No inflammation

No fibrosis

PIGMENTS IN THE LIVER

Pigment	Common Associations	Distribution	Special Stain
Haemosiderin	HH, secondary haemosiderosis	Periportal hepatocytes	Perl's Prussian blue
Lipofuscin	Aging, Wilson disease	Centrilobular	Ziehl–Neelsen
Bile	Cholestasis	Canaliculi, hepatocytes	Fouchet / Hall
Copper-associated protein	PBC, PSC, Wilson disease	Periportal	Orcein, Rhodanine
Dubin–Johnson pigment	Dubin–Johnson syndrome	Centrilobular	PAS+, ZN+
Haematoidin	Old hemorrhage	Kupffer cells	Unstained

Circulatory Disturbances of the Liver

Patterns

Hepatic venous outflow obstruction

Budd–Chiari syndrome

Sinusoidal obstruction syndrome

Intrahepatic circulatory impairment

Cirrhosis

Systemic circulatory failure

Portal inflow obstruction

Portal vein thrombosis

BUDD–CHIARI SYNDROME

Caused by thrombosis or obstruction of hepatic veins.

- Gross: swollen, congested liver
- Microscopy:
  - Central vein congestion
  - Centrilobular necrosis
  - Progressive centrilobular fibrosis
  - Regeneration of periportal hepatocytes

Associated with:

- Myeloproliferative disorders
- Antiphospholipid syndrome
- Pregnancy
- Polycythemia vera
- Protein C / S deficiency

SINUSOIDAL OBSTRUCTION SYNDROME (VENO-OCCLUSIVE DISEASE)

- Injury to sinusoidal endothelium and terminal hepatic venules
- Causes:
  - Pyrrolizidine alkaloids (bush tea)
  - Chemotherapy
  - Bone marrow transplantation

PELIOSIS HEPATIS

- Blood-filled cystic spaces lacking true endothelial lining
- Associated with:
  - Malignancy
  - Anabolic steroids
  - Oral contraceptives

NON-CIRRHOTIC PORTAL FIBROSIS (HEPATOPORTAL SCLEROSIS)

- Portal hypertension without cirrhosis
- Seen with:

Arsenic exposure

Vinyl chloride

Cytotoxic drugs

Histology:

Portal vein sclerosis and thrombosis

Recanalized venules

Preserved lobular architecture

CYSTS OF THE LIVER

A) Hydatid Cyst

Caused most commonly by *Echinococcus granulosus* and rarely by *E. multilocularis* (alveolar echinococcosis).

E. granulosus forms a unilocular, white, fluid-filled cyst, enlarging at 1–5 cm/year

Located superficial or deep to Glisson capsule

Microscopy

The cyst wall has **three layers**:

Germinal layer (endocyst)

Innermost, nucleated layer

Gives rise to brood capsules

Protoscolices develop within brood capsules

Detached brood capsules form daughter cysts

Ectocyst

Laminated, acellular, avascular membrane

Eosinophilic, refractile, chitinous (~1 mm thick)

PAS+, GMS+, Best mucicarmine+

Pericyst

Host-derived fibrovascular tissue

Chronic inflammatory infiltrate

Eggshell calcification in ~25% cases

Hydatid sand (FNA): free hooklets, protoscolices, daughter cysts

B) Amoebic Liver Abscess

Occurs in 3–9% of amoebiasis**,** predominantly in the right lobe.

Gross: well-circumscribed lesion with **"**anchovy sauce**"** necrotic material

Contents are bacteriologically sterile

Microscopy

Central necrosis with no neutrophils

Peripheral granulation tissue

Trophozoites seen at margins

Trophozoite morphology (*Entamoeba histolytica*)

Size: 10–60 μm

Central karyosome, beaded nuclear membrane

Cytoplasm with ingested RBCs

Stains

PAS: magenta cytoplasm

Iron hematoxylin: central karyosome

Diff-Quik: purplish cytoplasm

DD: macrophages (bean-shaped nucleus, fine chromatin)

C) PYOGENIC LIVER ABSCESS

Routes:

Biliary tract (30–70%)

Portal venous spread (15%)

Intra-abdominal sepsis

Septicemia

Usually right lobe

Single or multiple

Coalescence → honeycomb appearance

E. coli commonest organism; often polymicrobial

D) SIMPLE (CONGENITAL) HEPATIC CYST

Unilocular, non-neoplastic

Arises from ductal plate malformation remnants (von Meyenburg complexes)

Size: few cm to >40 cm

Lined by cuboidal to flattened biliary epithelium

Contents: clear, amber, occasionally bile-stained or hemorrhagic

Secondary changes: calcification, epithelial denudation

E) FIBRO-POLYCYSTIC LIVER DISEASES

These are **ductal plate malformations**.

Autosomal Dominant Polycystic Kidney Disease (ADPKD)

PKD1 / PKD2 mutations

Hepatic cysts in **70–90%**

Develop from dilated biliary microhamartomas

Caroli Disease

Segmental, communicating dilatation of large intrahepatic bile ducts

Congenital Hepatic Fibrosis / ARPKD

Portal fibrosis with anastomosing dilated bile ducts

No inflammation

No regenerative nodules

Portal hypertension common

F) NEOPLASTIC CYSTIC LESIONS (WHO 2019/2022)

i) Mucinous Cystic Neoplasm (MCN) of Liver

(WHO-preferred term replacing "biliary cystadenoma/cystadenocarcinoma")

Almost exclusively in women

Multiloculated cyst

Lined by mucin-secreting epithelium

Characteristic ovarian-type stroma (diagnostic)

Does NOT communicate with bile ducts

Intrahepatic (84%), rarely CBD or gallbladder

Size: 2.5–28 cm

Fluid: mucinous, gelatinous, brown or bilious

MCN with invasive carcinoma = malignant counterpart

Metastatic mucinous tumors (pancreas, ovary, appendix) must be excluded

ii) Serous Cystic Neoplasm

Rare

Multiple small cysts

Glycogen-rich, clear cells

Mucin-negative

Benign

MELD Score

The MELD Score helps to determine patients with end stage liver disease and is helpful in prioritising patients for liver transplants . Your MELD score is a number between 6 and 40 that's calculated to determine how soon one needs a liver transplant. The higher the MELD score, the higher the chances of end stage liver disease. There is a max score of 40 which assess transplant appropriateness.

HEPATOCELLULAR ADENOMA (HCA)

(WHO 2019 molecular classification)

Benign hepatocellular neoplasm arising in **non-cirrhotic liver**.

95% in women

Associated with:

- Oral contraceptives
- Anabolic steroids
- Metabolic disorders

AFP: normal

Gross

Solitary (70–80%), subcapsular

Right lobe

10 cm common

Sharply demarcated

No central scar

Hemorrhage and infarction frequent

Microscopy

- Plates 1–2 cells thick
- Bland hepatocytes
- No portal tracts or bile ducts
- Thin-walled veins
- Mitoses absent

WHO Molecular Subtypes

- HNF1α-inactivated HCA – steatotic
- Inflammatory HCA – sinusoidal dilation, inflammation
- β-catenin activated HCA – malignant risk
- Unclassified

β-catenin–activated HCA → risk of HCC

FOCAL NODULAR HYPERPLASIA (FNH)

- Second most common benign hepatic lesion
- 80–95% in women
- Peak: 3rd–4th decade
- Often incidental

Pathogenesis

Hyperplastic response to arterial malformation

Imaging

- Centrifugal hypervascularity
- Central scar

Gross

- Solitary (70–80%)
- Subcapsular
- Unencapsulated
- Tan nodular mass (<5 cm usually)
- Central stellate scar

Microscopy

Nodules of benign hepatocytes

Fibrous septa with:

Thick-walled arteries

Ductular reaction at interface

Portal veins absent

Bile ducts present only at margins

Telangiectatic variant: dilated vascular channels

DD -Fibrolamellar HCC (has bile, malignant cytology)

Comparative Features: HCA vs FNH vs Fibrolamellar HCC (FL-HCC)

Feature	Hepatocellular Adenoma (HCA)	Focal Nodular Hyperplasia (FNH)	Fibrolamellar HCC (FL-HCC)
Nature	Benign neoplasm	Benign hyperplastic lesion	Malignant hepatocellular carcinoma
Underlying Liver	Normal	Normal	Normal (non-cirrhotic)
Age / Sex	Young women (20–40 yrs)	Young women (20–50 yrs)	Adolescents & young adults
Hormonal Association	Strong (OCPs, anabolic steroids)	No strong hormonal link	None
Pathogenesis	Neoplastic proliferation	Hyperplastic response to vascular malformation	True malignancy
AFP Levels	Normal	Normal	Usually normal or mildly elevated
Number of Lesions	Solitary (70–80%); multiple in steroid use	Usually solitary	Solitary
Location	Subcapsular, often right lobe	Subcapsular	Variable
Gross Appearance	Well circumscribed or encapsulated	Unencapsulated	Firm, lobulated

Central Scar	**Absent**	**Present** (stellate scar)	**Present**, but dense & fibrous
Cut Surface	Variegated; hemorrhage & infarction common	Tan nodular with fibrous septa	Hard, fibrous with lamellae
Bile Ducts / Ductules	**Absent**	**Present at septa–parenchyma interface**	**Absent**
Portal Tracts	Absent	Absent (incomplete portal tracts)	Absent
Vascular Pattern	Thin-walled veins, unpaired arteries	Thick-walled arteries in septa	Variable, often fibrotic vessels
Hepatocyte Plates	1–2 cell thick	1–2 cell thick	Thickened plates
Cellular Atypia	Minimal / absent	Absent	Present
Fibrosis	Absent	Fibrous septa radiating from scar	**Lamellar fibrosis (hallmark)**
Bile Production	May be present	May be present	Often present
Calcification	Rare	Rare	Common
Metastasis	No	No	Yes
Prognosis	Excellent (risk of hemorrhage, rare malignant transformation)	Excellent	Better than conventional HCC but malignant

Key diagnostic clues:
– *Hemorrhage without scar* → ***HCA***
– *Scar with ductules* → ***FNH***
– *Scar with lamellar fibrosis and malignancy* → ***FL-HCC***

NODULAR REGENERATIVE HYPERPLASIA (NRH)

Nodular regenerative hyperplasia is a diffuse transformation of the liver into small regenerative nodules without fibrous septa, distinguishing it from cirrhosis.

Represents a non-cirrhotic cause of portal hypertension

Associated with:

Connective tissue diseases

Myeloproliferative disorders

Drugs (thiopurines, azathioprine)

Congestive cardiac failure

Pathogenetic basis: obliterative portal venopathy / portal vein thrombosis

Gross

Capsular surface: finely granular

Parenchyma: diffuse white–tan nodules

Nodule size: typically **1–3 mm**, occasionally larger

Congested internodular parenchyma

Microscopy

Nodules of hyperplastic hepatocytes, often periportal

Hepatocyte plates thickened (>1–2 cells)

Centrilobular atrophy in internodular regions

Lipofuscin absent in nodules but present in atrophic hepatocytes

Sinusoidal dilatation in internodular zones

No fibrous septa

DD (gross & microscopy): micronodular cirrhosis, metastatic carcinoma

REGENERATIVE AND DYSPLASTIC NODULES

(WHO / International Working Group on Hepatocellular Nodules)

Hepatic nodules in cirrhosis form a biological continuum from regeneration to malignancy.

A) Large (Macro) Regenerative Nodules (LRN / MRN)

Occur in cirrhotic or chronically injured liver

Incidence: 15–50% of cirrhotic livers

Size: 0.5–1.5 cm (may exceed 5 cm)

Multiple

Surrounded by thin fibrous septa

Microscopy

Hepatocytes resemble surrounding liver

Plates 1–2 cells thick

Multiple portal tracts (multiacinar)

Mild portal tract abnormalities

No cytological atypia

B) Dysplastic Nodules (DN)

(Previously "borderline nodules")

Occur in 5–15% of cirrhotic livers

Multiple

Usually <2 cm

Co-exist with regenerative nodules

Microscopy

Small cell change (small cell dysplasia):

- Reduced cell size
- Increased N:C ratio
- Basophilic cytoplasm

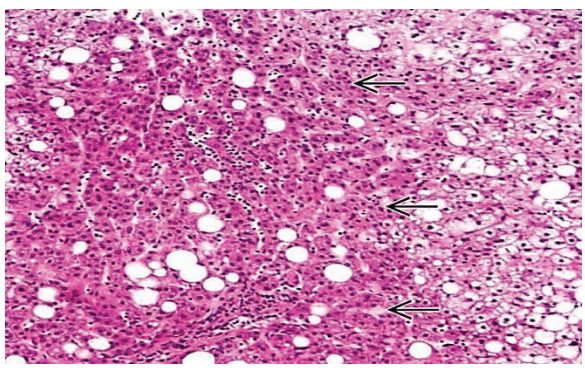
Intranodular (non-triadal) arteries

Portal tract abnormalities

Increased sinusoidal capillarization

Classified as:

- Low-grade DN
- High-grade DN (true premalignant lesion)



(Fig 10.7) Small cell change (left 2/3 of image) is characterized by small cells with high nuclear:cytoplasmic ratio leading to increased cell density. When present in a nodule, it is the hallmark of high-grade dysplastic nodule (HGDN).(basic medical key.com)

High-grade dysplastic nodules are the immediate precursors of HCC

HEPATOCELLULAR CARCINOMA (HCC)

(WHO 2019/2022)

HCC arises most commonly in a cirrhotic liver of any etiology.

Risk Association

Highest: chronic HBV

High: chronic HCV

Intermediate: alcoholic cirrhosis, hemochromatosis

Low: other causes

Gross (Eggel classification)

Multinodular

Massive

Diffuse

Grossly : Tumors are soft, yellow-green or reddish, often hemorrhagic.

Microscopy

HCC retains resemblance to normal liver architecture.

Patterns

Trabecular (sinusoidal) – most common

Plates >3 cells thick

Reduced Kupffer cells

Dilated sinusoids

Pseudoglandular (acinar)

Central degeneration of trabeculae

Lumina contain bile, debris, macrophages

Compact / solid / scirrhous

Compression artifact or therapy related

Fibrolamellar variant

Young patients

Polygonal cells with vesicular nuclei

Abundant eosinophilic granular cytoplasm

Lamellar fibrosis (diagnostic hallmark)

Better prognosis than conventional HCC

Other cytologic features:

Pleomorphism

Clear cell change

Oncocytic cells

Intranuclear inclusions

Bile pigment

Special Stains

Reticulin: loss or widening of framework

PAS: glycogen

Trichrome: fibrinogen inclusions

Enzyme Histochemistry

Canalicular alkaline phosphatase: reduced in HCC

Immunohistochemistry (summary)

HepPar-1: positive

Arginase-1: positive (most specific)

Glypican-3: positive (malignancy marker)

AFP: positive in ~25–30% (specific, insensitive)

Canalicular CEA: positive

WHO 5TH EDITION TUMOUR CLASSIFICATION

(Digestive System Tumours, WHO Blue Book Series; current global standard)

A. Tumours of the Liver and Intrahepatic Bile Ducts

Epithelial Tumours

Benign / Pre-neoplastic

Hepatocellular adenoma

Focal nodular hyperplasia (FNH)

Bile duct adenoma

Biliary adenofibroma

Biliary intraepithelial neoplasia (precursor lesion)

Intraductal papillary neoplasm of the bile ducts (IPNB)

Mucinous cystic neoplasm of the liver and biliary system

Malignant

Hepatocellular carcinoma (HCC)

Hepatoblastoma (pediatric)

Intrahepatic cholangiocarcinoma (iCCA)

Small-duct type and large-duct type (morphological subtypes)

Combined hepatocellular-cholangiocarcinoma (cHCC-CCA)

Undifferentiated primary liver carcinoma

(*Plus rarer epithelial carcinomas and subtypes as per WHO*)

Non-epithelial and Miscellaneous Tumours

Vascular: Haemangioma / epithelioid haemangioendothelioma / angiosarcoma

Mesenchymal and stromal: Mesenchymal hamartoma, PEComa/angiomyolipoma, embryonal sarcoma, etc.

Haematolymphoid neoplasms (lymphoma, etc.)

Secondary (metastatic) tumours to liver

B. Tumours of the Gallbladder and Extrahepatic Bile Ducts

Benign / Pre-neoplastic

Pyloric gland adenoma of gallbladder

Biliary intraepithelial neoplasia (low- and high-grade)

Intracholecystic papillary neoplasm (ICPN) (formerly intraductal/intracystic)

Intraductal papillary neoplasm of bile ducts

Malignant

Carcinoma of the gallbladder (adenocarcinoma and variants)

Carcinoma of extrahepatic bile ducts (cholangiocarcinoma)

Neuroendocrine neoplasms of gallbladder and bile ducts

Notes

Squamous and adenosquamous carcinomas are recognized variants.

Papillary invasive carcinomas emerging from ICPN are included in malignant categories.

TNM Staging

Use AJCC 8th edition TNM descriptors for HCC, iCCA, gallbladder carcinoma

Include stage groupings and definitions within your reporting templates**HCC vs Cholangiocarcinoma**

Feature	HCC	Cholangiocarcinoma (CC)
Cell of origin	Hepatocyte	Bile duct epithelium
Background liver	Cirrhosis common	Often non-cirrhotic
Gross	Soft, hemorrhagic	Firm, fibrotic
Architecture	Trabecular, solid, pseudoacinar	Glandular, cribriform
Sinusoids	Present	Absent
Mucin	Absent	**Present (always)**
Spread	Hematogenous	Lymphatic, perineural
AFP	±	−
HepPar-1 / Arginase-1	+	−
CK7 / CK19	Usually −	**Positive**
Canalicular CEA	+	−

SUMMARY : HEPATOCELLULAR CARCINOMA (HCC)

Definition

Hepatocellular carcinoma (HCC) is a primary malignant epithelial tumor of hepatocytes, most often arising in a background of chronic liver disease and cirrhosis.

Etiopathogenesis

Cirrhosis of any cause is premalignant

Major risk factors:

Chronic HBV (with or without cirrhosis)

Chronic HCV

Alcoholic cirrhosis

MASH/NASH

Hemochromatosis

Aflatoxin B1 exposure

Multistep hepatocarcinogenesis:

Regenerative nodules → Dysplastic nodules → Early HCC → Advanced HCC

Gross Patterns (Eggel classification)

Multinodular (most common; especially in cirrhosis)

Massive (single dominant mass)

Diffuse (rare, infiltrative)

Tumors are soft, yellow-green or reddish, often hemorrhagic; may show satellite nodules.

Microscopic Patterns

Trabecular (sinusoidal) – most common
(plates >3 cells thick, reduced reticulin)

Pseudo-glandular (acinar) – dilated canaliculi or degeneration

Solid / compact

Fibrolamellar variant

Young patients

No cirrhosis

Large polygonal cells with lamellar fibrosis

Better prognosis

Key Histologic Features

Thickened hepatocyte plates

Unpaired arteries

Loss or reduction of reticulin framework

Bile production within tumor cells

Vascular invasion (critical prognostic factor)

Precursor Lesions

High-grade dysplastic nodules

Small cell change

Capillarization of sinusoids

Special Stains

Reticulin: decreased or absent

PAS: glycogen

Trichrome: fibrinogen inclusions

Prognostic Factors

Tumor size and number

Vascular invasion (micro/macro)

Tumor differentiation

Background liver disease

Completeness of resection

Differential Diagnosis

Intrahepatic cholangiocarcinoma

Metastatic carcinoma

Hepatocellular adenoma (esp. well differentiated HCC)

IHC TABLE : HCC vs CHOLANGIOCARCINOMA vs METASTATIC CARCINOMA

Marker	HCC	Cholangiocarcinoma (CC)	Metastatic carcinoma
HepPar-1	Positive (diffuse)	Negative	Negative
Arginase-1	Strongly positive (most sensitive & specific)	Negative	Negative
Glypican-3	Positive (esp. moderate–poorly differentiated HCC)	Negative	Variable (some GI tumors +)
AFP	Positive (≈25–40%)	Negative	Negative
CEA (polyclonal)	Canalicular pattern	Cytoplasmic / luminal	Cytoplasmic
CK8 / CK18	Positive	Positive	Positive
CK7	Usually negative or focal	Positive	Variable
CK19	Usually negative	Positive	Variable

EMA	Usually negative	Positive	Positive
CD34	Diffuse sinusoidal capillarization	Negative	Negative
MUC1 / MUC5AC	Negative	Positive	Variable
SATB2	Negative	Negative	Positive in colorectal metastasis
TTF-1	Negative	Negative	Positive in lung metastasis
ER / PR	Negative	Negative	Positive in breast metastasis

Practical Diagnostic Tips

Canalicular CEA + HepPar-1 + Arg-1 (new IHC marker for hepatocytes) = HCC

CK7/CK19 + mucin + glands = Cholangiocarcinoma

Strong CK7/CK20 patterns ± organ-specific markers = Metastasis

Always interpret IHC with morphology and clinical context

V A LIVER BIOPSY – SYNOPTIC REPORTING

Report on hepatitis : (WHO 2019 / chronic hepatitis activity-based)

Specimen

Needle biopsy ☐

Wedge biopsy ☐

Adequacy

Length of biopsy core: _____ mm

Adequate ☐ Inadequate ☐

Portal tracts counted: ……

Hepatocyte Injury

Ballooning degeneration ☐

Feathery degeneration ☐

Mallory–Denk bodies ☐

Acidophil bodies ☐

Ground-glass hepatocytes ☐

Steatosis: ☐ <5% ☐ 5–33% ☐ 34–66% ☐ >66%

Intracellular bile ☐

Iron ☐ Lipofuscin ☐ Other ☐ Specify ……

Necro-inflammatory Activity

Spotty necrosis (0–4) ☐
Confluent necrosis (0–6) ☐
Interface hepatitis (0–4) ☐
Portal inflammation (0–4) ☐

Total HAI score: …… /18 **Fibrosis (Ishak)**

Stage: …… /6

Bridging fibrosis: ☐ Present ☐ Absent

Cirrhosis: Probable ☐ Definite ☐

Dysplastic foci: ☐ Present ☐ Absent

Dysplastic nodule: ☐ Present ☐ Absent

Additional Findings

Kupffer cell pigment ☐ Specify ……

Cholestasis ☐

Steatohepatitis (MASH / NASH) ☐

Vascular lesions ☐

Portal tract abnormalities: ☐ Present ☐ Absent

Bile duct injury: ☐ Present ☐ Absent

IHC (if performed)

HBsAg ☐

HBcAg ☐

Glypican-3 ☐ (if tumour suspected)

Others ☐ Specify……

Pattern of injury:

Hepatocellular ☐

Cholestatic ☐

Mixed ☐

Diagnosis (Impression):

- Primary diagnosis
- Grade / stage (if applicable)
- Etiology (if known)

LIVER RESECTION FOR MALIGNANCY

Specimen

Right hepatectomy ☐

Left hepatectomy ☐

Segmentectomy ☐

Explant liver ☐

Other ☐ Specify ……

Tumour

Solitary ☐ Multiple ☐

Location ……

Size (largest): …… cm

Satellites present ☐ Absent ☐

Tumour necrosis: ☐ Present ☐ Absent

Histologic Type (WHO 2019/2022)

Hepatocellular carcinoma, NOS ☐

Fibrolamellar HCC ☐

Combined hepatocellular–cholangiocarcinoma ☐

Intrahepatic cholangiocarcinoma ☐

Mucinous cystic neoplasm with invasive carcinoma☐

Hepatoblastoma ☐

Other ☐ Specify ……

Grade (Edmondson–Steiner)

G1 ☐ G2 ☐ G3 ☐ G4 ☐

Vascular Invasion

Microvascular invasion ☐ Present ☐ Absent

Macrovascular invasion ☐ Present ☐ Absent

Background Liver

Cirrhosis ☐

Fibrosis (stage ……) ☐

Steatohepatitis (MASH/NASH) ☐

Chronic hepatitis (HBV/HCV/other) ☐

Iron overload ☐

Portal hypertension features ☐

Margins

Parenchymal margin: Clear ☐ Involved ☐ Distance …… mm

Bile duct margin (if applicable): Clear ☐ Involved ☐

Closest margin: _____ mm

Lymph Nodes

Examined ……

Positive ……

Pathologic Stage (AJCC / UICC)

pT ……

pN ……

pM ……

KEY TERMINOLOGY CHANGES

Old term	Current WHO usage
MRN	Large regenerative nodule
Borderline nodule	Dysplastic nodule
Adenomatous hyperplasia	Not preferred
NASH	MASH (preferred; NASH acceptable)
HAI	Acceptable (Ishak)

TNM STAGING (Current Standard: AJCC/UICC 8th Edition)

The latest standardized TNM staging systems widely used in pathology and clinical practice as of now are from the **8th edition AJCC/UICC**, implemented since 2018. These remain the basis for synoptic reporting and clinical staging today pending newer revisions.

1) Hepatocellular Carcinoma (HCC) TNM

T (Primary Tumour)

T1a: Solitary ≤2 cm (± vascular invasion)

T1b: Solitary >2 cm, no vascular invasion

T2: Solitary >2 cm with vascular invasion OR multiple nodules none >5 cm

T3: Multiple tumours, ≥ one >5 cm

T4: Tumour(s) involving major vascular branches or invading adjacent organs (except gallbladder) or visceral perforation

N (Nodes)

N0: No regional lymph node metastasis

N1: Regional lymph nodes involved

M (Metastasis)

M0: No distant metastasis

M1: Distant metastasis

TNM stage groups (I–IV) follow these T/N/M combinations.

2) Intrahepatic Cholangiocarcinoma (iCCA)

Intrahepatic cholangiocarcinoma (iCCA) has a **separate TNM staging system distinct from hepatocellular carcinoma (HCC)** in the AJCC 8th edition.

Key features:

T1 subdivided into:

T1a: Tumour ≤5 cm

T1b: Tumour >5 cm

Periductal invasion is no longer included in the T4 category

Tumour growth patterns:

Mass-forming

Periductal infiltrating

Intraductal

Regional lymph nodes and metastasis:

N0 / N1

M0 / M1

Note: Perihilar and distal cholangiocarcinomas are staged separately.

3) Gallbladder Carcinoma TNM

T (Primary Tumour)

Tis: Carcinoma in situ

T1a: Invades lamina propria

T1b: Invades muscular layer

T2a: Invades perimuscular connective tissue (peritoneal side)

T2b: Invades perimuscular connective tissue (hepatic side)

T3: Perforates serosa and/or invades liver or one adjacent organ

T4: Invasion of main portal vein/hepatic artery or ≥2 extrahepatic organs

N (Regional lymph nodes)

N0: No lymph node metastases

N1: Metastases to 1–3 regional nodes

N2: Metastases to ≥4 regional nodes

M

M0: No distant metastasis

M1: Distant metastasis

Stage Grouping

Stage 0: Tis; N0; M0

Stage I: T1; N0; M0

Stage II: T2; N0; M0

Stage III: T3 and/or N1; M0

Stage IV: T4 or N2 or M1

1 B GALL BLADDER- ANATOMY

Location & Gross Anatomy

Pear-shaped organ attached to the undersurface of the liver.

Divided into fundus, body, and neck; neck continues as the cystic duct.

Function: Storage and concentration of bile.

Wall Structure

Mucosa: Highly folded, forming branching and anastomosing folds.

Muscular layer: Thin, irregularly arranged smooth muscle bundles.

Perimuscular connective tissue: Contains vessels and nerves.

Serosa/Adventitia: Serosa on the peritoneal surface; adventitia on the hepatic surface.

Epithelium

Tall columnar epithelial cells with apical microvilli forming a brush border.

Specialized for active absorption of water and electrolytes.

Distinctive Histological Features

Absence of muscularis mucosae.

Absence of submucosa.

Rokitansky–Aschoff sinuses: Mucosal invaginations extending into the muscular wall; common and physiologic but exaggerated in chronic cholecystitis.

Lamina Propria

Composed of loose connective tissue with lymphocytes and plasma cells.

May show lymphoid aggregates in normal adults.

II. B. GALL BLADDER- GROSSING TECHNIQUES

Measure the length of the gall bladder.

Open longitudinally along the long axis and drain the bile.

Fix bile in alcohol; in suspected malignancy, bile may be centrifuged for smears and cell block preparation.

If gallstones are present, wash and record the number and size of the largest stone. Cut one or two stones to indicate type (see fig). Strain bile to identify minute stones or cholesterol polyps.

Measure the maximum wall thickness.

Search for lymph nodes at the gall bladder neck.

Examine Hartmann's pouch for impacted calculi.

Describe:

- Serosal surface (fibrinous exudate, adhesions)
- Wall thickness variability due to fibrosis or tumour
- Mucosa (colour, ulcers, haemorrhage, cholesterolosis, yellow flecks)
- Cystic duct (dilatation, stones)
- Lymph nodes at the neck
- Colour and consistency of bile
- Stones: number, shape, size, colour, and cut surface

If a tumour is present, describe growth pattern (papillary, ulcerative, infiltrative), measure distance from the cystic duct margin, and assess for multicentricity.

Sampling (Fig 10.8)

Benign conditions:

A: Two sections from neck and Hartmann's pouch (include lymph node if present).

B: Three sections from body and fundus.

Carcinoma:

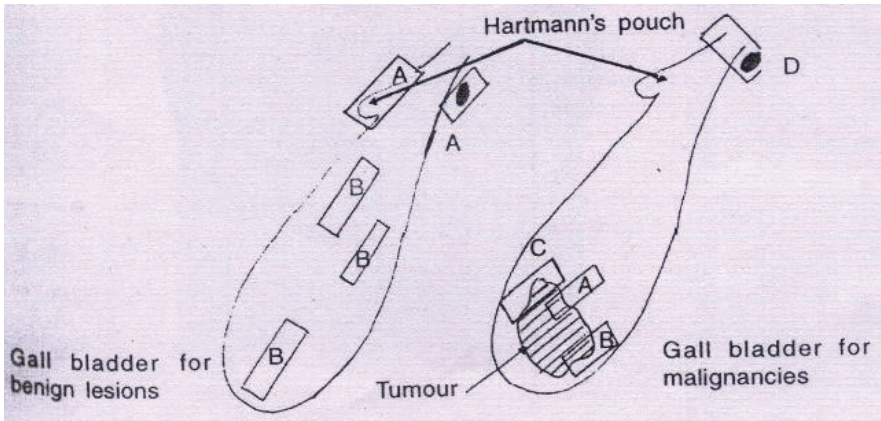


Fig 10.8: Sampling of gall bladder

A–C: Two to three tumour sections, including at least one full-thickness section up to serosa.

For carcinoma in situ, sample wider mucosal areas using two to three Swiss rolls.

D: Cystic duct surgical margin and lymph nodes at neck.

III B. GROSS PEARLS – GALL BLADDER

1) Gall bladder appears as a fibrous cord

Seen in Biliary atresia

Associated with obliteration or severe hypoplasia of extrahepatic bile ducts

Gall bladder may be absent or replaced by a fibrous remnant

2) Small gall bladder

Length < 2–3 cm

Seen in:

- Idiopathic neonatal hepatitis
- α_1-antitrypsin deficiency
- Cystic fibrosis

Reflects chronic cholestatic injury or ductal abnormality

3) Gall bladder diverticula

Can occur anywhere along the gall bladder wall

Usually acquired

Associated with chronic cholecystitis and increased intraluminal pressure

Must be distinguished from Rokitansky–Aschoff sinuses (intramural mucosal herniations)

4) Strawberry gall bladder

Seen in Cholesterolosis

Gross appearance:

- Multiple yellow to white punctate or streak-like deposits on the mucosal surface
- Background mucosa appears reddened or congested

May present as:

- Diffuse cholesterolosis
- A localized cholesterol polyp

5) Yellow, grumous, pus-like material in the lumen

Seen in Acute calculous cholecystitis, often with impaction of a stone in the cystic duct

Represents an emulsion of cholesterol crystals and calcium salts

May resemble pus but is chemically derived bile sludge

6) Thickened gall bladder wall

Wall thickening should be assessed for **pattern (uniform vs focal)**.

i) Chronic cholecystitis

Uniform, diffuse wall thickening

Wall appears fibrotic and firm

Often associated with mucosal ulceration and gallstones

ii) Adenomyomatous hyperplasia *(WHO preferred term)*

Focal or segmental wall thickening

Associated with prominent Rokitansky–Aschoff sinuses

Benign, non-neoplastic lesion

iii) Gall bladder carcinoma

Focal, asymmetric wall thickening, especially if irregular

May present as:

- Wall thickening
- Intraluminal mass

Often associated with cholelithiasis and porcelain gall bladder

7). Gall Stones (Fig 10.9)

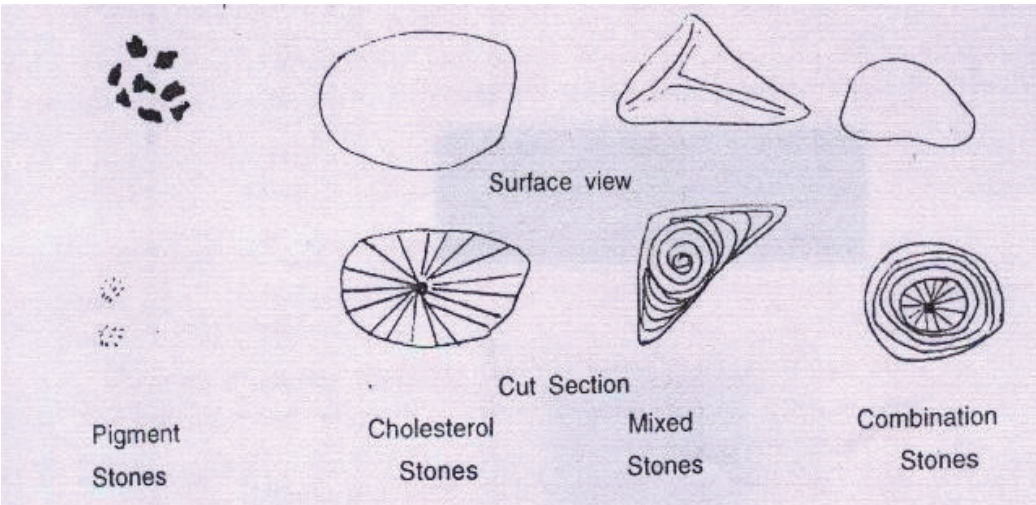


Fig 10.9 : Various gall stones -whole and cut section

Nature of Gall Stones

	Type of stone	Colour/Shape	Nature	Number/Size
1A. Pigment Stones -		Dark brown,	Friable,	Variable
	Calcium bilirubinate	concentric,	Crumbles at	
	stones (seen	dark and light	Pressure	
	in infections	areas on cut		
	and stasis)	section,		
		Irregular in shape		
1 B. Pure pigment		Black, shiny	Difficult	Many;
	stones (seen in	Irregular	to crush	3-5mm in
	Haemolytic			Diameter
	anaer:nias etc.)			
2.	Pure cholestrol stones	Pale, oval	Smooth	Single
		in shape;	surface;	2-5cm in
		cut surface has	cuts with	Diameter
		a radially	Difficulty	

			arranged structure;		
			may have a		
			pigmented nucleus		
3.	Mixed Stones		Greenish black;	Smooth	Variable
	(commonest,		variable in shape;	Surface	
	made up of		some are		
	cholesterol,		multifaceted with		
	bile pigments,		angular edges;		
	and calcium		Concentric		
	Carbonate & bilirubinate)		laminations on cut		
			Section		
4.	Combination stone(stone of one type surrounded by a shell of another type)		Depends on its constituent outer shelleg. cholesterol stone may be surrounded by a mixed stone	Variable	Single (barrel stone) may be associated with mixed stone

B IV GALL BLADDER MICROSCOPY

PRIMARY BILIARY CHOLANGITIS (PBC)

(formerly "primary biliary cirrhosis")

Epidemiology: Female predominance (~10:1)

Serology: Anti-mitochondrial antibodies (AMA) > 90% of cases

Clinical associations**:** Xanthelasmas, hypercholesterolemia, other autoimmune disorders (Sjögren's, scleroderma, thyroiditis)

Histology

Target ducts: Interlobular bile ducts (40–80 μm)

Early lesions: Focal non-suppurative destructive cholangitis

Epithelial swelling, stratification, eosinophilic condensation

Lymphoplasmacytic infiltrate; sometimes granulomas

Rupture of bile duct basement membrane

Progression:

Portal and periportal fibrosis → septal fibrosis → cirrhosis

Ductopenia develops

Regenerative hepatocytes may narrow portal veins → portal hypertension before overt fibrosis

Fibrosis pattern**:** May be micronodular (cholestatic) or macronodular (hepatitic features)

Staging (Ludwig et al., 1978):

Portal fibrosis

Periportal fibrosis

Septal fibrosis

Cirrhosis

OVERLAP SYNDROME

Features of AIH + PBC

AMA may be negative; ANA titres elevated

Histology shows interface hepatitis + duct injury

PRIMARY SCLEROSING CHOLANGITIS (PSC)

Epidemiology: Male predominance (~75%), often associated with IBD (ulcerative colitis, 70%)

Pathogenesis: Immune-mediated periductal inflammation; cytokine-mediated damage

Cholangiography: Characteristic “beading” of intrahepatic ducts

Histology

Fibrous obliterative cholangitis: “Onion-skin” periductal fibrosis

Epithelial degeneration and atrophy → disappearance of ducts

Duct loss: Small and medium portal tracts

PAS stain: Thickened, wrinkled basement membranes

Parenchymal changes: Nodular regenerative hyperplasia; cirrhosis occurs later

Neoplasia risk: Strong association with cholangiocarcinoma

VANISHING BILE DUCT DISEASE (VBDS)

Definition: Progressive inflammatory destruction of intrahepatic bile ducts → ductopenia

Diagnosis: Needle/wedge biopsy of ≥ 5 portal tracts

"Widow's artery" = portal tract without bile duct

Immunohistochemistry: CK7 or TPA to outline duct epithelium

Paediatric manifestations

Paucity of interlobular bile ducts (PILBD)

Ratio < 0.5 ducts/portal tract (normal 0.9–1.8)

Syndromic: Alagille syndrome (facial, vertebral, cardiac, renal anomalies)

Non-syndromic: Cystic fibrosis, α1-antitrypsin deficiency, isolated or viral

Extrahepatic biliary atresia (EHBA)

Pan-biliary destruction; prognosis depends on duct size at porta hepatis (>400 μm = good)

Histology similar across causes

Diagnosis confirmed with radiology; Kasai operation may be corrective

Adult examples of VBDS: PBC, PSC

CHOLESTASIS

Cholestasis = impaired bile formation or bile flow → retention of bile components.

Levels of cholestasis:

Intrahepatic: canalicular (hepatocytes, canals of Hering) or ductular damage

Causes: massive hepatocyte necrosis (sepsis, shock), bile duct injury (PBC, PSC, PFIC)

Extrahepatic: obstruction of large intrahepatic or extrahepatic ducts

Causes: EHBA, neoplasms, choledochal cysts

Obstruction:

Complete: retention of bile salts + bilirubin

Incomplete**:** retention of bile salts only

Mixed etiologies can occur (e.g., PSC + extrahepatic obstruction)

Histologic features

Bile pigment: hepatocytes, canaliculi, Kupffer cells

Bile plugs / bile lakes

Granules or clumps in hepatocytes or Kupffer cells

Localization:

Centrilobular cholestasis: acute hepatitis, drug-induced injury, large duct obstruction

Periportal cholestasis: chronic liver disease, recurrent obstruction, late-stage PBC

Acute complete cholestasis

Bilirubin in hepatocytes and canaliculi

Hepatocyte necrosis

Kupffer cell activation

Portal oedema with incipient ductular reaction

Chronic complete / incomplete cholestasis

Feathery degeneration (hydropic swelling with bile retention)

Cell rosettes with CK7-positive periportal hepatocytes (hepatocyte → bile duct phenotype)

Mallory–Denk bodies may be present

Xanthomatous change: lipid-laden histiocytes

Bile infarcts: necrotic foci around bile lakes (Charcot–Gombault infarcts)

Portal changes**:** ductular reaction, neutrophilic infiltrate, periductular fibrosis → biliary cirrhosis

NEONATAL CHOLESTASIS

Definition: Cholestasis within the first 3 months of life.

Causes

Extrahepatic biliary atresia (EHBA):

Pan-biliary obliteration (porta hepatis → duodenum)

Histology: giant cells less extensive than neonatal hepatitis, portal ductular proliferation, fibrosis may be minimal initially

Surgical correction**:** Kasai portoenterostomy if early (<10 weeks)

Neonatal hepatitis syndrome:

Viral (CMV, HBV, HSV, coxsackie), bacterial, parasitic

Metabolic / genetic: α1-antitrypsin deficiency, galactosemia, Niemann–Pick, Gaucher

Toxins / drugs / parenteral nutrition

Histology: lobular disarray, giant cell transformation, extramedullary hematopoiesis, ductular proliferation

Clinical correlation essential; histology alone cannot always distinguish EHBA from non-obstructive causes

GENETIC / METABOLIC CHOLESTASIS

α1-Antitrypsin deficiency:

Childhood cholestasis

Genotypes: PiZZ (severe, early), PiZM (intermediate), PiMM (normal)

Histology: hepatocellular PAS-D positive, diastase-resistant globules

Progression: micronodular → macronodular cirrhosis

Cystic fibrosis:

Neonatal cholestasis / steatosis

Histology: macrovesicular steatosis, ductular proliferation, focal portal fibrosis, inspissated PAS+ bile in ducts

Cirrhosis may develop late

Cholangitis lenta:

Dilated ducts with flattened or necrotic epithelium, inspissated bile

Seen in **septicemia**, poor prognosis

HEREDITARY HYPERBILIRUBINEMIAS

Crigler–Najjar syndrome

Type I: absent UGT1A1 → unconjugated hyperbilirubinemia

Type II: reduced UGT1A1 activity

Gilbert syndrome

Mild unconjugated hyperbilirubinemia

Normal liver histology; sometimes mild parenchymal iron

Dubin–Johnson syndrome

Conjugated hyperbilirubinemia

Hepatocytes dark brown / coarse pigment (pericanalicular), PAS + / acid-fast +

Compatible with normal life

Rotor syndrome

Conjugated hyperbilirubinemia

No pigment accumulation in hepatocytes

FAMILIAL INTRAHEPATIC CHOLESTASIS

Benign Recurrent Intrahepatic Cholestasis (BRIC):

Autosomal recessive

Intermittent cholestasis, hepatocytes show bland bile stasis

Progressive Familial Intrahepatic Cholestasis (PFIC):

PFIC 1, 2, 3 (BSEP / MDR3 / TJP2 mutations)

Histology: canalicular cholestasis, giant cell transformation, ductular proliferation

Impaired bile salt export, progressive fibrosis → cirrhosis

PFIC : Progressive familial intrahepatic cholestasis (PFIC) comprises autosomal recessive disorders caused by defects in canalicular bile transport proteins. PFIC-1 and PFIC-2 are characterized by low-GGT cholestasis, whereas PFIC-3 shows high-GGT cholestasis with prominent bile duct injury.

PFIC-1 (Byler disease)

Oldest described form

Due to FIC1 (flippase) defect → bile acids accumulate in hepatocytes

Low or normal GGT (important exam point)

Microscopy

Canalicular cholestasis

Giant cell transformation

Minimal duct injury initially

Extrahepatic features

Diarrhea

Pancreatitis

Growth failure

PFIC-2

Caused by defect in BSEP (bile salt export pump)

Leads to severe bile acid retention

Low or normal GGT

Microscopy

Severe lobular cholestasis

Hepatocyte injury and apoptosis

Rapid progression to cirrhosis

Note : Has Increased risk of **hepatocellular carcinoma and cholangiocarcinoma in childhood**

PFIC-3

Caused by MDR3 deficiency

MDR3 normally secretes phospholipids into bile → protects bile ducts from bile acids

High GGT (this differentiates PFIC-3 from PFIC-1 & 2)

Microscopy

Portal inflammation

Bile duct injury and ductular proliferation

Early portal fibrosis → biliary cirrhosis

Differential Diagnosis of Hyperbilirubinemia

Type	Mechanism / Category	Examples / Notes
Unconjugated (Indirect)	**Increased bilirubin production**	Hemolysis: intrinsic (membrane defects, enzyme defects, hemoglobinopathies), extrinsic (immune/non-immune) Infections: sepsis, TORCH Extravasation: bruising, cephalohematoma, delayed cord clamping, twin-twin transfusion Polycythemia Enterohepatic circulation: delayed feeding, dehydration, GI obstruction
	Decreased conjugation	Genetic / enzyme defects: Crigler–Najjar syndrome (Type I & II), Gilbert syndrome, Lucy–Driscoll syndrome Physiologic neonatal jaundice Breast milk jaundice
Conjugated (Direct)	**Intrahepatic cholestasis –**	Neonatal / inherited: Alagille syndrome (arteriohepatic dysplasia), BRIC (benign recurrent

	Persistent / Acquired / Genetic	intrahepatic cholestasis), Byler disease, PFIC, α1-antitrypsin deficiency, cystic fibrosis, Dubin–Johnson, Rotor, familial hepatosteatosis, TPN-induced Infection: CMV, HSV, rubella, hepatitis B (neonatal), toxoplasmosis, echovirus, leptospirosis, varicella Drug-induced
	Extrahepatic obstruction	Neonatal / infant: extrahepatic biliary atresia, bile plug syndrome, choledochal cyst, choledocholithiasis, extrinsic bile duct compression, spontaneous bile duct perforation
	Metabolic disorders	Carbohydrate metabolism: hereditary fructose intolerance, galactosemia, glycogen storage diseases (Types I, III, IV, VI, IX) Amino acid metabolism: tyrosinemia Lipid metabolism: Gaucher disease, Niemann–Pick, Wolman disease, cholesteryl ester storage disease

Recent Concepts :

- Prefer **"neonatal cholestasis syndrome"** over older terms like giant cell hepatitis.
- PFIC subtypes (1,2,3) are now recognized molecularly and have distinct histologic features.
- EHBA vs neonatal hepatitis: biopsy alone may not distinguish — clinical/radiologic correlation essential.
- Genetic / metabolic cholestasis: α1-antitrypsin, cystic fibrosis, and familial hepatosteatoses are increasingly diagnosed by genotyping.

B V GALL BLADDER SYNOPTIC REPORTING

Reporting for cholecystectomy

Cholecystectomy ☐

Other ☐ Specify ……

Measurements

Length × Width × Thickness: …… × …… × …… cm

Gross Examination

Gall bladder

Distended ☐ Contracted ☐

Opened previously: Yes ☐ No ☐

Mucosa

Unremarkable ☐

Ulcerated ☐

Congested ☐

Cholesterolosis (strawberry gall bladder) ☐

Cholesterol polyp (non-neoplastic polypoid lesion) ☐

Porcelain gall bladder ☐

Other ☐ Specify ……

Wall

Normal thickness ☐

Uniformly thickened ☐

Focally thickened (suspicious) ☐ Specify site ……

Maximum wall thickness: _____ mm

Serosa

Normal ☐

Congested ☐

Serosal involvement by tumour ☐

Lymph nodes

Number identified: ___

Not identified ☐

Gallstones:

Present ☐ Absent ☐ Type: _____

Microscopy

Acute cholecystitis ☐

Chronic cholecystitis ☐

Xanthogranulomatous cholecystitis ☐

Cholesterolosis ☐

Non-neoplastic polypoid lesion ☐

Dysplasia (low / high grade) ☐

Adenocarcinoma: ☐ In situ ☐ Invasive

Depth of invasion:

☐ Lamina propria

☐ Muscular layer

☐ Perimuscular connective tissue

☐ Serosa / liver

Lymph nodes

Not involved ☐

Reactive ☐

Metastasis present ☐

Number examined …… / Positive ……

Perineural invasion

Present ☐ Absent☐

Lymphovascular invasion

Present ☐ Absent☐

Cystic duct margin (if applicable)

Free ☐ Involved ☐

Pathological staging based on above findings: pT: ….. ;pN:…..;pM:……

Signature ……. Date ……

Note: **Adenomatous hyperplasia is now regarded as a microscopic diagnosis**; gross focal thickening raises suspicion for carcinoma.

ASSESS YOURSELF LIVER & GALL BLADDER

Essay type questions (10 marks each)

1. Discuss the etiologic agents involved in acute hepatitis.
2. Describe grossing techniques for a hepatocellular carcinoma Resection
3. What is MASH ? Describe the liver changes in a needle biopsy in MASH.

Short essays (5% marks each)

4. Briefly explain the concept of the "Liver Acinus" (Rappaport's zones) and why Zone 3 is most susceptible to ischemic injury.
5. Classify liver abscesses based on etiology. Discuss the clinical presentation, radiological features, and the current management protocols for Amoebic vs. Pyogenic liver abscesses.
6. What is the MELD Score for liver transplant ?

Short answer questions (3 marks each)

7. Describe the etiopathogenesis, classic "beaded" appearance on imaging, and the long-term risk of cholangiocarcinoma in patients with PSC.
8. Briefly discuss Hepatic Hemangiomas and Focal Nodular Hyperplasia (FNH). Mention the characteristic "central scar" and it's significance on imaging.
9. Dysplastic nodules in the liver.

MCQs

Which of the following is the primary stimulus for the contraction of the gallbladder?

A) Secretin
B) Gastrin
C) Cholecystokinin (CCK)
D) Somatostatin

In a patient with complete biliary obstruction, which of the following findings is most likely?

A) Dark-colored stools and tea-colored urine
B) Pale (clay-colored) stools and tea-colored urine
C) Pale stools and colorless urine
D) Increased levels of urobilinogen in the urine

Which vitamin's absorption is most significantly impaired in chronic cholestatic liver disease?

A) Vitamin B12
B) Vitamin C
C) Vitamin K
D) Vitamin B6

Answer Key for MCQs

1. C
2. B
3. C

Chapter 11
Thyroid And Parathyroid Glands

Chapter outline: A – Thyroid Gland ; B- Parathyroid Gland

A 1 Thyroid Gland Anatomy ; A II -Thyroid Gland Grossing Techniques ; III A – Thyroid Gland Gross pearls; A IV Thyroid Gland Microscopy ; A V Thyroid Gland Synoptic reporting.

B I Parathyroid Gland Anatomy ; B II Parathyroid Gland Grossing Techniques; B III Parathyroid Gross Pearls ; B IV Parathyroid Microscopy ; B V- Parathyroid Synoptic reporting

A I THYROID GLAND ANATOMY

Thyroid Gland

The thyroid gland is a bilobed endocrine organ located in the anterior neck, anterolateral to the upper trachea and lower larynx, with the two lateral lobes connected by a thin isthmus usually overlying the second to fourth tracheal rings.

Each lobe is enclosed by a thin fibrous capsule and consists of numerous follicles lined By cuboidal to low columnar follicular epithelial cells surrounding colloid. Parafollicular (C) cells are scattered within the interfollicular spaces and secrete calcitonin. The gland is richly vascular, supplied mainly by the superior and inferior thyroid arteries.

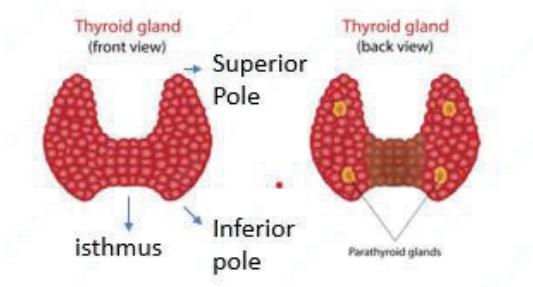


Fig 11.1: to show normal anatomy of the thyroid and parathyroid gland

II A — THYROID GLAND GROSSING TECHNIQUES

Types of Thyroid Specimens Received in the lab :

Lobectomy

Excision biopsy of a nodule

Isthmectomy

Subtotal thyroidectomy

(~8 g or approximately thumb-sized thyroid tissue left behind on each side)

Total thyroidectomy

Common Steps for ALL Thyroid Specimens

1. Type of Specimen

Clearly mention the type of specimen received as listed above.

2. Measurements

Record:

Weight (in grams)

Size in three dimensions (cm)

3. Orientation of the Specimen

Identify:

Anterior surface – *convex*

Posterior surface – *concave*

Superior pole is identified by a suture ligating the superior pedicle vessels.

Search for parathyroid glands:

Inferior parathyroids are more consistently found on the posterior aspect of the lower poles.

4. External Description

Describe:

Shape

Colour

Consistency

Surface – smooth / nodular

5. Sectioning of the Specimen

Bisect the specimen by passing the first cut through the greatest dimension.

Make subsequent parallel slices at 5 mm intervals.

Describe the **cut surface**:

Solid / cystic

Colour and texture

If **nodular**, describe:

Number of nodules

Size of smallest and largest nodule

Encapsulation

Solid or cystic nature

Areas of necrosis, haemorrhage, calcification

Invasion into adjacent thyroid tissue

Distance from the line of resection

Comment on adjacent thyroid parenchyma

6. Solitary Nodule

Examine the capsule carefully for evidence of capsular or vascular invasion.

SAMPLING (BITS) (Fig 11.2)

A. Inflammatory Conditions with Diffuse Enlargement

A, B, C, D: 4 bits – *2 from each lobe*

E: 1 bit – *from the isthmus*

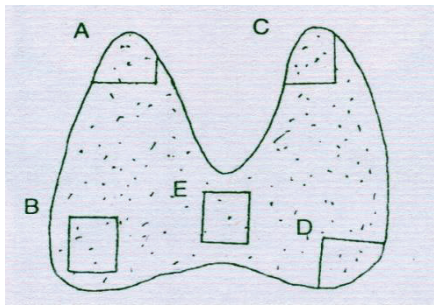


Fig 11.2 : Bits fron thyroid gland with diffuse enlargement

B. Thyroid follicular nodular disease (Multinodular Goitre)(Fig 11.3)

A, B, C, D, E:

Representative samples from at least 5 nodules

Include nodule rim and adjacent thyroid

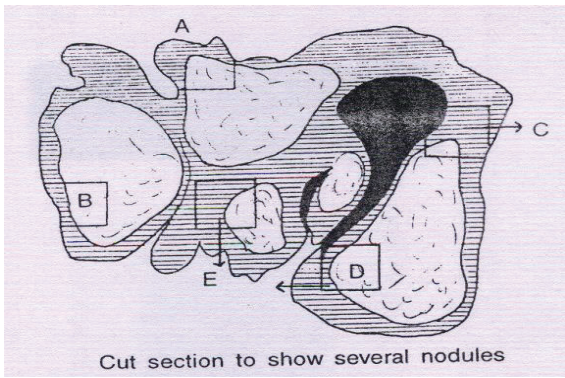


Fig 11.3 : Nodular thyroid gland with bits

If nodules are very large, sample adequately; may be 2 bits from same nodule.

C. Solitary Encapsulated Lesions (Fig 11.4)

(Solitary adenomatous nodule / follicular adenoma / follicular carcinoma)

≤ 5 cm:

Sample the entire circumference

Each bit must include capsule + adjacent thyroid

> 5 cm:

Entire circumferential sampling plus

One additional bit for every 1 cm increase in size

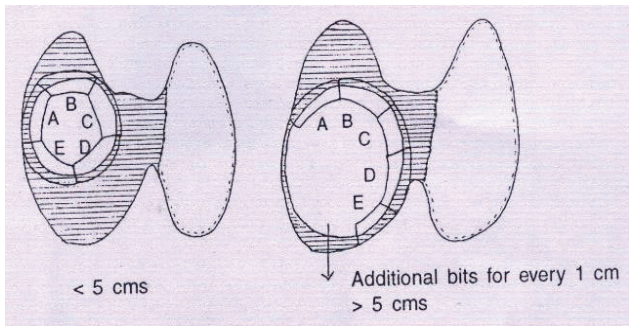


Fig 11.4 : Circumferential Capsular Sampling

D. Frank Carcinomas (Fig 11.5)

(Papillary, Follicular, Anaplastic, etc.)

A, B, C: 3 bits from the tumour

D: 1 bit from non-neoplastic thyroid

E, F: Line of resection (one or more blocks)

G: Extrathyroidal tissue, if present

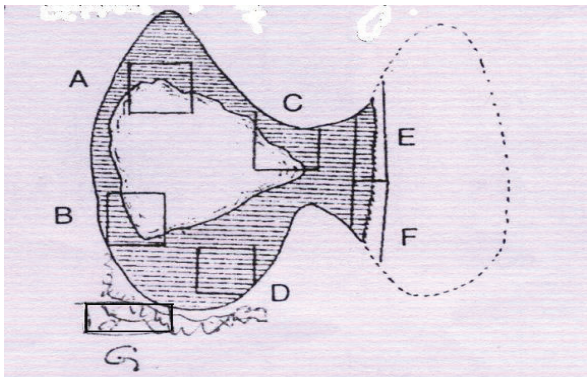


Fig 11.5 : Carcinoma Sampling

[A] [B] [C] → Tumour

[D] → Normal thyroid

[E][F] → Resection margin

[G] → Extrathyroidal tissue

In thyroid pathology, particularly carcinomas, the diagnosis often lies not in the nodule itself, but in the capsule, margins, and the so-called "normal" thyroid.

Associated lymph nodes:

Dissect and gross separately *(see lymph node grossing section)*

Papillary carcinoma: (Fig 11.6 A & B)

Sample extensively due to multicentricity

Always include line of resection

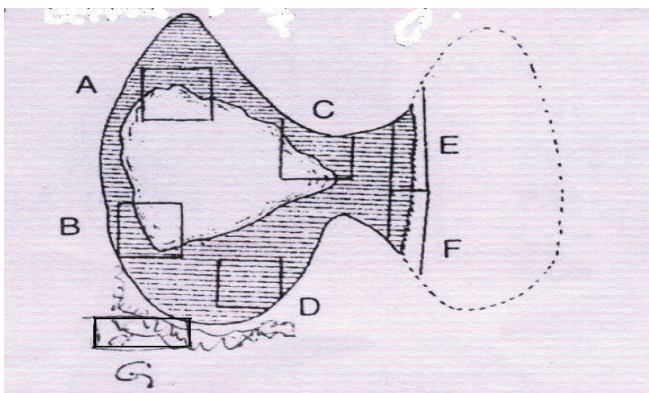


Fig 11.6 A Extensive sampling in papillary carcinoma

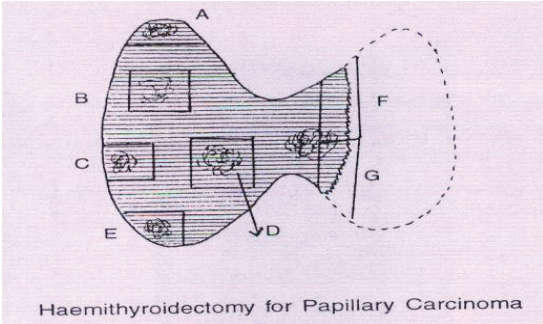


Fig 11.6B : Entensive sampling

Medullary carcinoma: (Fig 11.7)

Look for parafollicular (C-cell) hyperplasia in normal parenchyma

Suggests a genetic basis (MEN) if present

Parafollicular cells are concentrated:

Along a line perpendicular to the long axis of each lobe

Mainly in the upper and middle thirds

Submit parathyroid tissue separately if identified grossly

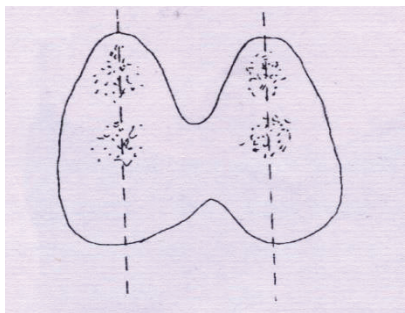


Fig 11.7 : Line of concentration of parafollicular cells

Distribution of Parafollicular Cells: Line of c-cell concentration ; along upper and middle thirds

E. Lymph Nodes

No identified ……No grossly involved …….

THYROID GROSSING – CHECKLIST

1. IDENTIFICATION

☐ Name / Age / Sex

☐ Hospital No. / Accession No.

☐ Clinical diagnosis / Indication for surgery

2. TYPE OF SPECIMEN (Tick one)

☐ Lobectomy (Right / Left)
☐ Excision biopsy – nodule
☐ Isthmectomy
☐ Subtotal thyroidectomy
☐ Total thyroidectomy

3. MEASUREMENTS

☐ Weight: _____ g
☐ Size (cm): _____ × _____ × _____

4. ORIENTATION & ANATOMY

☐ Anterior surface identified (convex)
☐ Posterior surface identified (concave)
☐ Superior pole identified (suture present ☐ Yes ☐ No)
☐ Parathyroid searched for ☐ Yes ☐ No
☐ Parathyroid identified ☐ Yes ☐ No → submit separately if identified.

5. EXTERNAL APPEARANCE

☐ Shape: _____
☐ Colour: _____
☐ Consistency: ☐ Soft ☐ Firm ☐ Hard
☐ Surface: ☐ Smooth ☐ Nodular

6. CUTTING & CUT SURFACE

☐ First cut through greatest dimension
☐ Serial sections at 5 mm intervals

Cut surface: ☐ Solid ☐ Cystic ☐ Solid–cystic
☐ Colour: _____

7. NODULES (If present)

☐ Number of nodules: _____
☐ Smallest nodule: _____ cm
☐ Largest nodule: _____ cm

For dominant / solitary nodule: ☐ Encapsulated ☐ Non-encapsulated
☐ Capsule examined for invasion ☐ Yes ☐ No

☐ Haemorrhage ☐ Necrosis ☐ Calcification ☐
☐ Invasion into adjacent thyroid ☐ Yes ☐ No
☐ Distance from margin: _____ cm

8. ADJACENT THYROID PARENCHYMA

☐ Normal appearing
☐ Diffuse enlargement / thyroiditis
☐ Nodular

9. SAMPLING (BITS) – TICK AS APPLICABLE

A. Diffuse / Inflammatory Conditions

☐ A,B,C,D – 2 bits from each lobe
☐ E – Isthmus

B. Thyroid follicular nodular disease (Multinodular Goitre)

☐ ≥ 5 nodules sampled
☐ Rim + adjacent thyroid included

C. Solitary Encapsulated Lesion

☐ Entire circumference sampled (≤ 5 cm)
☐ Additional 1 bit / cm if > 5 cm

D. Frank Carcinoma

☐ A,B,C – Tumour
☐ D – Non-neoplastic thyroid
☐ E,F – Resection margin
☐ G – Extrathyroidal tissue (if present)

10. LYMPH NODES

☐ Received ☐ Not received
☐ Number dissected: _____
☐ Gross appearance recorded

Central compartment nodes may be small and embedded in fat → search carefully.

11. SPECIAL NOTES (Tick if applicable)

☐ Papillary carcinoma – extensive sampling done

☐ Margins adequately sampled

☐ Medullary carcinoma suspected

☐ Looked for C-cell hyperplasia (upper & middle thirds)

12. FINAL CHECK BEFORE SUBMISSION

☐ Margins submitted

☐ Parathyroid submitted if identified

☐ All blocks labelled correctly

Grossed by: _____ Date: _____

III A THYROID GLAND -GROSS PEARLS

Diffusely enlarged thyroid gland, smooth external surface;

If C/S is brown with minute colloid filled spaces then it is diffuse colloid goiter;

if C/S is brown and homogenous (cut chiko appearance) then it is graves Disease.

Diffusely enlarged thyroid gland, smooth external surface; firm in consistency; C/S lobulated and meaty : Hashimoto's thyroiditis

Enlarged thyroid gland with nodular surface; C/S multiple nodules of varying sizes filled with colloid – thyroid follicular nodular disease or multinodular goiter.

Enlarged glands, nodular surface; C/S multiple nodules of varying sizes filled with colloid; fibrosis & haemorrhage : multinodular goiter or thyroid follicular nodular disease with secondary changes.

Single nodule :

C/S showing **colloid filled cyst** : Colloid cyst

C/S **colloid filled spaces , may or may not be encapsulated:** Dominant nodule of a Goiter

C/S **lobulated meaty appearance** : Nodular Hashimoto's thyroiditis.

C/S **fleshy encapsulated nodule** : Follicular neoplasm, adenomatous nodule of a goiter. (If obvious capsular invasion is seen at gross then it is most likely follicular carcinoma; has to be confirmed at microscopy).

Enlarged gland ; C/S white granular, single or multiple focus, with or without cystic change : papillary carcinoma

Cyst in gland : Cystic change in goiter

Cystic change in adenoma

Cystic papillary carcinoma

Enlarged gland C/S firm white with infiltration into surrounding tissue/capsule : Widely infiltrating follicular carcinoma, undifferentiated carcinoma

A IV THYROID GLAND MICROSCOPY

NON-NEOPLASTIC -GRAVES DISEASE (Diffuse Toxic Goitre)

Gross

Diffuse, smooth, symmetric enlargement involving both lobes and isthmus

Gland is soft and markedly vascular (bruit may be audible)

Pressure symptoms are uncommon

Pathogenesis

Autoimmune disorder mediated by thyroid-stimulating immunoglobulins (TSI) acting on TSH receptors

Associated ophthalmopathy due to accumulation of glycosaminoglycans in orbital tissues

Microscopy

Follicles are distended and hyperplastic

Lining epithelium is tall columnar with frayed (scalloped) luminal borders

Colloid shows scalloping with artefactual retraction

Papillary infoldings into the follicular lumen may be prominent

Interstitium shows increased vascularity

Exam Pearl

Tall columnar epithelium + scalloped colloid = Graves disease

THYROIDITIS – CLASSIFICATION

Chronic inflammatory disorders of the thyroid, autoimmune in several forms.

Hashimoto's thyroiditis

Subacute granulomatous (de Quervain's) thyroiditis

Subacute lymphocytic (painless) thyroiditis

Riedel's thyroiditis

HASHIMOTO'S THYROIDITIS (Chronic Lymphocytic Thyroiditis)

Clinical

Autoimmune disorder

Presents as diffuse firm enlargement or single nodular enlargement

Diagnosis often made on FNAC, supported by antibodies to:

- Thyroglobulin
- Thyroid peroxidase / membrane antigens

May be associated with other autoimmune disorders:

- Autoimmune gastritis
- Addison's disease

Microscopy

Dense lymphoid infiltrate with well-formed germinal centres (Fig 11.8)

Hürthle cell (Askanazy / oxyphil cell) metaplasia of follicular epithelium

Associated plasma cell infiltrate

Progressive follicular destruction

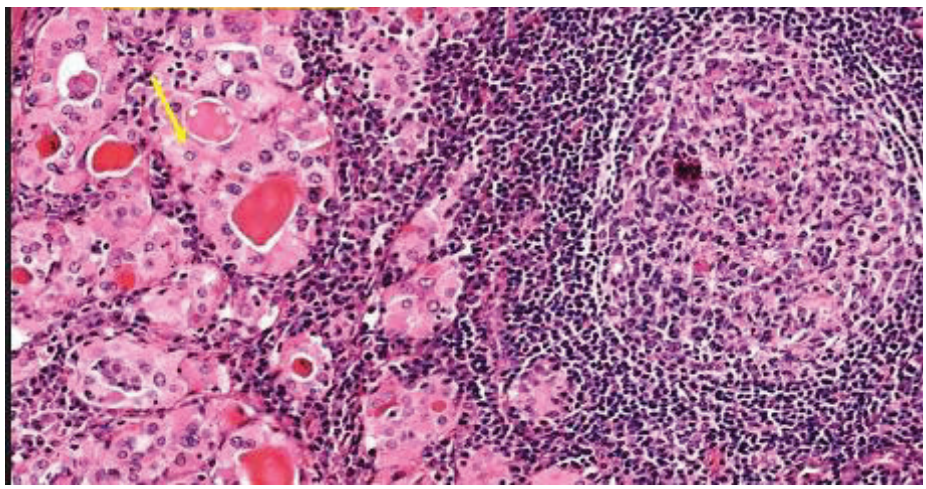


Fig 11.8 : H & E x400 showing Hashimoto's thyroiditis with a lymph follicle showing well formed germinal center. Thyroid follicles show hurthle cell change

Note

Chronic lymphocytic thyroiditis is considered an early stage of Hashimoto's thyroiditis

Hashimoto's thyroiditis is a risk factor for primary thyroid lymphoma

SUBACUTE GRANULOMATOUS THYROIDITIS (de Quervain's Thyroiditis)

Clinical

Female predominance, young adults

Often preceded by viral infection (mumps, influenza, etc.)

Presents with:

- Fever
- Painful thyroid enlargement
- Transient hyperthyroidism

Self-limited; recovery in 4–5 months

Microscopy

Early phase: follicular disruption with inflammatory infiltrate

Later phase:

- Collections of macrophages
- Multinucleated giant cells
- Formation of granulomas

Note : *Painful thyroid + granulomas = favours de Quervain's thyroiditis*

SUBACUTE LYMPHOCYTIC (PAINLESS) THYROIDITIS

Clinical

Painless thyroid enlargement

Transient hyperthyroidism

Often postpartum

Resolves spontaneously

Microscopy

Patchy lymphocytic infiltration

No granuloma formation

Minimal follicular destruction

Key Difference

Similar to de Quervain's **but without pain and granulomas**

RIEDEL'S THYROIDITIS (WOODY THYROIDITIS)

Clinical

Rare chronic inflammatory disorder of unknown etiology

Middle-aged to elderly, female predominance

Thyroid replaced by dense fibrosis extending beyond the gland

Mimics carcinoma clinically:

- Hard fixed gland
- Stridor
- Dysphagia
- Recurrent laryngeal nerve palsy
- Dyspnoea

Microscopy

Dense hyalinised fibrosis

Scattered lymphocytic infiltrate

Destruction of normal thyroid architecture

Note

Part of the IgG4-related disease spectrum (modern concept)

Congenital - THYROGLOSSAL DUCT CYST

Clinical

Most common clinically significant congenital neck anomaly

Midline neck swelling

Moves with deglutition and tongue protrusion

Microscopy

Lining epithelium:

- Stratified squamous epithelium (high neck)

Thyroid follicular epithelium (low neck)

Cyst contains mucus

Thyroid follicles present in the fibrous wall

Tumours of the thyroid are now derived depending on -

Cell of origin

Biological behaviour

Molecular and clinical risk

WHO 5th edition shifts thyroid pathology from pure morphology to biologic risk stratification, introducing low-risk categories (NIFTP), distinct oncocytic tumours, and a new differentiated high-grade carcinoma.

WHO (2017) - Thyroid gland

Tumours of the thyroid gland

Follicular adenoma

Hyalinizing trabecular tumour

Other encapsulated follicular patterned thyroid tumours

Follicular tumours of uncertain malignant potential

Well differentiated tumour of uncertain malignant potential

Noninvasive follicular thyroid neoplasm with papillary-like nuclear features

Papillary thyroid carcinoma

Papillary carcinoma

Follicular variant of PTC

Encapsulated variant of PTC

Papillary microcarcinoma

Columnar cell variant of PTC

Oncocytic variant of PTC

Follicular thyroid carcinoma (FTC), NOS

FTC, minimally invasive

FTC, encapsulated angioinvasive

FTC, widely invasive

- Hürthle (oncocytic) cell tumours
    - Hürthle cell adenoma
    - Hürthle cell carcinoma
- Poorly differentiated thyroid carcinoma
- Anaplastic thyroid carcinoma
- Squamous cell carcinoma
- Medullary thyroid carcinoma
- Mixed medullary and follicular thyroid carcinoma
- Mucoepidermoid carcinoma
- Sclerosing mucoepidermoid carcinoma with eosinophilia
- Mucinous carcinoma
- Ectopic thymoma
- Spindle epithelial tumour with thymus-like differentiation
- Intrathyroid thymic carcinoma
- Paraganglioma and mesenchymal / stromal tumours
    - Paraganglioma
    - Peripheral nerve sheath tumours (PNSTs)
        - Schwannoma
        - Malignant PNST
    - Benign vascular tumours
        - Haemangioma
        - Cavernous haemangioma
        - Lymphangioma
    - Angiosarcoma
    - Smooth muscle tumours
        - Leiomyoma
        - Leiomyosarcoma
    - Solitary fibrous tumour
- Hematolymphoid tumours
    - Langerhans cell histiocytosis

Rosai-Dorfman disease

Follicular dendritic cell sarcoma

Primary thyroid lymphoma

Germ cell tumours

Benign teratoma

9080/1 Immature teratoma

9080/3 Malignant teratoma

Secondary tumours

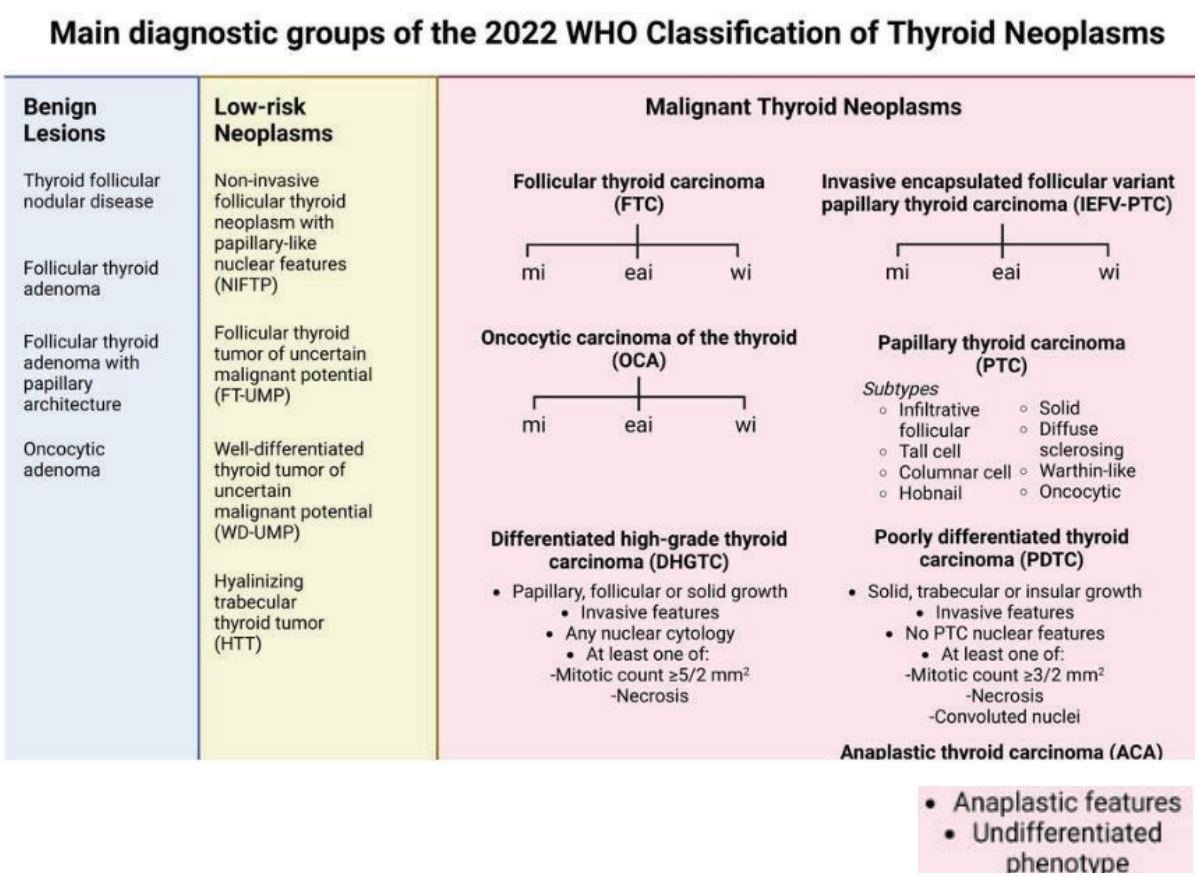
WHO (2017) - Parathyroid gland

Tumours of the parathyroid glands

Parathyroid carcinoma

Parathyroid adenoma

Secondary, mesenchymal and other tumours



Tall cell & hobnail variants = high-risk PTC.

Poorly differentiated carcinoma bridges differentiated and anaplastic tumours.

Differentiated high-grade carcinoma is a new WHO 5th edition category.

Oncocytic carcinoma is a distinct entity, not a subtype of follicular carcinoma.

1) Follicular Cell–Derived Neoplasms

Benign Tumours

Follicular adenoma

Oncocytic adenoma (formerly "Hürthle cell adenoma")

Follicular adenoma with papillary architecture

Thyroid follicular nodular disease (new terminology replacing "multinodular goitre")

Low-Risk Neoplasms (Not Carcinomas)

Non - invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP)

Tumours of uncertain malignant potential

Hyalinizing trabecular tumour

These are stratified as low-risk because they lack invasive features.

Malignant Tumours

Papillary Thyroid Carcinoma (PTC)

Classical

Tall cell

Columnar cell

Solid

Diffuse sclerosing

Hobnail

Follicular Thyroid Carcinoma (FTC)

Minimally invasive

Widely invasive

Oncocytic carcinoma (distinct morphological entity)

Poorly Differentiated Thyroid Carcinoma (PDTC)

Differentiated High-Grade Thyroid Carcinoma *(new category)*

Anaplastic Thyroid Carcinoma (ATC)

Other malignant categories include:

Salivary gland-type carcinomas (e.g., mucoepidermoid, secretory)

Thymic tumours within thyroid (e.g., SETTLE, CASTLE)

Tumours of uncertain histogenesis (e.g., cribriform-morular tumour)*

Key Conceptual Changes in WHO 5th Ed.

Oncocytic carcinoma is now defined as a distinct entity (≥75% oncocytic cells) separate from follicular carcinoma.

Differentiated high-grade carcinoma is newly recognized for aggressive differentiated tumours with high mitotic index / necrosis.

Papillary thyroid *micro*carcinoma is *no longer* recommended as a distinct subtype.

Cribriform-morular carcinoma is no longer classified under PTC subtypes; it's considered a tumour of uncertain histogenesis.

Follicular cell-derived tumours are grouped as benign, low-risk, and malignant.

Oncocytic carcinoma is distinct; not just "Hürthle."

NIFTP is a *low-risk neoplasm*, not carcinoma

Differentiated high-grade carcinoma is a *new malignant category*

OLD vs WHO 5th EDITION

1. FOLLICULAR CELL–DERIVED TUMOURS

Earlier WHO (2017 & before)	WHO 5th Edition (2022–current)	Important Pearl
Multinodular goitre	Thyroid follicular nodular disease	Non-neoplastic, clonal spectrum recognised
Follicular adenoma	Follicular adenoma	Benign neoplasm
Hürthle cell adenoma	Oncocytic adenoma	"Hürthle" terminology discouraged
Hürthle cell carcinoma	Oncocytic carcinoma	Separate entity (≥75% oncocytic cells)
Encapsulated FVPTC	NIFTP (if non-invasive)	Low-risk, *not carcinoma*
–	Tumours of uncertain malignant potential	Borderline category
Papillary microcarcinoma	*No longer a separate subtype*	Size descriptor only

2. MALIGNANT FOLLICULAR CELL TUMOURS

Earlier WHO	WHO 5th Edition	Exam / Viva Pearl
Papillary carcinoma (subtypes)	Papillary carcinoma (classical, tall cell, hobnail, etc.)	Subtypes retained
Follicular carcinoma	Minimally invasive / Widely invasive	Same concepts
Poorly differentiated carcinoma	Poorly differentiated carcinoma	Turin criteria retained
–	Differentiated high-grade thyroid carcinoma	**NEW ENTITY**
Anaplastic carcinoma	Anaplastic carcinoma	Still separate, undifferentiated
Cribriform-morular variant of PTC	Tumour of uncertain histogenesis	No longer PTC subtype

2) C-Cell (Parafollicular)–Derived Neoplasms

Medullary Thyroid Carcinoma (MTC)

The WHO 5th ed. includes a **grading system** based on:

Mitotic count

Tumour necrosis

Ki-67 index

Calcitonin-secreting hyperplasia (C-cell hyperplasia) remains a recognized precursor / associated lesion.

1. DETAIL ON PAPILLARY THYROID CARCINOMA (PTC)

PTC constitutes about 80% of all thyroid cancer cases and is the most prevalent thyroid cancer subtype in countries having an imbalance in the iodine content of the diet. It is usually detected in the third to fifth decades of the patients' life, with the mean age at 40 years. Women are more frequently affected than men, in ratios of 2:1 to 4:1 . The only known aetiologic factor is a previous history of radiation exposure. Other suggested risk factors include pre-existing benign thyroid disease like Hashimoto's thyroiditis or having a family history of PTC .

PTC is often characterized by *RET* chromosomal rearrangement or mutations of the *BRAF*, *RAS* or *RET* genes are found in nearly 70% of PTC cases.

A. DIAGNOSTIC NUCLEAR FEATURES *(KEY TO DIAGNOSIS)*

Diagnosis of PTC rests on nuclear features, not architecture

Enlarged, oval nuclei

Optically clear ("Orphan Annie eye") nuclei (Fig 11.9 inset)

Nuclear overlapping and crowding

Nuclear grooves

Intranuclear cytoplasmic inclusions

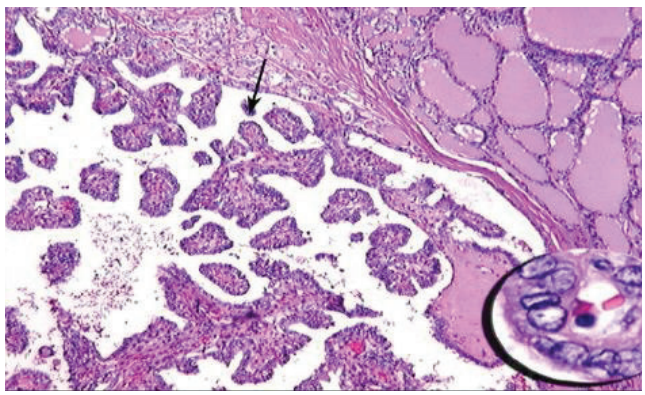


Fig 11.9 shows thyroid tissue with papillae in a cystic space . The cells ove papillae show typical nuclear change (inset) H &E x 100

(At least some of these must be present)

Architecturally papillary, follicular, solid, trabecular or mixed patterns are seen ; True papillae with fibrovascular cores (common but not mandatory), but nuclear features are more important.

B. OTHER FEATURES

Psammoma bodies (concentric lamellated calcifications)

Dense fibrous stroma (especially in sclerosing variants)

Lymphatic invasion common

C. VARIANTS

Classical – papillae + typical nuclei

Follicular variant – follicular architecture + PTC nuclei

Tall cell variant – cells ≥3× as tall as wide; aggressive

Hobnail variant – apically placed nuclei; aggressive

Diffuse sclerosing – extensive fibrosis, lymphoid infiltrate

D. IMMUNOHISTOCHEMISTRY (SUPPORTIVE)

CK19, HBME-1, Galectin-3 – positive

Thyroglobulin – positive

F. AGGRESSIVE VARIANTS OF PAPILLARY THYROID CARCINOMA

These variants retain papillary carcinoma nuclear features but show aggressive behaviour.

1. TALL CELL VARIANT

Definition

Tumour cells ≥ 3 times taller than wide

Tall cells constitute ≥ 30% of tumour

Microscopy

Elongated cells with abundant eosinophilic cytoplasm

Basally placed nuclei with classical PTC nuclear features

Papillary or solid architecture

Clinical Pearls

Occurs in older patients

Associated with **extrathyroidal extension and recurrence**

Considered **high-risk PTC variant**

2. HOBNAIL VARIANT

Microscopy

Cells show apically placed nuclei with loss of polarity

Hobnail or teardrop appearance

Papillary or micropapillary architecture

Clinical Pearls

Aggressive behaviour

Often associated with vascular invasion and metastasis

3. COLUMNAR CELL VARIANT

Microscopy

Tall columnar cells

Pseudostratified elongated nuclei

Resembles endometrial or intestinal epithelium

Pearls

Encapsulated tumours behave better

Infiltrative tumours are aggressive

4. DIFFUSE SCLEROSING VARIANT

Microscopy

Diffuse involvement of thyroid

Dense fibrosis

Numerous psammoma bodies

Prominent lymphoid infiltrate with germinal centres

Pearls

Common in children and young adults

Extensive lymph node metastasis common

FOLLICULAR NEOPLASMS

(Adenoma vs Carcinoma –

A. COMMON MICROSCOPIC FEATURES

Uniform follicles (microfollicular, normofollicular, trabecular)

Colloid usually scant

Tumour is encapsulated

No papillary carcinoma nuclear features

B. FOLLICULAR ADENOMA

Intact capsule

No capsular invasion

No vascular invasion

Compression of adjacent thyroid common

C. FOLLICULAR CARCINOMA

DEFINING FEATURE

Presence of capsular and/or vascular invasion

Capsular invasion: tumour penetrates capsule completely

Vascular invasion: tumour thrombus attached to vessel wall

TYPES

Minimally invasive – limited capsular ± vascular invasion

Widely invasive – extensive invasion into thyroid and beyond

D. IMPORTANT PEARLS

Cytology **cannot** distinguish adenoma from carcinoma

Diagnosis is **histological**, based on invasion

FLOW CHART : FOLLICULAR-PATTERNED THYROID TUMOUR

Follicular-patterned lesion

↓

Encapsulated?

↓

YES ---------- NO

↓ ↓

Capsular / vascular Infiltrative growth

invasion present? → Carcinoma

↓

NO → NIFTP / Adenoma

YES → Follicular carcinoma

SOLITARY NODULE OF THE THYROID

A clinical presentation rather than a diagnosis.

Common causes

Dominant nodule in a multinodular goitre (most common); now called follicular nodular disease

Follicular neoplasm (adenoma or carcinoma)

Nodular form of Hashimoto's thyroiditis

Important Pearl

A solitary thyroid nodule is malignant until proven otherwise.

MEDULLARY THYROID CARCINOMA (MTC)

(Parafollicular C-cell tumour)

A. CELL OF ORIGIN

Derived from parafollicular (C) cells

Produces calcitonin

B. ARCHITECTURE

Nests, sheets, trabeculae or cords

Cells may be spindle-shaped, plasmacytoid or polygonal

C. CYTOLOGICAL FEATURES

Moderate pleomorphism

Eccentric nuclei

Salt-and-pepper chromatin (neuroendocrine type)

D. STROMAL FEATURES (CLASSIC)

Amyloid deposition in stroma

Congo red positive

Apple-green birefringence under polarized light

E. IMMUNOHISTOCHEMISTRY (DIAGNOSTIC)

Calcitonin – positive

CEA – positive

Chromogranin, Synaptophysin – positive

Thyroglobulin – negative

F. ASSOCIATED FINDINGS

C-cell hyperplasia in surrounding thyroid (MEN-associated cases)

FLOWCHART : MEDULLARY CARCINOMA APPROACH

Medullary carcinoma diagnosed

↓

Search non-neoplastic thyroid

↓

C-cell hyperplasia present?

↓

YES → Suspect MEN (genetic basis)

NO → Likely sporadic

COMPARISON OF VARIOUS CARCINOMAS

Feature	Papillary CA	Follicular CA	Medullary CA
Cell of origin	Follicular	Follicular	C-cell
Architecture	Papillary/follicular	Follicular	Nests/trabeculae
Diagnostic hallmark	Nuclear features	Capsular/vascular invasion	Amyloid + calcitonin
Spread	Lymphatic	Hematogenous	Lymphatic & hematogenous
Thyroglobulin	Positive	Positive	Negative
Calcitonin	Negative	Negative	Positive

Papillary carcinoma: nuclear features are diagnostic.

Follicular carcinoma: invasion defines malignancy.

Medullary carcinoma: amyloid + calcitonin clinch the diagnosis.

NIFTP : Non-invasive Follicular Thyroid Neoplasm with Papillary-like Nuclear Features

Non-invasive follicular thyroid neoplasm with papillary like nuclear features was previously known as 'Encapsulated Non-invasive Follicular Variant Papillary Thyroid Carcinoma.' This is a low grade thyroid neoplasm and the cells in a NIFTP have features that look like papillary thyroid cancer; this finding alone does not mean NIFTPs are malignant. Because NIFTP tumours are not invasive and are contained within the tumour capsule, they are considered to

have an extremely low risk of adverse outcomes like tumour recurrence or spread (metastasis). For a correct diagnosis, NIFTP tumours must meet very stringent criteria microscopically -

The tumour should not show evidence of invasion or penetration into any part of its capsule, the blood vessel or the lymphatic channels within the tumour or outside.

The tumour must have a "follicular" growth pattern.

The tumour should not have any necrosis or increase in mitotic activity.

The tumour must have no or minimal (<1%) papillary appearance, and should NOT have the appearance of any other thyroid cancer variants like e insular, oncocytic, tall cell, columnar cell, diffuse sclerosing, etc

AGGRESSIVE VARIANTS OF THYROID CARCINOMAS

POORLY DIFFERENTIATED THYROID CARCINOMA (PDTC)

Diagnostic Criteria (Turin criteria)

Solid / trabecular / insular growth

Absence of PTC nuclear features

Plus at least one of the following:

≥ 3 mitoses / 10 HPF

Tumour necrosis

Convoluted nuclei

Pearls

Intermediate prognosis between differentiated and anaplastic carcinoma

DIFFERENTIATED HIGH-GRADE THYROID CARCINOMA (NEW – WHO 5th Ed.)

Definition

Differentiated carcinoma (PTC / FTC) with **high-grade features**

Microscopy

Retains differentiated architecture

≥ 5 mitoses / 10 HPF and/or tumour necrosis

Pearls

Worse prognosis than conventional PTC/FTC

Must be specifically mentioned in report

ONCOCYTIC (HÜRTHLE CELL) LESIONS

Oncocytic tumours are now recognised as distinct entities in WHO 5th edition.

1. ONCOCYTIC ADENOMA

Microscopy

Encapsulated tumour

Sheets, nests or follicles of oncocytic cells

Cells have:

- Abundant granular eosinophilic cytoplasm
- Central round nuclei
- Prominent nucleoli

Key Point

No capsular or vascular invasion

2. ONCOCYTIC CARCINOMA

Diagnostic Criteria

≥ 75% oncocytic cells

Capsular and/or vascular invasion present

Microscopy

Same cytology as adenoma

Invasion into capsule or vessels defines malignancy

Behaviour

More aggressive than follicular carcinoma

Increased risk of distant metastasis

FLOWCHART : ONCOCYTIC (HÜRTHLE CELL) LESION

Oncocytic tumour (>75%)

↓

Capsular / vascular invasion?

↓

NO → Oncocytic adenoma

YES → Oncocytic carcinoma

ONCOCYTIC VARIANT OF PTC

Microscopy

Oncocytic cytoplasm

Classical PTC nuclear features present

Pearl

Still classified as **papillary carcinoma**, not oncocytic carcinoma

COMPARISON – ONCOCYTIC vs FOLLICULAR

Feature	Oncocytic Tumours	Follicular Tumours
Cytoplasm	Abundant, granular, eosinophilic	Scant to moderate
Mitochondria	Numerous	Normal
Diagnosis of carcinoma	Capsular/vascular invasion	Capsular/vascular invasion
Behaviour	Generally more aggressive	Variable

A V–THYROID GLAND SYNOTIC REPORTING

THYROID BIOPSY / RESECTION SPECIMENS – SYNOPTIC REPORTING

(Aligned with WHO Classification of Tumours of Endocrine Organs, 2022 and AJCC/TNM 8th Edition)

A. NON-NEOPLASTIC LESIONS

Type of specimen (tick as applicable):

Wedge biopsy ☐

Lobectomy ☐ Side: Right ☐ Left ☐

Nodulectomy ☐ Side: Right ☐ Left ☐

Isthmectomy ☐

Right lobe + isthmus ☐

Left lobe + isthmus ☐

Subtotal thyroidectomy ☐

Total thyroidectomy ☐

Specimen size: …… × …… × …… cm
Weight (if available): …… g

Parathyroid included: Yes ☐ No ☐
If yes: Size …… × …… mm

Gross examination

Outer surface: Nodular ☐ / Smooth ☐ / Diffusely enlarged ☐

Configuration: Nodular ☐ / Globular ☐

Extrathyroid tissue identified: Yes ☐ No ☐

Consistency: Soft ☐ Firm ☐ Hard ☐

Cut surface:
Dark brown ☐ / Grey-white ☐ / Haemorrhagic ☐

Nodularity: Single nodule ☐ / Multiple nodules ☐ / Nil ☐

Cystic change: Yes ☐ No ☐

Microscopy (tick all that apply)

Diffuse colloid goitre ☐

Nodular (multinodular) colloid goitre ☐

Adenomatous nodule (hyperplastic nodule) ☐

Graves disease ☐

Chronic lymphocytic (Hashimoto) thyroiditis ☐

Non-specific chronic thyroiditis ☐

Subacute (granulomatous / De Quervain) thyroiditis ☐

Riedel thyroiditis ☐

Capsule: Intact ☐ Thickened ☐ Fibrosed ☐

Hurthle cell change: ☐ Present ☐ Absent

Fibrosis Mild ☐ Mod ☐ Severe ☐

Other (specify): ……

Comment: ……

B. NEOPLASTIC LESIONS

Gross examination

Type of specimen:
Lobectomy ☐ Side: Right ☐ Left ☐
Nodulectomy ☐ Side: Right ☐ Left ☐
Isthmectomy ☐
Right lobe + isthmus ☐
Left lobe + isthmus ☐
Subtotal thyroidectomy ☐
Total thyroidectomy ☐

Specimen size: …… × …… × …… cm
Tumour present: Yes ☐ No ☐ Indeterminate ☐
Tumour size (maximum dimension): …… cm

Encapsulation: Present ☐ Absent ☐
Circumscription: Well circumscribed ☐ Infiltrative ☐
Number of tumour foci: Solitary ☐ Multifocal ☐ (Number ……)

Tumour appearance: Solid ☐ Cystic ☐ Mixed ☐

Calcification: ☐ Present ☐ Absent

Distance to closest surgical margin: …… cm

Lymph nodes (if included)

Neck dissection: Central ☐ Selective ☐ Modified radical ☐ Radical ☐ Extended ☐

Side: Right ☐ Left ☐ Bilateral ☐

Number identified: ……

Largest node size: …… × …… cm

Other structures received:
Sternocleidomastoid muscle ☐ Size …… cm
Submandibular gland ☐ Size …… cm
Jugular vein ☐ Size …… cm

Gross extrathyroid invasion: Yes ☐ No ☐
If yes, structure involved: ……

Microscopy

Histological tumour type (WHO 2022):

Follicular adenoma ☐

Oncocytic (Hürthle cell) adenoma ☐

Hyalinising trabecular tumour ☐

NIFTP (Non-invasive follicular thyroid neoplasm with papillary-like nuclear features)

(Requires complete submission of tumour capsule for diagnosis))

Papillary thyroid carcinoma

Classical ☐ Follicular variant ☐ Tall cell ☐ Hobnail ☐ Columnar ☐ Solid/trabecular ☐ Other (specify) ……

Follicular carcinoma:

Minimally invasive ☐ Encapsulated angioinvasive ☐ Widely invasive ☐

Oncocytic (Hürthle cell) carcinoma ☐

Poorly differentiated thyroid carcinoma ☐

Anaplastic (undifferentiated) carcinoma ☐

With residual differentiated carcinoma ☐ Without residual component ☐

Medullary thyroid carcinoma ☐

C-cell hyperplasia: Present ☐ Absent ☐ (Calcitonin, CEA, amyloid; RET status if available)

Mixed carcinoma (specify): ……

Tumour parameters

Tumour location: Right lobe ☐ Left lobe ☐ Isthmus ☐ Both lobes ☐

Capsular invasion: Absent ☐ Present ☐

Vascular invasion: Absent ☐ Present ☐ Number of foci ……

Extrathyroidal extension:

Absent ☐ Microscopic ☐ Gross ☐ (structure involved ……)

Surgical margins:

Free ☐ Involved ☐ Site ……

Tumour multifocality: Yes ☐ No ☐

If yes, number of foci: ……

Tumour necrosis: ☐ Present ☐ Absent

Mitotic activity: ☐ Low ☐ Increased

Perineural invasion: ☐ Present ☐ Absent

Lymph node status (microscopic)

Metastasis: Absent ☐ Present ☐

Number examined: …… Number positive: ……

Size of largest metastatic deposit: …… cm

Extranodal extension: Present ☐ Absent ☐

Level of nodes involved: Central ☐ Lateral ☐

Pathologic staging (AJCC / TNM 8th Edition)

pT: ……

pN: ……

pM: ……

Associated pathology: ……

Parathyroid gland identified: Yes ☐ No ☐

If yes: Number …… Location …… Normal ☐ Abnormal ☐

Final integrated report:

Procedure

Tumour type

Tumour size

Grade / subtype

Extent

Margins

Lymph nodes

pTNM stage…….

Comments: ……

Pathologist's signature: ……

Date: ……

THYROID CARCINOMA – TNM (AJCC 8th Edition)

*(Applies to papillary, follicular, oncocytic, poorly differentiated carcinomas; **NOT** anaplastic)*

T – PRIMARY TUMOUR

T1 – Tumour ≤ 2 cm, limited to thyroid

T1a: ≤ 1 cm

T1b: > 1 cm but ≤ 2 cm

T2 – Tumour > 2 cm but ≤ 4 cm, limited to thyroid

T3a: Tumour >4 cm limited to thyroid
T3b: Gross extrathyroidal extension into strap muscles

T4 – Gross extrathyroidal extension

T4a (Moderately advanced): Invades subcutaneous soft tissue, larynx, trachea, oesophagus, or RLN

T4b (Very advanced): Invades prevertebral fascia, carotid artery, or mediastinal vessels

N – REGIONAL LYMPH NODES

N0 – No regional lymph node metastasis

N1 – Regional lymph node metastasis present

N1a: Level VI or VII (central compartment: pretracheal, paratracheal, prelaryngeal)

N1b: Lateral cervical (levels I–V), retropharyngeal, or superior mediastinal nodes

M – DISTANT METASTASIS

M0 – No distant metastasis
M1 – Distant metastasis present (lung, bone, etc.)

STAGE GROUPING (AGE-DEPENDENT)

Patients < 55 years

Stage I: Any T, Any N, M0

Stage II: Any T, Any N, M1

Patients ≥ 55 years

Stage I: T1–T2, N0/Nx, M0

Stage II: T1–T2 with N1 **OR** T3, M0

Stage III: T4a, Any N, M0

Stage IV: T4b, Any N, M0 **OR** Any T, Any N, M1

IMPORTANT THYROID TNM PEARLS

Minimal microscopic extrathyroidal extension is NOT staged (removed in AJCC 8th).

Age cut-off shifted from 45 → 55 years

Size-based staging applies only when tumour is confined to thyroid.

Always correlate with gross extrathyroidal extension, not microscopy alone.

All anaplastic carcinomas are Stage IV:

IVA: Intrathyroidal

IVB: Gross extrathyroidal extension

IVC: Distant metastasis

B I PARATHYROID GLAND ANATOMY

Anatomy

Two pairs: superior and inferior

Located on posterior surface of thyroid, near lower poles

Microscopy

Lobulated architecture

Cells arranged in cords**,** nests, sheets and pseudofollicles

Rich capillary network

Adipose tissue present (increases with age)

Cell Types

Chief cells

8–12 µm

Pale eosinophilic or amphophilic cytoplasm

Central round nuclei

Oxyphil cells

Larger (12–20 µm)

Abundant granular eosinophilic cytoplasm

Central pyknotic nuclei

Represent transformation of chief cells

Clear cells

Polygonal

Clear to amphophilic cytoplasm

Important Pearl

Presence of fat helps distinguish normal parathyroid from adenoma

B II PARATHYROID GLAND GROSSING TECHNIQUES

Specimens received

Frozen section

Excision biopsy of suspected parathyroid nodule

Incidentally removed glands in subtotal thyroidectomy

Excision biopsy :

State the number of glands sent.

Take the measurement of the single largest gland sent in 2 or 3 directions depending on the size.

Take the weight in toto

Description : shape , colour, consistency and surface

Bisect the specimen if more than 5 mm , else take it entirely

Descirbe the cutsurface : yellow brown or grey white, solid/cystic, areas of necrosis, haemorrhage and calcification. Comment on the surrounding thyroid tissue if present.

If sent for frozen section (FS) then process it entirely as FS, if the size is less than 8mm; if more, then one half may be taken for FS and the other retained for paraffin section processing.

Staining for intracytoplasmic fat may be done to differentiate normal from abnormal parathyroid gland.

Bits

The entire specimen should be taken in one or more blocks depending on the number and size of the specimen.-

Most parathyroid adenomas are found immediately posterior or inferior to the thyroid.

Superior gland adenomas lie in the tracheo-esophageal or paraesophageal space, while

inferior gland adenomas have more variable positions due to longer embryologic descent.

Most parathyroid adenomas are solitary (up to 87% [2]), well-circumscribed, and oval or

bean-shaped; larger lesions may be multilobulated. A thin interface separates it from the thyroid

Supernumerary glands (more than four) occur in up to 13% of individuals and may contribute to persistent or recurrent hyperparathyroidism.

Ectopic adenomas account for up to 5% of cases, with locations including :

mediastinum (usually superior mediastinum)

carotid sheath

retropharyngeal space

intrathyroidal

submandibular region (less common)

B IV MICROSCOPY OF PARATHYROID GLAND

PARATHYROID HYPERPLASIA

In primary hyperplasia, all four glands are involved, though enlargement is often asymmetric. Glands range from mildly enlarged to markedly nodular with pseudopod-like extensions in long-standing cases. Frequently associated with MEN syndromes.

Management

Subtotal parathyroidectomy: removal of 3½ glands, leaving ~50 mg tissue

Microscopy

Chief cell hyperplasia most common

Water-clear cell hyperplasia less frequent

Diffuse or nodular growth patterns

Fibrous septa, acinar formations and giant hyperchromatic nuclei may be present

Stromal fat is unreliable for distinguishing normal from hyperplastic glands

Familial hyperparathyroidism and MEN are typically associated with chief cell hyperplasia

Morphology alone cannot reliably distinguish primary from secondary hyperplasia

Primary

Autonomous PTH overproduction

Causes:

Adenoma ~85%

Hyperplasia ~10–15%

Carcinoma <5%

Secondary

Due to chronic hypocalcaemia

Most commonly **chronic renal disease**

Tertiary

Autonomous hyperparathyroidism developing after long-standing secondary hyperparathyroidism

Seen in chronic renal disease or malabsorption

Hyperplasia concept refined

Primary hyperplasia is typically re-classified as multiglandular parathyroid disease based on evidence of multi-gland clonal proliferation.

Secondary hyperplasia remains the dominant use of the term "hyperplasia."

Genetic and syndrome associations

Parathyroid carcinoma and atypical tumours are increasingly subclassified based on CDC73, MEN1, CDKN1B, MAX and related features to guide diagnosis and germline testing.

Note: WHO 5th edition itself does not provide an exhaustive staging for parathyroid hyperplasia but promotes standardized reporting with updated terminology.

PARATHYROID LESIONS – OLD vs WHO 5th EDITION

Earlier Terminology	WHO 5th Edition	Exam / Viva Pearl
Parathyroid hyperplasia	Multiglandular parathyroid disease	Reflects clonal biology
Parathyroid adenoma	Parathyroid adenoma	Commonest cause of PHPT
Atypical parathyroid adenoma	Atypical parathyroid tumour	Uncertain malignant potential
Parathyroid carcinoma	Parathyroid carcinoma	CDC73 mutation important

Feature	Adenoma	Hyperplasia	Carcinoma
No. of glands	One	Multiple	One
Capsule	Present	Absent	Invasive
Fat	Absent	Reduced	Absent
Invasion	No	No	Yes

PARATHYROID ADENOMA

Parathyroid adenoma is the commonest cause of primary hyperparathyroidism, accounting for approximately 85% of cases. About 10–15% are due to parathyroid hyperplasia, and <2–5% are due to parathyroid carcinoma. Adenomas occur more commonly in women.

Parathyroid adenomas may occur sporadically or as a component of MEN type **1** (and rarely MEN 2A).

Clinical features

Manifestations are due to hypercalcaemia, including:

- Renal calculi
- Skeletal involvement such as osteitis fibrosa cystica

Symptoms usually resolve following excision of the adenoma alone

Ethanol ablation under ultrasound guidance has been used in selected high-risk surgical candidates

Preoperative localisation

Ultrasonography

Technetium-99m sestamibi scintigraphy (SPECT/CT) — highly useful for localisation

Anatomical distribution

Most commonly involves the inferior parathyroid gland, followed by the superior

Ectopic adenomas may be found in the mediastinum, intrathyroidal location, oesophagus or carotid sheath, leading to failed explorations

DOUBLE ADENOMAS

An acknowledged but uncommon entity, accounting for 2–10% of primary hyperparathyroidism. Distinction from asymmetric hyperplasia is difficult.

Suggested diagnostic criteria

Two enlarged, histologically hypercellular glands

Remaining glands normal at surgery

No family history or clinical evidence of MEN or familial hyperparathyroidism

Permanent cure of hypercalcaemia after excision of the two enlarged glands alone

GROSS FEATURES

Weight usually <1 g, though tumours up to 50 g have been reported

Larger adenomas tend to be associated with more severe hypercalcaemia

Size: 1–several cm

Well circumscribed, soft

Colour: orange-brown to reddish-yellow

Haemorrhage, calcification and cystic change may occur

Oxyphil adenomas have a characteristic mahogany brown cut surface

MICROSCOPY

Usually encapsulated, sometimes lobulated or vaguely nodular

A rim of compressed normal parathyroid tissue is present at the periphery in ~60% of cases

Absence of this rim makes distinction from hyperplasia difficult

Composed of one or more parathyroid cell types:

- Chief cells (most common)
- Oxyphil cells
- Water-clear cells

Architectural patterns:

- Solid sheets
- Cords
- Acini
- Follicles and microcysts

Rich sinusoidal vascular network with palisading of nuclei along vascular septa

Nuclei may be enlarged, hyperchromatic or pleomorphic

Mitoses are absent

Bizarre hyperchromatic nuclei may be seen and do not indicate malignancy in the absence of mitoses

Areas of fibrosis, haemorrhage and infarction may occur and may cause spontaneous remission

Amyloid may be present in acini or septa

Best evidence of adenoma

Rim of normal parenchyma around the lesion **and**

Microscopically normal second gland

VARIANTS OF PARATHYROID ADENOMA

Lipoadenoma

Adipose tissue constitutes **20–90%**

Large size (1–15 cm)

Other glands normal

Papillary variant

May mimic papillary thyroid carcinoma

Water-clear cell adenoma

Predominantly clear cells

Follicular (acinar) variant

May mimic thyroid follicular neoplasm

Oxyphil adenoma

Composed entirely of oxyphil cells

Often less functional

Intrathyroidal lesions may mimic Hürthle cell tumours

IMMUNOHISTOCHEMISTRY

Positive for:

Cytokeratin

Neuroendocrine markers (chromogranin, synaptophysin)

Neurofilament

Parathyroid hormone (PTH)

Ki-67: focal positivity, not diffuse

ATYPICAL TERMINOLOGY AND NOMENCLATURE (WHO 5TH EDITION)

"Atypical parathyroid adenoma" is now termed "atypical parathyroid tumour" to reflect uncertain malignant potential rather than definitive carcinoma

FLOWCHART 4: PARATHYROID TUMOUR TERMINOLOGY

Parathyroid lesion

↓

Local invasion / metastasis?

↓

YES → Parathyroid carcinoma

NO → Adenoma / Atypical parathyroid tumour

PARATHYROID CARCINOMA

Classically presents with hyperparathyroidism; non-functional tumours behave aggressively. Patients are generally **younger** than those with adenomas.

Clinical features suggestive of carcinoma

Serum calcium **>14 mg/dL**

Palpable neck mass

Vocal cord paralysis

Combined renal and skeletal disease

Early recurrence after surgery

Behaviour

Sporadic in most cases

Rarely associated with MEN

Tendency to invade thyroid and adjacent soft tissues

Requires en bloc excision

Recurrences common within 3 years

Metastases: lymph nodes, mediastinum, lung, liver, bone

Hypercalcaemia often dominates the clinical course

Molecular

Loss of **RB tumour suppressor gene**

Cyclin D1 overexpression common

Gross

Grey-white, firm tumours

Size: 1–several cm

Average weight ~12 g

Thick capsule or infiltrative margins

Necrosis and calcification may be present

Microscopy

Thick fibrous capsule

Trabecular growth pattern

Broad fibrous bands separating tumour nodules

Cells may resemble adenoma but show:

- Macronucleoli
- Diffuse atypia
- Increased mitotic activity

Diagnostic criteria (WHO)

- Capsular invasion (full thickness)
- Vascular invasion with endothelial adherence
- Perineural or soft tissue invasion
- Metastasis

Immunohistochemistry

PTH positive (especially useful in ectopic tumours)

Ki-67 index increased (0.4–26%)

Differences between Parathyroid Adenoma and Parathyroid Carcinoma

Feature	Parathyroid Adenoma	Parathyroid Carcinoma
Incidence	Common	Rare (<1%)
Sex	F > M	F = M
Age (years)	56–62	45–54
Renal stones	4–30%	48–56%

Skeletal involvement	14–20%	63–91%
Renal + skeletal disease	Rare (<5%)	32–53%
Palpable neck mass	Rare (<2%)	38–45%
Serum calcium	Mild–moderate (≈2.75–3 mmol/L)	Marked (≥3.5–4 mmol/L)
Tumour weight	Usually <1 g	Often >3 g (mean 10–12 g)
Gross appearance	Well circumscribed	Firm, lobulated, adherent
Definitive malignant criteria	Absent	Invasion into thyroid/soft tissue/oesophagus/nerves or metastasis
Capsular invasion	Absent	May be present
Vascular invasion	Absent	May be present
Mitotic activity	1–5/10 HPF	Often >5/10 HPF
Broad fibrous bands	Absent	Common
Coagulative necrosis	Absent	May be present
Cellular atypia	Focal	Often diffuse
Macronucleoli	Rare	Frequent
Ki-67 index	Usually <5%	Often >5–10%
Parafibromin (CDC73)	Retained	Loss of nuclear staining
RB protein	Retained	Lost
Cyclin D1	Variable	Frequent overexpression
Flow cytometry	Usually diploid	Often aneuploid
Molecular genetics	MEN1, CCND1	**CDC73 (HRPT2)** mutations

WHO Classification of Tumours of Endocrine Organs (2022)

Parathyroid carcinoma is diagnosed **only** by:

Unequivocal invasive growth into adjacent structures
(thyroid, soft tissue, oesophagus, nerves, or vessels with extratumoral spread), and/or

Regional or distant metastasis

Histologic features alone are insufficient for diagnosis in the absence of invasion or metastasis

The former term **"atypical parathyroid adenoma"** has been replaced by **"Atypical parathyroid tumour"**

Loss of parafibromin expression strongly supports carcinoma or atypical parathyroid tumour

CDC73 mutations are characteristic of parathyroid carcinoma and HPT-JT syndrome

Atypical Parathyroid Tumour (WHO 2022)

Feature	Atypical Parathyroid Tumour
WHO category	Tumour of uncertain malignant potential
Gross features	Well circumscribed, may be firm
Invasive growth	Absent
Metastasis	Absent
Histologic features	Worrisome features present but not diagnostic
	(fibrous bands, increased mitoses, focal necrosis, cellular atypia)
Capsular/vascular invasion	Absent or equivocal
Ki-67 index	Often 3–10%
Parafibromin (CDC73)	Retained or lost
CDC73 mutation	May be present
Clinical behaviour	Usually indolent; requires close follow-up
Management implication	Complete excision and long-term surveillance

Note

Atypical parathyroid tumour represents a borderline category with histologic features suggestive of malignancy but lacking definitive invasive growth or metastasis.

B V PARATHYROID BIOPSY / RESECTION SPECIMENS – SYNOPTIC REPORTING

(Aligned with WHO Classification of Tumours of Endocrine Organs, 2022)

A. CLINICAL INFORMATION (if available)

Indication: Primary hyperparathyroidism ☐ Secondary ☐ Tertiary ☐ Incidental ☐

Serum calcium: …… mmol/L

Serum PTH: …… pg/mL

B. SPECIMEN DETAILS

Type of specimen (tick as applicable):
Parathyroidectomy ☐
Focused / single gland excision ☐
En bloc resection ☐
Re-exploration ☐

Intraoperative PTH drop: Yes ☐ No ☐ (if available)
Other (specify): ……

Number of glands received: ……

Specimen size: …… × …… × …… mm
Weight: …… g

Associated structures:
Thyroid tissue ☐ Soft tissue ☐ Skeletal muscle ☐ Other ……

C. GROSS EXAMINATION

External surface: Smooth ☐ Lobulated ☐ Adherent ☐

Consistency: Soft ☐ Firm ☐ Hard ☐

Cut surface: Tan-brown ☐ Grey-white ☐ Haemorrhagic ☐

Capsule: Thin ☐ Thick ☐ Absent ☐

Evidence of invasion (gross): Present ☐ Absent ☐

D. MICROSCOPY

Parathyroid lesion:

Normal parathyroid tissue ☐

Parathyroid hyperplasia ☐ (Diffuse ☐ Nodular ☐)

Parathyroid adenoma ☐

Atypical parathyroid tumour (WHO 2022) ☐

Parathyroid carcinoma ☐

E. HISTOLOGIC FEATURES (tick as applicable)

(Supportive features – not diagnostic in isolation)

Capsular invasion: Absent ☐ Present ☐ Equivocal ☐

Vascular invasion: Absent ☐ Present ☐

Mitotic activity: Low ☐ Increased (>5/10 HPF) ☐

Broad fibrous bands: Absent ☐ Present ☐

Coagulative tumour necrosis: Absent ☐ Present ☐

Cellular atypia: Mild ☐ Moderate ☐ Marked ☐

F. DEFINITIVE MALIGNANT CRITERIA (WHO 2022)

Invasion into adjacent structures: Yes ☐ No ☐

If yes, specify: Thyroid ☐ Soft tissue ☐ Oesophagus ☐ Nerve ☐ Vessels ☐

Regional lymph node metastasis: Present ☐ Absent ☐

Distant metastasis (if known): Present ☐ Absent ☐

G. IMMUNOHISTOCHEMISTRY (if performed)

Parafibromin (CDC73): Retained ☐ Lost ☐

Ki-67 index: …… %

RB protein: Retained ☐ Lost ☐

Cyclin D1: Positive ☐ Negative ☐

Galectin-3 ☐ / PGP9.5 ☐

H. FINAL DIAGNOSIS

I. COMMENT (if required)

Behavioural risk category: Benign ☐ Uncertain malignant potential ☐ Malignant ☐

Recommendation for clinical correlation / follow-up ☐

Pathologist's signature: ……
Date: ……

PARATHYROID CARCINOMA – TNM (AJCC 8th Edition)

*(TNM applies **only to parathyroid carcinoma**, not adenoma or hyperplasia)*

T – PRIMARY TUMOUR

T1 – Tumour ≤ 2 cm, limited to parathyroid

T2 – Tumour > 2 cm but ≤ 4 cm, limited to parathyroid

T3 – Tumour > 4 cm **OR** invasion into adjacent soft tissue

T4 – Tumour invades adjacent organs

Thyroid, trachea, oesophagus, RLN, or major vessels

N – REGIONAL LYMPH NODES

N0 – No regional lymph node metastasis
N1 – Regional lymph node metastasis present

M – DISTANT METASTASIS

M0 – No distant metastasis
M1 – Distant metastasis present

PARATHYROID TNM PEARLS

In thyroid carcinoma, TNM staging is age-dependent and gross extrathyroidal extension matters; in parathyroid carcinoma, invasion defines malignancy more than tumour size.

Parathyroid lesions use updated terms: multiglandular disease and atypical parathyroid tumour. In parathyroid lesions, TNM staging is rarely used, as carcinoma is uncommon.

Invasion (capsular, vascular, adjacent organs) is more important diagnostically than size.

Atypical parathyroid tumour is NOT staged.

Parathyroid adenoma**:** Well circumscribed encapsulated lesion composed of uniform chief cells with marked reduction of adipose tissue.

Parathyroid hyperplasia: Diffuse or multinodular proliferation of parathyroid cells involving multiple glands with reduced but present adipose tissue.

Parathyroid carcinoma**:** Parathyroid tumour showing capsular and/or vascular invasion with fibrous bands; diagnosis depends on invasive features rather than cytologic atypia.

ASSESS YOURSELF THYROID AND PARATHYROID GLANDS

Long Essay Questions (10 Mark Each)

1. 1.Discuss the management of a 4cm Papillary Thyroid Carcinoma in a 45-year-old male. Include the indications for total thyroidectomy versus lobectomy and how would u gross the resection?
2. Provide a comprehensive algorithm for evaluating a solitary thyroid nodule.
3. Discuss high grade carcinomas of the thyroid.

Short essays (5 Mark each)

4. Discuss the genetics of Medullary carcinoma of the thyroid (RET proto-oncogene), the significance of calcitonin levels. Writ briefly on the microscopy of the neoplasm.
5. Describe the clinical manifestations of a thyroid storm and the conditions of thyroid which give rise to this.
6. Retrosternal Goiter: Define a retrosternal goiter. What would be its gross and microscopy?
7. Parathyroid adenoma

Short answer Questions (3 marks Each)

8. Describe what a hurthle cell is., the histopathological appearance and it's significance.
9. What is lymphocytic thyroiditis ? What does it progress to?
10. Aberrant thyroid .

MCQs

1. Which type of thyroiditis is clinically mistaken for a thyroid carcinoma?

a. Hashmoto's thyroiditis
b. Reidel's thyroiditis
c. Subacute thyroiditis
d. Acut thyroiditis

2.Which carcinoma show nuclei to have "Orphan Anne Eye Appearance"?

a. Papillary Carcinoma thyroid
b. Medullary Carcinoma thyroid
c Anaplastic Carcinoma thyroid
d. Follicular Carcinoma thyroid.

3.Which of the following carcinomas classically spread through the lymphatics?

a. Papillary Carcinoma thyroid
b. Medullary Carcinoma thyroid
c Anaplastic Carcinoma parathyroid
d. Follicular Carcinoma thyroid.

Ans to MCQS 1. b.2. a. 3.a

Chapter 12
Pancreas

Chapter outline: I Pancreas Anatomy ; II Pancreas Grossing Techniques ; III Pancreas Gross Pearls ; IV Pancreas Microscopy ; V Pancreas Synoptic Reporting.

I PANCREAS ANATOMY

Anatomy of the Pancreas

The pancreas is an elongated organ (approximately 15 cm) which lies obliquely across the posterior abdominal wall, at the level of the **L1** and **L2 vertebral bodies**.

The pancreas is in contact with several surrounding organs in the **epigastric**, **left** hypochondriac**,** and umbilical region of the abdomen; anteriorly the stomach, lesser sac and the transverse mesocolon, posteriorly the aorta, inferior vena cava, both renal veins, superior mesenteric vessels and the left kidney; laterally it is in contact with the spleen; superiorly the splenic artery traverses it and medially the descending and transverse parts of the duodenum are in contact with it.

Apart from the tail, the rest of the organ is retroperitoneal, and divided for ease into five anatomical parts; the head, uncinate process, neck, body and tail.(Fig 12.1)

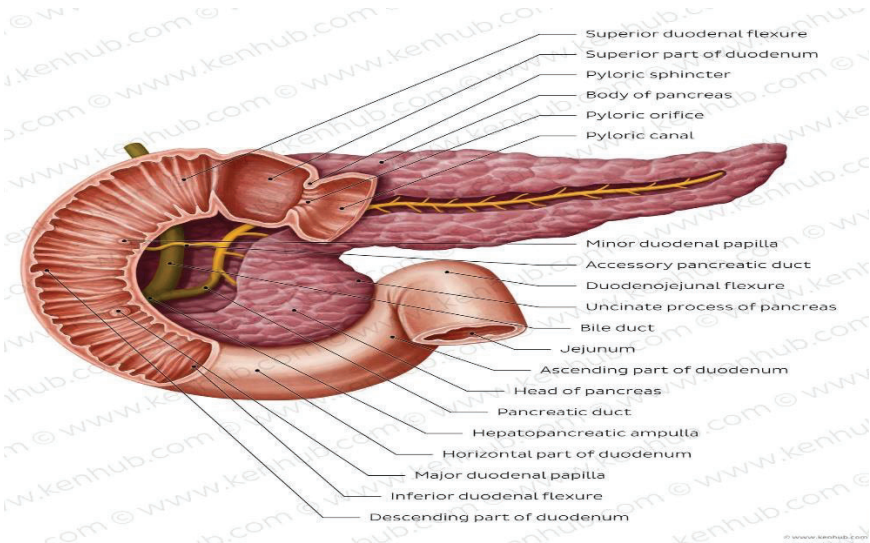


Fig12.1 : Shows pancreas with it's duct system.

The pancreas is an accessory and exocrine gland of the digestive system, as well as a hormone producing endocrine gland. It has an internal system of ducts.

The pancreas is supplied by pancreatic arteries stemming from surrounding vessels and is innervated by the vagus nerve , celiac plexus, and superior mesenteric plexus.

Traveling within the pancreas from the head to tail is the main pancreatic duct of Wirsung. It connects with the bile duct in the head of the pancreas to form the hepatopancreatic duct, otherwise called the ampulla of Vater. This opens into the descending part of the duodenum at the duodenal papilla. Flow through the ampulla of Vater is controlled by a smooth muscle sphincter called the sphincter of Oddi. It also prevents reflux of duodenal contents into the hepatopancreatic duct. The terminal parts of the main pancreatic and bile ducts also have sphincters, which play an important role in controlling the flow of pancreatic and bile fluids.

In addition to the main duct, the pancreas also contains an **accessory duct**. It communicates with the main pancreatic duct at the level of the pancreatic neck and opens into the descending part of the duodenum at the minor duodenal papilla.

The pancreas is an organ that fulfills both exocrine and endocrine roles. Its exocrine function includes the synthesis and release of digestive enzymes into the duodenum of the small intestine. Its endocrine function involves the release of insulin and glucagon into the bloodstream, two important hormones responsible for regulating glucose, lipid, and protein metabolism.

II PANCREAS GROSSING TECHNIQUES

TYPES OF SPECIMENS RECEIVED IN THE LAB

Small biopsies
- Core needle biopsy
- Wedge biopsy

Partial pancreatectomy
- Body / Tail

Pancreaticoduodenectomy (Whipple procedure)
- Pancreas + duodenum ± stomach ± bile duct ± gallbladder

Total pancreatectomy
- Entire pancreas ± stomach ± duodenum ± spleen

Extended / Regional pancreatectomy
- With vascular resection (portal / SMV / IVC / aorta)
- ± colon, mesocolon, omentum, lymph nodes

I PANCREATIC BIOPSIES

A. DESCRIPTION : State the number of tissue fragments ; Colour & consistency

B. MEASUREMENTS of the Largest and smallest fragment ; Third dimension if wedge or larger tissue

C. SUBMISSION - Core needle biopsy – submit entirely

Wedge biopsy

- **≥5 mm → bisect**
  - **– One half for histology**
  - **– One half for IF / EM (if indicated)**
- **<5 mm → submit entirely**

II. WHIPPLE PROCEDURE – GROSS EXAMINATION

A. IDENTIFICATION & MEASUREMENTS Components received (pancreas, duodenum, stomach, bile duct, spleen, gall bladder)

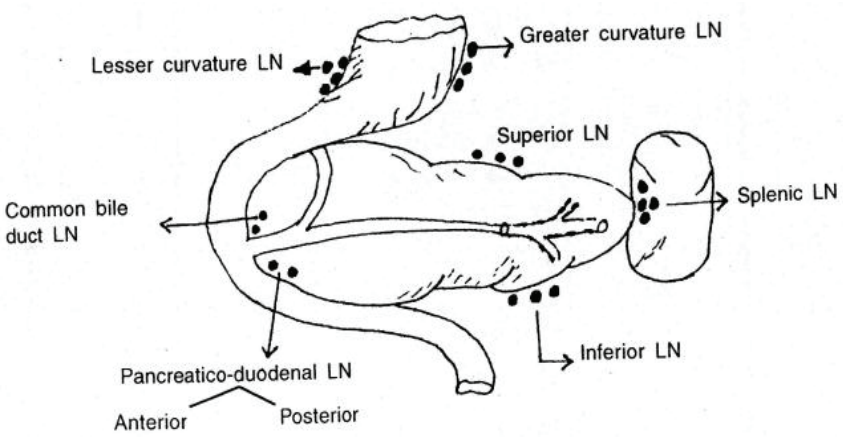


Fig 12.2 : Whipple's procedure and organs removed

B . MEASUREMENTS:

Pancreas (3 dimensions)

Length of duodenum

Length of stomach (greater & lesser curvature)

Length of CBD and pancreatic duct

C. LYMPH NODE DISSECTION (FRESH SPECIMEN)

Dissect lymph nodes before opening

Separate and label according to anatomical groups

D. MARGINS – IDENTIFY & INK

Pancreatic neck margin

Common bile duct margin

Duodenal margin

Gastric margins (if present)
Vascular margins (if included)

→ Take blocks immediately and keep aside

E. OPENING THE SPECIMEN

Open the stomach along the lesser curvature ; Extend the incision along duodenum opposite pancreatic attachment ; Lay stomach & duodenum open

Pass probes through

- Common bile duct
- Pancreatic duct

→ into Ampulla of Vater

F. SECTIONING

Bisect pancreas with duodenum into anterior & posterior halves
(Section passes just anterior to the probe at ampulla)

Serially slice pancreas “bread-loaf style”

- Keep slices attached by capsule
- Essential to detect multicentricity

F. TUMOUR ASSESSMENT

1. Location

Head / Body / Tail
Ampullary / Peri-ampullary / Intra-ampullary

2. Configuration

Whether Infiltrative; Circumscribed or Intraductal

3. Size

Maximum dimension (critical for staging & prognosis)

4. Extent

Confined to pancreas
Extension into:Duodenum; CBD;Peripancreatic tissue ;Stomach; Spleen ;Colon and Major vessels

5. Relationship to ducts

Pancreatic duct – dilated / narrowed
Common bile duct – compressed / infiltrated

7. Distance from margins

Pancreatic resection margin……;
CBD margin……. ;Proximal gastric margin……; Distal duodenal margin…..

8. Other organs

Pancreas (non-tumour)

Note whether Atrophied; Fibrosed ; Calcificied ;
Presence of Pancreatitis; Multifocality of tumour

Spleen (if present) Note its Weight ; Dimensions ; Cut surface – invasion / congestion

Stomach & Duodenum - Ulcers ; Haemorrhage ; Tumour infiltration

9. LYMPH NODES - Number of nodes per group; Largest dimension ; Cut surface – metastatic / reactive

VII. Bits (Fig 12.3)

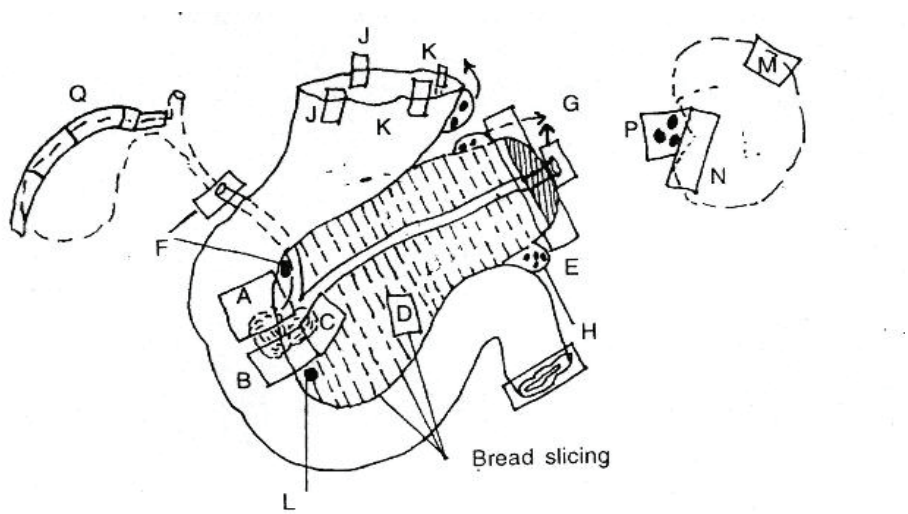


Fig 12.3 : Bits in Whipple's procedure

A–C: Tumour (include duodenum / ampulla / CBD / pancreas) D: Uninvolved pancreas
E: Pancreatic resection margin ; F: CBD margin + CBD lymph node
G: Pancreatic duct margin + superior LN group ; H: Duodenal margin + inferior LN group; J & K: Gastric margins (lesser & greater curvature) + gastric LNs
L: Pancreaticoduodenal LN group; M & N: Spleen (hilum + parenchyma with capsule)
P: Splenic LN group ; Q: Gall bladder (full length including cystic duct);

Additional blocks for:

Any suspicious lymph node

Additional lesions

Tumour: capsule + interface with adjacent parenchyma

Resection margins: pancreatic, bile duct, duodenal

Lymph nodes (peripancreatic, periduodenal, superior mesenteric)

Background pancreas (chronic pancreatitis, fatty change)

I. GROSS PEARLS -PANCREAS

Annular pancreas : encirclement of duodenum by pancreatic tissue/parenchyma; leads to constriction of the duodenal lumen. Embryonic abnormality in which the ventral primordium of the pancreas fails to rotate properly. May be seen in combination with other congenital abnormalities. The duct in annular pancreas originates anteriorly , courses to the right over the duodenum, then posteriorly and then to the left behind the duodenum passing near the common bile duct & finally joining the main pancreatic duct.

Swollen and oedematous pancreas : acute pancreatitis.

Yellow plaques & nodules seen within & outside the pancreas in the mesentery: areas of fat necrosis in pancreatitis.

Focal, segmental or diffuse irregular scarring; **cystic change** (dilated duct), calculi & calcification : chronic pancreatitis.

Hard poorly demarcated solitary mass fibrotic, **chiefly located in the head** of pancreas: ductal carcinoma.

Haemorrhage & necrotic lesion : pseudocyst, giant cell tumour of the pancreas, acute haemorrhagic pancreatitis.

Cysts & Cystic lesions of the pancreas

I. NON-NEOPLASTIC / BENIGN CYSTIC LESIONS

1. Pancreatic Pseudocyst

Most common cystic lesion of pancreas

Secondary to acute or chronic pancreatitis

No epithelial lining (key exam point). Cyst with thick irregular walls, behind or adjacent to pancreas, spreads beyond the pancreas with cloudy/haemorrhagic contents, shaggy inner surface .

Lined by granulation tissue and fibrosis

Contents: necrotic debris, blood, pancreatic enzymes (fluid shows high amylase content).

2. Retention Cysts / Duct Ectasia

Due to ductal obstruction (chronic pancreatitis, stones, tumour)

True cysts lined by flattened ductal epithelium

Often multiple, small

3. Serous Cystadenoma (Microcystic type)

(WHO: Serous cystic neoplasm – benign)

Numerous small cysts giving “honeycomb / sponge” appearance

Lined by glycogen-rich cuboidal cells

Central stellate scar may be present

Strong association with VHL syndrome

Almost always benign

4. Lymphoepithelial Cyst

Rare, benign

Squamous epithelial lining

Dense lymphoid tissue with germinal centres

Often mistaken radiologically for neoplasm

5. Lymphangioma

Congenital malformation of lymphatic vessels

Multiloculated cystic lesion

Lined by flattened endothelial cells

6. Cystic Degeneration in Mesenchymal Tumours

Schwannoma with cystic change (rare)

Usually incidental

7. Pancreatic Cysts in Genetic Syndromes

Von Hippel–Lindau disease

Multiple pancreatic cysts

Often associated with renal and hepatic cysts

Serous cystic neoplasms common

8. Parasitic Cysts

Hydatid cyst (*Echinococcus*)

Rare in pancreas

9. Developmental / Ectopic Cysts

Retroperitoneal bronchogenic cyst

Extremely rare, usually incidental

II. Cystic Neoplasms

A. BENIGN / VERY LOW MALIGNANT POTENTIAL

1. Serous Cystic Neoplasm

Benign

Does not require resection unless symptomatic

B. PREMALIGNANT / NEOPLASMS OF UNCERTAIN MALIGNANT POTENTIAL

1. Mucinous Cystic Neoplasm (MCN)

(Old term "mucinous cystadenoma / cystadenocarcinoma" replaced)

Occurs almost exclusively in women

Body & tail of pancreas

Does not communicate with pancreatic duct

Diagnostic feature: ovarian-type stroma

Spectrum:

- MCN with low-grade dysplasia
- MCN with high-grade dysplasia
- MCN with associated invasive carcinoma

2. Intraductal Papillary Mucinous Neoplasm (IPMN)

Arises from pancreatic ducts

Communicates with ductal system

Types:

- Main duct IPMN

Branch duct IPMN

Mixed type

Graded as:

Low-grade dysplasia

High-grade dysplasia

IPMN with invasive carcinoma

3. Solid Pseudopapillary Neoplasm (SPN)

(Old: Solid cystic papillary epithelial tumour / Gruber–Frantz tumour)

Young women

Large, well-circumscribed

Solid + cystic areas (due to degeneration)

Low-grade malignant potential

Excellent prognosis after resection

C. MALIGNANT CYSTIC TUMOURS

1. Pancreatic Ductal Adenocarcinoma with Cystic Change

Necrosis or dilated ducts may simulate cyst

Most common malignant tumour overall

2. Colloid (Mucinous Non-cystic) Carcinoma

Often arises from IPMN

Pools of extracellular mucin

Better prognosis than conventional PDAC

3. Acinar Cell Carcinoma (Cystic Variant)

Rare

May show cystic degeneration

III. TOPOGRAPHICAL DISTRIBUTION

Tumours Commonly Seen in the Head of Pancreas

Pancreatic ductal adenocarcinoma (and variants)

IPMN (especially main duct)

Ampullary carcinomas (periampullary region)

Tumours Common in Body & Tail

Mucinous cystic neoplasm (MCN)

Solid pseudopapillary neoplasm (SPN)

Tumours Occurring Anywhere in Pancreas

Serous cystic neoplasm

Acinar cell carcinoma

Anaplastic (undifferentiated) carcinoma

Pancreatoblastoma (children)

Neuroendocrine tumours

PEARLS

No epithelial lining → pseudocyst

Ovarian-type stroma → MCN

Duct communication → IPMN

Young female + solid–cystic → SPN

Microcystic + central scar → serous cystadenoma

VHL → multiple pancreatic cysts

IV PANCREAS MICROSCOPY-

1. WHO 5th Edition Classification (2022)

The **WHO classification of tumours of the pancreas** is part of the 5th edition WHO classification of digestive system tumours .

Epithelial tumours

Benign epithelial tumours and precursors

acinar cystic transformation of the pancreas

serous neoplasms of the pancreas

pancreatic intraepithelial neoplasia

pancreatic intraductal papillary mucinous neoplasm

pancreatic intraductal oncocytic papillary neoplasm

pancreatic intraductal tubulopapillary neoplasm

pancreatic mucinous cystic neoplasm

Malignant epithelial tumours

pancreatic ductal adenocarcinoma

pancreatic acinar cell carcinoma

pancreato -blastoma

solid pseudopapillary neoplasm of the pancreas

Pancreatic neuroendocrine neoplasms

non-functioning pancreatic neuroendocrine tumours

functioning pancreatic neuroendocrine tumours

insulinoma

gastrinoma

VIPoma

glucagonoma

somatostatinoma

ACTH-producing neuroendocrine tumour

serotonin-producing neuroendocrine tumour

pancreatic neuroendocrine carcinoma

pancreatic mixed neuroendocrine-non-neuroendocrine neoplasms (MiNENs)

Pancreatic cancer (Fig 12.4) most commonly occurs as an adenocarcinoma in the head of the pancreas.

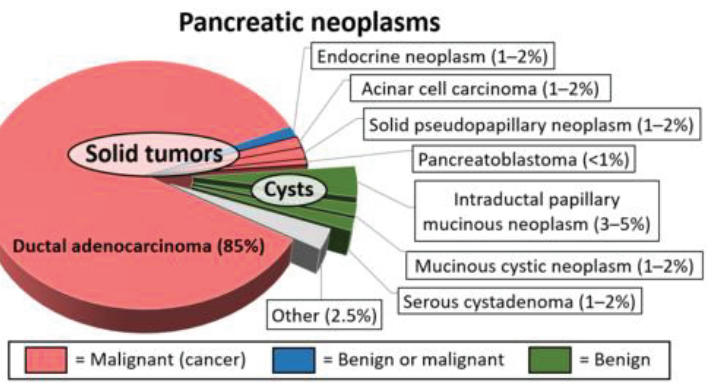


Fig12.4 : Relative incidences of various pancreatic neoplasms, with pancreatic cancers in red/pink color.

Pancreatic cancer rarely occurs in the younger age group and the median age of diagnosis is 71 years. Risk factors include chronic pancreatitis, older age, smoking, obesity, diabetes, and rare genetic conditions including multiple endocrine neoplasia type 1, hereditary nonpolyposis colon cancer and dysplastic nevus syndrome among others. About 5-10% of cases are related to genes. Pancreatic cancer involves both the endocrine and exocrine portions of the gland.

A.Exocrine Pancreas

Ductal adenocarcinoma (most common)

Variants: colloid carcinoma, medullary carcinoma, adenosquamous, undifferentiated, hepatoid

Cystic neoplasms:

Serous cystic neoplasm (SCN)

Mucinous cystic neoplasm (MCN)

Intraductal papillary mucinous neoplasm (IPMN)

Solid pseudopapillary neoplasm (SPN)

B. Endocrine Pancreas (NETs) : Pancreatic endocrine tumors are rare but the incidence is rising sharply; (not clear to what extent this reflects increased detection, especially through imaging as th ese tumours are slow to develop) .

C. Others : 1.Rare mesenchymal / hematolymphoid tumours

2.Secondary tumours (metastases)

Detail on commonly occurring tumours :

A. Ductal Adenocarcinoma of Pancreas (PDAC) : Most common (80 to 90%) form of pancreatic cancer, and is cancer arising from the exocrine part of the pancreas.

Commonly occurs in the head of the pancreas. 2/3rds occur in the head the rest in the body and tail. Symptoms occur late in the disease when they give rise to abdominal pain, weight loss, and painless jaundice. It is generally a solid & poorly demarcated tumour located in the head, hard in consistency, yellow white to grey; haemorrhage & cystic changes are uncommon. Scarring leads to a mistaken diagnosis of chronic pancreatitis.

Those situated in body & tail are more diffuse.

Microscopy – Classic Features

Architecture

Irregular, angulated, infiltrating glands (Fig 12.5)

Haphazard arrangement

Often small glands dissecting through stroma

Back-to-back glands may be seen

Cribriforming uncommon (think colorectal if prominent)

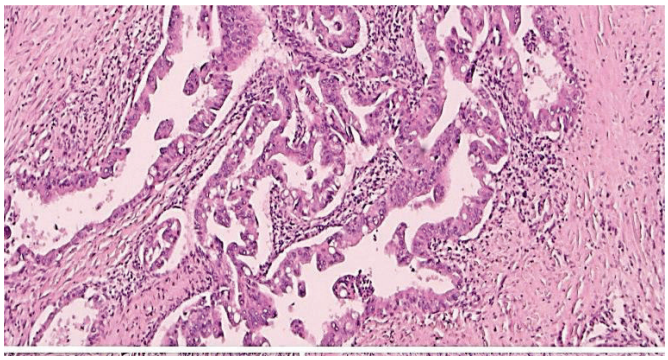


Fig 12.5 shows ductal carcinoma with irregular glands infiltrating stroma H&Ex 100

Cytology

Enlarged hyperchromatic nuclei

Irregular nuclear membranes

Prominent nucleoli

Loss of polarity

Moderate eosinophilic cytoplasm

Mitoses variable

Diagnostic Clues

"Hard tumour grossly = desmoplasia microscopically."

Perineural invasion – extremely common

Lymphovascular invasion

Pancreatic intraepithelial neoplasia (PanIN) nearby

Glands infiltrating fat without lobular architecture

Stroma - Desmoplastic reaction (dense fibrotic stroma) – hallmark; Activated fibroblasts; Inflammatory cells

IHC of PDAC

Maspin, placental S100 (S100P), IMP3 (all positive in > 90%) ; p53 expressed in most cases .

CK7, CK8, CK18, CK19, MUC1, MUC3, MUC4, MUC5AC, CEA, B72.3, CA19-9, CA125 (in 48% of cases)

Common Diagnostic Pitfall : Chronic pancreatitis vs PDAC

Chronic Pancreatitis	PDAC
Lobular architecture preserved	Disorganized
Ducts evenly spaced	Angulated, irregular
No perineural invasion	Frequent PNI
Bland nuclei	Nuclear atypia
Inflammation prominent	Desmoplasia dominant

If in doubt → look for **nerve invasion**.

Histologic Grading of PDAC

Based on gland formation:

G1 – Well differentiated

95% gland formation

G2 – Moderately differentiated

50–95% gland formation

G3 – Poorly differentiated

<50% gland formation

Solid sheets

Marked atypia

(G4 no longer used)

Important Variants of PDAC

Colloid (Mucinous non-cystic) carcinoma

Pools of extracellular mucin

Floating tumour cells

Often arises from IPMN

Better prognosis than conventional PDAC

Adenosquamous carcinoma

≥30% squamous component

More aggressive

Medullary carcinoma

Syncytial growth

Prominent lymphoid infiltrate

Often MSI-high

Better prognosis

Undifferentiated carcinoma

Pleomorphic giant cells

Highly aggressive

Undifferentiated carcinoma with osteoclast-like giant cells

Reactive multinucleated giant cells

Slightly better prognosis than pure undifferentiated

Cystic Neoplasms

MCN: mucinous epithelium + ovarian-type stroma, usually in body/tail, females

SCN: glycogen-rich clear cells, microcystic pattern

IPMN: papillary architecture, ductal connection, variable dysplasia

SPN: A solid pseudopapillary tumour is a low-grade malignant tumour of the pancreas of papillary architecture that typically afflicts young women.It shows pseudopapillary areas, hyaline globules, β-catenin nuclear positivity

COMPARISON TABLE

MCN vs IPMN vs Serous Cystic Neoplasm vs SPN

Feature	MCN	IPMN	Serous Cystic Neoplasm	Solid Pseudopapillary Neoplasm (SPN)
Typical patient	Middle-aged women	Elderly (M = F)	Elderly women	Young women
Location	Body & tail	Head > body	Anywhere	Body & tail; can occur elsewhere.
Duct communication	X -Absent	√ Present	X- Absent	X- Absent
Gross	Unilocular / multilocular large cysts	Dilated ducts with papillary excrescences	Microcystic “honeycomb”, central scar	Solid + cystic (degenerative)

Lining epithelium	Tall columnar mucin-secreting	Mucinous, papillary	Cuboidal, glycogen-rich, clear	Poorly cohesive cells. : solid portion contains sheets, cords & trabeculae of uniform rather small & fairly round cells
Nuclear features	Dysplasia (low → high)	Dysplasia graded	Bland nuclei	Grooved, uniform nuclei
Papillae	Rare	Prominent	Absent	Pseudopapillae
Ovarian-type stroma	**Present (diagnostic)**	Absent	Absent	Absent
Intracystic mucin	Abundant	Abundant	Absent	Absent
Background pancreas	Normal	Often chronic pancreatitis	Normal	Normal
Invasion	May be present	May be present	Absent	Rare
IHC (key)	CK7, MUC5AC; stroma: ER/PR	CK7, MUC5AC, MUC2 (intestinal)	CK7, inhibin	β-catenin (nuclear), CD10, vimentin
Behaviour	Premalignant	Premalignant	Benign	Low-grade malignant

Key : Mucinous cystic neoplasm (MCN); Intraductal Papillary Mucinous Neoplasm (IPMN); Solid pseudopapillary neoplasm (SPN)

Neuro endocrine Tumours

Nested / trabecular architecture

Salt-and-pepper chromatin

Immunostains: chromogranin, synaptophysin

Grading by Ki-67 and mitoses

Insulinomas (are largely benign) and gastrinomas are the most common types. For those with neuroendocrine cancers the number alive after five years is much better and varies with the subtype .

Morphologic features : Different tumour subtypes may show a predilection for the head, body or tail of the pancreas. Some subtypes (Gastrinomas, Stomatostatinomas, Vipomas) can also occur outside the pancreas. Majority of neoplasms appear well demarcated, but lack a well defined capsule. C/S : whitish yellow to pink-brown, firm in consistency. Some may be dark red, soft & cystic. Size ranges from 1-5cm. The size is not related to the severity of hormonally related symptoms. If more than one tumour is detected an MEN should be considered.

Micros : Majority are well differentiated. They are composed of relatively uniform cuboidal cells with finely granular eosinophilic cytoplasm and a centrally located round or oval nucleus with a distinct nucleolus. Mitoses are rare. Occasionally cells with large nuclei are seen. In rare cases mucin production, PAS +ve globules, clear cells, vacuolated lipid rich cells or oncocytic change may be seen. Two main histologic patterns may be observed - Trabecular pattern including ribbon like, gyriform & glandular structures; & a solid-medullary pattern. Mixed patterns may be seen. A vascular stroma with sclerosis (which may be prominent) is seen. Rare cases may show cytologic pleomorphism with nuclear hyperchromatism and their neuroendocrine nature can be detected only by immunohistochemistry.

IHC : NSE, protein gene product (PGP) 9.5, synaptophysin, chromogranins A,B, C , HSIL-19, proprotein convertases PC 2 & PC3 etc are +ve are reveal the neuroendocrine nature of the neoplasm.

Biologic behavior : Pancreatic endocrine tumours are potentially malignant neoplasms. Rate of malignancy varies among different tumour types. Insulinomas have the lowest rate of malignancy (5-15%), whereas most of the other types including non-functional tumours are malignant . Gross invasion of adjacent organs and metastasis are unequivocal evidence of malignancy.

MEN 1 associated tumours of the pancreas rarely metastasize in contrast to those of the duodenum. In general malignant metastasizing pancreatic endocrine tumours are slowly growing indolent neoplasms. Survival from the time of diagnosis ranges from 2-10 years.

Histologically they are graded as -

G1: Ki-67 ≤2%, mitoses <2/10 HPF

G2: Ki-67 3–20%, mitoses 2–20/10 HPF

G3: Ki-67 >20%, mitoses >20/10 HPF

Functioning vs non-functioning NETs

Functional tumours : Functional neoplasms account for 60-85% of pancreatic endocrine tumours. In this Insulinomas are the most frequent type (upto 70% of all cases), followed by gastrinomas. The remaining functional pancreatic tumours include Vipomas, Glucagonomas etc.

Insulinomas : Patients present with hypoglycemia, ie Whipple triad- mental confusion, weakness, fatigue & seizures while fasting; blood glucose < 50mg % and immediate relief

of symptoms following injection of glucose. The serum insulin levels are inappropriately elevated for the prevailing plasma glucose concentration & there is usually a greater proportion for proinsulin than normally found. Insulinomas can also arise in pancreas of non insulin dependant diabetics with amelioration of diabetes.

Zollinger – Ellison syndrome (ZES): Inappropriate gastrin secretion from gastrinomas causes Zollinger – Ellison syndrome. Characterised by gastrin acid hypersecretion (gastrin stimulates this hypersecretion), intractable peptic ulceration & occasionally severe diarrhea. In 60-75% of cases ZES occurs as an isolated phenomenon, in the remaining it is part of the MEN 1 syndrome.

Glucagonoma syndrome : includes a skin rash as necrolytic migratory erythema, mild glucose intolerance, normochromic normocytic anemia, weight loss, depression & a tendency to develop deep vein thrombosis. The symptomatology reflects the catabolism of excessively elevated glucagon levels.

Verner-Morrison syndrome : Excess secretion of vasoactive intestinal polypeptide (VIP) by vipomas causes the watery diarrhea, hypokalemia & achorhydria (WDHA) syndrome also called the Verner-Morrison syndrome. Besides VIP other substances like peptide, histidine & methinine may also be involved.

Ectopic hormone syndromes: are Cushing's syndrome, atypical carcinoid syndrome (due to serotonin), acromegaly (growth hormone releasing factor), paraneoplastic hypercalcemia (PTH and PTH related protein), etc

Acinar cell carcinoma :

constitutes < 5% of exocrine pancreatic tumours. More common in adults & in men. Rare in children when prognosis is better.

Occurs as a pure from or mixed with ductal or endocrine component.

Secretion of lipase & other enzymes causes multifocal fat necrosis, polyarthropathy and non bacterial thrombotic endocarditis.

Gross : occur anywhere in pancreas, 2-15 cm , well demarcated, soft, lobulated masses, pink to tan homogenous & fleshy. Necrosis & haemorrhage may be conspicuous. Invasion to adjacent structures is found at surgery.

Microscopically : Growth pattern is variable- acinar (microglandular), trabecular, solid & mixed. Acinar & solid patterns are more common. There is a rich network of thin walled vessels. Cytoplasm is abundant, eosinophilic and granular, containing PAS +ve diastase resistant zymogen granules. Nuclei are uniform, round & large with conspicuous nucleoli. Mitoses are frequent.

IHC: +ve for lipase, trypsin, chymotrypsin & α-1 antitrypsin. Amylase & CEA positivity is inconsistent.

Pancreatoblastoma : (Syn – pancreaticoblastoma, infantile pancreatic carcinoma)

Rare malignant tumour occurs exclusively in infants & children, more often in boys.

Morphology is analogous to nephroblastoma & hepatoblastoma.

Gross : Arises in head or body of pancreas as a large (5-20cm), soft wholly or partly encapsulated mass. C/S shows conspicuous haemorrhage, necrosis & cystic change.

Micros : Chiefly epithelial elements rarely mesenchymal. The former characterized by monomorphic polygonal cells with PAS +ve material in either cytoplasmic or glandular lumina, growing in solid, trabecular or acinar pattern. Mitoses is prominent and formation of squamoid nests or "corpuscles" is a characteristic feature. Mesenchymal component consists of spindle shaped cells with stromal hyalinization or myxoid change. Acinar differentiation is seen with +tivity for lipase, trypsin, chymotrypsin & α-1 antitrypsin. In addition scattered neuroendocrine or α-feto protein positive cells are identified.

Exam Pearls

Ductal adenocarcinoma: **desmoplasia + perineural invasion** hallmark

MCN: ovarian stroma diagnostic

IPMN: main-duct involvement = high-risk

SPN: young females, β-catenin nuclear positivity

NETs: G3 is high-grade, often aggressive

II. RADIOLOGY – PATHOLOGY CORRELATION

1. Mucinous Cystic Neoplasm (MCN)

Radiology

Large, solitary cyst

Body/tail

Thick wall ± septations

No duct communication

Pathology correlation

Large mucin-filled cysts

Ovarian-type stroma

Graded epithelial dysplasia

Radiology suspicion of MCN → resection recommended

2. IPMN

Radiology

Dilated main pancreatic duct (>5–10 mm)

“Fish-mouth” papilla (ERCP)

Branch duct IPMN: grape-like clusters

Pathology correlation

Papillary mucinous epithelium

Ductal communication

Intestinal / gastric / pancreatobiliary subtypes

Main duct IPMN = high malignancy risk

3. Serous Cystic Neoplasm

Radiology

Microcystic lesion

Central stellate scar ± calcification

“Honeycomb” appearance

Pathology correlation

Multiple small cysts

Clear glycogen-rich cells

No mucin, no atypia

Radiology diagnostic → surgery not needed most often

4. SPN (Solid and Cystic Pancreatic Neoplasm)

Radiology

Large, well-circumscribed mass

Mixed solid + cystic

Hemorrhagic degeneration common

Pathology correlation

Pseudo-papillae

Degenerative cystic spaces

β-catenin nuclear positivity

Young woman + solid-cystic pancreatic mass → SPN until proven otherwise

III. FROZEN SECTION APPROACH IN CYSTIC PANCREATIC LESIONS

A. WHEN FROZEN SECTION IS ASKED. For -

Pancreatic cyst of uncertain nature

Margin assessment in Whipple's

Suspicion of invasive carcinoma

B. WHAT QUESTIONS SHOULD BE ANSWERED -

Is this neoplastic or non-neoplastic?
Is it mucinous (premalignant)?
Is there invasion?
Are margins free?

C. WHAT CAN & CANNOT BE ANSWERED ON FROZEN -

√ CAN BE ANSWERED _

 Dubin–Johnson vs Rotor: histology + pigment (absent in Rotor) confirms diagnosis.

Presence of epithelial lining (vs pseudocyst)

Mucinous vs non-mucinous

Invasive carcinoma (if obvious)

Margin status (CBD, pancreatic neck)

X CANNOT RELIABLY BE ANSWERED -

Degree of dysplasia

Ovarian-type stroma (often missed)

IPMN subtype

Low-grade invasion

D. PRACTICAL TIPS FOR FROZEN SECTIOS -

Avoid freezing cyst wall only → sample solid areas/septa

Mucin alone ≠ malignancy

Bland epithelium ≠ benign (IPMN!)

Extensive sampling on permanent sections is mandatory

E. FROZEN SECTION PITFALLS

1. Serous cystadenoma may look “empty”
2. IPMN epithelium may denude
3. SPN may be misread as neuroendocrine tumour
4. Pseudocyst may show reactive epithelium mimicking neoplasia

ONE-LINE MEMORY PEARLS

MCN = mucin + ovarian stroma + no duct
IPMN = mucin + duct communication
Serous = microcystic + clear cells
SPN = young female + solid-cystic + β-catenin

V PANCREAS – SYNOPTIC REPORT

Specimen Type

☐ Core needle biopsy ☐ Wedge biopsy ☐ Distal pancreatectomy (body/tail)
☐ Pancreaticoduodenectomy (Whipple)
☐ Total pancreatectomy
☐ Specimen dimensions: ___ × ___ × ___ cm
☐ Other (specify): _____

Tumour Site

☐ Head
☐ Uncinate process
☐ Body
☐ Tail
☐ Periampullary
☐ Other / Not specified
☐ Ampullary carcinoma

Tumour Size

Greatest dimension: ___ cm
Additional dimensions: ___ × ___ cm

Histologic Type

Pancreatic ductal adenocarcinoma (PDAC)

☐ Conventional
☐ Colloid carcinoma (mucinous non-cystic carcinoma) ☐ Medullary carcinoma

☐ Adeno-squamous carcinoma
☐ Undifferentiated carcinoma (including anaplastic-type variants)
☐ Undifferentiated carcinoma with osteoclast-like giant cells

Cystic Neoplasms

☐ Intraductal papillary mucinous neoplasm (IPMN)
  ☐ Low grade
  ☐ Intermediate grade
  ☐ High grade
  ☐ With associated invasive carcinoma (specify type)

☐ Mucinous cystic neoplasm (MCN)
  ☐ Low grade
  ☐ High grade
  ☐ With associated invasive carcinoma

☐ Serous cystadenoma

☐ Solid pseudopapillary neoplasm (SPN)

Acinar Neoplasms

☐ Acinar cell carcinoma

Pancreatoblastoma

Pancreatic Neuroendocrine Tumour (PanNET)

Grade (WHO grading mandatory):
☐ G1 (Ki-67 <3%)
☐ G2 (3–20%)
☐ G3 (>20%)

Ki-67 index: ___ %

Mitotic count: ___ per 10 HPF

Histologic Grade (PDAC only)

☐ G1 well differentiated
☐ G2 moderately differentiated
☐ G3 poorly differentiated

(G4 no longer used for PDAC in modern classification)

Tumour Extension

☐ Limited to pancreas
☐ Extends into peripancreatic soft tissue
☐ Involves duodenum
☐ Involves bile duct
☐ Involves stomach
☐ Involves spleen
☐ Involves colon
☐ Involves major vessels (specify) ☐ Retroperitoneal (posterior) soft tissue involvement

Margins (CRITICAL in pancreas)

Pancreatic neck margin:
☐ Negative (distance ___ mm)
☐ Positive

Uncinate (SMA) margin:
☐ Negative (distance ___ mm)
☐ Positive

Posterior margin:
☐ Negative
☐ Positive

Common bile duct margin:
☐ Negative
☐ Positive

Proximal (gastric/duodenal) margin:
☐ Negative
☐ Positive

Distal margin (if applicable): Anterior surface involved ☐ Yes ☐ No

☐ Negative
☐ Positive

R status:
☐ R0 (no tumour within 1 mm of margin)
☐ R1 (tumour ≤1 mm from margin)
☐ R2 (gross residual disease) ***(1 mm rule is now widely adopted)***

Lymphovascular invasion

☐ Present

☐ Absent

Perineural invasion : ☐ Present ; SS☐ Absent

Lymph Nodes

Number examined: ___

Number positive: ___ Lymph node ratio: ___

Associated lesions

Highest grade PanIN: ___

Chronic pancreatitis ☐

Other: _____ IPMN-associated changes ☐

Final integrated report:

Procedure

Tumour type

Tumour size

Grade

Extent

Margins (R status)

Lymph nodes

pTNM stage

Signature ……. Date …….

AJCC 8th Edition TNM – PANCREATIC DUCTAL ADENOCARCINOMA-Pathologic staging

T – Primary Tumour (AJCC 8th)

T1 ≤ 2 cm : T1a ≤ 0.5 cm ; T1b >0.5–1 cm ; T1c >1–2 cm

T2 >2 cm and ≤4 cm ; T3 >4 cm

T4 Tumour involves celiac axis, superior mesenteric artery, and/or common hepatic artery (Unresectable disease)

Tumour Regression (if neoadjuvant therapy given):

☐ Not applicable

☐ Present (specify grade if used)

Extension beyond pancreas into peripancreatic soft tissue does NOT automatically make T3 anymore.

N – Regional Lymph Nodes (AJCC 8th)

NX – not assessed
N0 – no metastasis
N1 – 1–3 positive nodes
N2 – ≥4 positive nodes

Your older "N1a/N1b" classification is outdated.

M – Distant Metastasis

M0 – none
M1 – distant metastasis

Prognostic Factors Most important:

R1 margin (≤1 mm) ; Lymph node ratio ; Tumour size (>4 cm) ;Perineural invasion

Lymphovascular invasion ; N2 status (≥4 nodes) ; CA 19-9 elevated ; Poor differentiation

Flow cytometry, AgNOR, RB gene — no longer clinically relevant in modern reporting.

ASSESS YOURSELF PANCREAS

Long Essay Questions (10 marks each)

1. Discuss the pathology of pancreatic ductal adenocarcinoma (PDAC), including morphology, spread, and prognostic factors.
2. Describe the **c**ystic neoplasms of the pancreas, with emphasis on their classification and malignant potential.
3. Write an essay on pancreatic neuroendocrine tumours (PanNETs), including grading and prognostic factors.

Short Essays (5 marks each)

4. PanIN lesions and their significance in pancreatic carcinogenesis.
5. Grossing of Whipple specimen (pancreaticoduodenectomy).
6. Differences between IPMN and mucinous cystic neoplasm (MCN).

Short Answer / Point Questions (3 marks)

7. Enumerate the important margins in pancreaticoduodenectomy specimens.
8. List the prognostic factors in pancreatic ductal adenocarcinoma
9. Briefly describe the embryological basis and the clinical presentation of annular pancreas in adults versus neonates.

MCQs –

1.The most important prognostic factor in pancreatic ductal adenocarcinoma is:

a) Tumour location
b) Tumour size
c) Margin status
d) Presence of cystic change

2. The grading of pancreatic neuroendocrine tumours is based primarily on:

a) Tumour size
b) Ki-67 index
c) Presence of necrosis
d) Lymph node status

3. Which of the following is characteristic of mucinous cystic neoplasm (MCN)?

a) Occurs predominantly in males
b) Communicates with pancreatic duct
c) Ovarian-type stroma
d) Always malignant

Answer Key for MCQs

1. .c
2. b
3. C

Chapter 13
Paediatric Tumours

A Renal Childhood tumours ; B Neuroblastoma; C Hepatoblastoma; D Retinoblastoma; E Germ Cell Tumours

AI Renal Childhood tumours Anatomy ;AII Renal Childhood Tumours Grossing Techniques;AIII Renal Childhood Tumours Gross Pearls; AIV Renal Childhood Tumours Microscopy; AV Renal Childhood Tumours Synopptic Reporting.

B I Hepatoblastoma Anatomy; B II Hepatoblastoma Grossing Techniques; B III Hepatoblastoma Gross Pearls ; B IV Hepatoblastoma Microscopy ; B V Hepatoblastoma Synoptic Reporting.

C I Neuroblastoma Anatomy; CII Neuroblastoma Grossing Techniques ; C III Neuroblastoma Gross Pearls; C IV Neuroblastoma Microscopy; C V Neuroblastoma Synoptic Reporting.

D I Retinoblastoma Anatomy; D II Retinoblastoma Grossing Techniques; D III Retinoblastoma Gross Pearls; D IV Retinoblastoma Microscopy; D V Retinoblastoma Synoptic Reporting

E I Paed Germ Cell Tumours Anatomy (relevant to grossing) ; E II Paed Germ Cell Tumours Grossing Techniques; E III Paed Gross Pearls; E IV Paed Germ Cell Tumours Microscopy; E V Paed Germ Cell Tumours Synoptic Reporting.

A I RENAL CHILDHOOD TUMOURS ANATOMY

Given in chapter 9

A II RENAL CHILDHOOD TUMOURS GROSSING TECHNIQUES

General Principles in Grossing Paediatric Neoplasms

Grossing of paediatric neoplasms requires special attention due to the following factors:

Embryonal Nature and Heterogeneity

Many paediatric solid tumours are embryonal and demonstrate divergent differentiation (epithelial, mesenchymal, neural, etc.). Since histologic subtype and percentage of differentiation directly influence prognosis and risk stratification, *generous and representative sampling is mandatory.*

Small Round Blue Cell Tumours (SRBCTs)

Diagnostic grey zones frequently occur in small round blue cell tumours. Accurate diagnosis may require:

Immunohistochemistry

Molecular studies

Cytogenetics

Flow cytometry (in selected cases)

Need for Fresh Tissue

Whenever feasible, paediatric tumour specimens should be submitted fresh and intact to the surgical pathology laboratory to allow appropriate triage.

Fresh tumour tissue (from viable, non-necrotic areas) may be allocated for:

Molecular studies (cytogenetics, FISH, PCR, NGS)

Snap freezing (liquid nitrogen) for DNA/RNA analysis

Flow cytometry (RPMI medium, when indicated)

Touch imprints

Biobanking, where applicable

Sampling for ancillary studies should never compromise:

Evaluation of resection margins

Tumour staging

Assessment of tumour rupture

A II Renal Childhood Tumours - Wilms Tumour – Grossing Protocol

1. Specimen Types

Radical nephrectomy

Nephrectomy with adrenal

Nephrectomy with IVC thrombus

Post-chemotherapy resection

Bilateral tumours (partial resections)

2. Orientation and Inking

Ink:

Renal capsule surface

Perirenal fat margin

Renal vein margin

Ureteric margin

Gerota's fascia

Open renal vein longitudinally to check for tumour thrombus.

3. External Examination

Specimen size and weight

Capsule:

- Intact?
- Ruptured?

Surface tumour deposits?

4. Tumour Description

Size (3 dimensions)

Location (pole/central)

Replacing entire kidney?

Cut surface:

- Tan-grey, lobulated
- Necrosis
- Hemorrhage
- Cystic change
- Post-chemotherapy changes

5. Extent of Tumour

Carefully assess:

Capsular invasion

Perirenal fat invasion

Renal sinus involvement (very important)

Pelvicalyceal involvement

Renal vein involvement

IVC extension (measure length)

Adrenal involvement

Gerota's fascia involvement

Adjacent organ invasion

6. Tumour Rupture

Document clearly:

Preoperative rupture

Intraoperative spill

Capsular breach with surface tumour

(Critical for Stage III)

7. Lymph Nodes

Sample hilar and para-aortic nodes

Submit all identified nodes

Record number

8. Sampling Protocol

Minimum recommended:

Tumour with capsule (×2) : The capsule should never be stripped in paediatric tumour, as this would distort any morphology relating to infiltration of the capsule. Any nephrogenic rests normally situated in the subcapsular region may not be detected microscopically if they have been peeled away with the capsule.

Tumour with perirenal fat

Tumour with renal sinus : *Method of sampling the sinus:*

The bits taken should enable histological examination of the sinus and its resected soft tissue margin. Overnight fixing after the specimen is bisected enables adequate fixation for subsequent sectioning of the sinus as a horizontal slice which may be then divided and given in two or more bits with the soft tissue resected margin being inked. In the fresh state however the margin may be inked and taken separately, and the contents of the sinus may be scooped out in a separate capsule with portions of any adherent vessels or ureter.

Tumour with pelvis

Tumour with renal vein

Renal vein margin

Ureteric margin

Adrenal (if present)

Gerota's fascia (if suspicious)

Representative viable areas

Necrotic areas

All lymph nodes

The possible sections that could be taken for a routine Wilm's Tumour are shown below. The general rule followed should be one generous bit per one cm of the greatest tumour dimension.

Bits (Fig 13.1)

A to D - neoplasm with renal capsule and adjacent parenchyma.

E - neoplasm and portion of hilar sinus; tumour thrombus in renal vein with portion of vein (adjacent renal artery and ureter if necessary may be included).

F - soft tissue - hilar sinus (if not included in E).

G - Soft tissue surgical margin at hilar sinus.

H & J - uninvolved kidney to include nephrogenic rests.

K - Renal artery and vein surgical margin.

L - Ureteric surgical margin.

M &N - Lymph nodes - perihilar, others.

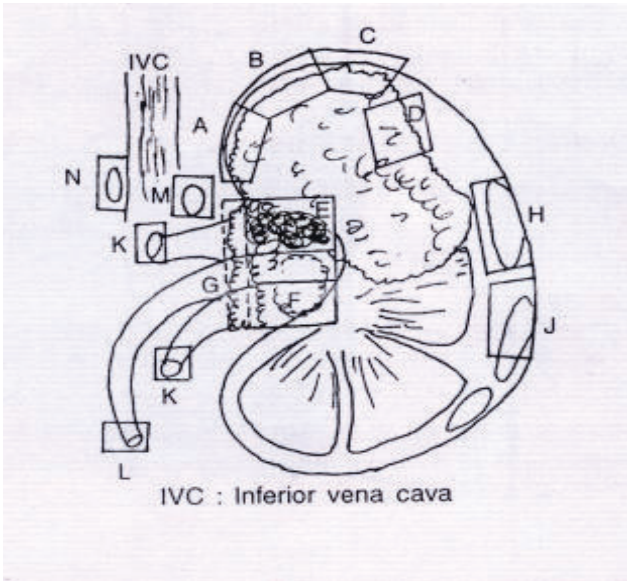


Fig 13.1

15.Post-chemotherapy:

Extensive sampling to assess:

Viable blastemal component

Percentage necrosis

Stromal differentiation

A III RENAL CHILDHOOD TUMOURS GROSS PEARLS

Homogenous and pink, well circumscribed kidney mass : Wilms tumour

Medullary-based tumour: Collecting duct carcinoma or renal medullary carcinoma.

Single large homogenous mass, average 10cms in size, in medulla with cyst formation & haemorrhage : Clear cell rhabdoid sarcoma

Light tan to yellow colored tumor mass situated within the lower pole of the kidney with a fibrous, whorled cut section reminiscent of a leiomyoma- mesoblastic nephroma.

Tumour with lack of encapsulation (as seen in Wilm's seen in Wilm's and clear cell sarcoma,

located medially in the kidney with infiltration of the renal sinus and pelvis , typically yellow grey or light tan crumbly tumours. Haemorrahge and necrosis are common- rhabdoid tumour

A IV RENAL CHILDHOOD TUMOURS MICROSCOPY

Classification of kidney tumours in infants & children

Nephroblastoma (Wilms tumour)

- Unfavourable histology
- Favourable histology

Cystic partially differentiated nephroblastoma

Mesoblastic nephroma

Clear cell sarcoma

Rhabdoid tumour

Rare tumours

Wilms Tumour (Nephroblastoma)

Epidemiology

- Most common renal tumour in children
- Peak age: 2–5 years
- Bilateral in 5–10%

Associated Syndromes

- WAGR (WT1)
- Denys–Drash syndrome (WT1)
- Beckwith–Wiedemann syndrome (11p15, WT2 locus)

Gross

Large, well-circumscribed mass

Soft, tan-grey with hemorrhage and cysts

Microscopy (Fig 13.2

Triphasic pattern:

Blastemal

Epithelial

Stromal

Anaplasia defines unfavourable histology

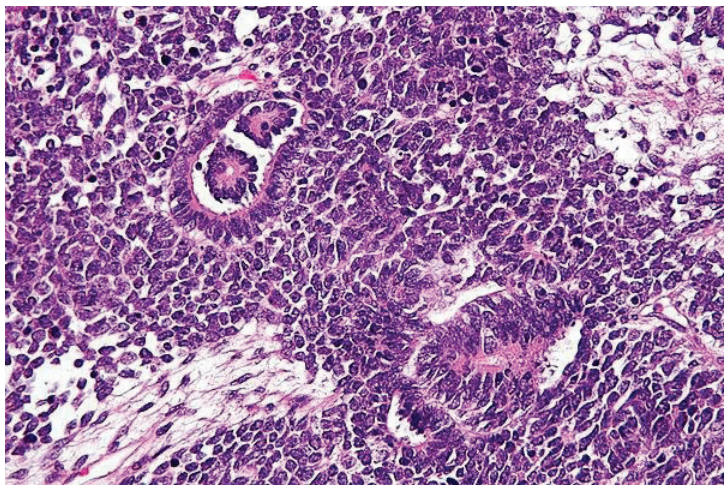


Fig 13.2 – (Libre pathology)shows Wilm's tumour with blastema and epithelial elements. H& Ex 100

NWTS Classification

Favourable histology

Unfavourable histology (anaplasia)

Anaplasia (defines unfavourable histology):

Markedly enlarged hyperchromatic nuclei

Atypical multipolar mitoses

Nuclei ≥3× size of blastemal cells

Classification (NWTS/COG):

Favourable histology

Unfavourable histology (anaplasia)

Cystic Partially Differentiated Nephroblastoma

Resembles cystic nephroma grossly

Wilms tumour elements confined to septa

Elements typical of Wilms' are contained in the septa; may be inconspicuous, therefore tumours should be sampled excessively.

Mesoblastic Nephroma

Most common renal tumour in neonate

Comprises < 3% of primary renal tumours in children.

Occur in first 3 months of life and are uncommon in children.

Associated with polyhydramnious and prematurity.

Abdominal mass is the presenting symptom and vast majority are cured by surgical excision.

Benign but locally infiltrative

Grossly large with non encapsulation.

Micros : moderately cellular proliferation of interlacing bundles of spindle shaped cells. Entrapment of glomeruli and tubules is common.Cellular variant is more aggressive

Stromal-predominant Wilms tumour

Clear Cell sarcoma of the kidney :

originally called "bone metastasizing renal tumour of childhood" is a highly malignant neoplasm comprising 6% of paediatric renal tumours. Occurs in the same age range as Wilm's tumour.

"Characterized by **BCOR gene alterations"**

Gross : well circumscribed, cut surface is variable-homogenous, grayish lobular, pink soft areas or firm and whorled. Occasionally abundant mucin production is seen as well as cystic change.

Microscopy : monotonous array of cells with lightly staining or vacuolated cytoplasm and indistinct borders. Nuclei contain finely dispersed chromatin. In the classic pattern the cells are arranged in sheets with intervening small blood vessels. An infiltrative border is seen. Other patterns include spindle, hyaline sclerosis, cystic change and palisading.

The propensity for metastasis to bone is marked, atleast 10 times more likely as compared to other paediatric tumours.

Rhabdoid tumor of kidney :

Most malignant of the renal neoplasms of childhood , this tumour usually metastasizes widely and causes death within 12 months of diagnosis

The patients are very young at the time of diagnosis (rare after 3years) and there is a 1.5 : 1 male preponderance.

Microscopy : diffuse and monotonous pattern of polygonal cells with abundant eosinophilic cytoplasm and spheroidal nuclei with thick nuclear membranes and prominent large nucleoli. Often the cytoplasm contains large eosinophilic globular inclusions which displaces the nucleus.

Exam Pearls

Triphasic pattern → diagnostic of Wilms tumour

Anaplasia → adverse prognosis

Clear cell sarcoma → bone metastasis

Rhabdoid tumour → worst prognosis

Mesoblastic nephroma → neonatal tumour

"Loss of **INI1 (SMARCB1)** expression → diagnostic hallmark" →rhabdoid tumour

"Characterized by **BCOR gene alterations"** → clear cell sarcoma

V RENAL CHILDHOOD TUMOURS SYNOPTIC REPORTING

For kidney childhood Tumours

Gross

Total nephrectomy □ Partial □ Portion of kidney□ Other□

Side: Right □ Left □ Not specified □

Size of kidney with tumour : ……..(length) x ……. x……..cms.

Location of tumour : Upper pole □ Lower pole □ Medulla □ Other □

Multicentric Yes □ No□

Size of tumour : …..x…..x…..cms (largest dimension if multifocal).

Adrenal attached Yes □ No □

Tumour infiltrates the renal capsule □

Tumour infiltrates the perirenal tissue /fat □

Tumour infiltrates Gerota's fascia □

Tumour infiltrates adrenal gland □

Cut surface : Variegated □ Homogenous □ Cystic□

Microscopy :

Presence of tumour Yes □ Benign □ Malignant□

No□

Histological subtype : Specify ……

Histologic grade ……

Tumour : confined to kidney □

infiltrates renal capsule□

Histology type in Wilm's :…… (triphasic / blastemal predominant / etc.)

Anaplasia in Wilm's : Present□ Absent □

Tumour margins : infiltrates parenchymal cut margin (perinephric fat, in partial nephrectomy) □

infiltrates perirenal fat /renal sinus fat □

infiltarates Gerota's fascia□

infiltrates Adrenal gland□

infiltrates renal vascular cut margin□

ureteric cut margin □

Lymphovascular invasion : Present □ Absent □

Renal vein invasion Yes □ No□

Regional lymphnodes : No of nodes examined …….

No of nodes involved……..

Distant metastasis : Cannot be assessed

Known , specify site……

Staging criteria for Wilms tumor in SIOP and COG Systems ("Two commonly used staging systems"*)*

Stage	SIOP (pre-operative chemotherapy)	COG (primary surgery)
I	Tumor is limited to the kidney and completely resected	Tumor is limited to the kidney and is completely resected
	Tumor is present in the perirenal fat but is surrounded by a fibrous (pseudo)capsule; the (pseudo)capsule might be infiltrated by viable tumor, which does not reach the outer surface	Renal capsule intact; not penetrated by tumor
	Tumor might show protruding ('botryoid') growth into the renal pelvis or ureter but does not infiltrate their walls	Tumor not ruptured or biopsied prior to removal
	The vessels or the soft tissues of the renal sinus and not involved by tumor; intrarenal vessel involvement might be present	No tumor invasion of veins or lymphatics of renal sinus
	** Fine needle or tru-cut biopsy do not upstage tumor*	No nodal or hematogenous metastases
		No rupture or biopsy prior to removal
II	Viable tumor is present in the perirenal fat and is not covered by a (pseudo)capsule but is completely resected (resection margins are clear)	Tumor in the perirenal fat but completely resected
	Viable tumor infiltrates the soft tissues of the renal sinus	Tumor infiltrates the renal sinus or blood and lymphatic vessels outside the renal parenchyma but is completely resected
	Viable tumor infiltrates blood or lymphatic vessels of the renal sinus or of the perirenal tissue	Tumor infiltrates adjacent organs or vena cava but is completely resected
	Viable tumor infiltrates the wall of the renal pelvis or the ureter	
	Viable tumor infiltrates the vena cava or adjacent organs (except the adrenal gland) but is completely resected	

III	Viable tumor present at resection margin(s)	Residual tumor or nonhematogenous metastases confined to abdomen
	Abdominal lymph nodes contain viable or nonviable tumor	Involved abdominal lymph nodes
	Viable or nonviable thrombus present at resection margins of ureter, renal vein or inferior vena cava	Peritoneal tumor implants
	Viable or nonviable tumor thrombus in the inferior vena cava removed piecemeal by a surgeon	Tumor spillage before or during surgery
	Preoperative or intraoperative tumor rupture, if confirmed by microscopic examination (viable tumor at the surface of the specimen at the area of rupture)	Gross residual tumor in abdomen
	Wedge or open biopsy before preoperative chemotherapy or surgery	Biopsy of tumor (including fine needle biopsy) prior to removal of kidney
	Tumor implants (viable or nonviable) in the abdomen	Resection margins involved by tumor or transection of tumor during resection (i.e. piecemeal excision of tumor)
	Tumor (viable or nonviable) penetrated through the peritoneal surface	
IV	Hematogenous metastases (lung, liver, bone, brain) or lymph node metastases outside the abdominopelvic region	Hematogenous metastases or spreads beyond abdomen
V	Bilateral tumors at diagnosis; each side should be substaged according to the above criteria	Bilateral tumors at diagnosis; each side should be substaged according to the above criteria

Risk Stratification for Wilm's Tumour Is based on:

Stage

Histology (favourable vs unfavourable)

Molecular alterations (e.g., 1q gain)

B I LIVER- ANATOMY (FOR HEPATIC RESECTION - HEPATOBLASTOMA)

The liver is the largest solid organ in the body, located in the right upper quadrant and extending into the epigastrium and left hypochondrium. It is covered by a thin fibrous capsule called as the Glisson's capsule.

External Anatomy

Divided into right and left lobes by the falciform ligament.

Inferior surface shows the porta hepatis, through which pass:

- Portal vein
- Hepatic artery
- Bile duct

The gallbladder lies in a fossa on the inferior surface.

The bare area lies posteriorly in contact with the diaphragm.

The inferior vena cava (IVC) runs in a groove on the posterior surface.

Functional Anatomy

Functionally the liver is divided into 8 independent segments, each with:

Independent portal venous inflow

Hepatic arterial supply

Biliary drainage

Venous outflow

This segmental anatomy forms the basis of hepatic resections.

Vascular Anatomy

It has a dual blood supply:

- Portal vein (≈75% of blood flow)
- Hepatic artery (≈25%)

Venous drainage via three major hepatic veins (right, middle, left) into the IVC.

Lymphatic Drainage

Hilar (periportal) nodes

Peripancreatic nodes

Celiac nodes

Relevance for Hepatoblastoma Resection & Grossing

Assessment must include:

Segmental involvement

Capsular breach

Vascular invasion (portal vein/hepatic veins/IVC)

Hilar structure involvement

Resection margins

Lymph nodes

B II LIVER - HEPATOBLASTOMA –-GROSSING TECHNIQUES

General Principals -The basic description and sectioning follow standard hepatic resection protocols. It is important to know the staging of the tumour before grossing. Gross photographs are recommended when facilities permit.

Grossing in Special Situations

Post-embolization / in cases where preoperative therapy is given :
Sample the *periphery* of tumour nodules, as viable tumour may be limited to these areas.

Large feeder vessels or vascular abnormalities:
Include sections demonstrating vascular relationships, particularly when vascular origin or influence is suspected.

Staging of Paediatric Liver Tumours

The Children's Cancer Study Group (CCSG) staging system has largely been replaced by:

PRETEXT (Pre-treatment Extent of Disease) system

Used by SIOPEL/International Paediatric Liver Tumour Strategy Group and presently incorporated into current risk stratification protocols.

PRETEXT = PRE-Treatment Extent of disease

The liver is divided into **4 sections**:

Left lateral (II, III)

Left medial (IV)

Right anterior (V, VIII)

Right posterior (VI, VII)

PRETEXT Groups (hepatoblastoma)

Group	Definition
PRETEXT I	3 contiguous sections free of tumour
PRETEXT II	2 contiguous sections free
PRETEXT III	1 section free
PRETEXT IV	All 4 sections involved

Annotation Factors can be used when doing Grossing-

V – Hepatic vein/IVC involvement

P – Portal vein involvement

E – Extrahepatic extension

F – Multifocality

R – Tumour rupture

C – Caudate lobe involvement

N – Nodal disease

M – Distant metastasis

Gross findings must help assess these parameters.

Hepatoblastoma – Grossing Protocol

1. Specimen Types

Partial hepatectomy

Lobectomy

Extended resection

Post-chemotherapy resection

Explant (rare)

2. Orientation and Inking

Identify anatomical landmarks.

Ink:

Parenchymal resection margin

Vascular margins

Bile duct margin

- Capsular surface (if concern for rupture)

Allow ink to dry before sectioning.

3. External Examination

- Measure specimen (3 dimensions)
- Weight
- Capsule:
  - Intact?
  - Ruptured?
  - Tumour on surface?

4. Tumour Description

- Location (segment/section)
- Size (3 dimensions)
- Number (solitary/multifocal)
- Cut surface:
  - Fetal/embryonal appearance
  - Cystic areas
  - Necrosis
  - Hemorrhage
  - Calcification
  - Post-chemotherapy changes

5. Extent of Tumour

Document:

- Capsular invasion
- Extrahepatic extension
- Vascular invasion:
  - Portal vein
  - Hepatic veins
  - IVC
- Bile duct involvement

Caudate lobe involvement

Adjacent organ invasion

Tumour rupture

6. Lymph Nodes

Sample hilar and periportal nodes

Record number submitted

7. Sampling Protocol / Bits

General rule:
At least 1 section per cm of greatest tumour dimension

Section *perpendicular to the resection margin* at approximately 1 cm intervals.

Submit the margin entirely when close or grossly involved.

Prepare a diagram indicating:

Tumour location

Relation to margins

Sections submitted

Include:

Tumour with capsule

Tumour with adjacent normal liver

Tumour with resection margin (perpendicular)

Vascular invasion (if present)

Necrotic areas

Viable tumour areas

All margins

All lymph nodes

The possible sections that could be taken for a routine hepatic neoplasm are shown below (Fig 13.3). The general rule followed should be one generous bit per one cm of the greatest tumour dimension.

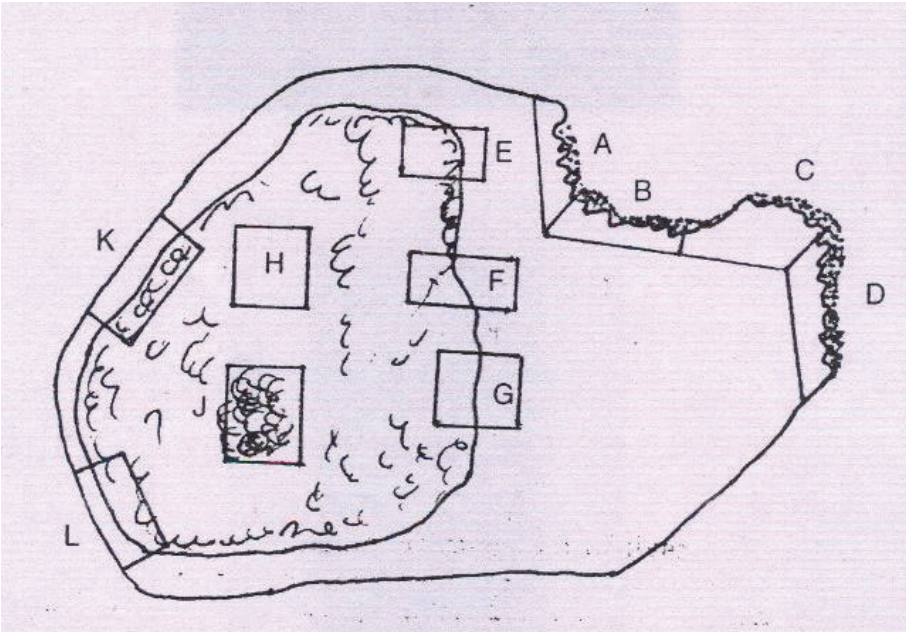


Fig 13.3 Hepatic resection in neoplasm

A-D - Inked surgical margin *to* evaluate the completeness of the resection for staging purposes.

E-G - Sections- of tumour with adjacent normal liver to ensure comparison of tumour morphology with normal liver.

H, J, K & L- Varied tumour areas representing necrosis etc. and capsular study.

Other bits from multifocal lesions if any (see fig 13.3)

Post-chemotherapy cases:
Extensive sampling to assess percentage necrosis.

B III GROSS PEARLS – HEPATOBLASTOMA

Grossly-

Single mass, often very large when detected and may weigh >1000g, solid and well circumscribed .

Cut surface is variegated according to the proportion of its histologic components brown to green, fibrous or calcified.

Often shows areas of necrosis, cystic change and haemorrhage.

Wilms Tumour vs Hepatoblastoma – Grossing & Staging Highlights

Feature	Wilms Tumour (Nephroblastoma)	Hepatoblastoma
Typical Age	2–5 years	<3 years (peak <2 yrs)
Organ	Kidney	Liver
Bilaterality	5–10% (Stage V in COG)	Rare (multifocality possible)
Capsule	Often well circumscribed, pseudo capsule	May be well circumscribed; can show capsular breach
Key Gross Concern	Renal sinus & vein invasion	Vascular invasion (portal/hepatic vein/IVC)
Critical Margin	Renal vein margin	Parenchymal resection margin
Important Spread	Renal vein → IVC → RA	Portal vein/hepatic veins
Lymph Nodes	Hilar, para-aortic	Hilar, periportal
Tumour Rupture	Major staging factor (Stage III)	Important PRETEXT annotation (R)
Post-chemo Changes	Common in SIOP protocol	Common in PRETEXT-based protocols
Sampling Rule	≥1 section/cm	≥1 section/cm
Special Sampling	Renal sinus, thrombus	Tumour-normal interface, vascular invasion
Present Staging	COG (I–V) / SIOP	PRETEXT (I–IV + annotations)
Unique Staging Feature	Bilaterality = Stage V	Section-based (4 liver sections)
Syndromic Associations	WAGR, Denys-Drash, BWS	BWS association possible
Prognostic Histology	Anaplasia (focal vs diffuse)	Epithelial vs mixed; small cell undifferentiated variant important

BWS- Beckwith Weidman Syndrome

B IV HEPATOBLASTOMA MICROSCOPY

Hepatoblastoma

accounts for 0.2 to 5.8% of all malignancies in childhood and 25 to 45% of all primary hepatic tumours.

Hepatoblastoma is a malignant liver tumor that reiterates the hepatic development and has variable epithelial and mesenchymal components

Vast majority occur in patients under 5 years and 66% in the first 2 years of life, primarily in infants.

One third of patients have some form of congenital anomaly- hemihypertrophy, cleft palate, talipes, cardiac and renal malformations, Beckwith Weidemann and Down's syndromes and nephroblastomas.

Trisomy of chromosomes 2 & 20 are common.

Raised serum and urinary levels of hCG observed . Serum levels of α feto-protein are often elevated.

Tumours composed exclusively of immature hepatocytic elements. Epithelial variant may show- embryonic subtype, fetal subtype, or mixtures of both. Foetal component consists of hepatocytes arranged in irregular laminae, two cell thick recapitulating those of foetal liver. Foci of extramedullary hemopoiesis is seen commonly with the fetal pattern.

The embryonal component is more immature with a solid pattern of growth but may also exhibit ribbons, rosettes, papillary formations etc.

Others may be composed of small anaplastic cells and still others a cholangiolar pattern with tubules. Some tumours show cells resembling liver cell carcinoma ie a macro trabecular pattern.

Mixed epithelial and mesenchymal elements may be seen in $1/4^{th}$ of hepatoblastomas. The mesenchymal component is spindle celled or osteoid, rarely cartilage and neural tissue.

Rare variants are the teratoid, mucoid and rhabdoid types.

IHC : hCG, CEA, EMA, hepatic cytokeratins 8 &18, biliary cytokeratins 7 & 19,

α-1 antitrypsin, AFP and Hep Par (negative in small cell type) ferritin, S-100

protein, glutamine synthetase, chromogranin A and NSE in varying proportions.

Cytogenetics : trisomies 2, 8 & 20; Fetal type usually diploid, embryonal often aneuploid.

Spread : occurs to regional lymph nodes, lung, brain and other organs. Fetal pattern is associated with a better prognosis.

B V -HEPATOBLASTOMA SYNOPTIC REPORTING

Specimen

Right hepatectomy ☐

Left hepatectomy ☐

Segmentectomy ☐

Explant liver ☐

Other ☐ Specify ……

Tumour

Solitary ☐ Multiple ☐

Location ……Segment …….

Size (largest): …… cm

Vascular Invasion

Microvascular invasion ☐ Present ☐ Absent

Macrovascular invasion ☐ Present ☐ Absent

Histology

Epithelial……

Foetal……..

Embryonic ……..

Mesenchymal ……. (type……..)

Mixed ……..

Extra medullary haemopoiesis ……..

PRETEXT Groups ……..

Margins

Parenchymal margin: Clear ☐ Involved ☐ Distance …… mm

Bile duct margin (if applicable): Clear ☐ Involved ☐

Lymph Nodes

Examined ……

Positive ……

Comments ……..

Signature……. Date ……

C I NEUROBLASTOMA ANATOMY

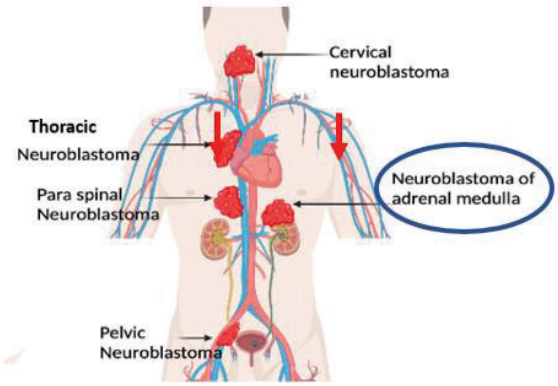


Fig 13.4 : Sites of occurrence of neuroblastoma in the sympathetic chain

Anatomy (Relevant to Grossing)

Neuroblastoma is a malignant embryonal tumour arising from primitive neural crest cells forming the sympathetic nervous system.

1. Sites of Origin (Fig 13.1)

Adrenal medulla – most common site (≈35–40%)

Extra-adrenal sympathetic chain, including:

Paraspinal retroperitoneum

Posterior mediastinum

Cervical sympathetic chain

Pelvis

The tumour follows the **distribution of sympathetic ganglia**.

2. Adrenal Anatomy (Important for Adrenal Neuroblastoma)

Adrenal gland consists of:

Cortex (zona glomerulosa, fasciculata, reticularis)

Medulla (chromaffin cells derived from neural crest)

Relations:

Right adrenal: adjacent to IVC and liver

Left adrenal: related to spleen, pancreas, and left kidney

Rich vascularity:

Central adrenal vein (short on right → IVC; longer on left → renal vein)

Relevance:
Neuroblastoma frequently:

Envelops major vessels (aorta, IVC)

Extends into renal hilum

Invades kidney or liver

Shows vascular encasement without frank luminal invasion (a characteristic feature)

3. Paraspinal Anatomy

Tumours may extend through neural foramina into spinal canal → "dumbbell tumour"

Possible spinal cord compression

Posterior mediastinal masses common in infants

4. Pattern of Spread

Lymph nodes (regional and distant)

Bone marrow (very common)

Cortical bone (skull, long bones)

Liver (especially in infants – stage MS)

Skin (blueberry muffin lesions)

C II NEUROBLASTOMA – GROSSING TECHNIQUES

Sample:

Viable solid areas

Necrotic areas

Areas with different texture

Interface with kidney/adrenal capsule

Vascular margins

Lymph nodes

Look for:

Nodules of maturation (may indicate ganglioneuroblastoma)

Distinct nodular pattern (nodular neuroblastoma)

Calcified foci

Always correlate with:

- Imaging (vessel encasement)
- Bone marrow findings
- Clinical staging system (e.g., International Neuroblastoma Staging System)

Take adequate samples.

Submit tumour–vessel interface to assess encasement vs invasion.

C III NEUROBLASTOMA – GROSS PEARLS

1. General Appearance

Large, lobulated, soft mass

Grey-tan to variegated cut surface

Frequent areas of:

- Hemorrhage
- Necrosis
- Cystic degeneration
- Calcification (gritty feel on cutting)

Calcification is common and often seen radiologically.

2. Adrenal Tumours

Replace and distort adrenal gland

Adrenal cortex often thinned and stretched over tumour

Tumour may:

- Encase aorta/IVC
- Envelop renal vessels
- Invade kidney

Renal capsule may be adherent but true invasion must be carefully assessed

3. Extra-Adrenal Tumours

Paraspinal masses may show:

- Extension through neural foramina
- Dumbbell configuration

Posterior mediastinal masses in infants are common

4. Consistency

Soft and friable in poorly differentiated tumours

Firmer in tumours with:

Schwannian stroma

Maturation (ganglioneuroblastoma areas)

5. Differential Gross Diagnosis

Wilms tumour (More renal-based, pseudo-capsulated, less vessel encasement)

Adrenocortical carcinoma (Often more yellow, cortical origin)

Pheochromocytoma (Usually well circumscribed, less necrotic in children)

Classic Gross Clues Suggesting Neuroblastoma

Adrenal mass in infant

Calcification present

Vessel encasement without occlusion

Extensive necrosis

Early bone marrow metastasis

Gross with Risk Stratification Relevance

A. Major Vascular Relationships (Critical in Imaging and Grossing)

Neuroblastoma characteristically:

Envelops aorta, IVC

Encases renal vessels

Surrounds celiac axis, SMA

Encases carotid or subclavian vessels (cervical/mediastinal tumours)

Vessel encasement without luminal invasion is typical.

Presence of vascular encasement = IDRF positive → higher surgical risk.

B. Intraspinal Extension

Extension through neural foramina

“Dumbbell tumour”

Cord compression

Intraspinal extension with cord compromise = IDRF positive.

C. Organ Invasion

Kidney invasion

Liver infiltration

Diaphragmatic involvement

True organ invasion increases complexity and may affect staging.

INRG Staging (Pre-Treatment) : The present global standard is the International Neuroblastoma Risk Group Staging System (INRGSS) shown above.

Based on imaging and metastasis:

Stage	Definition
L1	Localised tumour, confined to one body compartment, **no IDRFs**
L2	Locoregional tumour with ≥1 IDRF
M	Distant metastasis
MS	Metastatic disease in children <18 months limited to skin, liver, and/or bone marrow

MS has special biologic behaviour and may regress spontaneously.

B. INSS (Older Surgical Staging- based on extent of Resection)

Stage	Definition
Stage 1	Localized, completely excised
Stage 2A	Unilateral, incomplete excision
Stage 2B	Ipsilateral nodes positive
Stage 3	Unresectable or contralateral nodes
Stage 4	Distant metastasis
Stage 4S	<1 year old with limited metastasis (skin, liver, marrow)

Stage 4S ≈ MS in INRG (but age cutoff differs slightly).

Image-derived Risk Factors (IDRF) -positive neuroblastoma patients have a higher risk of incomplete surgical resection, surgical complications, and 5-year mortality and/or relapse. IDRFs remain a useful prognostic tool for neuroblastoma patients both for short and long-term outcomes.

Gross Clues Suggesting IDRF Positivity (Important for Pathologist to Document)

While IDRFs are radiologic, correlation should be done grossly for:

- Tumour completely encasing renal vessels
- Aorta/IVC circumferential involvement
- Dense adherence to pancreas or liver
- Foraminal extension (paraspinal tumours)
- Diaphragm infiltration

Document:

- Vessel margins
- True invasion vs encasement
- Renal capsule breach

Biology-Based Risk Stratification (Beyond Stage)

Final risk grouping combines:

- INRG stage
- Age
- Histology (Shimada/INPC)
- MYCN amplification
- 11q aberration
- DNA ploidy

High-risk category often includes:

- MYCN amplification (any stage)
- Stage M disease in older children

Gross Features That May Correlate with Biology

Gross Feature	Possible Implication
Extensive necrosis	High-grade tumour
Soft, friable mass	Poor differentiation
Firm, fibrous areas	Schwannian stroma-rich
Nodular architecture	Nodular neuroblastoma
Maturation areas	Better prognosis

Special Entity: Stage MS

Infants (<18 months):

Massive hepatomegaly

Small primary tumour

Skin nodules

Despite metastasis, may show spontaneous regression.

C IV NEUROBLASTOMA MICROSCOPY

Neuroblastoma :

Microscopy : vaguely nodular due to presence of delicate incomplete fibrous septa.

Tumour cells are small and regular with round deeply staining nuclei slightly larger than lymphocytes. There is little cytoplasm and cytoplasmic outlines are poorly defined.

Homer –Wright rosettes are present in $1/4^{th}$ to $1/3^{rd}$ cases- these are collections of tumour cells not related to blood vessels arranged around a central area filled with fibrillary material which is a tangled mass of neuritis as shown by the silver stain . Rosettes do not show a central lumen (in contrast to those seen in retinoblastoma, ependydomas and carcinoid tumours).

The lobular pattern may be prominent with a Zellballen like pattern.

Haemorrhage is seen as also calcification which may occur as dust like or basophilic stippling.

A pleomorphic variant of neuroblastoma has also been described.

IHC : NSE, neurofilaments, chromogranin, synaptophysin, secretogranin II, vasoactive intestinal peptide, cell surface ganglioside GD2 are all +ve.

Histologic classification systems

Shimada Classification

Histologic classification system first proposed in 1984 with prognostic implications

International Neuroblastoma Pathology Classification System (INPC)

Original Shimada classification system was modified and was renamed in 1999.

This had prognostic significances-

3 subtypes of neuroblastoma: undifferentiated, poorly differentiated, differentiating

Subtype	Description
Undifferentiated	Tumor cells small to medium, indiscernible to small amount of cytoplasm, vague cytoplasmic borders Nuclei round to elongated, salt and pepper chromatin, distinct nucleoli No background neuropil Need ancillary studies to establish diagnosis
Poorly differentiated	Background neuropil present ≤ 5% of tumor cells are differentiating neuroblasts
Differentiating	Abundant background neuropil ≥ 5% of tumor cells are differentiating neuroblasts % of differentiating neuroblasts is more important criteria than amount of neuropil If present, Schwannian stromal development with mature / maturing ganglion cells <50% of tumor with a continuous transition zone to neuroblastomatous areas

NOTE: undifferentiated and poorly differentiated neuroblastoma may have focal / diffuse areas with large, spindled, anaplastic, pleomorphic or even rhabdoid undifferentiated cells

Detail on Categories INPC, 1999 :

i) Undifferentiated neuroblastoma :

small to medium sized cells with a thin rim of cytoplasm and indistinct cytoplasmic borders

nuclei round to oval, coarse granular chromatin (salt and pepper), indistinct nucleoli

neuropil absent

No ganglion cell differentiation

No Schwannian stroma

Occasional immature ganglion cells (vesicular nucleus & prominent nucleolus) is a clue to

differentiating these tumours from other small blue cell tumours

ii) Poorly differentiated neuroblastoma :

most cells similar to those described above

neuropil : present

ganglionic differentiation < 5% of neoplastic cells (enlarged eccentric nucleus, vesicular chromatin, single prominent nucleolus & cytoplasm that is eosinophilic or amphophilic.

Cell diameter : Nucleus diameter is 2:1)

nil or minimal ganglioneuromatous stroma ie Schwannian stroma

iii) Differentiating neuroblastoma :

> 5% of cells show ganglionic differentiation more abundant neuropil than poorly differentiated neuroblastomas

< 50% of Schwannian stroma & < 50% of ganglion cells ganglionic differentiation & Schwannian stroma seen at the periphery more often than in the center of the neoplasm transition zone between neuroblastoma & ganglioneuroblastoma component is poorly defined (as compared to nodular neuroblastoma)

iv) Ganglioneuroblastoma, Nodular : characterized by gross presence of haemorrhagic neuroblastoma

nodules that are present in a background which may resemble Ganglioneuroblastoma, intermixed or the glistening tan pink whorled cut surface of Ganglioneuroblastoma. The neuroblastoma nodules develop as result of evolution of one or more aggressive clones of cells within the tumour.

Microscopy : abrupt demarcation of the neuroblastic (stroma poor) component from the ganglioneuroblastoma, intermixed (stroma rich) or ganglioma neuroma (stroma dominant) component.

nodules have a pushing border or may even have a fibrous pseudocapsule. the stroma rich/ stroma dominant component is often located at the periphery but can appear as septa between tumour nodules. examination of the periphery is essential in identifying this tumour type

v) Ganglioneuroblastoma, intermixed (Schwannian stroma rich neuroblastic tumour):

- random intermingling of neuroblastic nests within the stroma rich (ganglioneuromatous) component.
- differs from ganglioneuroblastoma, nodular in lacking a macroscopic distinct haemorrhagic nodule; and microscopically lacks the distinct interface between the stroma poor and stroma rich components ie.edge is infiltrative and not pushing.
- Ganglioneuroblastoma, intermixed unlike ganglioneuroblastoma, nodular, is classified as a favourable histology neuroblastic tumour.
- distinguished from differentiating neuroblastoma on the basis of the extent of ganglioneuromatous component which exceeds 50% of the total volume of the microscopic fields examined.

i) Ganglioneuroma : In the INPC classification Ganglioneuroma (GN) is classified into GN maturing and GN mature.

the GN maturing subtype is composed predominantly of ganglioneuromatous stroma with a minor component of scattered evenly/unevenly distributed component of differentiating neuroblasts and or maturing ganglion cells in addition to fully mature ganglion cels. Separation from GNB intermixed is based on the fact that neuroblastomatous foci do no form distinct microscopic nests, but instead individual neuroblastic cells merge into ganglioneuroblastic stroma.

GN mature subtype can occur in adrenal and extra adrenal sites like retroperitoneum. They are sharply circumscribed but do not have a true capsule. Cut surface is grey to tan.

Microscopically this subtype contains varying numbers of mature ganglion cells and Schwann cells with collagen . A few multinucleated ganglion cells may be seen. They lack a neuroblastoma component.

IHC : i) Neuroblastoma component is +ve for 68 kd neurofilament protein, sometimes +ve for 150 and

200 kd neurofilament protein. GNBs and GNs are +ve for all three.

NSE, chromogranin, secretogranins, synaptophysin +ve

Trk receptor protein : +tivity denotes neuroblastoma with favourable histology

NB 84 +ve (this is an antibody raised against NB cells. It is not specific as it is also +ve in Ewing's

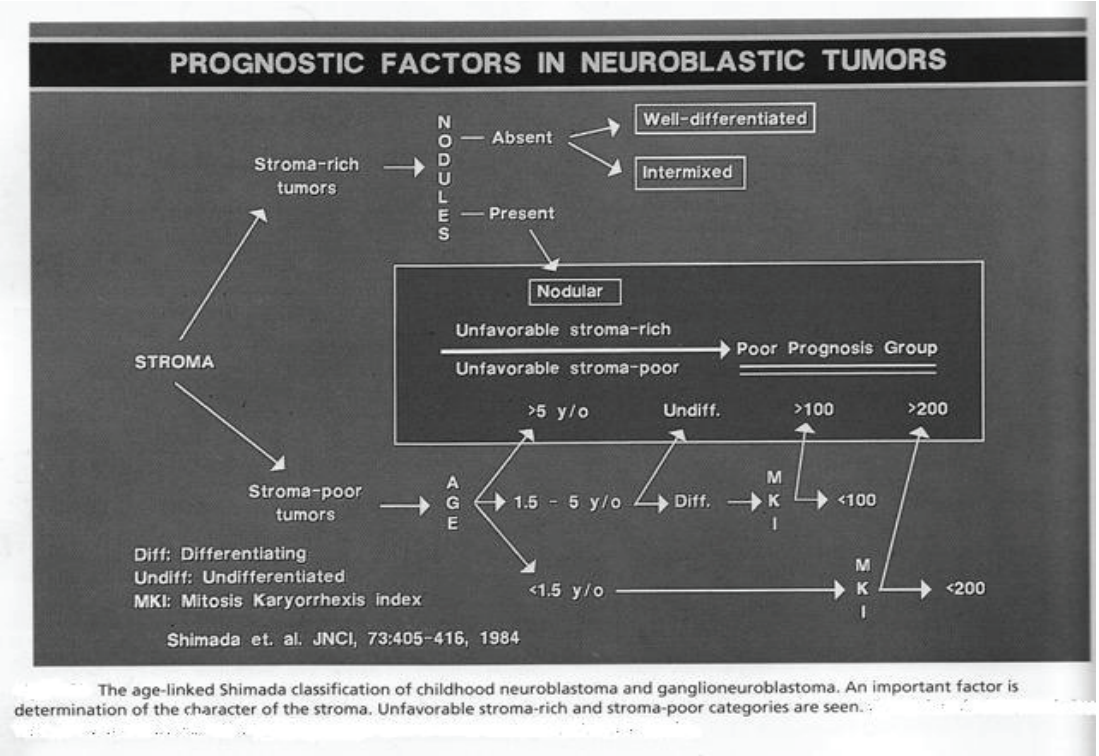
Sarcoma, PNET and desmoplastic small round cell tumours).

INI1	Retained (loss suggests rhabdoid tumour)

GD2 Positive

Genetics : deletion of chromosome 1p is the most characteristic cytogenetic abnormality found in neuroblastomas.

Grading of neuroblastoma : Shimada classification : (Fig 13.5)



The age-linked Shimada classification of childhood neuroblastoma and ganglioneuroblastoma. An important factor is determination of the character of the stroma. Unfavorable stroma-rich and stroma-poor categories are seen.

(Fig13.5: taken from Fletcher C D M : Diagnostic histopathology of Tumours page 1727.)

Mitosis-Karyorrhexis Index (MKI):

Low: < 100 per 5000 cells

Intermediate: 100–200 per 5000 cells

High: > 200 per 5000 cells

This index reflects proliferative and apoptotic activity and is tightly linked to prognosis.

Age Cut-offs:

< 18 mo: generally favourable biology if differentiation and MKI criteria are met.

≥ 18 mo to < 5 yr: intermediate group where MKI and differentiation drive classification.

≥ 5 yr: unfavourable if neuroblastoma subtype predominates. These age cut-offs reflect fundamental differences in tumour behaviour.

GNBn Subset Classification:

If multiple nodules are present, the **least favourable nodule's histology** determines overall INPC

classification.

"International Neuroblastoma Pathology Classification (INPC): Age-linked Histologic Risk Stratification."

Histologic Category / Subtype	Age Criteria	Mitosis-Karyorrhexis Index (MKI)	Favourable Histology?	Comments / Recent Notes
Ganglioneuroma (GN)	Any age	Any MKI	Favourable	Fully Schwannian stroma-dominant tumour.
Ganglioneuroblastoma, Intermixed (GNBi)	Any age	Any MKI	Favourable	Stroma-rich component; no adverse features.
Neuroblastoma (Schwannian stroma-poor) Undifferentiated	Any age	Any MKI	Unfavourable	Immature tumour regardless of MKI/age.
Neuroblastoma, Poorly Differentiated	**< 18 mo**	**Low or Intermediate MKI**	Favourable	Age-linked favourable subset.
	< 18 mo	High MKI	Unfavourable	High MKI overrides age.
	≥ 18 mo	Any MKI	Unfavourable	Older age carries worse prognosis.
Neuroblastoma, Differentiating	< 18 mo	Low/Intermediate MKI	Favourable	Reflects maturation potential.
	< 18 mo	High MKI	Unfavourable	High proliferative activity → adverse.
	18 mo – < 5 yr	Low MKI	Favourable	Age and MKI both favourable.
	18 mo – < 5 yr	Intermediate/High MKI	Unfavourable	MKI drives risk upward.
	≥ 5 yr	Any MKI	Unfavourable	Increased age risk across subtypes.
Ganglioneuroblastoma, Nodular (GNBn)	Any	Any	Variable	Prognosis determined by the **neuroblastomatous nodule component** — favourable or unfavourable based on age/MKI criteria applied to that nodule (*least favourable nodule dictates classification*).

Recent Advances & Refinements

Concept	Modern Insight
Integration with Risk Systems	New approaches start with INPC (favourable vs unfavourable) and integrate INRG staging to define Risk Groups I–IV (better stratification than older RNRC models).
Digital/AI Assessment	Computational pathology and radiomics models are emerging to assist INPC prediction and reduce subjectivity.
Biologic Correlates	Favorable histology often correlates with specific molecular signatures (e.g., Whole chromosome gains), while Unfavourable correlates with partial gains/losses — highlighting genomic interaction with pathology.

Molecular markers in Neuroblastoma

.Molecular abnormalities are critical for **risk stratification, prognosis, and therapy selection** and are integrated with stage and histology in modern classification systems such as the International Neuroblastoma Risk Group Staging System

Key Molecular Markers in Neuroblastoma

Marker	Method of Detection	Frequency	Prognostic Significance	Comments for Reporting
MYCN amplification	FISH (standard), PCR	~20–25%	Very poor prognosis	High-risk regardless of stage or histology
11q deletion	FISH / CGH / NGS	30–40%	Unfavourable	Often mutually exclusive with MYCN amplification
1p deletion	FISH	20–35%	Poor prognosis	Common in aggressive tumours
17q gain	CGH / NGS	~50%	Adverse factor	Most frequent chromosomal abnormality
DNA ploidy (hyperdiploidy)	Flow cytometry	Common in infants	Favourable	Seen in low-risk tumours

MYCN Amplification : Is amplification of the MYCN oncogene located on chromosome 2p24.

Clinical Significance

Strongest adverse prognostic factor.

Assigns tumour to high-risk category regardless of stage or age.

Associated with:

- Rapid progression
- High mitotic activity
- Poor differentiation
- Advanced stage disease

Even a small amplified clone is clinically significant.

11q Deletion : is the loss of long arm of chromosome 11.

Clinical Significance

Independent poor prognostic marker.

Frequently seen in:

- Older children
- Advanced-stage disease

Usually mutually exclusive with MYCN amplification.

Suggests chromosomal instability phenotype.

DNA Ploidy : Measured by flow cytometry.

Ploidy Pattern	Prognosis
Hyperdiploid (DNA index >1)	Favourable (especially infants)
Diploid	Less favourable

Hyperdiploidy correlates with:

Whole chromosome gains

Better response to therapy

Other Emerging / Relevant Molecular Markers

Marker	Clinical Relevance
ALK mutation	Seen in familial and sporadic cases; targetable (ALK inhibitors)
TERT rearrangements	Associated with aggressive disease
ATRX mutation	Seen in older children; linked to chronic disease course
Segmental chromosomal alterations	Worse prognosis than whole chromosome gains

Integration with Risk Stratification

Modern risk assignment combines:

INRG Stage (L1, L2, M, MS)

Age

INPC histology

MYCN status

11q status

DNA ploidy

Example:

Scenario	Risk Group
Stage L1, no MYCN amp, hyperdiploid	Low risk
Stage M, MYCN amplified	High risk
Stage M, no MYCN amp, 11q deletion	Intermediate to High risk

MYCN amplification = high risk regardless of age or stage.

11q deletion = adverse, often MYCN-negative tumours.

Hyperdiploidy = favourable biology in infants.

Segmental chromosomal aberrations worse than whole chromosome gains.

ALK mutations → targeted therapy available.

C V NEUROBLASTOMA- SYNOPTIC REPORTING

Reporting on Neuroblastoma resection specimens

Site of biopsy :

Adrenal □

Extra adrenal □ Specify□

Other tissue sent □ Specify □

Gross :

Measurement ……x ……x……..cm

Cut surface : variegated □

Haemorrhage □

cystic □

solid □

Microscopy : Histologic subtype :

Undifferentiated neuroblastoma : □

Poorly differentiated neuroblastoma : □

Differentiating neuroblastoma : □

Ganglioneuroblastoma, Nodular□

Ganglioneuroblastoma, intermixed□

Ganglioneuroma maturing □

Ganglioneuroma mature □

Favourable histology □

Unfavourable histology □

MKI index ……

Surgical margin involvement : Yes □ No □

No of lymph nodes sent ……

No of lymph nodes involved……

Molecular Section:

MYCN amplification: Yes □ No □

11q deletion status: Yes □ No □

1p deletion status: Yes □ No □

17q gain: Yes □ No □

DNA ploidy: Yes □ No □

ALK mutation (if tested): Yes □ No □

Final integrated comment: …….

"Molecular profile consistent with low / intermediate / high risk neuroblastoma in conjunction with clinicopathologic parameters."

Comments ……..

TNM / Staging (AJCC / SIOP / COG)

Neuroblastoma: INSS / INRG (depends on imaging and resection)

D I RETINOBLASTOMA ANATOMY RELEVANT TO GROSSING

See chapter 17 – Eye and Orbit

D II RETINOBLASTOMA GROSSING TECHNIQUES

See chapter 17- Eye and Orbit

D III RETINOBLASTOMA GROSS PEARLS

General Features

Most common primary intraocular malignancy of childhood.

Arises from primitive retinal neuroectoderm.

Median age: 18 months.

Rare after 5 years of age.

Gross Pathology

Clinical features : presents as leukocoria (white pupillary reflux). Rarely presents with extra ocular extension with formation of an orbital mass.

Bilateral in 30% of all cases and in over 90% of familial cases.

Appears as a white, friable, chalky tumour arising from the retina.

Often shows areas of necrosis and calcification, producing a gritty sensation on cutting.

Growth patterns:

Endophytic (into vitreous)

Exophytic (subretinal)

Diffuse infiltrating (rare)

Multifocal tumours common in hereditary cases.

Tumour may grow towards the vitreous cavity (it is endophytic) or towards the choroid (exophytic).

Examine carefully for optic nerve invasion, particularly at the lamina cribrosa and surgical margin.

Massive choroidal invasion is an important adverse prognostic feature

D IV RETINOBLASTOMA MICROSCOPY

Genetics & Epidemiology of Retinoblastoma

~60% sporadic

~40% hereditary

Bilateral in:

~30% overall

90% hereditary cases

Caused by biallelic inactivation of the RB1 tumour suppressor gene.

Clinical Presentation

Leukocoria (most common)

Strabismus

Decreased vision

Secondary glaucoma

Rarely presents with orbital mass (advanced disease)

Microscopy

Microscopic examination is essential in confirming the diagnosis of ocular and orbital lesions. Many ocular diseases show characteristic histologic patterns that correlate closely with their gross appearance. Eg in retinoblastoma the appearance is of

Small round blue cell tumour

Hyperchromatic nuclei

Scant cytoplasm

High mitotic rate

Necrosis common

Dystrophic calcification frequent

Differentiation Patterns

Flexner–Wintersteiner rosettes (true rosettes)

Homer Wright rosettes

Fleurettes (well-differentiated)

Azzopardi effect may be seen in necrotic areas.

High-Risk Histopathologic Features

(Critical for Adjuvant Therapy Decision)

Post-laminar optic nerve invasion

Tumour at optic nerve surgical margin

Massive choroidal invasion

Scleral invasion

Extrascleral extension

Anterior chamber involvement

These significantly increase metastatic risk.

Routes of Spread

Along optic nerve to brain

Via subarachnoid space

Hematogenous dissemination

Uveal invasion

D V RETINOBLASTOMA SYNOPTIC REPORTING

3) Retinoblastoma Report on Resected Specimens

Specimen type : Enucleation □

Limited exenteration □

Complete exenteration □

Other Specify ……

Laterality : Right □ Left □ Both□

Not specified □

Specimen size : For enucleation :

Anteroposterior diameter……cm

Horizontal diameter ….cm

Vertical diameter ….cm

Length of optic nerve….cm

Diameter of optic nerve…cm

Cannot be determined □

For exenteration :

Greatest diameter……cm

Additional diameter….cm

Cannot be determined

Tumour site : Cannot be determined □

Supero-temporal quadrant of globe □

Supero-nasal quadrant of globe □

Infero-temporal quadrant of globe □

Infero-nasal quadrant of globe □

Tumour extent : anterior chamber□

Extrascleral extension □

Optic nerve □

Tumour diameter after sectioning :

Cannot be determined □

Base from the cut edge……mm

Height from the cut edge…….mm

Maximum tumour height……mm

Distance from anterior edge of tumour to limbus at the cut edge….mm

Distance of posterior margin of tumour base from edge of optic disc…mm

Histological features : Cannot be determined □

Undifferentiated □

Differentiated □

Homer Wright rosettes □

Flexner- Wintersteiner rosettes □

Fleurettes □

Necrotic□

Growth pattern : Endophytic □

Exophytic □

Both □

Tumour involvement of other ocular structures/other pathology

cannot be determined□

optic disc□

choroid, minimal (Bruch's membrane destroyed by 3 or more microscopic

cell clusters without deeper penetration) □

choroid, massive □ vitreous□

retinal detachment □ Vortex vein □

ciliary body □ iris□

lens □ anterior chamber□

angle □ sclera □ cornea□

Extent of optic nerve invasion : None □

Not to end of nerve □

to cut end of optic nerve □

Surgical margins : Cannot be assessed □

Free □ Distance from closest resection margin ……

Specify margin ……..

Involved □ Specify margin…….

TMN Staging :

pTNM :

pTx : primary tumour cannot be assessed

pT0 : no evidence of primary tumour

pT1 : tumour confined to retina, vitreous, or subretinal space. No optic nerve or choroidal invasion

pT2 : minimal invasion of the optic nerve and /or optic coats

pT3: significant invasion of the optic nerve and /or optic coats

pT4 : extra-ocular tumour extension that includes any of the following : invasion of the optic nerve to the line of resection; invasion of orbit through the sclera; extension both anteriorly or posteriorly through the orbit; extension into brain extension to chiasma; extension into brain beyond the chiasma.

Regional lymph nodes :

pNX : cannot be assessed

pN0 : no Regional lymph node metastasis

pN1 : Regional lymph node metastasis

Distant metastasis (pM)

pMX : distant metastasis cannot be assessed

pM0 : No distant metastasis

pM1a: Bone marrow

pM1b : Distant metastasis

Signature…….. Date…….

E I PAEDIATRIC GERM CELL TUMOURS ANATOMY

Anatomical Considerations Relevant to Gross Examination

Midline location in extragonadal tumours.

Relationship to coccyx in sacrococcygeal lesions.

Capsule integrity in ovarian tumours.

Tunica and cord margin status in testicular specimens.

Extent of pelvic/abdominal extension in large congenital tumours.

Understanding these relationships ensures accurate orientation, margin assessment

Embryologic Basis

Paediatric germ cell tumours (GCTs) arise from primordial germ cells, which originate in the yolk sac endoderm and migrate along the midline through the dorsal mesentery to reach the developing gonadal ridges. Migration occurs along hindgut/ dorsal mesentery pathway.Arrestresults in extragonadal midline tumours.

Failure of normal migration or aberrant persistence along this pathway explains the characteristic midline distribution of extragonadal tumours in children.

This embryologic basis is essential to understanding:

Sacrococcygeal tumours in neonates

Mediastinal and retroperitoneal tumours

Pineal and suprasellar germ cell tumours

Common Anatomical Sites in Children

1. Sacrococcygeal Region

Most common site in neonates and infants.

Tumours arise anterior to the coccyx.

May have:

External component

Pelvic extension

Abdominopelvic extension

Altman classification (types 1 to IV) based on external vs internal components

Complete excision requires coccygectomy due to origin from the primitive streak region.

2. Testis (Prepubertal)

Usually pure yolk sac tumour in young boys.

Important anatomical structures for assessment:

Tunica albuginea

Tunica vaginalis

Rete testis

Epididymis

Spermatic cord margin

Lymphatic drainage: para-aortic nodes.

3. Ovary (Children and Adolescents)

Often large unilateral masses.

Capsule integrity is critical for staging.

Surface involvement and rupture influence stage.

Peritoneal dissemination may occur.

4. Mediastinum

Typically anterior mediastinum.

May compress great vessels, airway, or pericardium.

Association with haematologic disorders in rare cases.

5. Retroperitoneum

Along para-aortic region.

Important to distinguish primary tumour from nodal metastasis.

6. Central Nervous System

Pineal and suprasellar regions.

Midline location consistent with germ cell migration pathway.

E II GROSSING OF PAEDIATRIC GERM CELL TUMOURS

General Grossing Principles

Sacro-coccygeal Tumours

Confirm whether the coccyx is included.

Coccygectomy is mandatory to reduce recurrence. In a sacro-coccygeal tumour the pathologist should determine whether the coccyx is received with the specimen. If this has not been surgically removed, the pathologist should notify the surgeon that coccygeal excision is part of the recommended surgical procedure for resection of such tumours. The soft tissue immediately adjacent to the coccyx needs to be sampled as this can be the site of an occult yolk sac tumour.

Sample soft tissue adjacent to coccyx carefully (possible occult yolk sac tumour).

Extragonadal Malignant Germ Cell Tumours

Often lack a true capsule. Malignant extragonadal tumours frequently do not appear to have an intact capsule therefore it may not be possible to determine whether they have been completely resected.

Complete resection may be difficult to assess grossly.

Careful margin inking and perpendicular sectioning required.

Exclude metastasis from a ‘ burnt out primary”

Extensive Sampling Required

At least 1 generous section per cm of tumour is the minimun standard.

Therapy is directed toward the most malignant component.

Multiple histologic subtypes may coexist.

Choriocarcinomas are often haemorrhagic

Haemorrhagic Areas Must Be Sampled

Unlike most tumours, haemorrhagic areas in germ cell tumours should be sampled.

Choriocarcinoma components are often haemorrhagic.

Testicular Tumours

Sample adjacent testis to evaluate for:

Germ cell neoplasia in situ (GCNIS)

Important for risk of contralateral tumour.

Ovarian tumours : every nodule from the omentum and peritoneal surface and all lymph nodes should be sampled. If these nodules or nodes contain mature teratomatous elements (gliomatosis peritonii) the ovarian tumour stage is unchanged. If on the other hand malignant elements are found in these tissues, the tumour becomes a stage II or III tumour and requires more aggressive therapy.

E III GROSS PEARLS – PAEDIATRIC GERM CELL TUMOURS

Paediatric germ cell tumours are frequently heterogeneous. Careful inspection and generous sampling are critical.

1. Always Suspect Mixed Histology

A largely cystic tumour may harbour small solid malignant foci.

Mature teratoma may contain:

Yolk sac tumour

Embryonal carcinoma

All solid areas must be thoroughly sampled.

2. Solid Nodules Within a Cystic Mass Is a Red Flag

Particularly in:

Sacrococcygeal tumours

Ovarian teratomas

Even small mural nodules require multiple sections.

3. Haemorrhage and Necrosis Suggest Malignancy

Extensive haemorrhage or necrosis raises suspicion for:

Yolk sac tumour

Embryonal carcinoma

Choriocarcinoma component

These areas must be extensively sampled.

4. Sacrococcygeal Tumours

Confirm presence of coccyx in specimen.

If absent, document clearly.

Sample:

Solid areas

- Areas adjacent to coccyx
- Deep pelvic extension

Large tumours require mapping and block indexing.

5. Testicular Tumours (Prepubertal)

Usually homogeneous and solid.

Sample:

- Tumour–tunica interface
- Rete testis
- Epididymis if grossly involved
- Spermatic cord margin

Multifocality is uncommon but should be excluded.

6. Ovarian Tumours

Record:

- Capsule status
- Surface deposits
- Rupture

Sample:

- Solid areas
- Papillary projections
- Areas of haemorrhage

Large cystic tumours require adequate representation of mural nodules.

7. Post-Chemotherapy Specimens

Look for:

- Residual viable tumour
- Mature teratoma (growing teratoma syndrome)
- Necrosis/fibrosis

Extensive sampling is required even if the mass appears necrotic.

8. Correlate With Tumour Markers

Always correlate gross findings with:

AFP (suggests yolk sac component)

β-hCG (suggests choriocarcinoma component)

Ovarian Germ Cell Tumours

Sample all omental/peritoneal nodules.

Sample all lymph nodes.

Mature teratomatous implants (gliomatosis peritonii) do NOT upstage tumour.

Malignant implants → higher stage → aggressive therapy.

Paediatric ovarian GCT sampling broadly follows FIGO principles with modifications incorporating tumour markers and paediatric oncology protocols.

Modern Concept

Still broadly similar:

Stage I: Ovary confined

Stage II–III: Peritoneal/lymph node spread

Stage IV: Distant metastasis

But consider briefly mentioning:

FIGO staging (for completeness)

Importance of peritoneal washings

Extragonadal –Term still acceptable

Paediatric germ cell tumours – staging

Paediatric germ cell tumours (GCTs) are staged using systems developed by the Children's Oncology Group (COG). Tumour markers (AFP, β-hCG) are integral to staging and risk stratification.

A. TESTICULAR GERM CELL TUMOURS (Prepubertal)- Staging

Stage I

Tumour confined to testis

Completely resected by high inguinal orchiectomy

No lymphovascular invasion

Tumour markers normalize appropriately (AFP decline according to half-life)

No radiologic evidence of metastasis

Stage II

Microscopic residual disease OR

Scrotal violation/spillage OR

Persistent elevation of tumour markers after appropriate half-life decline OR

Small volume retroperitoneal nodal disease

Stage III

Gross retroperitoneal lymph node involvement

Stage IV

Distant metastasis (lung, liver, bone, brain)

Important Point

Tumour marker kinetics (AFP half-life ~5–7 days) are critical.

Retroperitoneal lymph node sampling is not routinely performed in all cases now (imaging-based staging common).

High inguinal orchidectomy is essential; transscrotal approach upstages disease.

B. OVARIAN GERM CELL TUMOURS (Children/Adolescents)

Based broadly on FIGO principles but adapted in paediatric oncology.

Stage I

Tumour confined to ovary (one or both)

Capsule intact

No surface tumour

Negative peritoneal washings

Markers normalize post-resection

Stage II

Microscopic residual OR small nodal involvement

Washings negative

Stage III

Gross residual disease OR

Large lymph node involvement OR

Peritoneal implants OR

Positive washings

Stage IV

Distant metastasis (e.g., liver parenchymal, lung)

Mature teratomatous implants (gliomatosis peritonii) **do not upstage** disease.

C. EXTRAGONADAL GERM CELL TUMOURS

Stage I

Complete resection

Negative margins

Markers normalize

Stage II

Microscopic residual

Nodes negative

Stage III

Gross residual disease OR nodal involvement

Stage IV

Distant metastasis

E IV GERM CELL TMOURS MICROSCOPY

Paediatric germ cell tumours – General and Key Microscopic features of Importance

Tumour	Microscopy	Marker
Yolk sac tumour	Reticular/microcystic pattern; Schiller-Duval bodies	AFP+, Glypican-3+, SALL 4+.
Embryonal carcinoma	Primitive epithelial cells; marked atypia	CD30+, OCT4+
Immature teratoma	Immature neuroepithelium	Variable
Dysgerminoma / Seminoma	Sheets of large cells; fibrous septa with lymphocytes	OCT4+, PLAP+C KIT (CD117) +ve

One-Line PG Comparison Table (Spotters)

Tumour	Key Histology	Risk / Prognosis	Notes
Wilms	Blastema + epithelium + stroma	Anaplasia = high	Most common renal tumour in children
Hepatoblastoma	Fetal/embryonal epithelium, mesenchyme	Small cell undiff = poor	AFP elevated
Neuroblastoma	Small blue cells, rosettes, neuropil	N-myc amp = poor	Adrenal > sympathetic chain
Rhabdomyosarcoma	Embryonal / alveolar / spindle	Alveolar = worse	IHC: desmin/myogenin

SMALL ROUND BLUE CELL TUMOURS OF CHILDHOOD- Differential Grid

Tumour	Key Morphology	Rosettes	IHC Profile	Molecular
Wilms tumour (blastemal)	Primitive small cells; may be triphasic	Rare	WT1+, PAX8+	WT1 alterations
Neuroblastoma	Neuropil; Homer-Wright rosettes; calcification	Homer-Wright	Synapto+, Chromogranin+, PHOX2B+	MYCN amp
Ewing sarcoma	Uniform small cells; glycogen-rich cytoplasm	Rare pseudo-rosettes	CD99 diffuse membranous+, FLI1+	EWSR1–FLI1
Rhabdomyosarcoma (embryonal)	Strap cells; eccentric eosinophilic cytoplasm	No true rosettes	Desmin+, Myogenin+, MyoD1+	Variable
Lymphoblastic lymphoma	Diffuse sheets; starry sky	None	LCA+, TdT+	Ig/TCR rearrangement
Desmoplastic small round cell tumour	Small nests in desmoplastic stroma	Rare	Desmin (dot-like)+, WT1 (C-term)+	EWSR1–WT1
Malignant rhabdoid tumour	Large cells; hyaline inclusions	None	INI1 loss	SMARCB1 loss
Clear cell sarcoma (kidney)	Clear cytoplasm; vascular network	None	Cyclin D1+, BCOR+	BCOR ITD

PRACTICAL DIAGNOSTIC APPROACH

Neuropil present? → Think of Neuroblastoma

Diffuse CD99 membranous? → Ewing

INI1 loss? → Rhabdoid tumour

Myogenin nuclear strong? → Rhabdomyosarcoma

WT1 nuclear positive in renal mass? → Wilms

Desmoplastic stroma + dot desmin? → DSRCT

A structured report incorporating tumour type, extent, margin status and tumour markers correlation is recommended for optimal clinical management.

ASSESS YOURSELF PAEDIATRIC TUMOURS OF CHILDHOOD

Essay Questions (10 marks each)

1. **Compare Wilms tumour and Neuroblastoma** with respect to:

   Site of origin ; Gross features ; Microscopy ;Pattern of spread and Prognostic factors

2. **Discuss Neuroblastoma** under the following headings:

   Anatomy relevant to grossing ; Gross features ; Microscopy and classification (INPC) and Molecular markers and risk stratification

3. **3.Describe Wilms tumour (Nephroblastoma)** with rgard to:

   Clinical features and syndromic associations ; Gross morphology ; Microscopy (triphasic pattern) and NWTS classification and prognostic factors

4. **Write an account of Retinoblastoma**, highlighting:

   Genetics (RB1 pathway) ;Gross and growth patterns ; Microscopy (rosettes, fleurettes)

   And High-risk histopathological features

5. **Discuss Paediatric Germ Cell Tumours** with emphasis on:

   Embryologic basis and anatomical distribution ;Gross features and sampling strategy;

   Microscopic subtypes and tumour markers as well as

   Staging principles in children

Short Answer Questions (5 marks each)

6. Describe the triphasic pattern of Wilms tumour.
7. What are Image Defined Risk Factors (IDRFs) in neuroblastoma?
8. Enumerate the high-risk histologic features in retinoblastoma.
9. Write short notes on MYCN amplification in neuroblastoma.

10. Describe the gross features of hepatoblastoma.
11. List the common sites of paediatric germ cell tumours.
12. What are Flexner–Wintersteiner rosettes?
13. Write short notes on mesoblastic nephroma.
14. Describe the microscopic features of yolk sac tumour.
15. What is the significance of massive choroidal invasion in retinoblastoma?

Short Answers (3 marks each)

16. Gross differences between Wilms tumour and neuroblastoma
17. Homer Wright rosettes
18. Fleurettes in retinoblastoma
19. Schiller–Duval bodies
20. "Dumbbell tumour" in neuroblastoma
21. Role of AFP in paediatric tumours
22. Calcification in neuroblastoma vs retinoblastoma
23. Ganglioneuroblastoma vs ganglioneuroma
24. Gliomatosis peritonii

MCQs

1.The most common renal tumour in children is:

A. Neuroblastoma
B. Wilms tumour
C. Mesoblastic nephroma
D. Rhabdoid tumour

2.MYCN amplification in neuroblastoma indicates:

A. Favourable prognosis
B. Benign behaviour
C. High-risk tumour
D. Spontaneous regression

3.Flexner–Wintersteiner rosettes are seen in:

A. Neuroblastoma
B. Retinoblastoma
C. Hepatoblastoma
D. Wilms tumour

4.Most common site of neuroblastoma:

A. Kidney
B. Liver
C. Adrenal gland
D. Brain

5.Schiller–Duval bodies are characteristic of:

A. Embryonal carcinoma
B. Dysgerminoma
C. Yolk sac tumour
D. Teratoma

Answers (for MCQs)

1. 1.B
2. C
3. 3.B
4. 4.C
5. 5.C

Chapter 14
Skin

Chapter outline I Skin Anatomy; II Skin Grossing Techniques; III Skin Gross Pearls; IV Skin Microscopy; V Skin Synoptic Reporting

I SKIN ANATOMY

The skin is the **largest organ** of the body and serves as a protective, sensory, and regulatory interface between the body and the environment.

1. Layers of the Skin

Epidermis

Outermost layer composed of stratified squamous keratinized epithelium.

Thickness varies: thin skin (eyelids) 0.05 mm, thick skin (palms, soles) up to 1.5 mm.

Sub-layers (from deep to superficial):

Stratum basale – basal keratinocytes, melanocytes

Stratum spinosum – prickle cells, Langerhans cells

Stratum granulosum – keratohyalin granules

Stratum lucidum – present in thick skin

Stratum corneum – terminally differentiated keratinized cells

Dermis

Connective tissue layer beneath the epidermis.

Two sublayers:

Papillary dermis – loose connective tissue, dermal papillae, capillaries, sensory nerve endings

Reticular dermis – dense irregular connective tissue, sweat glands, hair follicles, sebaceous glands

Hypodermis (Subcutis)

Composed of adipose tissue and connective tissue.

Provides insulation, cushioning, and energy storage.

2. Appendages

Hair follicles – tubular structures producing hair shafts

Sebaceous glands – secrete sebum, usually associated with hair follicles

Sweat glands – eccrine (thermoregulation) and apocrine (axilla, groin)

Nails – specialized keratinized structures

3. Vascular Supply

Dermal plexus: superficial and deep networks supplying epidermis via diffusion

Rich capillary network facilitates nutrient supply, thermoregulation, and healing

4. Nerve Supply

Sensory endings: Meissner's corpuscles (touch), Pacinian corpuscles (pressure/vibration), free nerve endings (pain/temperature)

Autonomic fibers regulate sweat glands and arrector pili muscles

Key Surgical-Pathology Note

Depth of biopsy is crucial: epidermis only, epidermis + dermis, or full-thickness (including hypodermis) depending on clinical suspicion.

Knowledge of layers guides grossing, sectioning, and margin assessment.

II SKIN GROSSING TECHNIQUES

Specimen Types

Skin biopsies include:

Punch biopsy

Shave biopsy

Excision biopsy

1. Punch Biopsy

Description

Assess shape, thickness, presence of surface bullae, and presence of subcutaneous tissue.

Measurements

Record size in three dimensions.

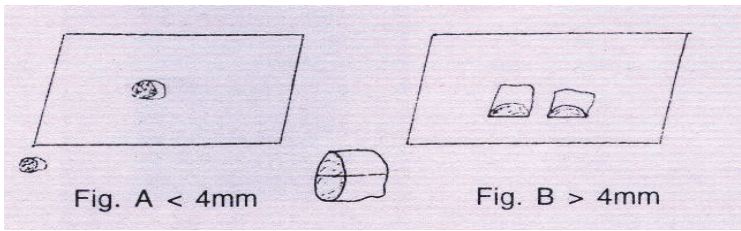


Fig 14.1 Bits in Punch Biopsy

Processing / Blocking (Fig 14.1)

≤4 mm → embed entire tissue.

4 mm → slit tissue and embed to include representative areas.

Do not cut through the bullae.

Tips

Label microtome sections in serial order (1, 2, 3…) with 4–5 sections per slide.

2. Shave Biopsy

Description

Thin, round or oval tissue- typically done for keratosis, basal cell carcinoma, or graft viability.

Note number of fragments and surface features.

Measurements

Record the two dimensions.

Processing / Blocking

≤4 mm → embed entire tissue on edge.

5 mm–1 cm → slice into 2–3 mm thick parallel bits, orient on edge, embed in one or two cassettes.

1 cm → select representative sections for embedding.

Tips

Ensure proper orientation on the block for accurate histology.

3. Excision Biopsy

Description

Used for lesions ≤2 cm (naevi, keratosis, small basal cell carcinoma).

Paint margins with India ink if malignancy is suspected.

Note regularity or irregularity of the margin.

Record shape, surface, color, and size of specimen in pigmented lesions.

Note distance from closest surgical margin.

Processing / Blocking (14.2)

≤4 mm → orient on edge and embed.

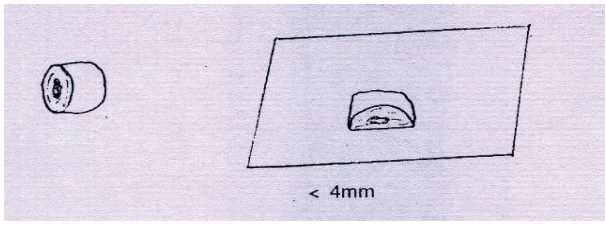


FIG 14.2 ; Blocking of excision biopsy of skin, < 4 mm

4–8 mm → make one cut including lesion and embed.

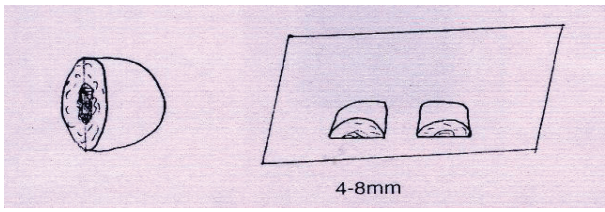


Fig 14.3 : Blocking of 4-8 mm of excision biopsy

8 mm → make parallel sections:

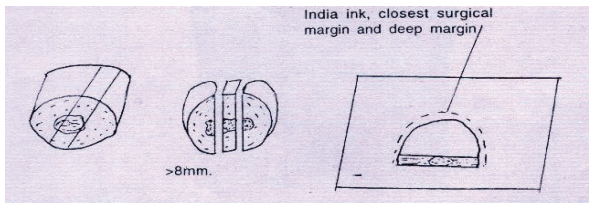


Fig 14.4 : a > 8mm

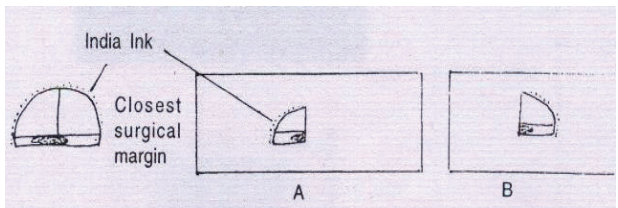


Fig 14.4 b : Specimen > 8mm , blocking including surgical margins.

First slice: lesion with closest skin and deep margins (see figs 14.4 a &b)

More than one block may be used if both margins cannot be represented in one slice.

Additional slices: remaining tissue preserved in formalin.

Large specimens (< 5and more than >5 cm): multiple vertical "bread slices"; select slices showing closest surgical margin and maximum depth of infiltration for embedding.(fig14. 5)

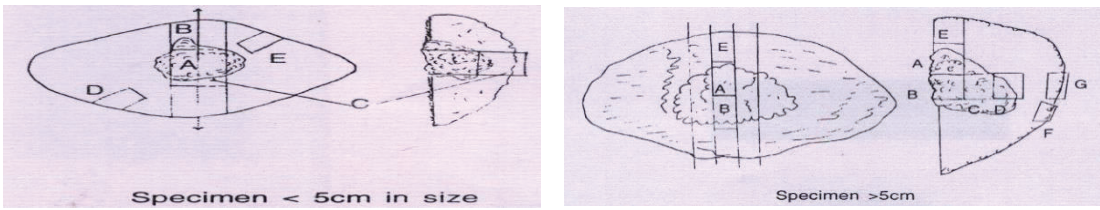


Fig 14.5 A & B : Specimen > 5 cm

Tips

Use multiple blocks if one block cannot represent all critical margins.

Label and orient blocks clearly for margin assessment.

III SKIN GROSS PEARLS

Polypoid, soft raised lesion composed of and covered by skin. Composed of skin and underlying soft tissue measuring 3 mm to 6 mm in size- fibroepithelial polyp

Warty lesion- viral

Raised smooth lesions less than 3-4 mm with central crater- Molluscum Contagiosum

Pigmented flat lesion with regular margins- Naevus

Pigmented, nodular, ulcerated or intact skin surface-Melanoma

Nodular lesion, firm, with or without surface ulceration -carcinoma

"**Lesions above a line from angle of mouth to tragus"** are more likely basal cell carcinoma

IV SKIN MICROSCOPY

Microscopic evaluation of skin lesions relies on **pattern recognition at scanning and low power magnification**, followed by cytological assessment at higher power. A disciplined approach—assessment of epidermal architecture, dermoepidermal junction, dermal infiltrate, growth pattern, cytology, stromal response, adnexal relationship, and special features—is essential for accurate diagnosis in surgical pathology.

I. EPIDERMAL LESIONS

A. Viral Infections

Verruca vulgaris (HPV 2; seen chiefly on hands)

Characterised by focal epidermal hyperplasia with marked hyperkeratosis and parakeratosis. The rete ridges are elongated and converge toward the centre, producing a cup-shaped lesion.

Papillomatosis with a “church-spire” configuration is typical. Koilocytosis with perinuclear vacuolation is seen in the upper Malpighian layer.

Verruca plantaris (soles)
Shows pronounced epidermal hyperplasia with inward growth, coarse basophilic intracytoplasmic keratohyaline granules, and surface hyperkeratosis. Lesions tend to be endophytic due to pressure effects.

Verruca plana (HPV 10; face, hands)
Flat lesions with mild epidermal hyperplasia, prominent granular layer, minimal papillomatosis, and occasional intranuclear inclusions.

Condyloma acuminatum (HPV 6/11)
Exhibits marked acanthosis, papillomatosis, and koilocytosis. Dysplasia may coexist, and association with Bowen disease or invasive squamous cell carcinoma should be excluded.

Molluscum contagiosum
Lobulated epidermal hyperplasia with central crater formation. Large eosinophilic intracytoplasmic inclusion bodies (molluscum bodies) expand keratinocytes and displace nuclei peripherally, accompanied by a surrounding dermal inflammatory response.

B. Bacterial Infections

Hidradenitis suppurativa
Shows apocrine gland involvement with abscess formation, sinus tracts, dense chronic inflammation, and fibrosis in long-standing disease.

Cutaneous tuberculosis

Lupus vulgaris: Epidermal hyperplasia with well-formed epithelioid granulomas, with or without caseation. Acid-fast bacilli may be scanty.

Tuberculids (papulonecrotic, erythema induratum, lichen scrofulosorum): Granulomatous inflammation without demonstrable bacilli, often perifollicular or perivascular.

Leprosy
Histology varies across the spectrum from tuberculoid to lepromatous forms. Tuberculoid leprosy shows well-formed granulomas with nerve involvement and scant bacilli, whereas lepromatous leprosy demonstrates sheets of foamy histiocytes with numerous acid-fast bacilli (globi). Reactional states show oedema, neutrophilic infiltrates, vasculitis, and bacillary debris.

C. Inflammatory Dermatoses

Psoriasis
Regular acanthosis with elongated rete ridges, suprapapillary thinning, parakeratosis, absent granular layer, dilated capillaries in dermal papillae, and Munro microabscesses composed of neutrophils.(Fig 14.6)

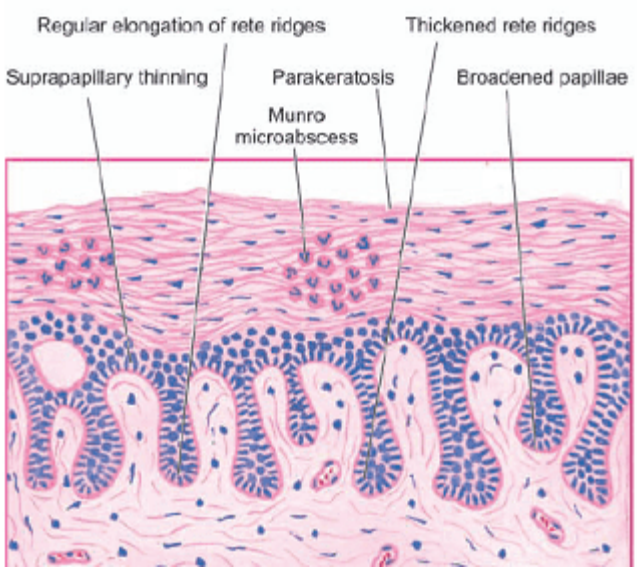


Fig 14.6 : Diagrammatic representation of microscopy in psoriasis

Lichen planus

Compact hyperkeratosis, wedge-shaped hypergranulosis, basal cell vacuolar degeneration, and a dense band-like lymphocytic infiltrate at the dermo-epidermal junction. Civatte bodies and subepidermal clefts may be present.

D. Bullous Disorders (Based on Level of Split)

Intraepidermal bullae

Subcorneal: Pemphigus foliaceus, IgA pemphigus

Suprabasal: Pemphigus vulgaris, Darier disease

These show acantholysis with loss of intercellular adhesion.

Subepidermal bullae

Seen in bullous pemphigoid, dermatitis herpetiformis, linear IgA disease, and epidermolysis bullosa. Blister formation results from basal keratinocyte damage or basement membrane zone disruption, often with eosinophilic or neutrophilic infiltrates.

E. Hyperkeratotic and Hyperplastic Lesions

Epidermal nevus shows orthokeratosis with papillomatous epidermal hyperplasia and flat-topped papillary projections.

Seborrhoeic keratosis shows marked proliferation of basal-type keratinocytes with multiple histologic patterns, the commonest being the acanthotic type with pseudohorn cysts. Other patterns include hyperkeratotic, adenoid and acantholytic forms. Irritated seborrhoeic keratosis shows squamous metaplasia. Tumour cells express low-molecular-weight cytokeratins.

Actinic keratosis shows cytologic atypia of keratinocytes. The atrophic form shows only a few layers of atypical cells; the acantholytic form shows loss of intercellular cohesion; the

hypertrophic form shows massive hyperkeratosis with alternating parakeratosis and orthokeratosis. Bowenoid actinic keratosis shows full-thickness atypia, while pigmented variants show melanin-laden atypical keratinocytes.

F. Endophytic Hyperkeratotic Lesions

Warty dyskeratoma shows a cup-shaped epidermal invagination filled with keratinous material, numerous acantholytic dyskeratotic cells, elongated villi and corps ronds resembling Darier's disease.

Keratoacanthoma (Fig 14.7) shows a hemispherical architecture with overhanging edges and a keratin-filled crater. The epithelium is well differentiated with pushing borders and a dense inflammatory infiltrate rich in eosinophils. Despite cytologic atypia, architectural features are most useful in distinguishing it from squamous cell carcinoma.

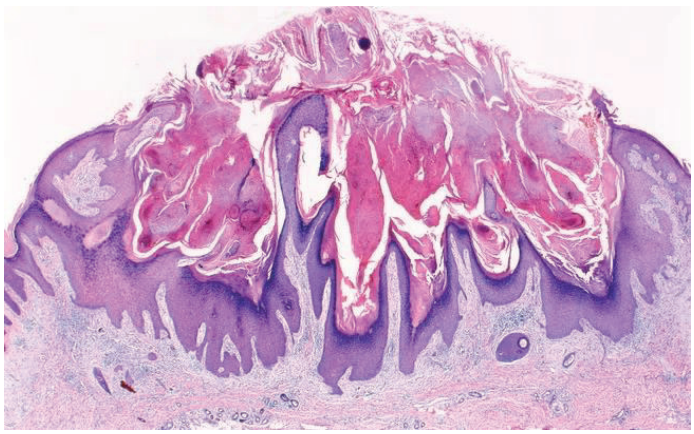


Fig 14.7 : Keratoacanthoma with epidermis showing overhanging edges to a crateriform cup of keratin, H& E x100

II. STROMAL (MESENCHYMAL) TUMOURS OF THE DERMIS

Benign fibrous histiocytoma (Dermatofibroma)

Poorly circumscribed dermal lesion composed of spindle cells in a storiform pattern, collagen trapping at the periphery, and overlying epidermal hyperplasia with basal hyperpigmentation.

Dermatofibrosarcoma protuberans

Uniform spindle cells in a prominent storiform pattern with lace-like infiltration into subcutaneous fat. Epidermis is attenuated and a grenz zone is absent.

Atypical fibroxanthoma

Superficial dermal tumour composed of pleomorphic spindle and epithelioid cells with atypical mitoses, confined to the dermis.

Neurofibroma

Non-encapsulated lesion with spindle cells having wavy nuclei, infiltrating dermis and subcutis with entrapment of adnexal structures.

Schwannoma
Encapsulated tumour with Antoni A and Antoni B areas, nuclear palisading, Verocay bodies, and hyalinised blood vessels.

III. OTHERS

Cysts and Pilar Tumours

Epidermal cysts are lined by stratified squamous epithelium with a granular layer and contain laminated keratin.
Pilar (trichilemmal) cysts show abrupt keratinization without a granular layer and contain non-laminated keratin with frequent calcification.
Pilar tumours show solid lobules with pushing borders and trichilemmal keratinization; although generally benign, rare malignant transformation and metastasis may occur.

Adnexal Tumours

Eccrine, follicular and sebaceous tumours show varied morphology.
Eccrine poroma shows sharp demarcation from adjacent epidermis, composed of small keratinocytes resembling acrosyringium; EMA is positive.
Eccrine acrospiroma shows solid and cystic nodules with clear cells, squamous metaplasia and positivity for CK, EMA, CEA, S-100 and vimentin.
Syringomas show small ducts lined by two layers of cells, often with comma-shaped tails.
Chondroid syringoma resembles mixed tumour of salivary glands with epithelial and myoepithelial differentiation.
Cylindroma shows jigsaw-puzzle lobules surrounded by thick basement membrane material.
Spiradenoma shows two cell populations with prominent lymphocytic infiltrates and increased vascularity.
Papillary syringadenoma shows papillary glandular proliferations with dense plasma cell infiltrates.
Aggressive digital papillary adenoma shows tubulo-alveolar and papillary patterns with potential for recurrence and malignancy.
Clear cell acanthoma shows psoriasiform acanthosis with glycogen-rich clear keratinocytes.

Malignant Tumours

Basal cell carcinoma (Fig 14.8)shows nests of basaloid cells with peripheral palisading, stromal retraction and multiple histologic patterns including nodular, infiltrative and morpheaform variants.

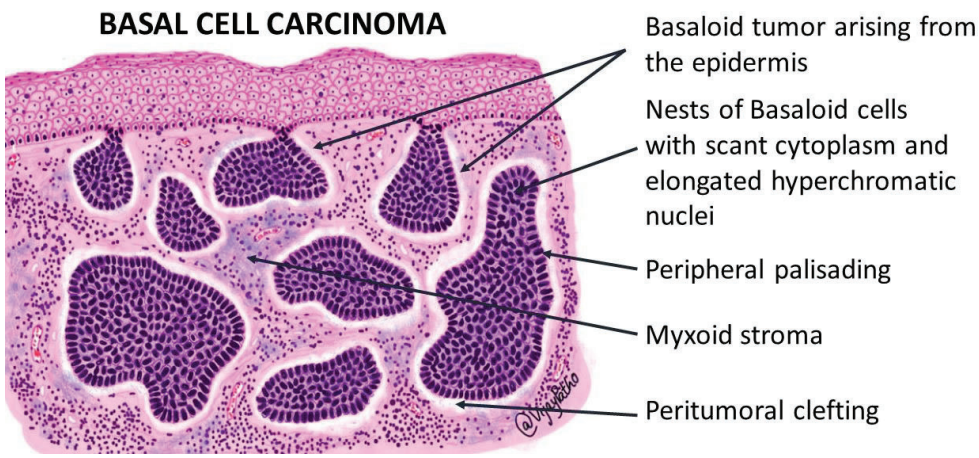


Fig 14.8 : Basal cell carcinoma Diagrammatic - H & E x 100

Merkel cell carcinoma is a dermal neuroendocrine carcinoma composed of small round cells with scant cytoplasm, high mitotic activity and positivity for CK20 and neuroendocrine markers

Melanocytic Lesions

Benign melanocytic nevi

Junctional, compound, and intradermal nevi represent stages of maturation, with progressive decrease in cell size and pigmentation with depth. Spitz and blue nevi show characteristic architectural and cytological features but maintain symmetry and circumscription.

Malignant melanoma

Asymmetric, poorly circumscribed lesion with atypical melanocytes, pagetoid spread, dermal invasion without maturation, frequent mitoses, and possible regression. Microstaging is assessed using Clark levels and Breslow thickness.

Pattern Recognition Tables – Skin Microscopy

1. Epidermal Reaction Patterns

Pattern	Key Microscopic Features	Important Examples
Acanthotic	Regular epidermal hyperplasia, elongated rete ridges	Psoriasis, Seborrhoeic keratosis
Papillomatous	Upward projections of epidermis, church-spire pattern	Verruca vulgaris, Epidermal nevus
Hyperkeratotic	Thickened stratum corneum ± parakeratosis	Actinic keratosis, Lichen simplex
Interface dermatitis	Basal vacuolar change, apoptotic keratinocytes	Lichen planus, Erythema multiforme

Spongiotic	Intercellular edema of epidermis	Eczema, Hailey–Hailey disease

2. Viral Infections – Pattern Clues

Lesion	Hallmark Feature
Verruca vulgaris	Church-spire papillomatosis, vacuolated keratinocytes
Verruca plana	Flat epidermis, prominent granular layer
Condyloma acuminatum	Acanthosis + epithelial nests
Molluscum contagiosum	Large intracytoplasmic molluscum bodies

3. Granulomatous Dermatoses

Pattern	Microscopy	Likely Diagnosis
Well-formed granulomas ± necrosis	Epidermal hyperplasia, AFB may be seen	Lupus vulgaris
Granulomas without bacilli	Perifollicular / perivascular	Tuberculids
Granulomas + nerve involvement	Variable AFB, foam cells	Leprosy
Diffuse foamy macrophages	Numerous bacilli, globi	Lepromatous leprosy

4. Psoriasiform vs Lichenoid Pattern

Feature	Psoriasis	Lichen planus
Rete ridges	Regular, elongated	Irregular
Granular layer	Absent	Wedge-shaped hyper-granulosis
Suprapapillary plates	Thinned	Preserved
Inflammation	Neutrophils (Munro abscesses)	Band-like lymphocytes
Basal damage	Minimal	Prominent (Civatte bodies)

5. Bullous Disorders – Level of Split

Level of Blister	Diseases
Subcorneal	Pemphigus foliaceus, IgA pemphigus
Suprabasal	Pemphigus vulgaris, Darier's disease

Spongiotic	Hailey–Hailey disease
Subepidermal (basal necrosis)	EM, LP (interface disorders)
Subepidermal (BMZ destruction)	Bullous pemphigoid, DH, Linear IgA

Mnemonic:
Acantholysis = Pemphigus
BMZ antibodies = Pemphigoid

6. Hyperkeratotic & Crateriform Lesions

Feature	Keratoacanthoma	Squamous Cell Carcinoma
Architecture	Symmetrical, crateriform	Irregular, infiltrative
Margins	Pushing	Infiltrative
Inflammation	Prominent, eosinophils	Variable
Cytology: Behavior	Bland May regress	Atypical Progressive

7. Cysts – Infundibular vs Pilar

Feature	Epidermal Cyst	Pilar Cyst
Granular layer	Present	Absent
Keratin	Laminated	Compact
Calcification	Rare	Common
Site	Anywhere	Scalp

8. Adnexal Tumours – Key Clues

Tumour	Diagnostic Microscopy
Eccrine poroma	Sharp epidermal junction, EMA +
Cylindroma	Jigsaw-puzzle lobules
Spiradenoma	Two cell populations + lymphocytes
Chondroid syringoma	Mixed tumour pattern
Clear cell acanthoma	Abrupt glycogen-rich clear cells

9. Basal Cell Carcinoma Variants

Variant	Hallmark
Nodular	Peripheral palisading
Morpheaform	Dense fibrosis, infiltrative
Superficial	Lateral growth, trunk
Fibroepithelial (Pinkus)	Thin epithelial strands, abundant stroma

10. Small Round Cell Tumours of Skin

Tumour	Key Feature
Merkel cell carcinoma	CK20 dot-like positivity
Lymphoma	LCA +
Metastatic small cell carcinoma	TTF-1 +

V SKIN SYNOPTIC REPORTING

Synoptic reporting in skin pathology ensures completeness, clarity, and reproducibility, particularly in neoplastic lesions where margin status, depth, and prognostic parameters influence management. While not all skin biopsies require synoptic reporting, it is mandatory for excisions of malignant and potentially malignant lesions.

A. General Requirements (Applicable to All Skin Specimens)

Patient details

Anatomic site

Type of specimen

Punch biopsy

Shave biopsy

Excision biopsy

Laterality (if applicable)

Clinical diagnosis / indication

Previous biopsy or treatment (if known)

B. Synoptic Reporting – Non-Neoplastic Lesions

(Inflammatory, infectious, bullous disorders)

Essential elements

- Nature of lesion (inflammatory / infectious / bullous)
- Predominant histologic pattern
  - Psoriasiform
  - Lichenoid
  - Interface
  - Spongiotic
  - Vesiculobullous (level of split specified)
- Presence of:
  - Granulomas (± necrosis)
  - Organisms (AFB / fungi / viral inclusions)
  - Vasculitis
- Correlation with clinical diagnosis

Note: Margin status is not required for non-neoplastic conditions.

C. Synoptic Reporting – Premalignant Lesions

Actinic Keratosis

- Type:
  - Atrophic
  - Hypertrophic
  - Acantholytic
  - Bowenoid
  - Pigmented
- Degree of atypia:
  - Partial thickness
  - Full thickness (Bowenoid)
- Margins (if excised):
  - Free / involved

D. Synoptic Reporting – Basal Cell Carcinoma (BCC)

Mandatory elements

- Histologic type:
  - Nodular

- Superficial
- Infiltrative
- Morphea -form
- Pigmented
- Keratotic

Tumour size (greatest dimension, mm/cm)

Depth of invasion:

- Confined to dermis
- Involvement of subcutis

Margins:

- Peripheral margins – free / involved
- Deep margin – free / involved

Perineural invasion: Present / Absent

Ulceration: Present / Absent

Comment: Morpheaform and infiltrative types have a higher risk of recurrence.

E. Synoptic Reporting – Squamous Cell Carcinoma (SCC)

Mandatory elements

Histologic grade:

- Well differentiated
- Moderately differentiated
- Poorly differentiated

Tumour size

Depth of invasion:

- Measured from granular layer to deepest point (mm)

Anatomic level:

- Dermis
- Subcutis
- Deeper structures

Margins:

- Peripheral margins – free / involved (distance in mm)

Deep margin – free / involved

Perineural invasion: Present / Absent

Lympho-vascular invasion: Present / Absent

Associated lesion:

Actinic keratosis

Bowen's disease

F. Synoptic Reporting – Melanoma

Breslow thickness

Ulceration

Mitotic rate

F. Synoptic Reporting – Keratoacanthoma

Architectural features consistent with keratoacanthoma

Margins:

Free / involved

Comment:

Distinction from SCC based primarily on architecture

Complete excision advised despite potential regression

G. Synoptic Reporting – Adnexal Tumours

Essential elements

Tumour type (eccrine / apocrine / follicular / sebaceous)

Benign vs malignant features

Growth pattern:

Pushing / infiltrative

Margins:

Free / involved

Perineural / vascular invasion (if present)

H. Synoptic Reporting – Merkel Cell Carcinoma

Mandatory elements

Tumour size

Depth of invasion

Margins:

- Peripheral and deep

Mitotic activity

Necrosis

Lymphovascular invasion

IHC profile:

- CK20 (dot-like)
- Neuroendocrine markers

Comment on aggressive behaviour

I. Margin Reporting

Margins free of tumour

Tumour involves peripheral margin

Tumour involves deep margin

Closest margin: ___ mm

J. Suggested Final Line (Template Style)

"The histomorphological features are consistent with _____.
The surgical margins are free / involved.
Clinical correlation advised."

ASSESS YOURSELF SKIN

A. Long Essay Questions (10 marks each)

1. Describe the pathogenesis and microscopy of "Psoriasis".
2. Describe the histopathology of vesiculobullous disorders of the skin. Classify them based on the level of blister formation.
3. Describe Basal Cell Carcinoma of the skin under the following headings :Etiology and risk factors ; Gross features ; Microscopy ; Variants ;Differential diagnosis

B. Short Essay Questions (5 markseach)

4. Seborrhoeic keratosis – microscopy and variants.
5. Leprosy – histopathological spectrum with special reference to lepra reactions.
6. Keratoacanthoma – histologic features and its differentiation from squamous cell carcinoma.

C. Short Answer Questions (3 marks each)

7. Munro microabscess – define and mention its significance.
8. Molluscum bodies – mention the disease and histologic appearance.
9. Civatte bodies – define and state its significance.

D. Multiple Choice Questions (MCQs)

1. **A subepidermal blister with eosinophil-rich infiltrate is most characteristic of:**

    a. Pemphigus vulgaris
    b. Bullous pemphigoid
    c. Dermatitis herpetiformis
    d. Hailey–Hailey disease

2. **The most characteristic histologic feature of psoriasis is:**

    a. Hyper-granulosis
    b. Civatte bodies
    c. Munro microabscesses
    d. Subepidermal cleft

3. **CK20 positivity in a dot-like pattern is characteristic of:**

    a. Basal cell carcinoma
    b. Squamous cell carcinoma
    c. Merkel cell carcinoma
    d. Sebaceous carcinoma

Answer Key for MCQs

1. 1.c
2. 2.c
3. 3.c

Chapter 15
Soft Tissue

Chapter outline- I Soft Tissue Anatomy ; II Soft Tissue Grossing Techniques; III Soft Tissue Gross Pearls; IV Soft Tissue Microscopy; V Soft Tissue Synoptic Reporting

I SOFT TISSUE ANATOMY

Applied Surgical Anatomy (Relevant to Gross Examination)

Definition:
Soft tissue comprises nonepithelial, extraskeletal tissues of mesenchymal origin, excluding bone, cartilage, hematopoietic tissue, viscera, and the central nervous system. It includes adipose tissue, skeletal muscle, fibrous tissue, blood vessels, peripheral nerves, and supporting connective tissue.

1. Anatomic Compartments

Soft tissues is organized into compartments bounded by investing fascia and intermuscular septa.

A. Superficial Compartment

Located above the deep (investing) fascia

Includes subcutaneous fat and superficial fascia

Tumours here are termed *superficial*

B. Deep Compartment

Located beneath the deep fascia

Includes skeletal muscle groups, neurovascular bundles, and intermuscular septa

Tumours here are termed *deep.*

Practical importance:
Depth (superficial vs deep) is a key prognostic and staging parameter in soft tissue sarcomas.

2. Fascial Planes

Investing (deep) fascia: Dense fibrous layer enclosing muscle groups

Intermuscular septa: Fibrous partitions separating muscle compartments

Epimysium/perimysium: Connective tissue sheaths within muscle

Neurovascular bundles: Often run within defined fascial planes

Grossing relevance:

Sarcomas often extend longitudinally within compartments.

Fascial planes may act as temporary barriers to spread.

Margin assessment must document relationship to fascia and major neurovascular structures.

3. Relationship to Bone and Joint

Although classified as soft tissue, many lesions:

Abut periosteum

Encase joints

Involve tendon sheaths

It is essential to document:

Bone invasion

Periosteal involvement

Intra-articular extension

4. Site-Specific Considerations

Extremities

Well-defined fascial compartments

Compartmental resection common

Depth and relation to fascia critical

Trunk

Less distinct compartments

Proximity to body cavities

Retroperitoneum

No true compartmental confinement

Tumours often large at presentation

Margins frequently close or positive

Head & Neck

Dense concentration of vital structures

Narrow surgical margins common

II SOFT TISSUE GROSSING TECHNIQUES

AMPUTATION FOR SOFT TISSUE TUMOURS

External Examination

Type of amputation and side.

Total length of limb segment.

Tumour size and location (distance from proximal and distal margins).

Skin changes (ulceration, scar, biopsy site).

Dissection

Open skin longitudinally along a plane that preserves tumour integrity.

Avoid cutting directly through the tumour unless necessary to assess bone involvement.

Dissect tumour en bloc with surrounding compartment.

If bone involvement is suspected (clinically or radiologically):

Saw through bone in line with tumour to assess cortical and medullary extension.

Description of Tumour

Size (3 dimensions)

Exact plane of origin (if identifiable):

- Intramuscular
- Intermuscular
- Subcutaneous
- Fascial
- Tendon sheath
- Nerve
- Vessel

Relationship to:

- Major neurovascular bundles
- Bone and joint
- Fascia

Percentage necrosis (if post-therapy specimen)

If large:

Serially section and fix overnight before detailed sampling.

Examination of Rest of Limb

Skin and soft tissues

Joint involvement (if present, open joint and sample synovium and adjacent structures)

Vascular Involvement

Open major vessels.

Submit sections if:

Tumour thrombus suspected

Vessel wall invasion present

Recommended Sampling (Sarcoma Amputation) (Fig 15.1)

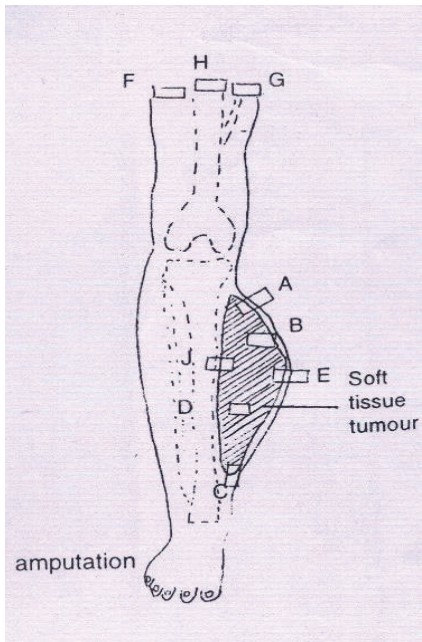


Fig 15.1 : Sampling in a sarcoma

A–D : Multiple sections from tumour including periphery with adjacent fat, muscle, and fascia
E : Previous biopsy site / tract
F : Proximal soft tissue margin
G : Closest radial margin (including neurovascular bundle if near)
H : Proximal bone margin
J : Tumour with underlying bone (if involved)
K–M : Lymph nodes (if present or submitted)

AMPUTATION FOR OCCLUSIVE VASCULAR DISEASE (Lower Limb)

These specimens are usually for peripheral arterial disease.

External Description

Type of amputation and side.

Length and circumference.

Skin:

- Colour changes
- Gangrene (dry/wet)
- Ulcers (size and depth)
- Infection

Muscle viability (if visible).

Dissection of Major Arteries

Modern practice does not require extensive block excision of large rectangles of tissue. Instead:

Systematically expose major vessels.

Open longitudinally.

Examine for:

- Atherosclerotic plaques
- Thrombus (recent vs organised)
- Calcification
- Occlusion
- Embolic material

Major vessels to examine: see fig 15.2 and 15.3

Femoral

Popliteal

Anterior tibial

Posterior tibial

Peroneal

Dorsalis pedis

Plantar vessels

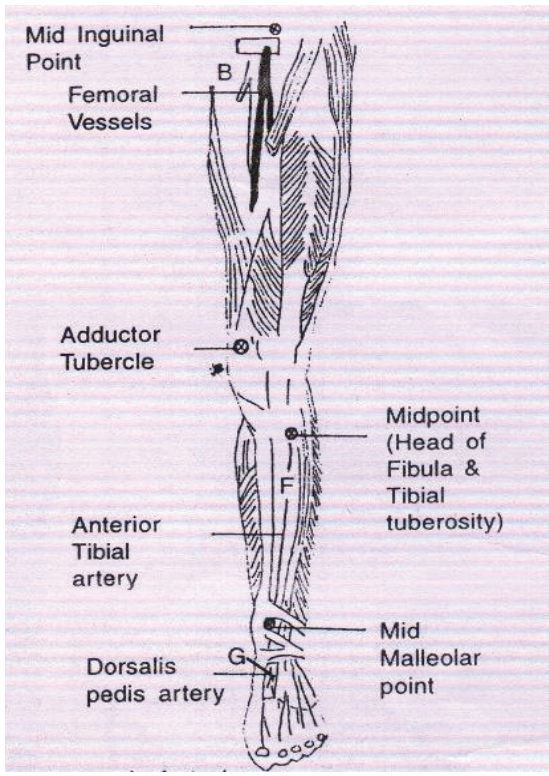


Fig 15.2 : Major blood vessels in lower limb

Describe: degree of luminal occlusion

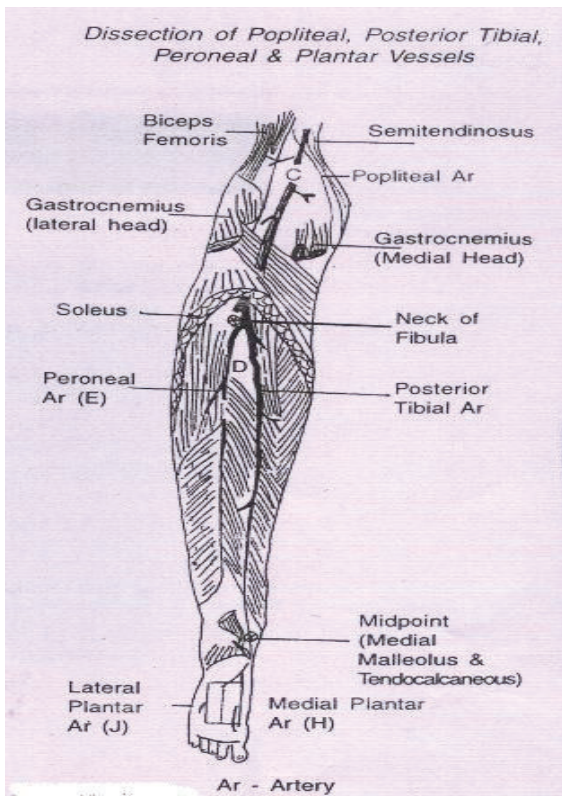


Fig 15.3 Dissection of Popliteal, posterior tibial and peroneal vessels

III SOFT TISSUE – GROSS PEARLS

Circumscribed cystic and haemorrhagic lesion with fibrotic periphery → Cysticercal granuloma *(* Look carefully for a scolex; often mistaken for necrotic tumour).

Vascular Lesions : Cystic, multiloculated, thin-walled lesion → Lymphangioma (lymphatic malformation)

Modern terminology: Lymphatic malformation preferred.

Haemorrhagic, poorly circumscribed infiltrative mass → Angiosarcoma

Ill-defined margins

Extensive haemorrhage and necrosis

Often in scalp (elderly) or post-radiation setting

Spongy lesion with large vascular channels → Arteriovenous malformation

Fibro-histiocytic & Dermal Tumours: Bosselated nodular mass;

overlying skin stretched and adherent → Dermatofibrosarcoma protuberans (DFSP)
Modern gross clue: Infiltrative "tentacle-like" extensions into subcutaneous fat. Always sample radial margins widely

Well-defined dermal/subcutaneous lesion → May be solitary neurofibroma
→ Leiomyoma

Adipocytic tumours Gross Appearance – Lipoma & Atypical Lipoma

Lipoma – Gross Features

- Well-circumscribed, often encapsulated tumour
- Soft, lobulated, and compressible
- Yellow cut surface resembling mature adipose tissue
- Lobules separated by delicate fibrous septa
- Size varies from a few millimetres to several centimetres
- Usually easily shelled out from surrounding tissue

Cut surface:

- Uniform, greasy, bright yellow
- No significant hemorrhage or necrosis

Key Gross Pearl:
Homogeneity is the hallmark—any variation should raise suspicion

Atypical Lipomatous Tumour (ALT) / Well-differentiated Liposarcoma – Gross Features

- Larger than conventional lipoma (often >10 cm, especially in deep sites)
- Less well circumscribed; may appear lobulated rather than encapsulated
- Firm areas interspersed with fatty tissue

Cut surface:

• Predominantly yellow (fatty), BUT:

Thick fibrous septa

Focal whitish or firm areas

Possible nodularity

• May show variation in texture (unlike lipoma)

Important Suspicious Features:

• Thickened septae
• Nodular non-fatty areas
• Size and deep location (retroperitoneum, thigh)

Key Gross Pearl:

Any "lipoma" that is large, deep, or heterogeneous → sample extensively to rule out ALT

Neural Tumour : Encapsulated, eccentric to nerve, firm, tan-white, whorled

cut surface → Schwannoma

Pearl: May show cystic degeneration . Antoni A/B areas may correlate

grossly with firm vs myxoid zones

Fusiform enlargement of nerve, infiltrative → Malignant

peripheral nerve sheath tumour (MPNST)

Red flag: Necrosis; Haemorrhage ; Rapid enlargement in NF1 patient

Muscle Tumours : Intramuscular, infiltrative mass → Intramuscular lipoma (benign but infiltrative)

Fleshy, soft to firm, grey-white tumour with necrosis → Rhabdomyosarcoma

Modern note: In children/young adults

Often poorly circumscribed

Rapid growth

Firm, whorled, well-defined lesion in dermis/subcutis → Leiomyoma. **Soft to firm subcutaneous tumour with necrosis** → Cutaneous leiomyosarcoma

Red flag: Irregular margins ; Infiltrative growth into deeper tissue

Lipoma & Atypical Lipoma

Lipoma – Gross Features

- Well-circumscribed, often encapsulated tumour, edge slips on palpation
- Soft, lobulated, and compressible
- Yellow cut surface resembling mature adipose tissue
- Lobules separated by delicate fibrous septa
- Size varies from a few millimetres to several centimetres
- Usually easily shelled out from surrounding tissue

Cut surface:

- Uniform, greasy, bright yellow
- No significant hemorrhage or necrosis, homogenous appearance

Atypical Lipomatous Tumour (ALT) / Well-differentiated

Liposarcoma

- Larger than conventional lipoma (often >10 cm, especially in deep sites)
- Less well circumscribed; may appear lobulated rather than encapsulated
- Firm areas interspersed with fatty tissue

Cut surface:

- Predominantly yellow (fatty), BUT:

  Thick fibrous septa (Important feature)

  Focal whitish or firm areas

  Possible nodularity

  May show variation in texture (unlike lipoma)

Key Gross Pearl:
Any "lipoma" that is large, deep, or heterogeneous → sample extensively to rule out ALT.

Retroperitoneal Tumours : Large, Deep seated fatty, mass →
Liposarcoma **(most common retroperitoneal sarcoma)**
Modern gross pearl: Retroperitoneal fatty tumours are malignant until proven otherwise; Often extremely large at presentation; Dedifferentiated areas appear firm, grey-white, non-fatty

Fat + haematopoietic elements → Extra-adrenal myelolipoma

Highly vascular mass near great vessels → Paraganglioma

Soft, fleshy, homogeneous mass → Lymphoma

Heterogeneous tumour with alternating myxoid and solid areas → Consider:
Myxoid liposarcoma

Myxofibrosarcoma

Low Grade fibromyxoid sarcoma

Large (>5 cm), deep, rapidly growing mass → Always suspect sarcoma

Gelatinous, multinodular tumour in elderly extremity → Myxofibrosarcoma
Pearl: Often infiltrative with poorly defined margins . High local recurrence

Nodular lesion along tendon sheath of hand → Tenosynovial giant cell tumour ;
;*Gross:* Brown-yellow due to hemosiderin

Deep-seated tumour with biphasic cystic and solid areas in young adult →
Synovial sarcoma

Any soft tissue tumour that is: Deep to the fascia; 5 cm ; Heterogeneous;

Shows necrosisand is ill-defined → Requires extensive sampling and careful margin assessment.

IV SOFT TISSUE MICROSCOPY

(Updated as per WHO 2020 Soft Tissue Tumours)

IMPORTANT CLASSIFICATION UPDATES

"Malignant fibrous histiocytoma (MFH)" → now termed
Undifferentiated Pleomorphic Sarcoma (UPS)

Hemangiopericytoma → merged under
Solitary Fibrous Tumour (SFT)

Round cell liposarcoma → now considered
High-grade Myxoid Liposarcoma

Adult fibrosarcoma → now very rare diagnosis (diagnosis of exclusion)

Many entities now defined by **specific molecular alterations**

I. ADIPOCYTIC TUMOURS – MICROSCOPY PEARLS

Lipoma

Mature adipocytes

Uniform nuclei

No atypical stromal cells

No lipoblasts

Atypical Lipomatous Tumour / Well-Differentiated Liposarcoma

Mature fat with:

- Hyperchromatic stromal cells
- Atypical cells in fibrous septa
- Scattered multivacuolated lipoblasts (may be rare)

Thick fibrous septa

Nuclear enlargement and irregularity

Key molecular marker:

MDM2 and CDK4 amplification (IHC/FISH diagnostic)

Important: Retroperitoneal fatty tumours are almost never simple lipomas.

Dedifferentiated Liposarcoma

Abrupt transition:

- Well-differentiated component
- High-grade non-lipogenic sarcoma

Dedifferentiated area may resemble UPS

Molecular hallmark:

MDM2 amplification retained in both components

Myxoid Liposarcoma

Abundant myxoid matrix

Uniform oval cells

Delicate “chicken-wire” capillary network

Lipoblasts with nuclear scalloping

Molecular hallmark:

FUS–DDIT3 fusion (t(12;16))

High-grade areas = increased cellularity (formerly “round cell”).

Pleomorphic Liposarcoma

High-grade pleomorphic sarcoma

Multivacuolated pleomorphic lipoblasts essential

No MDM2 amplification

Spindle Cell / Pleomorphic Lipoma

Mature fat + bland spindle cells

Ropey collagen

Floret giant cells (pleomorphic lipoma)

Genetics:

13q/RB1 deletion

Hibernoma

Brown fat differentiation

Multivacuolated eosinophilic granular cytoplasm

No atypia

II. FIBROBLASTIC / MYOFIBROBLASTIC TUMOURS

Nodular Fasciitis

Tissue culture–like fibroblasts

Myxoid stroma

Extravasated RBCs

Frequent mitoses (normal)

No significant atypia

Molecular hallmark:

USP6 rearrangement (confirms reactive neoplasm concept)

Myositis Ossificans

Zonation phenomenon:

- Central immature fibroblasts
- Peripheral mature bone

Reverse zoning suggests osteosarcoma

Desmoid-type Fibromatosis

Long sweeping fascicles

Infiltrative margins

Minimal atypia

Rare mitoses

Molecular hallmark:

CTNNB1 (β-catenin) mutation

Nuclear β-catenin positivity

No metastatic potential but high recurrence.

Solitary Fibrous Tumour (SFT)

Pattern less pattern

Alternating cellularity

Staghorn vessels

Keloid-like collagen

Molecular hallmark:

NAB2–STAT6 fusion

STAT6 nuclear positivity (diagnostic)

(Hemangiopericytoma term obsolete)

Inflammatory Myo-fibroblastic Tumour

Spindle myofibroblasts

Plasma cell-rich infiltrate

Variable atypia

Molecular:

ALK rearrangement (subset)

ALK IHC positive in ~50%

Myxofibrosarcoma

Myxoid nodules

Curvilinear vessels

Pleomorphic cells

Pseudolipoblasts

Common in elderly extremities; high local recurrence.

Low-Grade Fibromyxoid Sarcoma

Alternating fibrous and myxoid zones

Bland spindle cells

Whorled growth

"Collagen rosettes"

Molecular:

FUS–CREB3L2 fusion

Infantile Fibrosarcoma

Cellular spindle tumour

Herringbone-like areas

High mitotic rate

Hemangiopericytoma-like vessels

Molecular hallmark:

ETV6–NTRK3 fusion
(Targetable with TRK inhibitors)

Adult Fibrosarcoma

True cases rare

Classic herringbone pattern

Diagnosis of exclusion

III. "FIBROHISTIOCYTIC" CATEGORY (Updated Concept)

Most lesions now reclassified.

Undifferentiated Pleomorphic Sarcoma (UPS)

(former MFH)

Marked pleomorphism

Tumour giant cells

High mitotic activity

Necrosis

No line of differentiation after IHC

Diagnosis of exclusion.

Tenosynovial Giant Cell Tumour

Mononuclear cells

Osteoclast-like giant cells

Hemosiderin

Molecular:

CSF1 rearrangement

IV. SMOOTH MUSCLE TUMOURS

Leiomyoma (Fig 15.4)

Intersecting fascicles

Blunt-ended nuclei

No atypia or necrosis

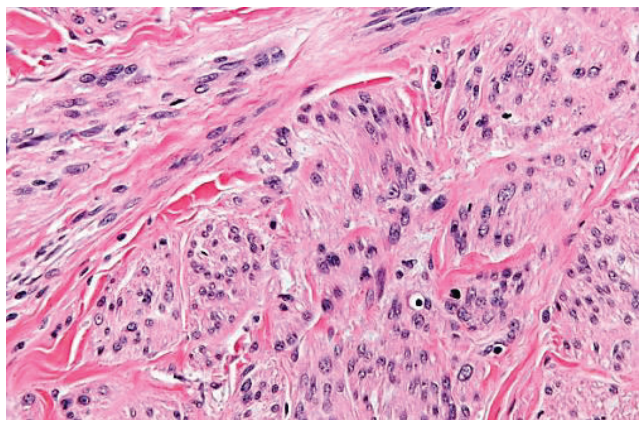


Fig 15.4 Leiomyoma with spindle -shaped cells showing bland nuclei H&E x 400

Leiomyosarcoma

Nuclear atypia

Tumour cell necrosis

Mitotic activity

Eosinophilic cytoplasm

IHC: SMA, desmin, h-caldesmon positive

V. SKELETAL MUSCLE TUMOURS

Embryonal Rhabdomyosarcoma

Small round to spindle cells

Cambium layer (botryoid variant)

Rhabdomyoblasts

Alveolar Rhabdomyosarcoma

Fibrous septa

Discohesive cells

Central loss forming alveolar spaces

Molecular:

PAX3/7–FOXO1 fusion

Pleomorphic Rhabdomyosarcoma

High-grade pleomorphic tumour

Rhabdomyoblasts

Adult patients

IHC: Myogenin, MyoD1 positive

VI. VASCULAR TUMOURS

Epithelioid Hemangioendothelioma

Cords and nests

Myxohyaline stroma

Intracytoplasmic lumina

Molecular:

WWTR1–CAMTA1 fusion

Angiosarcoma

Anastomosing vascular channels

Marked atypia

Multilayering

Necrosis

IHC: CD31 (most specific), ERG positive

VII. TUMOURS OF UNCERTAIN DIFFERENTIATION

Synovial Sarcoma

Biphasic or monophasic

Uniform spindle cells

Hemangiopericytoma-like vessels

Molecular hallmark:

SS18–SSX fusion (t(X;18))

Epithelioid Sarcoma

Nodular growth

Central necrosis

Epithelioid cells

Loss of INI1 (SMARCB1)

Clear Cell Sarcoma

Nests of clear cells

Prominent nucleoli

Melanocytic markers positive

EWSR1–ATF1 fusion

Alveolar Soft Part Sarcoma

Alveolar nests

Abundant eosinophilic cytoplasm

PAS-positive crystals

TFE3 rearrangement

Desmoplastic Small Round Cell Tumour

Small round blue cells

Desmoplastic stroma

EWSR1–WT1 fusion

IMPORTANT MODERN DIAGNOSTIC CONCEPTS

Molecular testing is now central in many sarcomas.

Many entities once diagnosed morphologically now require confirmation by:

FISH

RT-PCR

NGS panels

"Diagnosis of exclusion" category (UPS) should only be used after adequate IHC.

V SOFT TISSUE SYNOPTIC REPORTING

The pathology report of a soft tissue tumour should include the following parameters:

1. Specimen Details
   • Type of specimen (biopsy, wide local excision, compartmental resection, amputation, etc.).
   • Anatomical site and laterality.
2. Tumour Characteristics
   • Histological type according to the current WHO classification.
   • Tumour size (greatest dimension and additional dimensions in centimetres).
   • Depth/location (superficial, subcutaneous, intramuscular, deep-seated, retroperitoneal, visceral, etc.).
   • Histological grade (where applicable).
3. Microscopic Features
   • Tumour differentiation.
   • Mitotic activity (mitoses per 10 high-power fields or per mm^2).
   • Percentage of necrosis.
   • Presence of vascular or lymphatic invasion.
   • Other significant pathological features specific to the tumour type.
4. Surgical Margins
   • Status of margins (negative or positive).
   • Distance of tumour from closest margin.
   • Margin involved, if positive.
5. Ancillary Studies
   • Immunohistochemical findings.
   • Molecular or cytogenetic studies, where performed.
6. Treatment Response (if neoadjuvant therapy given)
   • Percentage of viable tumour.
   • Percentage of necrosis/fibrosis.
   • Assessment of treatment effect.
7. Pathological Stage
   • TNM stage, where applicable, according to the current staging system.

Essential reporting elements for malignant soft tissue tumours include tumour type, size, grade, extent, margin status, and pathological stage, as these parameters have major prognostic and therapeutic significance.

ASSESS YOURSELF SOFT TISSUE

A. Long Essay Questions *(10 marks each)*

1. Classify soft tissue tumours and describe the clinicopathological features of liposarcoma.
2. Describe fibromatosis (desmoid tumour) under the following headings:

   Etiopathogenesis ;Clinical features ; Gross features ; Microscopy and

   Behaviour and prognosis
3. Discuss rhabdomyosarcoma with emphasis on subtypes, histopathology, and prognostic factors.

B. Short Essay Questions *(5 marks each)*

4. Lipoma vs atypical lipomatous tumour – gross and microscopic differences.
5. Synovial sarcoma – histology and molecular features.
6. Malignant peripheral nerve sheath tumour (MPNST) – key histologic features and differential diagnosis.

C. Short Answer Questions *(3 marks each)*

7. What is the significance of MDM2 amplification in soft tissue tumours?
8. Define “herringbone pattern” and mention the tumour in which it is seen.
9. What are the characteristic features of myxoid stroma in soft tissue tumours?

D. Multiple Choice Questions (MCQs)

1. **The most common benign soft tissue tumour is:**

   a. Fibroma
   b. Lipoma
   c. Haemangioma
   d. Neurofibroma

2. **A tumour showing a biphasic pattern with epithelial and spindle cell components is characteristic of:**

   a. Fibrosarcoma
   b. Synovial sarcoma
   c. Liposarcoma
   d. Leiomyosarcoma

3. **Which of the following is associated with t(X;18) translocation?**

    a. Ewing sarcoma
    b. Synovial sarcoma
    c. Rhabdomyosarcoma
    d. Fibrosarcoma

Answer Key for MCQs

1. b
2. b
3. b

Chapter 16
Bone

Chapter outline : 1 – Bone Anatomy II- Bone Grossing Techniques III- Bone Gross Pearls; IV – Bone Microscopy ; VBone - Synoptic Reporting.

I BONE ANATOMY

Bone is a specialized mineralized connective tissue that provides structural support, protection of vital organs, locomotion, haematopoiesis, and mineral homeostasis. The human skeleton consists of 206 bones, broadly divided into the axial and appendicular skeleton.

1. Types of Bones

Based on shape:

Long bones – e.g., femur, tibia, humerus

Short bones – carpals, tarsals

Flat bones – skull bones, ribs, sternum

Irregular bones – vertebrae, pelvis

Sesamoid bones – patella

In surgical pathology, long bones and flat bones are most frequently encountered in tumour resections and biopsies.

2. Gross Structural Organization

(a) Long Bone

A long bone is divided into:

Epiphysis – expanded ends, composed predominantly of cancellous (trabecular) bone and covered by articular cartilage

Metaphysis – region between epiphysis and diaphysis; site of active growth in children and common site for primary bone tumours

Diaphysis – shaft composed mainly of compact (cortical) bone surrounding a medullary cavity

The growth plate (physis) in children separates epiphysis from metaphysis and is composed of orderly zones of endochondral ossification.

3. Microscopic Architecture

Bone exists in two major architectural forms:

(a) Cortical (Compact) Bone

Dense outer shell

Organized into osteons (Haversian systems)

Provides mechanical strength

(b) Cancellous (Trabecular) Bone

Interconnected trabeculae

Marrow-filled intertrabecular spaces

Predominates in vertebrae, pelvis, ribs, and epiphyses of long bones

4. Cellular Components

Osteoblasts – bone-forming cells derived from mesenchymal stem cells

Osteocytes – mature bone cells within lacunae

Osteoclasts – multinucleated cells responsible for bone resorption

5. Bone Marrow

Two types:

Red marrow – haematopoietically active (axial skeleton, metaphysis of long bones in adults)

Yellow marrow – fatty marrow (diaphysis of long bones in adults)

Knowledge of normal marrow distribution is essential in evaluating marrow-based lesions and metastatic disease.

6. Blood Supply

Nutrient artery (enters diaphysis)

Metaphyseal and epiphyseal arteries

Periosteal vessels

The metaphyseal region has sluggish blood flow, explaining its predisposition to hematogenous infection and tumour localization.

7. Periosteum and Endosteum

Periosteum – outer fibrous layer and inner osteogenic layer; important in reactive bone formation and periosteal reactions in tumours/infection

Endosteum – lines medullary cavity and trabeculae

8. Development

Bone forms by:

Intramembranous ossification – flat bones of skull

Endochondral ossification – long bones

Most primary bone tumours recapitulate stages of endochondral differentiation.

Applied Surgical Anatomy of Bone Tumours

Bone tumours show a predilection for specific anatomical compartments of bone—epiphysis, metaphysis, and diaphysis—which directly influences surgical planning, margin assessment, and reconstruction.

The metaphysis is the most common site for primary malignant bone tumours such as Osteosarcoma, whereas the diaphysis is classically involved in Ewing sarcoma. Pure epiphyseal lesions are characteristic of tumours like Giant cell tumour of bone.

Understanding:

Relationship to the physis (growth plate) in skeletally immature patients

Cortical breach and periosteal reaction

Medullary spread

Neurovascular proximity

Joint involvement

is essential for limb-salvage resections versus amputation and for accurate pathological margin evaluation.

The anatomical compartmentalization of bone (medulla, cortex, periosteum, and surrounding soft tissue) forms the basis of oncologic resection margins.

II BONE GROSSING TECHNIQUES

1. Specimens Received

Bone biopsy

Bone marrow needle biopsy

Curettage specimen

Femoral head excision

Resection specimen of tumour

Amputation / Disarticulation specimen

A. Bone Biopsy / Curettage / Needle Biopsy

Types

Open biopsy

Curettage specimen

Core/needle biopsy

Bone marrow needle biopsy

Grossing Protocol

Measurements

Measure all fragments in 2–3 dimensions.

Weigh curettage specimens.

Description

Number of fragments

Shape

Colour

Consistency

Cystic change

Necrosis (if appreciable)

Separate soft tissue and calcified tissue and process separately.

Fixation

Fix bony tissue in formalin for 1 hour before decalcification.

Decalcification

(a) Formic acid solution

Formic acid – 125 ml

40% formalin – 25 ml

Distilled water – 350 ml

(b) Nitric acid solution

Concentrated nitric acid – 5 ml

Distilled water – 95 ml

(c) EDTA (Preferred for IHC & molecular studies)

Best for immunohistochemistry, enzyme studies, and electron microscopy.

Bits

Submit entire material.

Bilateral biopsies must be submitted separately.

B. Femoral Head Excision

1. Identification

Mention side (right/left).

2. Measurements

Diameter and thickness.

3. External Description

Articular surface: smooth/irregular

Osteophytic lipping

Synovial membrane: hypertrophic/papillary

4. Sectioning

First cut through the **fovea**, medial to lateral.

Divide into anterior and posterior halves.

Serial parallel cuts at 5 mm intervals.

5. Cut Surface Description

Cartilage thickness

Cartilage integrity (intact/eroded)

Eburnation (marble-like polished subchondral bone)

Cysts (size and contents)

Necrosis

Evidence of fracture

Appearance of remaining bone

6. Fixation

Select representative slice.

Fix in formalin (2 hours).

Decalcify.

Bits

A, B – 2 bits from lesion (include cartilage + synovium).

C. Amputation / Disarticulation Specimens (Extremities)

Review clinical details, radiology (X-ray, MRI), and prior biopsy before grossing.

Types of Procedures

Upper Limb

Forequarter amputation

Shoulder disarticulation

Above/below elbow amputation

Hand amputation

Lower Limb

Hip disarticulation

Above/below knee amputation

Foot/toe amputations

General Principles

Handle immediately; if delayed, store at 4°C.

Orient properly.

Ink margins before sectioning.

Description and Dissection

Mention side and type of procedure.

Measure length and circumference at tumour level.

Note previous biopsy site (position & size).

External tumour characteristics:

- Skin colour
- Ulceration
- Scars
- Size, borders, consistency

Take surgical margins:

- Neurovascular bundle
- Soft tissue
- Skin

- Dissect and separately label regional lymph nodes (if present).
- Soft tissue dissection:
  - Dissect tumour up to periosteum.
  - Maintain continuity if soft tissue invasion present.
  - Document plane of spread.
- Joint Handling:
  - If joint uninvolved → disarticulate through joint.
  - If joint involved → cut through uninvolved bone 5–10 cm beyond.
- Longitudinal section through tumour:
  - Divide bone into anterior/posterior or medial/lateral halves.

Cut Surface Description

- Size of tumour
- Location:
  - Epiphysis
  - Metaphysis
  - Diaphysis
- Medullary involvement
- Cortical destruction
- Physeal involvement (has tumour crossed growth plate?)
- Periosteum:
  - Uninvolved
  - Elevated
  - Invaded
- Soft tissue extension
- Distance from nearest articular surface
- Gross appearance:
  - Shape

- Colour
- Consistency
- Encapsulation
- Necrosis
- Myxoid change
- Cystic areas
- Bone/cartilage formation

Rest of limb:

- Skin
- Muscles
- Vessels
- Nerves
- Other joints

Lymph nodes:

- Number
- Size
- Cut surface

Bits (Standard Tumour Sampling)

Tumour

A – Tumour + cortex + periosteum

B – Tumour + medulla

C – Tumour + epiphyseal line + cartilage

D – Tumour + soft tissue

Additional

E – Previous biopsy site

F – Osseous surgical margin

G – Neurovascular margin

H/J – Skin and soft tissue margins

K – Uninvolved bone (8–10 cm away)

L – Any abnormal area elsewhere

M+ – All lymph nodes

Minimum: 4 tumour blocks; more if heterogeneous.

Given below is a diagram (Fig 16.1) showing sample bits taken for an amputation of tumour

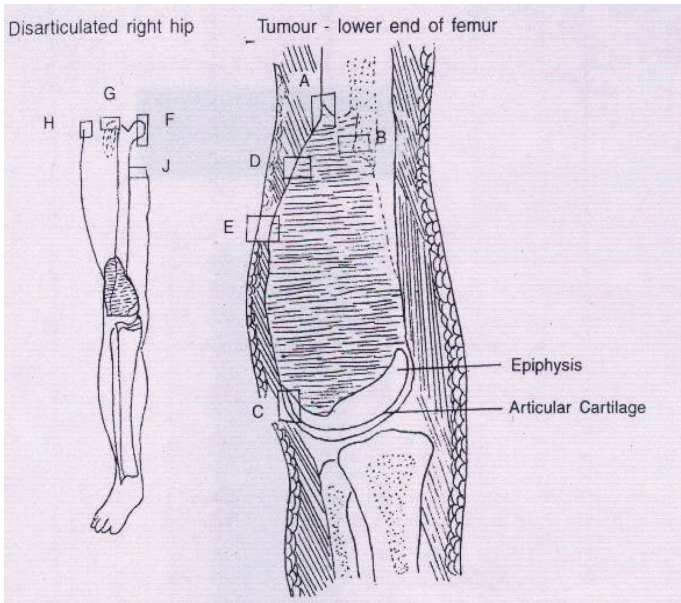


Fig 16.1

A - One to include tumour with adjacent cortex and periosteum

B - One to include tumour with medulla

C - One to include tumour with epiphyseal line and articular cartilage

D - One to ihclude tumour with soft tissue

Extra bits are taken depending on the requirement

E - 1 bit from previous biopsy site

F - 1 bit from the osseous surgical margin

G - 1 bit surgical margin, neurovascular bundle

H,J - 2 bits surgical margin of skin and soft tissue.

K : - one bit uninvolved bone, 8-10 cm away from joint which appears grossly involved.

L - bits from abnormal looking areas elsewhere in the limb (not shown).

M and more - submit the lymph nodes depending on the groups and number (not shown).

Response to Chemotherapy in Bone Tumours

In many instances chemotherapy is given before surgery or amputation in cases of osteogenic sarcoma and Ewing's sarcoma. In such cases in addition to the description and dissection of the specimen as outlined above, a slight variation in the sampling of the tumour has to be made This necrosis is recorded as 10 percentile increments. E.g. more than 90% necrosis, more than 80% necrosis etc., and should be entered in the report with a copy of the grid diagram included in the final report. The bits taken should be indicated by a figure as shown (Fig 16.2).

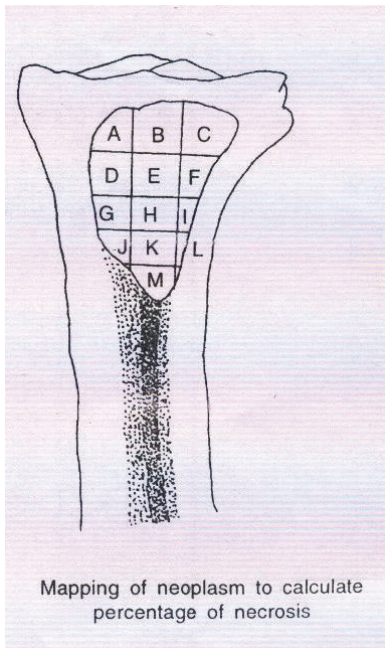


Fig 16.2

Neoadjuvant chemotherapy is commonly used in:

Osteosarcoma and Ewing sarcoma

Special Sampling Requirement

Sample the entire largest tumour surface sequentially.

Map blocks in grid fashion.

Assess percentage necrosis per block. For this the percentage of the necrosis in each bit is added and an average taken. Though the calculation of this percentage of necrosis is quite subjective, it has been observed that more than 90% necrosis (calculated for the entire surface) denotes a good response to chemotherapy.

Calculate average necrosis and express as a percentile : it has been observed that more than 90% necrosis (calculated for the entire surface) denotes a good response to chemotherapy.

Reporting

Record necrosis in 10% increments.

90% necrosis → Good response.

Include grid diagram copy in final report.

BONE TUMOUR RESECTION GROSSING CHECKLIST

1. Pre-Grossing Review

☐ Clinical details
☐ Age & skeletal maturity
☐ Site of tumour
☐ Imaging reviewed (X-ray / MRI)
☐ Neoadjuvant chemotherapy given? (Yes/No)
☐ Previous biopsy site identified

2. Specimen Identification

☐ Side (Right / Left)
☐ Type of procedure
  ☐ Limb salvage resection
  ☐ Amputation
  ☐ Disarticulation
☐ Length of specimen (cm)
☐ Circumference at tumour level (cm)

3. External Examination

☐ Skin changes (ulcer/scar/sinus)
☐ Previous biopsy site (measure & ink)
☐ Soft tissue swelling
☐ Neurovascular bundle identified

4. Margin Inking

☐ Proximal bone margin
☐ Distal bone margin
☐ Radial soft tissue margin
☐ Neurovascular margin
☐ Skin margin

5. Sectioning

☐ Longitudinal section through tumour
☐ Additional parallel slices (1–2 cm)
☐ Joint involvement assessed
☐ Growth plate involvement assessed (if applicable)

6. Tumour Description (Cut Surface)

☐ Size (3 dimensions)
☐ Location
    ☐ Epiphysis
    ☐ Metaphysis
    ☐ Diaphysis
☐ Medullary involvement
☐ Cortical destruction
☐ Periosteal elevation / invasion
☐ Soft tissue extension (measure depth)
☐ Distance to nearest articular surface (cm)
☐ Distance to closest margin (cm)

Gross Appearance

☐ Colour
☐ Consistency
☐ Necrosis (%) estimated
☐ Cystic change
☐ Hemorrhage
☐ Cartilage/bone formation

7. Lymph Nodes (if present)

☐ Number identified
☐ Largest dimension
☐ Submitted entirely

8. Standard Tumour Blocks

Minimum 4–6 tumour sections

☐ Tumour + cortex + periosteum
☐ Tumour + medulla

☐ Tumour + soft tissue
☐ Tumour + growth plate / cartilage
☐ Closest bone margin
☐ Closest soft tissue margin
☐ Neurovascular margin
☐ Previous biopsy site
☐ Uninvolved bone (remote)

9. Post-Chemotherapy Cases

☐ Entire largest tumour surface sampled sequentially
☐ Grid mapping performed
☐ Necrosis % assessed
☐ Average necrosis calculated

90% necrosis = Good response (e.g., in **Osteosarcoma** and **Ewing sarcoma**)

III BONE GROSS PEARLS

General Principles

Radiograph the specimen (whole or sliced) whenever possible.

Helps define tumour extent and margins.

Essential for identifying nidus in Osteoid osteoma.

Correlate with MRI for marrow and soft tissue extension.

Necrotic bone: opaque, yellow, dry.
Viable bone: translucent, pink.

Characteristic Gross Appearances

1. Gritty to cut, grey-yellow lesion

→ Fibrous dysplasia
(Classic "ground glass" radiology; firm, fibrous, curvilinear trabeculae microscopically.)

2.Well-circumscribed shaft/skull lesion, greenish-brown hue

→ Langerhans cell histiocytosis

Colour due to abundant eosinophils.

Skull: "hole-in-hole" appearance (asymmetric table destruction).

Shaft: lucent lesion with reactive periosteal new bone.

3. Well-circumscribed lesion in muscle

Center: oedematous skeletal muscle
Periphery: calcification
→ Myositis ossificans (zonal phenomenon)

4.Granular brown-yellow metaphyseal lesion

→ Non-ossifying fibroma / metaphyseal fibrous defect

5. Small red granular nidus, soft vs surrounding sclerotic bone → Osteoid osteoma

Red granular nidus WITHOUT sclerotic rim → Osteoblastoma

7. Bony protruberance at metaphysis covered by cartilage→ Osteochondroma

8.Central cyst at metaphysis with clear yellow fluid → Simple (solitary) bone cyst

9. Eccentric metaphyseal lytic lesion, "blown-out", hemorrhagic → Aneurysmal bone cyst (Fluid levels on imaging).Important differential:→ Telangiectatic Osteosarcoma

10.Soft dark-brown tumour at epiphyseal end of long bone

Cystic change common → Giant cell tumor of bone

11.Small bone filled with cartilage; cortex paper-thin → Enchondroma

12.White firm tumour at epiphysis → Chondroblastoma

13.White lobulated tumour→ Chondromyxoid fibroma

14.Large axial cartilaginous tumour

Light blue / pearly white
Gritty calcified areas
Myxoid change → **Chondrosarcoma**

12. Expanded swelling at end of long bone

"Leg of mutton" appearance
Cut surface: bony hard to cystic, friable, hemorrhagic
Cortical breach with symmetrical soft tissue extension→ Conventional **Osteosarcoma**

Common Lesions by Location in Long Bone

Epiphysis

- Giant cell tumor of bone
- Chondroblastoma
- (Rarely) Osteoid osteoma

Metaphysis

- Osteoid osteoma
- Osteoblastoma
- Osteochondroma
- Osteosarcoma
- Chondromyxoid fibroma
- Peripheral chondrosarcoma
- Fibrous dysplasia
- Non-ossifying fibroma
- Simple bone cyst
- Aneurysmal bone cyst

Diaphysis

- Ewing sarcoma
- Primary lymphoma of bone
- Osteoblastoma
- Enchondroma
- Central chondrosarcoma
- Mesenchymal chondrosarcoma
- Fibrous dysplasia

VI BONE – MICROSCOPY

NON-NEOPLASTIC LESIONS OF BONE

Disorders of Bone Matrix (Collagen Defects)

Osteogenesis imperfecta

Pathogenesis

Caused by mutations in COL 1 A1/COL 1A2 genes.

Defective type I collagen synthesis → fragile osteopenic bone.

Most forms autosomal dominant; some recessive variants recognized.

Clinical Pearls

Recurrent fractures

Blue sclera

Hearing loss (otosclerosis)

Dentinogenesis imperfecta

Short stature

Radiology Osteopenia

Multiple fractures

Wormian bones in skull

"Popcorn calcification" near epiphyses (severe forms)

Microscopy

Markedly reduced and disorganized lamellar bone.

Predominance of woven bone.

Thin cortices and sparse trabeculae.

Crowded osteocytes within thin trabeculae.

Under polarized light: poor organization of collagen bundles.

In milder forms: lamellar bone present but thin.

Key Diagnostic Clue

→ Abnormal collagen architecture rather than mineralization defect.

Defects in Mineralization

Rickets / Osteomalacia

Etiology

- Vitamin D deficiency
- Impaired vitamin D metabolism
- Renal phosphate wasting (e.g., hypophosphatemic rickets)
- Chronic renal disease

Important Practical Pearl
Routine decalcified sections may obscure excess osteoid — clinic-radiologic correlation is essential.

Radiology

- Widened, irregular growth plate
- Metaphyseal cupping and fraying
- Looser zones (pseudofractures) in adults

Microscopy

- Increased unmineralized osteoid (wide osteoid seams).
- Thickened growth plate in children.
- Disorganized endochondral ossification.
- “Tongues” of uncalcified cartilage extending into metaphysis.
- Increased osteoblastic activity.
- In adults: abundant osteoid lining trabeculae.

Key Diagnostic Clue
→ Increased osteoid thickness (>10–15 μm).

Disorders Due to Increased Bone Resorption

Hyperparathyroidism

Types

- Primary (usually adenoma)
- Secondary (chronic renal failure)
- Tertiary

Radiology

Subperiosteal resorption (radial aspect of phalanges)

"Salt and pepper" skull

Brown tumours (osteitis fibrosa cystica)

Microscopy

Marked osteoclastic activity → tunneling resorption.

Peritrabecular fibrosis.

Increased bone turnover.

Brown tumour:

- Numerous osteoclast-type giant cells
- Hemorrhage
- Hemosiderin deposition
- Fibroblastic stroma

Key Diagnostic Clue
→ Giant cell lesion + clinical hypercalcemia.

Paget disease of bone

Pathogenesis

Increased osteoclastic activity followed by disorganized osteoblastic repair.

Viral hypothesis historically proposed; not definitively proven.

Common Sites

Pelvis

Spine

Skull

Femur

Biochemistry

Elevated alkaline phosphatase.

Microscopy

Mosaic pattern of lamellar bone.

Prominent cement lines.

Mixed lytic and sclerotic phases.

Enlarged osteoclasts (often multinucleated).

Thickened but structurally weak trabeculae.

Complication

Secondary sarcoma (≈1%) — usually osteosarcoma.

Key Diagnostic Clue
→ "Jigsaw puzzle" mosaic pattern.

Osteoporosis

Definition

Reduced bone mass with normal mineralization.

Common Types

Postmenopausal

Senile

Secondary (steroids, endocrine)

Radiology

Vertebral compression fractures

"Fish mouth" vertebrae

Microscopy

Thinned trabeculae.

Loss of trabecular connectivity.

Increased resorption surfaces.

Marrow fat prominence.

Key Diagnostic Clue
→ Quantitative loss, not qualitative defect.

5.Fracture Healing

Early (Days)

Necrotic bone.

Haemorrhage.

Ghost osteocytes.

1–2 Weeks

Granulation tissue.

Hyper-vascular fibrous tissue.

Reactive woven bone.

Cartilage formation.

May mimic sarcoma (pseudosarcomatous appearance).

Callus Formation

More exuberant in unstable fractures.

Complications

Delayed union.

Non-union.

Pseudoarthrosis (fibrous union).

Bone Infarction

Common in:

Sickle cell disease

Gaucher disease

Steroid therapy

Connective tissue disorders

Femoral head involvement:
→ 'Legg-Calve-Perthes disease'

Gross

Chalky, opaque area.

Hyperemic rim.

Microscopy

Empty lacunae (nonviable bone).

Necrotic marrow fat.

Ghost hematopoietic cells (early).

Peripheral granulation tissue.

Later fibrosis and repair.

Dystrophic calcification common.

Key Diagnostic Clue
→ Dead trabeculae + reparative rim.

Polarized Light Examination of Bone

Useful for:

Differentiating woven vs lamellar bone.

Demonstrating abnormal collagen architecture (e.g., osteogenesis imperfecta).

Highlighting mosaic pattern in Paget disease.

UPDATED WHO CLASSIFICATION OF BONE TUMOURS

I. CARTILAGINOUS TUMOURS

Benign

Osteochondroma

Enchondroma

Periosteal chondroma

Chondroblastoma

Chondromyxoid fibroma

Intermediate (locally aggressive)

Atypical cartilaginous tumour (ACT)
(= Grade 1 chondrosarcoma of appendicular skeleton)

Malignant

Conventional chondrosarcoma (Grade 1–3)

Dedifferentiated chondrosarcoma

Clear cell chondrosarcoma

Mesenchymal chondrosarcoma

II. OSTEOGENIC TUMOURS

Benign

Osteoma

Osteoid osteoma

Osteoblastoma

Intermediate

Aggressive osteoblastoma

Malignant

Conventional osteosarcoma

Telangiectatic osteosarcoma

Small cell osteosarcoma

Low-grade central osteosarcoma

Parosteal osteosarcoma

Periosteal osteosarcoma

High-grade surface osteosarcoma

Secondary osteosarcoma

III. FIBROGENIC / FIBRO-OSSEOUS TUMOURS

Desmoplastic fibroma

Fibrous dysplasia

Osteofibrous dysplasia

Low-grade central osteosarcoma (overlaps)

(Malignant fibrous histiocytoma is obsolete → now undifferentiated pleomorphic sarcoma)

IV. GIANT CELL–RICH LESIONS

Giant cell tumour of bone

Malignancy in giant cell tumour

V. EWING SARCOMA FAMILY

Ewing sarcoma (no longer separated from PNET)

VI. NOTOCHORDAL TUMOURS

Benign notochordal cell tumour

Chordoma

Poorly differentiated chordoma

VII. VASCULAR TUMOURS

Haemangioma

Epithelioid haemangioma

Epithelioid haemangioendothelioma

Angiosarcoma

VIII. HAEMATOPOIETIC TUMOURS

Plasma cell myeloma

Primary lymphoma of bone

IX. OTHER MESENCHYMAL TUMOURS

Adamantinoma

Leiomyosarcoma

Liposarcoma

Undifferentiated pleomorphic sarcoma

X. TUMOUR-LIKE LESIONS

Aneurysmal bone cyst

Simple bone cyst

Non-ossifying fibroma

Langerhans cell histiocytosis

Erdheim–Chester disease

DETAIL ON IMPORTANT TUMOURS- CARTILAGE TUMOURS

Osteochondroma

Most common benign bone tumour

Pathogenesis

EXT1 / EXT2 mutations (hereditary multiple exostoses)

Developmental growth plate abnormality

Radiology

Cortex and medullary cavity continuous with parent bone.

Grows away from joint.

Gross

Cartilage cap over bony stalk.

Microscopy

Cartilage cap with orderly columnar chondrocytes.

Endochondral ossification at base.

Marrow in stalk continuous with parent bone.

Red Flags for Malignant Transformation

Cap thickness > 2 cm (adult)

Pain

Irregular cap

Soft tissue mass

→ Secondary peripheral chondrosarcoma

Enchondroma

Small bones of hands/feet.

IDH1 / IDH2 mutations common.

Microscopy

Lobules of hyaline cartilage.

Hypocellular.

Minimal atypia.

No permeation.

Important
Small bone enchondromas may be hypercellular — correlate radiology.

Chondrosarcoma

Now divided into:

1. Conventional

IDH1/2 mutations common.

Graded 1–3.

Microscopy

Hypercellularity.

Nuclear enlargement.

Myxoid matrix.

Marrow permeation (most important sign).

Grade 1 in appendicular skeleton =
→ **Atypical Cartilaginous Tumour (ACT)**

2. Clear Cell Chondrosarcoma

Epiphyseal.

Clear cytoplasm.

Osteoclast-type giant cells.

Low grade behaviour.

3. Dedifferentiated Chondrosarcoma

Abrupt transition:
Low-grade cartilage → high-grade sarcoma.

Very poor prognosis.

4. Mesenchymal Chondrosarcoma

Biphasic:
Small round cells + islands of cartilage.

HEY1-NCOA2 fusion.

Aggressive.

OSTEOGENIC TUMOURS

a) Osteoid osteoma

<1.5–2 cm nidus.

Night pain relieved by NSAIDs.

High prostaglandin levels.

Microscopy

Interlacing osteoid trabeculae.

Plump osteoblasts.

Vascular stroma.

b) Osteoblastoma

2 cm.

Spine common.

Less reactive sclerosis.

May be aggressive.

Osteosarcoma

Definition

Malignant tumour producing osteoid directly from tumour cells.

Molecular

RB and TP53 pathway alterations.

Complex karyotype.

Microscopy

Malignant spindle cells.

Lace-like osteoid.

High mitotic activity.

Subtypes

Conventional

Telangiectatic

Small cell

Parosteal (MDM2 amplified)

Periosteal

Low-grade central (MDM2 amplified)

Post-chemotherapy

90% necrosis = good response.

EWING SARCOMA

t(11;22) → EWSR1-FLI1 fusion.

CD99 diffuse membranous.

Microscopy

Sheets of small round cells.

Glycogen positive (PAS).

Necrosis common.

DD

Lymphoma (CD45+)

Small cell osteosarcoma (osteoid present)

PNET is no longer separated.

GIANT CELL TUMOUR

H3F3A mutation (G34W).

Epiphyseal.

Microscopy (Fig 16.3)

Uniformly distributed osteoclast-type giant cells.

Mononuclear stromal cells are neoplastic component.

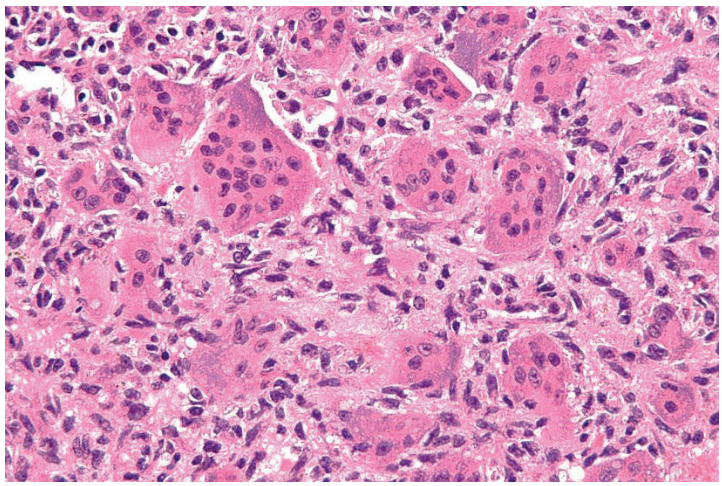


Fig 16.3 Giant cell tumour with uniform distribution of giant cells in stroma H & E x 100

Important

Benign histology does NOT exclude lung metastasis.

ADAMANTINOMA

Tibia.

Cytokeratin positive.

Epithelial nests in fibrous stroma.

Associated with osteofibrous dysplasia.

MULTIPLE MYELOMA

LYMPHOMA OF BONE

Usually, diffuse large B-cell lymphoma is more common..
CD20+, CD45+.

TUMOUR-LIKE LESIONS

Aneurysmal bone cyst

USP6 rearrangement.

Blood-filled spaces.

No endothelial lining.

Fibrous dysplasia

GNAS mutation.

“Chinese character” woven bone.

No osteoblastic rimming.

Osteofibrous dysplasia

Tibial cortex.

Osteoblastic rimming present.

Cytokeratin negative (distinguishes from adamantinoma).

SMALL ROUND CELL TUMOURS OF BONE – RAPID COMPARISON TABLE

Feature	Ewing sarcoma	Small cell osteosarcoma	Primary lymphoma of bone	Mesenchymal chondrosarcoma	Metastatic neuroblastoma
Age	Children/Adolescents	Adolescents	Adults	Young adults	Infants/Children
Pattern	Sheets of uniform small cells	Small cells + focal osteoid	Diffuse lymphoid infiltrate	Biphasic (small cells + cartilage islands)	Small blue cells
Cytoplasm	Scant	Scant	Moderate	Scant	Scant
Necrosis	Common	Common	Variable	Common	Common
Special Feature	PAS+ glycogen	Osteoid present	Lymphoglandular bodies	Abrupt cartilage islands	Homer-Wright rosettes
CD99	Diffuse membranous	Variable	Negative	Variable	Negative
CD45	Negative	Negative	**Positive**	Negative	Negative

NKX2.2	Positive	Negative	Negative	Negative	Negative
Cytokeratin	Negative	Negative	Negative	Rare	Negative
Molecular	EWSR1-FLI1	No EWSR1	B-cell clonality	HEY1-NCOA2	N-MYC amplified

Key Microscopic Pearls

If osteoid is present → Osteosarcoma,
even if tumour looks like Ewing.

If CD45 positive → Lymphoma.

If biphasic with cartilage → Mesenchymal chondrosarcoma.

RADIOLOGY–PATHOLOGY CORRELATION (always correlate the 2)

Location (Epiphysis / Metaphysis / Diaphysis)

Pattern (Lytic / Sclerotic / Mixed)

Periosteal reaction

Soft tissue extension

Bone tumour diagnosis is **radiologic–pathologic** — never histology alone.

Osteosarcoma

Radiology

Sunburst pattern

Codman triangle

Mixed lytic–sclerotic lesion

Metaphyseal

Pathology

Malignant osteoid

Tumour permeation through cortex

Soft tissue extension

Chondrosarcoma

Radiology

Ring and arc calcification

Endosteal scalloping

Cortical breach

Pathology

Hypercellular cartilage

Marrow permeation

Myxoid change

Ewing sarcoma

Radiology

Onion-skin periosteal reaction

Diaphyseal lesion

Permeative destruction

Pathology

Sheets of small round cells

Necrosis

CD99 positivity

Giant cell tumor of bone

Radiology

Epiphyseal

Expansile lytic lesion

Non-sclerotic margin

Pathology

Uniform giant cells

Stromal mononuclear cells

Multiple myeloma

Radiology

Multiple punched-out lesions

Diffuse osteopenia

Pathology

Sheets of plasma cells

Light chain restriction

IHC QUICK REFERENCE – MALIGNANT BONE TUMOURS

Tumour	Key Positive Markers	Helpful Negative Markers	Molecular
Osteosarcoma	SATB2	CD45	MDM2 (low-grade types)
Chondrosarcoma	S100	Cytokeratin	IDH1/2 mutations
Ewing sarcoma	CD99, NKX2.2	CD45	EWSR1-FLI1
Primary lymphoma of bone	CD45, CD20	SATB2	Ig gene rearrangement
Giant cell tumor of bone	H3F3A (G34W)	CD99	H3F3A mutation
Adamantinoma	Cytokeratin	CD45	—
Mesenchymal chondrosarcoma	SOX9	CD45	HEY1-NCOA2

V BONE – SYNOPTIC REPORTING

1. Patient Details

Name: _____ Age: ____ Sex: ____
Hospital No: _____
Specimen No: _____
Date: _____

2. Specimen Type (Tick ✓)

☐ Core biopsy
☐ Curettage
☐ Excision
☐ Limb salvage resection
☐ Amputation
☐ Disarticulation

Site: _____
Side: ☐ Right ☐ Left

A. FOR BIOPSY / CURETTAGE SPECIMENS

Gross

Aggregate size: _____ cm

Weight (if applicable): _____ g

Description: _____

Microscopy

Histologic Diagnosis (WHO): _____

Tumour Grade:
☐ Low grade
☐ Intermediate
☐ High grade
☐ Not applicable

Matrix Produced:
☐ Osteoid
☐ Cartilage
☐ Fibrous
☐ Small round cell
☐ Other: _____

Necrosis: ☐ Present ☐ Absent
% (if applicable): _____

Ancillary Studies:
IHC: _____
Molecular/Cytogenetics: _____

B. FOR RESECTION / AMPUTATION SPECIMENS

Gross Examination

Specimen length: _____ cm
Circumference at tumour level: _____ cm

Tumour size (3 dimensions): ___ × ___ × ___ cm

Location within bone:
☐ Epiphysis
☐ Metaphysis
☐ Diaphysis
☐ Transphyseal extension

Extent of Involvement:
☐ Medullary cavity
☐ Cortex destroyed
☐ Periosteal elevation
☐ Soft tissue extension (____ cm)

☐ Joint involvement

☐ Neurovascular involvement

Distance to nearest articular surface: _____ cm

Margins

Margin	Status	Distance (cm)
Proximal bone	☐ Neg ☐ Pos	____
Distal bone	☐ Neg ☐ Pos	____
Soft tissue	☐ Neg ☐ Pos	____
Neurovascular	☐ Neg ☐ Pos	____
Skin	☐ Neg ☐ Pos	____

Closest margin: _____ cm

Microscopy

Histologic Type (WHO): _____
(e.g. Conventional osteosarcoma, chondrosarcoma, etc.)

Histologic Grade:

☐ G1

☐ G2

☐ G3

Tumour Necrosis (Post-therapy cases):
____ %

Response:

☐ Good responder (>90%)

☐ Poor responder (<90%)

Lymphovascular invasion: ☐ Yes ☐ No

Perineural invasion: ☐ Yes ☐ No

Lymph nodes examined: ____
Lymph nodes involved: ____

Pathologic Staging

Primary tumour (pT):

☐ pT1 (≤8 cm)

☐ pT2 (>8 cm)

☐ pT3 (discontinuous tumours)

Regional nodes (pN):

☐ pN0

☐ pN1

Distant metastasis (pM):

☐ pM0

☐ pM1a (lung)

☐ pM1b (other sites)

Histologic grade (G): ☐ G1 ☐ G2 ☐ G3

Final Pathologic Stage: _____

Signature ……. Date ……..

TNM STAGING – PRIMARY MALIGNANT BONE TUMOURS

(AJCC 8th Edition; Union for International Cancer Control -UICC; Current oncologic orthopedic practice)

T – Primary Tumour

Category	Definition
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
T1	Tumour ≤ 8 cm in greatest dimension
T2	Tumour > 8 cm
T3	Discontinuous tumours in the primary bone site

Tumour size is measured in **greatest dimension**.

Multifocal tumour within same bone → **T3**.

Soft tissue extension does NOT change T category (unlike soft tissue sarcoma).

N – Regional Lymph Nodes

Category	Definition
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis

Lymph node metastasis is rare but automatically worsens stage grouping.

M – Distant Metastasis

Category	Definition
M0	No distant metastasis
M1a	Lung metastasis only
M1b	Metastasis to other distant sites (± lung)

Lung is most common metastatic site (especially osteosarcoma).

Bone-to-bone metastasis = M1b.

G – Histologic Grade

Grade	Definition
G1	Well differentiated (low grade)
G2	Moderately differentiated
G3	Poorly differentiated
GX	Grade cannot be assessed

Most osteosarcomas and Ewing sarcomas are high grade (G3).

STAGE GROUPING

Stage	Criteria
Stage IA	T1, N0, M0, G1
Stage IB	T2 or T3, N0, M0, G1
Stage IIA	T1, N0, M0, G2–3
Stage IIB	T2, N0, M0, G2–3
Stage III	T3, N0, M0, G2–3
Stage IVA	Any T, N0, M1a
Stage IVB	Any T, N1 OR M1b

REPORTING PEARLS

Size cutoff is **8 cm**

Multifocal tumour in same bone → Stage III

Histologic grade

Margin status

Lymph nodes (if sampled)

% necrosis (post-chemotherapy osteosarcoma)

Lung metastasis = Stage IVA

Non-lung metastasis = Stage IVB

Lymph node metastasis automatically Stage IVB

ASSESS YOURSELF – BONE

A. Long Essay Questions *(10 marks each)*

1. Classify bone tumours and describe the clinicopathological features of osteosarcoma.
2. Describe the pathology of giant cell tumour of bone under the following headings: Clinical features ;Radiology ;Gross features ; Microscopy ;Differential diagnosis
3. Discuss Ewing sarcoma with emphasis on molecular pathology, histology, and differential diagnosis from other small round cell tumours.

B. Short Essay Questions *(5 marks each)*

4. Chondrosarcoma – types and histologic features.
5. Osteoid osteoma vs osteoblastoma – differences.
6. Aneurysmal bone cyst – pathology and differential diagnosis.

C. Short Answer Questions *(3 marks each)*

7. What is Codman triangle? Mention its significance.
8. Define “sunburst appearance” in bone tumours.
9. What are the characteristic molecular alterations in Ewing sarcoma?

D. Multiple Choice Questions (MCQs)

1. **The most common primary malignant bone tumour is:**

   a. Chondrosarcoma
   b. Osteosarcoma
   c. Ewing sarcoma
   d. Fibrosarcoma

2. **Onion-skin periosteal reaction is classically seen in:**

    a. Osteosarcoma
    b. Ewing sarcoma
    c. Chondrosarcoma
    d. Osteoid osteoma

A tumour composed of malignant osteoid is:

a. Chondrosarcoma
b. Osteosarcoma
c. Ewing sarcoma
d. Giant cell tumour

Answer Key for MCQs

1. b
2. b
3. b

E. Case-Based Questions (Applied Pathology)

Case 1
A 15-year-old boy presents with pain and swelling around the knee. X-ray shows a metaphyseal lesion in the distal femur with a sunburst pattern and Codman triangle.

Questions:

What is the most likely diagnosis?

What is the key histologic feature required for diagnosis?

Mention two important differential diagnoses.

Case 2
A 12-year-old child presents with fever, bone pain, and a diaphyseal lesion in the femur. X-ray shows an onion-skin periosteal reaction.

Questions:

What is the most likely diagnosis?

Name the characteristic molecular abnormality.

List two differential diagnoses of small round cell tumours.

Case 3
A 30-year-old female presents with a lytic, epiphyseal lesion around the knee. Curettage specimen shows numerous multinucleated giant cells evenly distributed in a stromal background.

Questions:

What is the diagnosis?

What is the biological behaviour of this tumour?

Mention one important complication.

Case 4

A 40-year-old male presents with a slowly enlarging pelvic mass. Imaging shows a lobulated lesion with calcifications.

Questions:

What is the most likely diagnosis?

What is the characteristic histologic matrix?

How does it differ from osteosarcoma histologically?

Chapter 17
Eye and Orbit

Chapter outline – I -Eye and Orbit. Anatomy ; II -Eye and Orbit GrossingTechniques ; III and IV Eye and Orbit Gross Pearls and Microscopy-; V. –Eye and Orbit Synoptic Reporting

I EYE AND ORBIT ANATOMY

1. The Globe (Eyeball)

The eye is a specialized sensory organ situated within the orbit and is approximately 24 mm in anteroposterior diameter in adults. It is composed of three concentric layers:

A. Outer Fibrous Coat

Sclera – Dense collagenous tissue forming the posterior 5/6th of the globe; provides structural integrity and attachment for extraocular muscles.

Cornea – Transparent and in the anterior 1/6th; avascular and highly organized lamellar stroma permitting optical clarity.

B. Middle Vascular Coat (Uvea)

Iris – Controls pupillary size; contains sphincter and dilator muscles.

Ciliary body – Produces aqueous humour; and contains ciliary muscle for accommodation.

Choroid – Is the highly vascular, pigmented layer supplying the outer retina.

C. Inner Neural Coat

Retina – Is the multilayered neurosensory tissue lining the posterior segment.

Macula and fovea centralis are specialized for central vision.

Optic disc represents exit of the optic nerve (physiological blind spot).

2. Chambers of the Eye

Anterior chamber – Is between the cornea and iris.

Posterior chamber – Between iris and lens.

Vitreous cavity –Situated posterior to the lens and filled with vitreous humour.

Aqueous humour is produced by the ciliary body and drains via the trabecular meshwork and Schlemm canal at the irido-corneal angle.

3. Lens

A biconvex, avascular structure suspended by zonular fibres from the ciliary body. It is enclosed within a capsule and consists of anterior lens epithelium and elongated lens fibres.

4. Optic Nerve

Is formed by the retinal ganglion cell axons and exits posteriorly from the eyeball through the lamina cribrosa. It is surrounded by meninges and cerebrospinal fluid within the optic nerve sheath.

5. The Orbit

A pyramidal bony cavity formed by fusion of seven bones (frontal, zygomatic, maxillary, ethmoid, lacrimal, sphenoid, palatine). It's contents are :

The Globe of eyeball

Extraocular muscles (four recti, two obliques)

Optic nerve

Ophthalmic artery and veins

Lacrimal gland

Orbital fat and connective tissue

The orbital septum separates the eyelid structures from the deeper orbital contents.

6. Blood Supply and Lymphatics

Arterial – Ophthalmic artery (branch of internal carotid artery).

Venous – by the superior and inferior ophthalmic veins draining into the cavernous sinus.

Choroid has one of the highest blood flows per gram of tissue in the body.

The globe proper lacks true lymphatics; the lymphatic drainage is present in conjunctiva and eyelids.

7. Clinical–Pathological Relevance

The uveal tract (especially choroid) is the commonest site for primary intraocular melanoma.

Retina is the site of paediatric embryonal tumours (e.g., retinoblastoma).

Optic nerve involvement has staging implications in intraocular tumours.

The orbit is a common site for inflammatory, lymphoproliferative and metastatic lesions.

Applied Anatomy (Staging Importance)

1.Nerve Segments:

Intraocular (optic disc)

Intraorbital

Intracanalicular

Intracranial

The lamina cribrosa is the key anatomical landmark and if a tumour extension occurs beyond the lamina cribrosa it is associated with a higher metastatic risk.

2. Uveal Tract Surgical Relevance

The uvea includes: Iris ;Ciliary body and the Choroid

Important points to remember :

Ciliary body involvement indicates more advanced intraocular melanoma.

Tumour thickness and basal diameter are critical prognostic measurements.

Scleral emissary canals may act as routes for extrascleral spread.

3. Retina:

Retina is firmly attached at:

Optic disc

Ora serrata

Subretinal space is a potential space (site of retinal detachment).

Endophytic vs exophytic growth patterns are important in paediatric retinal tumours.

4. Anterior Chamber Angle

Components of the chamber are the trabecular meshwork ; Schlemm canal and the peripheral iris and it should be kept in mind that

Tumour may seed into angle structures

Secondary glaucoma may occur as also neovascularization

5. Orbit – Compartmental Considerations. The orbit may be divided into:

Intraconal (within extraocular muscle cone)

Extraconal

Cavernous haemangioma is typically intraconal.

Lacrimal gland tumours are supero-temporal and extraconal.

6. Lymphatic and Vascular Considerations. As the globe lacks true lymphatics.

Conjunctival tumours may metastasize via regional lymph nodes.

Choroid is a common site for metastases due to its rich vascularity .

7. Important Routes of Tumour Spread are -

Direct scleral extension

Along the optic nerve

Through the emissary canals

Perineural spread (especially lacrimal gland malignancies)

Hematogenous dissemination (uveal melanoma)

II EYE AND ORBIT GROSSING TECHNIQUES

Specimens received:

Enucleation of eye ball

Exenteration of orbital contents (for lid, ocular and orbital tumours)

Enucleation Specimen : Enucleation involves removal of the globe with a variable length of optic nerve.

General Principles :

Always orient the globe using the optic nerve, cornea, and extraocular muscle insertions.

Measure the anteroposterior, horizontal, and vertical diameters of the globe.

Record the length of the optic nerve stump, as this is important in intraocular tumours.

Note the condition of the cornea, sclera, and conjunctiva.

Sectioning is usually done through the pupil and optic nerve (pupil–optic nerve **section)** to demonstrate the posterior segment and optic nerve simultaneously.

Cornea – Transparent anterior dome; should be examined for invasion in anterior segment tumours.

Limbus – Junction of cornea and sclera; important for spread of conjunctival and limbal tumours.

Sclera – Assess for extra-scleral extension.

Optic nerve stump – Measure it's length; margin status is critical in intraocular malignancies and margin involvement in enucleation specimens must be documented.

Rectus muscle insertions – May be attached; useful orientation markers.

Pathological Relevance:

Optic nerve invasion (prelaminar, laminar, postlaminar) significantly alters staging in retinoblastoma.

Extra-scleral extension worsens prognosis in uveal melanoma.

Anterior chamber angle involvement has implications in certain tumours and glaucomatous changes.

2. Exenteration Specimen (may be total, subtotal or lid sparing)

Orbital exenteration removes the :

Globe

Extraocular muscles

Orbital fat

Lacrimal gland

± Eyelids and conjunctiva

Pathological Relevance:

Margins (soft tissue and bone) must be identified and inked.

Perineural invasion is common in lacrimal gland carcinomas.

Lymphoproliferative lesions often diffusely infiltrate orbital fat.

I. Enucleation- Method

1. Fix the whole eyeball in formalin for 24-48 hours
2. DO NOT freeze, or inject with formalin as artefacts are produced.
3. Wash in a gentle stream of tap water to prevent detachment of retina. Keep in 70% alcohol for 2 hours to restore colour.
4. **Specimen Orientation** (see fig17.1 below)-

Identify as right or left eye by locating the positions of insertion of the superior and, inferior oblique muscles and insertion of the optic nerve which is infero-medial.

5. Longest optic nerve segment should be measured and sampled en face for surgical margin if tumour is suspected.
6. Always measure tumour size in three dimensions and note its relationship to:

Optic disc
Macula

Ciliary body
Sclera

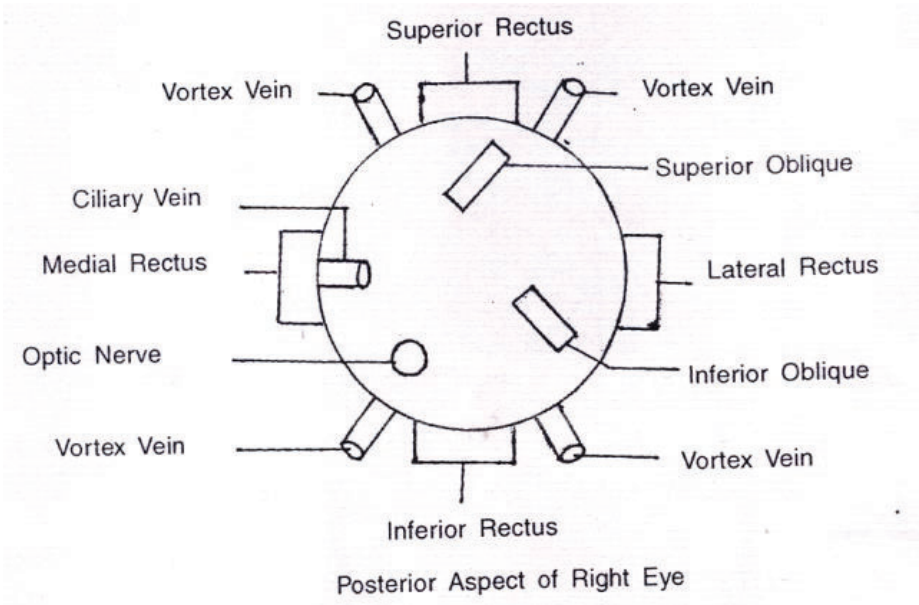


Fig 17.1 Eye ball and it's attachments

7. Sample at least one of the vortex veins from each of the four quadrants, if a tumour particularly a malignant melanoma is suspected.
8. Transilluminate the eye ball and look for tumour.
9. 1st cut: With a razor blade, cut the eye ball postero-anteriorly, starting adjacent to the optic nerve to the periphery of the cornea. If a lesion has been detected include that in the plane of section. If no lesion is seen, cut along a horizontal plane.
10. *2nd cut:* Place the eye flat on its cut surface and make a second plane of section, parallel to the first, to include the neoplasm, if any, between the two cuts.

Both horizontal cuts are made in such a *way that the neoplasm is included in the central slice.*

11. The central disc-shaped slab should contain the cornea, pupil, lens and optic nerve and should be about 7-8 mm in thickness.
12. Record the following:
    (a) Corneal thickness
    (b) Anterior chamber depth
    (c) Configuration of anterior chamber angle
    (d) Condition of iris
    (e) Condition of ciliary body
    (f) Condition of lens

(g). Condition of choroid

(h) Condition of retina

(i) Condition of vitreous body

(j) Condition of optic disc

(k) If a tumour is present – record the location

- size
- colour
- edges
- consistency

haemorrhage present/absent

- necrosis present/absent

occular structures involved

- extension into optic nerve

Bits (Fig 17.2)

A - One bit, eye slab (entire bit is to be taken as one block).

B - One bit, surgical margin of optic nerve in tumours.

C, D, E & F - Four bits, one each, from vortex veins of each of the four quadrants in suspected malignant melanoma (not shown in figure).

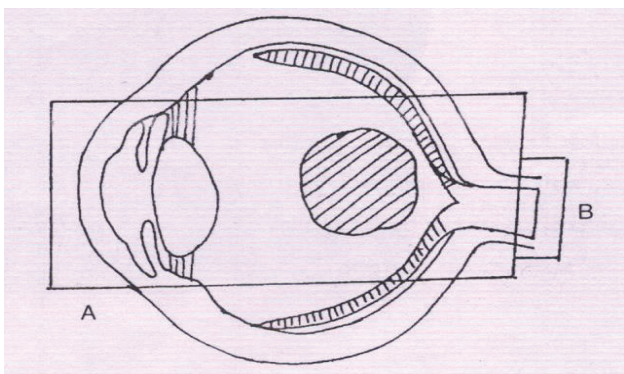


Fig 17.2

II. Orbital Exenteration

1. Pin the elliptical piece of orbicular skin on a cork or wax board and fix it overmight in formalin with the eyeball in front or behind depending on the specimen.

2. Paint the skin and *soft* tissue surgical margins with India ink.

3.Take surgical margins - skin

- *soft* tissue

- optic nerve - mark embedding surface with eosin

4. Cut skin, soft tissue and eyeball at right angles to the lid margins for lid lesions.(Fig 17.3)

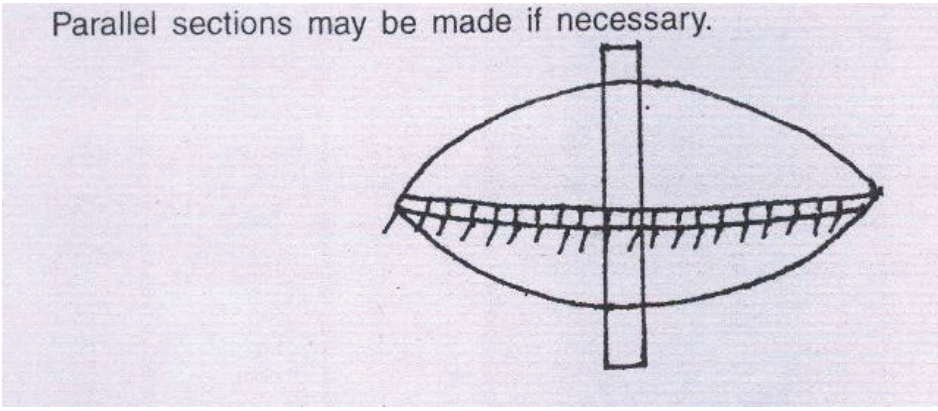


Fig 17.3 : cut passes right angles to lids

5. Description: a) Skin - shape and measurements

- appearance

-lesion - size

- shape

-depth of invasion

- colour

b) Soft tissues

c) Eyeball - dimensions

- appearance - as in enucleation

- length of optic nerve

6. For ocular tumours section as described previously.

Bits (Fig 17.4)

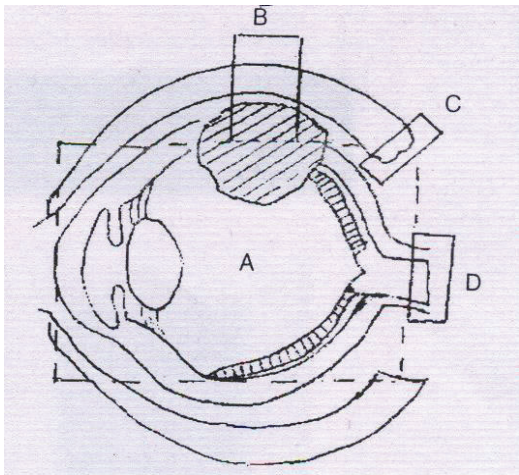
For ocular tumours.

One bit,slice of entire eyeball with tumour.

One to two bits, soft tissue adjacent to tumour.

One bit, closest soft tissue surgical margin.

One bit, optic nerve surgical margin.



17.4 : Bits for ocular tumours

For skin tumours: (No figure given)

Three bits, tumour.

One to two bits, superior skin surgical margin.

One to two bits, inferior skin surgical margin.

One to two bits, inferior (medial) skin surgical margin.

One to two bits, exterior (lateral) skin surgical margin.

Two bits, closest soft tissue surgical margin (more blocks if necessary)

One bit, optic nerve surgical margin.

One bit,eyeball.

III and IV Eye and Orbit Gross Pearls and Microscopy -

Eyelids :

Dermoid Cyst : typically involve the upper eyelid and represent forward extension of a mass that is primarily intra-orbital. They are attached firmly to the periosteum of the intra-orbital rim. Gross: soft, oval or round about 1 cm in diameter.

Microscopy : lined by well differentiated epidermis; the underlying dermis contains skin appendages such as hair follicles and sebaceous glands.

The lumen is filled with keratin, sebum and hair. A foreign body giant cell response may observed when the contents extrude into the surrounding tissues.

Inflammations : of the eyelids may be viral, rickettsial, bacterial, mycotic or parasitic. These are rarely biopsied but if so, then the presence of either fungi or parasites should give a clue to the diagnosis.

Chalazian : an extremely common lesion received by the surgical pathologist. It represents a lipogranuloma that develops in and around the Meibomian gland (sebaceous glands seen in the eyelid) as a consequence of obstruction and non specific infection of the excretory passages of the gland. Generally erupts through the conjunctival surface of the eyelid. The sebaceous material provokes an intense granulomatous reaction .

Microscopy shows foci of granulomatous inflammation with the center showing fat globules which appear as empty vacuolated spaces.

Cysts : of the skin of the eyelid and lid margin are common. These are i) keratinous or epidermal inclusion cysts. ii) cysts of the gland of Moll : called sodoriferous cysts or simple ductal cysts seen as thin walled translucent vesicles at the lid margin. Microscopically the cyst is lined by atrophic cuboidal or flattened epithelial cells with an empty lumen.

Tumours and tumour like lesions : i) Basal cell carcinoma : most frequent neoplasm, arises from the cutaneous surface of the eyelid. When excised it shows the classic morphology as seen elsewhere. Rarely invades the orbit, nose or both.

ii) Conjunctival papilloma : arises from the palpebral conjunctiva and shows an exophytic growth of well differentiated epithelial cells supported by a prominent central fibrovascular core. The complex pattern of growth may simulate a carcinoma.

iii) Keratotic lesions : may be squamous papilloma, pseudoepitheliomatous hyperplasia, kerato acanthoma, inverted follicular keratosis, seborrhoiec keratosis and actinic keratosis. These may mimic squamous cell carcinomas.

iv) Adnexal tumours : a) Sebaceous gland adenoma and adenocarcinoma-arise from the cutaneous sebaceous glands, glands of Zeis or Meibomian glands. Some are seen subsequent to radiotherapy for retinoblastoma. Meibomian gland carcinomas show considerable histologic and cytologic variation with well differentiated to poorly differentiated forms. These can be aggressive neoplasms with metastasis. Features indicative of poor prognosis are orbital or vascular invasion, involvement of both eyelids, poor differentiation, multic entric origin , large size and highly infiltrative pattern.

b) Trichilemmoma : features are similar to skin tumours occurring elsewhere.

c) Benign sweat gland tumours : apocrine hydrocystoma, papillary oncocytoma Malignant tumours include apocrine hydrocystadenocarcinoma and

d) mucinous adenocarcinoma (the latter is homologous to the adenoid cystic carcinoma of the skin seen in other locations). Local recurrences are common and distant metastasis are rare.

e) Melanocytic naevi : may be seen on the cutaneous or conjunctival surface of the eyelid. Most are of the junctional or compound type. May give rise to malignant melanoma but this is rare. Nevus of Ota : (congenital oculo- dermal melanosis)is a form of extra-scaral mongoloid spot involving the face in areas supplied by the first and second branches of the trigeminal nerve.

Seen more frequently in the orientals and the blacks who have also an increased incidence of ocular and orbital melanomas.

f) Mesenchymal tumours : Angiomas may be confined to the eyelid or extend deep into the orbit. Haemangiomas are commonly seen. Port wine stain (Nevus flammeus) of the face may be associated with choroidal haemangiomas of the eye. Sturge

Weber Syndrome (facial haemangioma) may be associated with choroidal haemangioma, glaucoma and a meningeal haemangioma on the ipsilateral side.

g) Xanthelasma : elevated yellow plaques situated on the medial aspect of the upper and lower eyelids; usually removed for cosmetic purposes.Patients may or may not have familial hypercholesterolaemia. Microscopically these lesions show large pale staining fat laden histiocytes throughout the subepithelial tissue.

LACRIMAL GLAND

1. Chronic Dacryoadenitis

Inflammatory reaction may be suppurative, granulomatous or necrotizing.

Lymphoplasmacytic infiltrate.

Sinus tracts may lead to the skin surface at the base of the nose.

May be associated with systemic disease.

2. IgG4-related Disease

(Modern replacement for “Mikulicz disease”)

Dense lymphoplasmacytic infiltrate

Storiform fibrosis

Obliterative phlebitis

Increased IgG4-positive plasma cells

Mikulicz’s Syndrome : chronic dacroadenitis associated with enlargement of the parotid or other salivary gland. This may be due to Mikulicz diasese or a variety of other specific diseases including sarcoidosis, tuberculosis, syphilis, mumps, Graves disease , malignant lymphoma and leukaemia.

When Mikulicz ‘s disease is accompanied by failure of lacrimal and conjunctival secretions and consequent kerato-conjunctivitis sicca the term Sjogren’s Syndrome is used. Most cases are seen in the post menopausal women.

3. Pleomorphic Adenoma

Biphasic tumour:

Ductal epithelial cells

Myoepithelial cells

Chondromyxoid stroma

4. Adenoid Cystic Carcinoma

Cribriform ("Swiss cheese") pattern

Perineural invasion common

Poor prognosis

CONJUNCTIVA

Cysts : Benign epithelial lined cysts .

Arise after trauma or rarely in the absence of it.

They are lined by stratified epithelium 2-5 layers in thickness.

Pinguecula

Actinic elastosis beneath epithelium.

Yellowish elevated lesion.

No true dysplasia.

Pterygium

Actinic elastosis

Fibrovascular proliferation

Extension onto cornea

Conjunctival Nevus

Junctional or compound pattern

Characteristic epithelial inclusion cysts

Inclusion cysts favor benignity

Conjunctival Melanoma

Atypical melanocytes with pagetoid spread

Often arises from primary acquired melanosis with atypia

Inclusion conjunctivitis

Seen in adults as an acute and chronic infection produced by Chlamydia group of organisms.

Microscopy shows follicular conjunctivitis and the finding of the so called "Halberstaedter Prowazek inclusions" in the conjunctival epithelium.

Cornea

Corneal Ulcer

Loss of corneal epithelium with underlying stromal necrosis and inflammatory infiltrate.

May show perforation in severe cases.

Corneal Opacity (Leucoma)

Cornea becomes opaque, white and scarred due to fibrosis. Associated with loss of transparency.

"Buttons" of cornea are generally submitted to the laboratory after corneal transplantation. The most common cause for these are a) endothelial decompensation : chronic edema of the stroma and epithelium which breakdown causing severe pain and eventually diffuse scarring and reduced vision. When the process is primary it is called "Fuch's dystrophy" and occurs in the females more than 50 years of age. It is bilateral but asymmetric. When it occurs after intraocular surgery, especially after cataract extraction it is clinically referred to as aphakic bullous keratopathy. Clinical features and histopathology are the same for both.

Normal Layers of cornea

Consists of 6 layers . Epithelium – Bowman layer – Stroma – Descemet membrane – Endothelium (Pre-Descemet layer may be identified in modern descriptions)

Fuchs Endothelial Dystrophy

Endothelial cell loss

Thickened Descemet membrane

Guttata (excrescences)

Stromal edema

Epithelial bullae

Keratoconus

Central stromal thinning

Breaks in Bowman layer

Fibrosis in advanced cases

Failed Graft

Stromal fibrosis

Neovascularization

Chronic inflammation

Retrocorneal membrane

LENS - Cataract

Lens appears opaque or milky white instead of transparent.

Nuclear sclerosis may give the lens a yellow to brown discoloration.

INTRAOCULAR LESIONS

1. Phthisis Bulbi

End-stage ocular degeneration.

Microscopy:

Disorganized globe

Retinal detachment

Osseous metaplasia of choroid

Cataract

Extensive fibrosis

2 Retinoblastoma

General Features

Most common primary intraocular malignancy of childhood.

Arises from primitive retinal neuroectoderm.

Median age: 18 months.

Rare after 5 years of age.

Genetics & Epidemiology

~60% sporadic

~40% hereditary

Bilateral in:

~30% overall

90% hereditary cases

Caused by biallelic inactivation of the RB1 tumour suppressor gene.

Clinical Presentation

Leukocoria (most common)

Strabismus

Decreased vision

Secondary glaucoma

Rarely presents with orbital mass (advanced disease)

Gross Pathology

Appears as a white, friable, chalky tumour arising from the retina.

Often shows areas of necrosis and calcification, producing a gritty sensation on cutting.

Growth patterns:

Endophytic (into vitreous)

Exophytic (subretinal)

Diffuse infiltrating (rare)

Multifocal tumours common in hereditary cases.

If tumour may grow towards the vitreous cavity (it is endophytic) or towards the choroid (exophytic).

Examine carefully for optic nerve invasion, particularly at the lamina cribrosa and surgical margin.

Massive choroidal invasion is an important adverse prognostic feature

Microscopy

Microscopic examination (Fig 17.5) is essential in confirming the diagnosis of ocular and orbital lesions. Many ocular diseases show characteristic histologic patterns that correlate closely with their gross appearance. Eg in retinoblastoma the appearance is of

Small round blue cell tumour

Hyperchromatic nuclei

Scant cytoplasm

High mitotic rate

Necrosis common

Dystrophic calcification frequent

Differentiation Patterns

Flexner–Wintersteiner rosettes (true rosettes)

Homer Wright rosettes

Fleurettes (well-differentiated)

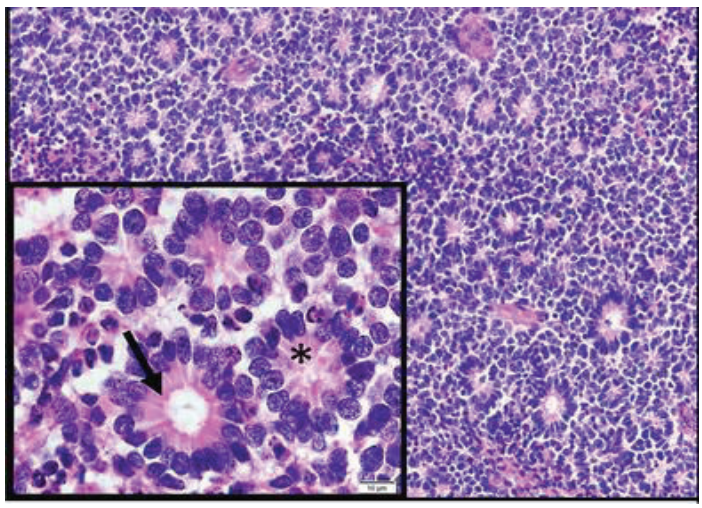


Fig 17.5 : Section shows hyperchromatic round cells and rosettes (inset). H&E x100

Azzopardi effect may be seen in necrotic areas.

High-Risk Histopathologic Features

(Critical for Adjuvant Therapy Decision)

Post-laminar optic nerve invasion

Tumour at optic nerve surgical margin

Massive choroidal invasion

Scleral invasion

Extrascleral extension

Anterior chamber involvement

These significantly increase metastatic risk.

Routes of Spread

Along optic nerve to brain

Via subarachnoid space

Hematogenous dissemination

Uveal invasion

3. Retinal Detachment

Retina appears elevated and folded, often forming a funnel-shaped configuration.

Subretinal fluid or hemorrhage may be present.

4.Proliferative Retinopathy

Fibrovascular membranes may be seen on the retinal surface or in the vitreous cavity.

5. Diabetic Retinopathy

Shows microaneurysms, capillary basement membrane thickening, and neovascularization.

Advanced cases may demonstrate fibrovascular proliferation on the retinal surface.

6. Uveal Melanoma

Arises from melanocytes of iris, ciliary body or choroid.

Usually presents as a **dark brown to black mass** arising from the **choroid or ciliary body**.

Tumour may have a **dome-shaped or mushroom-shaped configuration** due to rupture of Bruch's membrane.

Some tumours may appear **amelanotic (grey or pale)**.

Associated findings include:

- Retinal detachment
- Subretinal fluid
- Hemorrhage

Extraocular extension should always be looked for.

Histologic Types:

Tumour composed of spindle cells, epithelioid cells, or mixed cell types.

Cells contain melanin pigment, though some tumours may be amelanotic.

Epithelioid cell type is associated with poorer prognosis.

Mitotic activity, tumour size, and extrascleral extension are important prognostic factors.

Key Microscopic Clues:

Spindle cells → better prognosis

Epithelioid cells → worse prognosis

Mitotic activity correlates with risk

Closed vascular loops (poor prognosis)

IHC:

S100 positive

HMB45 positive

Melan-A positive

SOX10 positive

(Cytokeratin typically negative – helps exclude metastasis)

The **Callender classification**, originally proposed by George R. Callender in 1931, is a histological system used to categorize **uveal melanomas** (melanomas of the eye, specifically the choroid and ciliary body) based on their cell types.

It remains a cornerstone of ophthalmic pathology because the cell type is a significant predictor of the tumor's aggressiveness and the patient's long-term prognosis.

The Classification Categories

The system divides tumors into several distinct types based on the shape and features of the cells:

Cell Type	Description	Prognosis
Spindle A	Slender cells with flattened, elongated nuclei; no distinct nucleoli.	**Excellent** (Lowest mortality)
Spindle B	Robust, oval cells with prominent nucleoli.	**Good**
Fascicular	Spindle cells arranged in ribbons or palisades.	Similar to Spindle B
Epithelioid	Large, round/polygonal cells with abundant cytoplasm and prominent nucleoli; resemble epithelial cells.	**Poor** (Most aggressive)
Mixed	A combination of spindle and epithelioid cells.	**Guarded**
Necrotic	Tumors so damaged by cell death that the type cannot be determined	

Spread:

Hematogenous (liver most common)

Optic nerve extension possible

7. Cavernous Hemangioma

Composed of large dilated vascular channels lined by flattened endothelial cells.

Vascular spaces are separated by fibrous septa.

8. . Metastatic Tumours

Often appear as yellowish or creamy infiltrative lesions in the choroid.

Usually multifocal and poorly circumscribed.

Bilateral involvement may occur.

Lymphoma -appears as a soft, grey-white infiltrative mass involving orbital soft tissue or lacrimal gland.

Rhabdomyosarcoma -Commonest malignant orbital tumour in children. Grossly presents as a rapidly growing, soft, grey-white mass.

WHO CLASSIFICATION OF TUMOURS OF THE EYE AND ORBIT

(Adapted from the WHO Classification of Tumours – Eye and Orbit volume)

I. Eyelid Tumours

A. Benign

Squamous papilloma

Seborrhoeic keratosis

Melanocytic naevus

Hidrocystoma

Pilomatricoma

B. Malignant

Sebaceous carcinoma

Basal cell carcinoma

Squamous cell carcinoma

Melanoma

Merkel cell carcinoma

II. Conjunctival Tumours

A. Epithelial Tumours

Conjunctival intraepithelial neoplasia

Squamous cell carcinoma

B. Melanocytic Tumours

Naevus

Primary acquired melanosis (with / without atypia)

Conjunctival melanoma

C. Lymphoid Tumours

Extranodal marginal zone lymphoma (MALT type)

Diffuse large B-cell lymphoma

III. Intraocular Tumours

A. Uveal Tumours (Iris, Ciliary Body, Choroid)

Melanocytic

Naevus

Uveal melanoma

- Spindle cell type
- Epithelioid type
- Mixed type

Melanocytoma

Other Tumours

Metastatic carcinoma (most common intraocular malignancy in adults)

B. Retinal Tumours

Retinoblastoma

Retinoma (retinocytoma)

Astrocytic hamartoma

C. Ciliary Body Tumours

Medulloepithelioma

Melanoma

IV. Optic Nerve Tumours

Optic nerve glioma

Optic nerve sheath meningioma

Metastatic tumours

V. Lacrimal Gland Tumours

A. Epithelial

Pleomorphic adenoma

Adenoid cystic carcinoma

Carcinoma ex pleomorphic adenoma

Mucoepidermoid carcinoma

B. Lymphoid

Extranodal marginal zone lymphoma

Diffuse large B-cell lymphoma

VI. Orbital Soft Tissue Tumours

Benign

Cavernous venous malformation

Schwannoma

Dermoid / epidermoid cyst

Malignant

Rhabdomyosarcoma

Lymphoma

Solitary fibrous tumour

Metastases

VII. Secondary (Metastatic) Tumours to Eye and Orbit

Breast carcinoma

Lung carcinoma

Prostate carcinoma

Neuroblastoma (children)

Correlation of Gross and Microscopy – Eye and Orbit

Gross Appearance	Likely Diagnosis	Microscopic Features
White friable tumour with chalky calcified areas	Retinoblastoma	Small round blue cells with Flexner–Wintersteiner rosettes, necrosis and calcification
Dark brown or black dome-shaped mass arising from choroid	Uveal melanoma	Spindle or epithelioid melanocytic cells containing melanin pigment
Yellowish infiltrative lesion in choroid	Metastatic carcinoma	Infiltration by malignant epithelial cells, often adenocarcinoma
Elevated folded retina with subretinal fluid	Retinal detachment	Separation of neurosensory retina from retinal pigment epithelium
Grey-white corneal opacity	Corneal scar (leucoma)	Dense fibrous tissue replacing normal corneal stroma
Opaque or milky lens	Cataract	Degeneration and fragmentation of lens fibres
Well-circumscribed reddish-blue orbital mass	Cavernous hemangioma	Large dilated vascular spaces lined by endothelium
Cystic lesion containing hair or keratinous material	Dermoid cyst	Stratified squamous epithelium with skin appendages
Soft grey-white infiltrative orbital mass	Orbital lymphoma	Diffuse infiltration by monomorphic lymphoid cells
Rapidly growing soft orbital mass in children	Rhabdomyosarcoma	Primitive mesenchymal cells with rhabdomyoblasts

MOLECULAR PATHOLOGY OF OCULAR TUMOURS

1. RB1 Gene (Retinoblastoma)

Located on chromosome 13q14.

Function

Tumour suppressor gene regulating G1-S cell cycle transition.

Pathogenesis

Both alleles must be inactivated (“two-hit hypothesis”).

Hereditary cases:

- Germline mutation in one allele
- Second somatic hit

Sporadic cases:

- Two somatic hits

Clinical Implications

Germline mutation → risk of:

- Bilateral disease
- Trilateral retinoblastoma (pineal tumour)
- Secondary malignancies (osteosarcoma, soft tissue sarcoma)

2. MYCN-Amplified Retinoblastoma

Small subset (~1–2%)

- Occurs without RB1 mutation
- Often unilateral
- Early onset
- More aggressive behaviour
- High MYCN amplification

Important emerging molecular subtype.

3. Uveal Melanoma – Key Molecular Alterations

Unlike cutaneous melanoma, uveal melanoma has distinct mutations.

A. GNAQ and GNA11

- Activating mutations
- Early oncogenic events
- Present in ~80–90% of cases
- Mutually exclusive
- Activate MAPK pathway

These are initiating mutations but not prognostic markers.

B. BAP1 (Chromosome 3)

Most important prognostic gene.

Loss of BAP1 associated with:

- Monosomy 3
- Epithelioid morphology
- High metastatic risk
- Poor prognosis

Germline mutation → BAP1 tumour predisposition syndrome.

C. SF3B1

- Associated with intermediate prognosis
- Late-onset metastasis

D. EIF1AX

- Associated with good prognosis
- Rarely metastasizes

Molecular Risk Stratification in Uveal Melanoma

High Risk:

- Monosomy 3
- BAP1 mutation
- Class 2 gene expression profile

Low Risk:

- Disomy 3
- EIF1AX mutation
- Class 1 gene expression profile

Practical Diagnostic Relevance

Retinoblastoma:

RB1 mutation testing for:

- Genetic counseling
- Screening siblings
- Detecting germline mutation

Uveal Melanoma:

BAP1 IHC useful surrogate

Cytogenetics (monosomy 3)

Gene expression profiling for metastasis prediction

Key Features for a Quick Revision:

Tumour	Key Gene	Prognostic Significance
Retinoblastoma	RB1	Germline → bilateral & secondary cancers
Retinoblastoma (subset)	MYCN amplification	Aggressive, RB1 intact
Uveal melanoma	GNAQ / GNA11	Early driver mutation
Uveal melanoma	BAP1 loss	Poor prognosis
Uveal melanoma	SF3B1	Intermediate prognosis
Uveal melanoma	EIF1AX	Good prognosis

TNM STAGING (AJCC 8th Edition)

(As per American Joint Committee on Cancer, 8th Edition)

1. RETINOBLASTOMA – TNM (AJCC 8th Edition)

Primary Tumor (pT)

pTX – Primary tumor cannot be assessed
pT0 – No evidence of primary tumor

pT1 – Tumor confined to retina

No invasion of optic nerve or choroid

pT2 – Minimal invasion

Focal choroidal invasion (≤3 mm)

Pre-/intralaminar optic nerve invasion

pT3 – Significant local invasion

One or more of the following:

Massive choroidal invasion (>3 mm)

Retrolaminar optic nerve invasion (not to cut end)

Anterior segment invasion

Scleral invasion

pT4 – Extraocular extension

Tumor at optic nerve cut end

Tumor beyond sclera

Orbital invasion

Regional Lymph Nodes (N)

N0 – No regional lymph node metastasis
N1 – Regional lymph node metastasis present

Distant Metastasis (M)

M0 – No distant metastasis

M1 – Distant metastasis

M1a – Distant non-CNS metastasis

M1b – CNS metastasis

2. UVEAL MELANOMA – TNM (AJCC 8th Edition)

(Choroid / Ciliary body)

Tumor staging is based mainly on **tumor size category (T1–T4)** determined by:

Largest basal diameter

Tumor thickness

Ciliary body involvement

T1 – Small tumor

T2 – Medium tumor

Extraocular extension

Primary Tumor (T)

T3 – Large tumor

T4 – Very large tumor

Each T category is further subclassified:

a – No ciliary body involvement, no extraocular extension

b – With ciliary body involvement

c – Extraocular extension ≤5 mm

d – Extraocular extension >5 mm

Regional Nodes

N0 – No regional lymph node metastasis
N1 – Regional lymph node metastasis present

Distant Metastasis

M0 – No distant metastasis

M1 – Distant metastasis (most commonly liver)

M1a – Small metastasis

M1b – Medium metastasis

M1c – Large metastasis

3. CONJUNCTIVAL SQUAMOUS CELL CARCINOMA – TNM (AJCC 8th Edition)

Primary Tumor (T)

Tis – Carcinoma in situ

T1 – Tumor ≤5 mm, limited to conjunctiva

T2 – Tumor >5 mm or invades adjacent conjunctival structures

T3 – Invades adjacent ocular structures
(cornea, sclera, eyelid, orbit)

T4 – Invades orbital tissues, bone, or beyond

Nodes

N0 – No regional lymph node metastasis
N1 – Regional lymph node metastasis

Metastasis

M0 – No distant metastasis
M1 – Distant metastasis present

V -EYE AND ORBIT SYNOPTIC REPORTING

Small biopsies

Sit of biopsy : Eyelid □ Upper □ Lower □

Conjunctiva □ Palpebral □ Bulbar □

Corneal button □

Measurements ……mm x …… mm

Microscopy : **A. Conjunctival & eyelid biopsies**

Inflammation : Acute □ Chronic □ Other □

Granulation tissue□

Chalazion □

Ptyregium □

Pingeucula □

Benign cyst □

Xanthelasma □

Other Specify…….

B . Corneal button : shows paucity of endothelial cells □

thickening of the Decemet's membrane □

excrecences of the Decemet's membrane □

changes in the epithelium

-oedema of basal cells □

bulla formation □

pannus formation □

Comments …….

Signature …….. Date…….

Synoptic Reporting – Retinoblastoma

Specimen Type

Enucleation / Exenteration / Biopsy

Laterality

Right / Left / Bilateral

Tumour Location

Retina (specify quadrant if possible)

Involvement of:

Macula: Yes / No

Optic disc: Yes / No

Anterior segment: Yes / No

Tumour Size

Largest basal diameter: ___ mm

Thickness: ___ mm

Growth Pattern

Endophytic

Exophytic

Mixed

Histologic Features

Tumour differentiation:

Well differentiated / Moderately differentiated / Poorly differentiated

Rosettes:

Flexner–Wintersteiner: Present / Absent

Homer Wright: Present / Absent

Necrosis: Present / Absent

Calcification: Present / Absent

Extent of Tumour (Key Prognostic Factors)

Optic Nerve Involvement

Not involved

Prelaminar

Laminar

Retrolaminar

Involvement of surgical margin: Yes / No

Choroidal Invasion

None

Focal (<3 mm)

Massive (≥3 mm or full thickness)

Scleral Involvement

Absent

Present

Extrascleral Extension

Absent

Present

Anterior Segment Involvement

Iris: Yes / No

Ciliary body: Yes / No

Anterior chamber: Yes / No

Vitreous Seeding

Present / Absent

Other Findings

Retinal detachment: Present / Absent

Tumour necrosis: Present / Absent

Pathologic Staging (pTNM)

(According to current staging system – optional to detail in full)

pT category: _____

pN category: _____ (if applicable)

pM category: _____ (if applicable)

Final Diagnosis

Retinoblastoma, (unilateral/bilateral),
with (mention key high-risk features, e.g., retrolaminar optic nerve invasion, massive choroidal invasion, etc.)

Presence of **high-risk features** (optic nerve beyond lamina cribrosa, massive choroidal invasion, extrascleral spread) should be highlighted, as these influence adjuvant therapy.

Choroidal invasion (massive vs focal)

Extrascleral extension

Signature……. Date …….

ASSESS YOURSELF EYE & ORBIT

Long Essay Questions (10 Marks Each)

1. Discuss the "Two-Hit" hypothesis of Knudson as it pertains to retinoblastoma. Describe the macroscopic and microscopic features, including the various types of rosettes, and discuss the pathways of tumour spread.
2. Classify uveal melanomas using the Callender classification. Explain the prognostic significance of cell types (Spindle A, Spindle B, and Epithelioid) and describe the extraocular routes of metastasis.
3. Provide a detailed pathological classification of orbital masses. Discuss the clinical and histopathological features of Thyroid Eye Disease (Graves' Ophthalmopathy) and Idiopathic Orbital Inflammation (Pseudotumor).

Short Essay Questions (5 Marks Each)

4. Describe the ocular and systemic pathological features of Neurofibromatosis Type 1 (NF1) and Sturge-Weber Syndrome.
5. What is Sympathetic Ophthalmia? Define this condition, explain the underlying autoimmune mechanism (hypersensitivity type), and describe the characteristic histological findings in the "exciting" and "sympathizing" eye.
6. Give the staging system for retinoblastoma

III. Short Answer Questions (3 Marks Each)

7. Fleischer Ring vs. Kayser-Fleischer Ring: Differentiate between these two based on their chemical composition, location in the cornea, and associated clinical conditions.
8. What is leukocorea? What is its clinical importance?
9. Optic Nerve Glioma: Briefly describe its association with NF1 and its typical "hair-like" appearance on histopathology (Pilocytic Astrocytoma).

Multiple Choice Questions (MCQs)

1. Which of the following histopathological features is pathognomonic for Retinoblastoma?

A. Homer-Wright Rosettes
B. Flexner-Wintersteiner Rosettes
C. Psammoma Bodies
D. Touton Giant Cells

2. The most common primary intraocular malignancy in adults is:

A. Retinoblastoma
B. Choroidal Melanoma
C. Squamous Cell Carcinoma of Conjunctiva
D. Lymphoma

3. In Keratoconus, the iron deposition in the epithelial basement membrane is known as:

A. Stocker's Line
B. Ferry's Line
C. Fleischer Ring
D. Hudson-Stahli Line

Answer Key for MCQs

1. B
2. B
3. C

Chapter 18
Miscellaneous

Chapter outline A Brain; B Muscle ; C Temperal artery

A I Brain biopsy Anatomy; A II Brain Grossing Techniques ;A III Brain Gross Pearls; A IV Brain Microscopy ; AV Brain Synoptic Reporting

B I Muscle Anatomy; B II Muscle Grossing Techniques ; B III Muscle Gross pearls ; B IV Muscle Microscopy; B V Muscle Synoptic Reporting

C I Temporal artery Anatomy ; C II Temporal artery Grossing Techniques ; C III Temporal Artery Gross Pearls ; CIV Temporal Artery Microscopy ; C V Temporal Artery Synoptic reporting.

A I BRAIN BIOPSY ANATOMY

Applied Anatomy (Brief)

The brain consists of:

Supratentorial compartment

Cerebral hemispheres (frontal, parietal, temporal, occipital lobes)

Basal ganglia

Ventricular system

Infratentorial compartment

Cerebellum

Brainstem (midbrain, pons, medulla)

Meninges

Dura

Arachnoid

Pia

Understanding location is critical because:

Deep-seated lesions → stereotactic biopsy

Cortical-based lesions → open biopsy

Extra-axial lesions → often meningioma/metastasis

Radiologic correlation (MRI with contrast) is mandatory before grossing interpretation.

A II BRAIN GROSSING TECHNIQUES

Indications for Brain Biopsy (Adult)

Primary CNS tumours

- Diffuse gliomas
- Meningiomas
- Primary CNS lymphoma
- Metastases

Infective lesions

- Fungal abscess
- Tuberculoma
- Toxoplasmosis (immunocompromised)

Inflammatory / demyelinating lesions

- Tumefactive demyelination
- Vasculitis

Undiagnosed mass lesions

Neurodegenerative disorders are rarely diagnosed via biopsy in modern practice.

Types of Brain Biopsies

- Burr hole needle biopsy
- Stereotactic biopsy (CT/MRI guided) – most common for deep lesions
- Open biopsy / excision at craniotomy

Most adult biopsies are small stereotactic cores.

Specimen Handling & Transport

Brain tissue is soft and small → triage is essential.

General Principles

- Send specimen fresh and unfixed
- Immediate communication with pathologist
- Avoid drying
- Do not immerse in saline

Triage of Tissue

Depending on size:

Portion	Purpose
10% neutral buffered formalin	Routine histology + IHC
Fresh sterile sample	Microbiology (if infection suspected)
Snap frozen (liquid nitrogen / isopentane)	Molecular studies
2.5% Glutaraldehyde	Electron microscopy (rarely needed today)
Smear/squash prep	Intraoperative diagnosis

Modern practice prioritizes:

- Molecular studies (IDH, MGMT, 1p/19q etc.)
- Frozen tissue banking where possible

Intraoperative Diagnosis

A. Squash (Crush) Preparation

Technique

- 1 mm tissue fragment
- Gentle compression between slides
- Fix in 95% alcohol + 5% acetic acid
- Stain with H&E or Toluidine blue

Advantages

- Excellent nuclear detail
- Preserves glial processes
- Rapid diagnosis

Useful in

- Diffuse gliomas
- Lymphoma
- Metastasis
- Pituitary adenoma

B. Touch Preparation

Gently touch slide to tissue surface

Air dry

Stain with Giemsa

Useful in:

Lymphoma

Metastatic carcinoma

In many centres, squash cytology alone provides intraoperative diagnosis.

Gross Examination & Grossing Protocol

Most biopsies are tiny fragments.

A. Small Stereotactic Biopsy

Measure in 3 dimensions.

Describe:

- Colour (grey-white, tan, haemorrhagic)
- Consistency (soft, friable)

Submit **entire specimen**.

No need for slicing due to small size.

B. Large Resection Specimen

Record:

- Weight
- Dimensions
- Meningeal attachment (if any)

Note:

- Solid vs cystic
- Necrosis
- Haemorrhage
- Infiltrative margins

Serially section at 3–5 mm intervals.

Sample:

Tumour centre

Tumour margin

Necrotic areas

Adjacent brain

Dura (if present)

A III GROSS PEARLS (ADULT CNS)

Grey, soft, infiltrative lesion → diffuse glioma

Well-circumscribed, dural-based firm mass → meningioma

Soft, necrotic, haemorrhagic mass with peripheral enhancement → glioblastoma

Well-demarcated grey-white lesion at grey-white junction → metastasis

Soft, friable lesion in immunocompromised patient → consider lymphoma

Radiology-pathology correlation is mandatory.

A IV BRAIN MICROSCOPY

Evaluate:

1. Tumour vs Non-tumour

Reactive gliosis vs infiltrative glioma

2. Growth pattern

Diffuse infiltrative

Well circumscribed

Perivascular pattern

Sheets of discohesive cells

3. Cytology

Nuclear atypia

Mitoses

Necrosis

Microvascular proliferation

4. Specific Clues

Feature	Suggests
Perivascular lymphoid cuffing	Lymphoma
Rosenthal fibres	Pilocytic astrocytoma
Pseudopalisading necrosis	Glioblastoma
Whorls & psammoma bodies	Meningioma
Clear cell with perinuclear halo	Oligodendroglioma (confirm molecularly)

5. Immunohistochemistry (Adult CNS Core Panel)

Initial panel often includes:

- GFAP
- OLIG2
- IDH1 R132H
- ATRX
- p53
- Ki-67
- EMA
- Cytokeratin
- LCA (CD45)

Further molecular studies:

- IDH mutation
- 1p/19q co-deletion
- MGMT methylation
- TERT promoter mutation
- BRAF (selected cases)

Modern CNS diagnosis is **integrated histology + molecular**.

A V BRAIN SYNOPTIC REPORTING TEMPLATE

For Tumours

Specimen Type:
Stereotactic biopsy / Excision / Resection

Tumour Location:
Frontal lobe / Temporal lobe / Cerebellum etc.

Histologic Diagnosis:
(e.g., Diffuse astrocytoma)

WHO Grade:
Grade 2 / 3 / 4

Integrated Diagnosis:
(e.g., Astrocytoma, IDH-mutant, WHO Grade 4)

Histologic Features:

Cellular atypia: Present/Absent

Mitotic activity: Low/High

Necrosis: Present/Absent

Microvascular proliferation: Present/Absent

Immunohistochemistry:

GFAP: Positive

IDH1 R132H: Positive/Negative

ATRX: Retained/Lost

p53: Overexpressed

Ki-67 index: %

Molecular Findings:

IDH mutation: Present/Absent

1p/19q co-deletion: Present/Absent

MGMT methylation: Methylated/Unmethylated

Final Integrated Diagnosis:
(According to current WHO CNS classification)

Practical Points

Molecular classification now determines tumour entity.

"Oligodendroglioma" requires both IDH mutation and 1p/19q co-deletion.

Glioblastoma diagnosis in adults may be molecular (TERT mutation + EGFR amplification + chromosome 7 gain/10 loss).

Primary CNS lymphoma is usually diffuse large B-cell lymphoma.

Always correlate with imaging before issuing final diagnosis.

B I MUSCLE BIOPSY RELEVANT ANATOMY

Applied Anatomy (Brief & Practical)

Skeletal muscle is composed of:

- Muscle fibres (multinucleated cells)
- Organized into fascicles
- Surrounded by:
  - Endomysium
  - Perimysium
  - Epimysium

Each fibre contains:

- Myofibrils (actin & myosin)
- Mitochondria
- Sarcoplasmic reticulum

Muscle fibres are classified into:

- Type 1 (slow oxidative)
- Type 2 (fast twitch)
  - 2A
  - 2B
  - 2C (regenerating/intermediate)

Orientation is critical:

- **Transverse sections** → fibre size, type grouping
- **Longitudinal sections** → necrosis, regeneration, inclusions

B II MUSCLE BIOPSY GROSSING TECHNIQUES

Pre-Biopsy Clinical Correlation (Essential)

Muscle biopsy must be interpreted along with:

- **Serum muscle enzymes**
  - Creatine kinase (CK)
  - Aldolase

LDH

AST/ALT

Electromyography (EMG)

Myopathic vs neurogenic pattern

Nerve conduction studies

Autoimmune profile

ANA, myositis-specific antibodies

Serum immunoglobulins

Metabolic tests

Ischaemic lactate test (e.g., McArdle disease)

Serum potassium (periodic paralysis)

Thyroid function tests

Hypothyroid / thyrotoxic myopathy

Urine myoglobin

Rhabdomyolysis

ECG / Echocardiogram

Cardiomyopathy in muscular dystrophy

Muscle biopsy is **never interpreted in isolation**.

Indications for Muscle Biopsy

Suspected inflammatory myopathy (before therapy)

Proximal muscle weakness of unclear cause

Suspected muscular dystrophy

To exclude treatable myopathy in suspected motor neuron disease

Suspected metabolic myopathy

Systemic vasculitis (e.g., polyarteritis nodosa)

Sarcoidosis

Steroid-induced myopathy assessment

Carrier detection in Duchenne dystrophy

Malignant hyperthermia testing

Research

Selection of Muscle

Preferred sites:

- Quadriceps femoris (most common)
- Biceps brachii
- Deltoid
- Occasionally gastrocnemius or triceps

Biceps is often preferred due to balanced fibre type distribution.

Precautions Before Biopsy

Avoid:

- Severely atrophic muscle
- Muscle recently used for EMG
- Injection sites
- Muscle near tendinous insertion
- Excessive handling, squeezing, stretching
- Injection of local anaesthetic into muscle (skin/subcut only)

Choose:

- Moderately weak muscle (for neurogenic disorders)

Types of Muscle Biopsy

1. Needle Biopsy

Advantages:

- Minimally invasive
- Repeatable
- Good for comparison studies

Disadvantage:

- Small sample

2. Percutaneous Conchotome Technique

- Improved needle method
- Larger sample
- Less invasive than open biopsy

3. Open Biopsy

Preferred when:

- Large sample required
- Paediatric cases
- Complex diagnostic workup

Usually 2 cm length × 1 cm diameter sample.

Transport & Specimen Triage (Critical)

Muscle must reach lab **fresh**.

Keep:

- Wrapped in saline-moistened gauze
- On ice
- Process within 4–6 hours

Minimum 1 cm length required.

Specimen must be divided:

Portion	Purpose
Formalin	Routine histology
Snap frozen	Enzyme histochemistry
Glutaraldehyde	Electron microscopy (if required)
Fresh	Molecular / genetic testing

Formalin alone is inadequate for full diagnosis.

Gross Examination & Grossing

Description

- Measure length and thickness
- Colour (red-brown, pale)
- Texture (firm, fatty, fibrotic)

Gross appearance is rarely diagnostic.

Grossing Protocol

- Orient fibres under hand lens.

Cut perpendicular to long axis for transverse sections.

Submit:

Half for formalin

Half for snap freezing

Avoid crushing during embedding.

Entire biopsy is usually processed.

Processing Methods

A. Formalin Fixation

Fix in 10% buffered formalin (1–2 hours).

Routine stains:

H&E

Masson trichrome

PTAH

Used for:

Necrosis

Inflammation

Fibrosis

Fatty replacement

B. Snap Freezing (Essential)

0.5 cm cube
Freeze in isopentane cooled by liquid nitrogen (-160°C).

Cryostat sections cut at -18°C to -20°C.

Used for:

Enzyme histochemistry

Fibre typing

Early structural abnormalities

Avoid ice crystal artefact.

C. Enzyme Histochemistry Panel

NADH-TR

SDH

Myosin ATPase (pH 9.4, 4.6, 4.3)

Phosphorylase

COX (modern addition)

D. Electron Microscopy

Reserved for:

Mitochondrial myopathies

Congenital structural myopathies

B III MUSCLE GROSS PEARLS (ADULTS)

Marked fatty replacement → muscular dystrophy

Patchy pallor → inflammatory myopathy

Severe atrophy with fibre grouping → neurogenic process

Type 2 selective atrophy → steroid effect

Gross findings are supportive, not diagnostic.

B IV MUSCLE MICROSCOPY

Fibre Typing

Type 1 Fibres

Slow twitch

Oxidative

Fatigue resistant

Type 2 Fibres

Fast twitch

Glycolytic

Subtypes: 2A, 2B, 2C

Key ATPase Reactions

Reaction	Type 1	Type 2
ATPase pH 9.4	Light	Dark
ATPase pH 4.6	Dark	Light
NADH-TR	Dark	Variable

Important Patterns

Type grouping → neurogenic atrophy

Fibre type predominance → inherited pattern or disease

Type 2 atrophy → steroid myopathy

Ragged red fibres → mitochondrial disease

Assess:

1. Fibre Size Variation

Uniform?

Atrophic?

Hypertrophic?

2. Necrosis & Regeneration

Myophagocytosis

Basophilic regenerating fibres

3. Inflammation

Endomysial (polymyositis)

Perifascicular (dermatomyositis)

Perivascular

4. Fibre Type Distribution

Random (normal)

Grouping (neurogenic)

5. Structural Abnormalities

Central cores

Nemaline rods

Rimmed vacuoles

Mitochondrial aggregates

Major Diagnostic Patterns

Pattern	Suggests
Fibre necrosis + regeneration	Dystrophy
Endomysial lymphocytes	Polymyositis
Perifascicular atrophy	Dermatomyositis
Fibre grouping	Chronic denervation
Ragged red fibres	Mitochondrial disease
Vacuoles with inclusions	Inclusion body myositis

B V MUSCLE SYNOPTIC REPORTING

Specimen:
Needle / Open biopsy

Site:
Quadriceps / Biceps etc.

Adequacy:
Adequate / Limited

Processing:
Formalin + Frozen sections performed

Histologic Findings

Fibre size variation: Mild / Moderate / Marked

Necrosis: Present / Absent

Regeneration: Present / Absent

Inflammation: Type and distribution

Fibrosis: Present / Absent

Fatty replacement: Present / Absent

Fibre type grouping: Present / Absent

Structural abnormalities: Specify

Enzyme Histochemistry

ATPase typing: Normal / Type grouping

NADH-TR: Normal / Abnormal

SDH: Normal / Increased

COX: Normal / Deficient fibres

Final Impression

Myopathic process / Neurogenic process

Inflammatory myopathy

Muscular dystrophy

Metabolic myopathy

Non-specific changes

Correlate with clinical and genetic findings.

C I TEMPORAL ARTERY BIOPSY ANATOMY

(Giant Cell Arteritis Workup)

1. Applied Anatomy

The superficial temporal artery is a branch of the external carotid artery.

It runs subcutaneously over the temporal region and divides into:

Frontal branch

Parietal branch

It is a **medium-sized muscular artery** with well-developed elastic lamina — the target of granulomatous inflammation in giant cell arteritis.

C II TEMPERAL ARTERY GROSSING TECHNIQUES

Indications for biopsy

Suspected **Giant Cell Arteritis**

New-onset headache in elderly

Jaw claudication

Visual disturbances

Raised ESR / CRP

Biopsy should ideally be performed **before or within 1–2 weeks of steroid therapy**, but treatment must not be delayed for biopsy.

Specimen Requirements

Minimum length: **2–3 cm** (skip lesions common)

Entire specimen should be submitted

Measure length and diameter

Ink ends if orientation required

Grossing Protocol

Measure entire length.

Serially section at 2–3 mm intervals.

Submit **entire specimen in multiple blocks**.

Avoid over-handling (small fragile vessel).

Step sections are recommended if initial sections are negative.

C IV TEMPERAL ARTERY GROSS PEARLS

Classic finding: jaw claudication, typically in a patient older than 50 years.

Other findings: headache (very common), vision loss or diplopia, scalp tenderness, polymyalgia, weight loss, chills, fever.

C IV TEMPERAL ARTERY MICROSCOPY

Evaluate:

Intimal thickening

Disruption of internal elastic lamina

Granulomatous inflammation

Multinucleated giant cells

Luminal narrowing

Skip lesions

Special stain:

Elastic stain (e.g., Verhoeff–Van Gieson) to demonstrate elastic lamina fragmentation.

Microscopy Pearls

Giant cells may be absent — diagnosis still possible.

Inflammation may be focal → examine multiple levels.

Healed arteritis shows fibrosis + elastic lamina fragmentation.

C V TEMPERAL ARTERY SYNOPTIC REPORT – TEMPORAL

Specimen length: ___ cm
Adequacy: Adequate / Limited

Inflammation: Present / Absent
Type: Granulomatous / Lymphocytic

Giant cells: Present / Absent

Elastic lamina disruption: Present / Absent

Luminal narrowing: Mild / Moderate / Severe

Final Impression:
Positive for Giant Cell Arteritis / No evidence of arteritis (comment on adequacy)

ASSESS YOURSELF MISCELLANEOUS

Long Essays (10 marks each) What is the condition Temperal arteritis ? How is the diagnosis confirmed histologically? 2.What are the various muscle fibre types seen in the human body? Describe the difference between them regarding their "twitch" speed and primary metabolic pathway.

3. 3. What are the indications for a brain biopsy? What are the various preparations and biopsies done in the diagnosis of brain lesions?

Short essays (5 marks each)

- What is muscle fibre typing? How is it done?
- 5. Types of brain biopsies
- 6. Snap freezing in muscle biopsies

Short answers (3 marks each)

- **7.** Which special stain is essential to demonstrate fragmentation of the vessel wall in Giant Cell Arteritis (GCA)?
- 8.When is Electron Microscopy (EM) specifically indicated for muscle samples?
- 9. To diagnose a true "Oligodendroglioma," which two molecular alterations must be present?

MCQs

1.'Fried egg' appearace is characteristic of-

A. Glioblastoma
B. Pilocytic astrocytoma
C. Meningioma
D. Oligodendroglioma

2. What is the 'optimal length of doing a 'temperal artery biopsy'?

A. 1 cm ; B. < 1 cm ; C . 2 -3 cms ; D. 3 cms

3.The superficial temperal artery is a branch of –

A External carotid artery
B. Internal carotid artery
C. Popliteal artery
D. Subclavian artery

Answer Key for MCQs

1. D.
2. C
3. A.

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