Telegram - @nextpreladdernotes

1 CELL INJURY

Insight of Cell Injury

- The most common cause of cell injury is Hypoxia.
- The most common cause of Hypoxia is ischemia
- The most sensitive cell to Hypoxia is the brain/neurons.
- The most resistant cell to Hypoxia is a fibroblast.

Cell injury

Injury
↓
Adaptation
↓ fails
Reversible cell injury
↓injury continues
Irreversible (cell death) cell injury

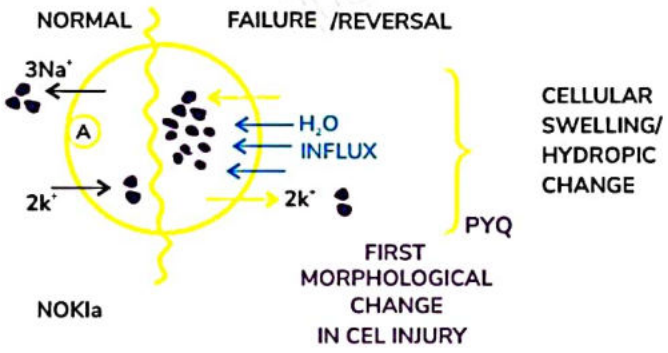
Types of Cell Injury 00:03:28

There are two types of cell injury:
1. Reversible Cell Injury
2. Irreversible Cell Injury

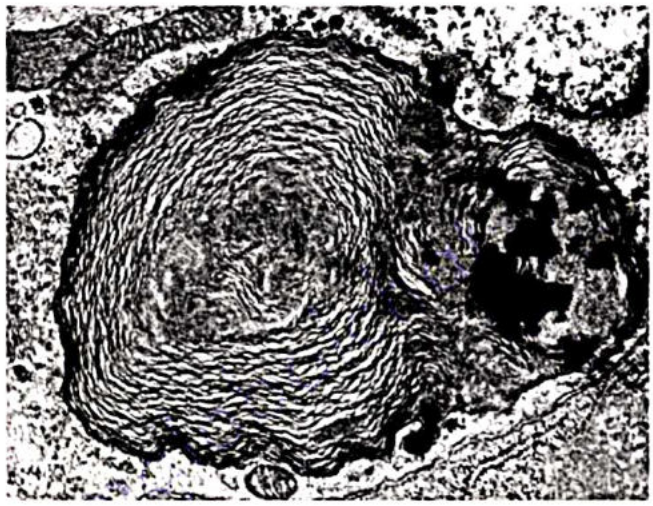
Reversible Cell Injury 00:05:22

Mitochondrial Dysfunction occurs causing the decrease in ATP production.
The process of ATP failure includes:

1. Failure of Na^+ K^+ ATPase Pump



- There is H2O influx.
- It is referred to as Hydropic Change.
- This is responsible for the following:
  - Cellular swelling
  - Endoplasmic reticulum swelling
  - Flattening of the microvilli
  - Formation of cytoplasmic blebs
  - Formation of Myelin Figures, also called Concentric Lamellation.
    → Composition: Phospholipids (primarily) and Ca^{2+}



2. Anaerobic Glycolysis

- The pH will become acidic, causing the nuclear chromatin to clump.

3. Decrease in Protein Synthesis

- The ribosome detachment is seen, leading to a decrease in protein synthesis.
- This is also associated with fatty change

Irreversible Cell Injury 00:22:28

The two defining moments of irreversible cell injury are:
1. **Severe Membrane Damage:** The inflow of calcium occurs. It causes the activation of three enzymes:
   a. Phospholipase
   b. Protease
   c. Nuclease

2. **Severe Mitochondrial Damage:** The calcium sits on the mitochondria causing Amorphous Flocculent Densities.

Important Information

Myelin Figures:
- Myelin Figures are also called concentric lamellation.
- Myelin figures are seen in reversible as well as irreversible cell injuries.
- More Myelin figures are seen in irreversible cell injury.

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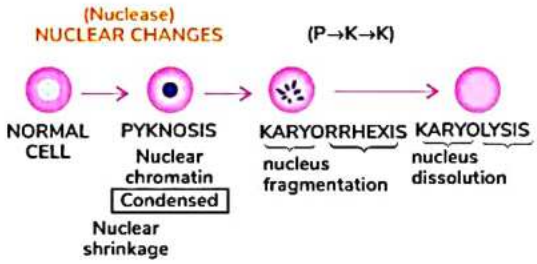
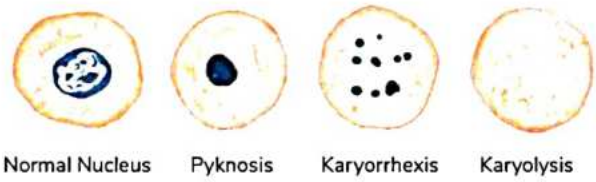
Nuclear Changes in the Cell Injury:

The three stages of nuclear changes in cell injury are:

- **Pyknosis**: The nucleus becomes small and dark due to the nuclear chromatin edit = condensation
- **Karyorrhexis**: There will be Nuclear Fragmentation.
- **Karyolysis**: The nucleus gets dissolved.





Cell Death - Necrosis

00:33:12

Definition

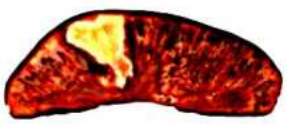
- It is a Pathological Cell Death.
- It is associated with inflammation.

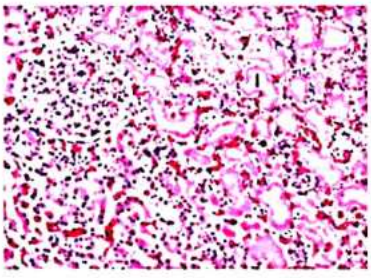
Microscopic Examination

- It appears pink (Eosinophilic)
- Amorphous
- Inflammation

Types of Necrosis

1. **Coagulative Necrosis:**
   - It is the most commonly occurring Necrosis.
   - There is denaturation of the proteins.
   - It occurs in the solid organs (kidney, liver, heart)
   - Occurrence of infarct (Wedge shaped structures) in the organs.
   - Leading to the formation of multiple ghost cells, causing a Tombstone Appearance.
   - The tissue architecture is preserved even after the death of the cells.





Important Information

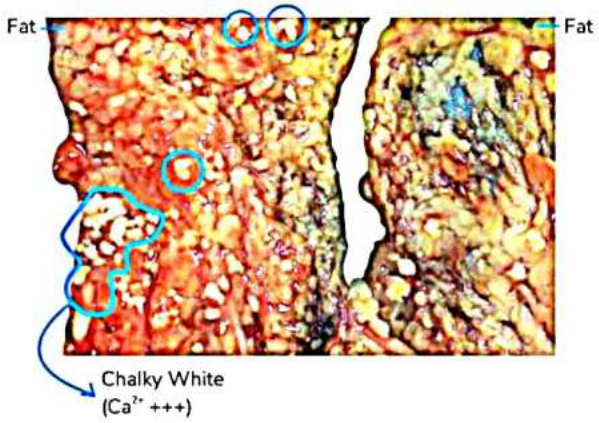
Ghost Cells:

- Ghost cells are associated with coagulative Necrosis.
- Occurs on a skin-generated tumor, Pilomatrixoma.

The row of Tombstone appears in the Pemphigus (Pemphigus Vulgaris)

2. **Liquefactive Necrosis:**
   - It mostly occurs in CNS and Pancreas.
   - This occurs because they are rich in Hydrolytic Enzymes.
   - It is also called Colliquative Necrosis.
   - The tissue architecture is not preserved.
3. **Caseous Necrosis:**
   - Associated with the high lipid content in the cell walls.
   - There is cheese-like appearance of the organs.
   - It is the combination of Coagulative Necrosis and Liquefactive Necrosis.
   - Most common examples include tuberculosis, fungal infection (histoplasmosis; coccidioidomycosisis), syphilis, etc.

4. **Fat Necrosis:**
   - It occurs in the fat-rich organs e.g., breasts; omentum, etc.
   - It causes the breakdown of fats into fatty acids.
   - A Chalky White appearance is seen.
   - It also occurs around the pancreas due to the presence of peripancreatic fat.



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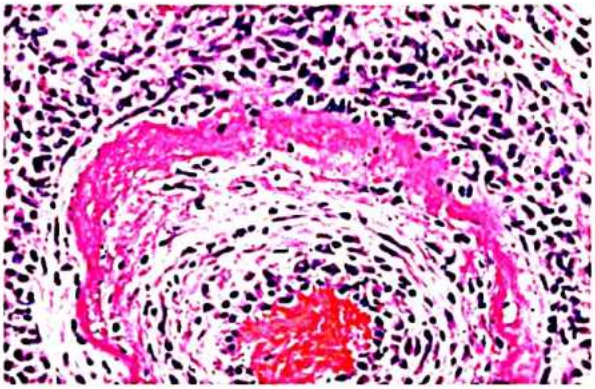
5. **Fibrinoid Necrosis:**
   - Associated with the antigen - antibody reactions (immune complex deposition).
   - Some examples are:
     → Polyarteritis Nodosa (PAN)
     → Rheumatic Heart Disease (RHD)
     → SLE
     → Malignant Hypertension (HTN)



6. **Gangrene:**
   - Blackish discoloration of the organs.
   - Foul smelling.
   - Dry gangrene is classified as Coagulative Necrosis with a line of demarcation.
   - Wet gangrene is classified as Liquefactive Necrosis without any line of demarcation.

7. **Zenker's Degeneration:**
   - It is a type of Coagulative Necrosis.
   - It occurs in typhoid or Enteric fever.
   - It is a complication seen in the skeletal muscles:
     → Rectus Abdominis
     → Diaphragmatic Muscle

MCQ:

Q. Myelin figures are derived from?

a. Cytoplasm
b. Nucleus
c. Cell Membrane
d. Ribosomes

Q. A 61-YEAR-OLD female patient presents with left-sided chest pain radiating to the left arm and jaw. The patient explains that the pain has increased severely over the past 40 minutes. She is immediately rushed to the hospital. Cardiac enzymes are elevated. The patient was admitted and started on thrombolytic therapy. However, on the fifth day of observation, she suddenly collapses and dies. Which of the following Necrosis are you most likely to find in the heart of this patient?

a. Liquefactive Necrosis
b. Coagulative Necrosis
c. Fat Necrosis
d. Fibrinoid Necrosis

Q. A 45-year-old female patient complained of being hit in the chest by a football while passing by a garden 4 weeks back. Initially, her left breast was tender and swollen. But over the weeks, the tenderness has subsided. However, she now notices a lump in the peri-areolar region which is firm to hard in consistency. Radiological investigations reveal calcific deposits. Which of the following best describes the phenomenon above?

a. Liquefactive necrosis
b. Coagulative necrosis
c. Fat necrosis
d. Fibrinoid necrosis

Cell Death – Apoptosis

01:04:15

Definition:

- Apoptosis is a greek word that means "falling off."
- It is both physiological as well as pathological cell death.
- Mitochondria play a pivotal role in apoptosis.
- It is a programmed cell death, now known as Caspase Dependent Programmed Cell Death.

ATP Usage:

- There will be the usage of ATP.
- Occurs as an Active Process

Inflammation:

- No inflammation will occur.

Examples of Apoptosis

- **Physiological Apoptosis**
  - Organogenesis (Embryogenesis), For example, fingers and hand formation.
  - Neutrophils Clearance
  - Endometrial Shedding

- **Pathological Apoptosis**
  - Councilman Bodies, seen in viral Hepatitis (Hepatitis C-Virus)
  - Civatte/ Colloid/ Cytoid Bodies, seen in Lichen Planus.

Important Information

Chemotherapy:

- It causes cancer cell death.
- It's a combination of Apoptosis (80%) and Necrosis(20%).

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Caspase 01:12:24

- It consists of Cysteine Residue.
- It breaks after aspartic acid residues.
- It is an Enzyme.

Mechanism of Apoptosis

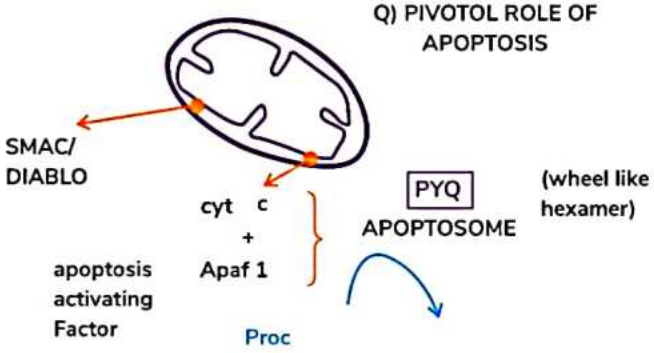
The mechanism is classified into two processes.

1. **Initiation**

   It is dependent on Caspase 8, 9, and 10. There are two pathways to apoptosis initiation.

   a. **Extrinsic Pathway:**
      i. It happens on the surface of the cell.
      ii. The cell wanting to die will have CD95 / Fas, and the supporting T cell will have CD95 Ligand / Fas Ligand.
      iii. It leads to the occurrence of Trimerisation of CD95/ Fas
      iv. The Fas Associated Death Domain (FADD) will activate Procaspase 8, 10 into Caspase 8, 10.
      v. The process is inhibited by FLIP (anti-apoptotic molecule)
      vi. It is also called Death receptor pathway.

   b. **Intrinsic Pathway:**
      i. It is also called Mitochondrial Pathway
      ii. It happens within the cell.
      iii. It occurs when the cells undergo stress.
      iv. The stress is detected by the stress sensors: BIM, BID, BAD, NOXA and PUMA.
      v. The sensor increases the pro-apoptotic factor and decreases the anti-apoptotic factors.
      vi. The Apoptosome activates Procaspase 9 into Caspase 9.



Pro-Apoptotic	Anti-Apoptotic
• p53	• BCl2
• BAC	• MCl1
• BAX	• BClXL
• BCLXS	

2. **Execution:**
   - It occurs after the Initiation process.
   - It is dependent on Caspase 3, 6, and 7.
   - It activates three enzymes:
     - Phospholipase
     - Protease

Important Information

Endonuclease:
- It breaks the DNA after 180-200 bp
- It is called Internucleosomal Cleavage

Once the execution takes place, the cell falls off into small bodies called apoptotic bodies. These apoptotic bodies give an "Eat me" signal to Macrophages, causing Phagocytosis.

Efferocytosis 01:38:10

- The apoptotic bodies giving the eat-me signals, expressed in the form of C1q, Thrombospondin, and PS (Phosphatidyl Serine)
- In the normal cell, the Phosphatidyl Serine is present in the inner leaflet, but in the apoptotic cell, the Phosphatidyl Serine comes out.
- There is Phosphatidyl Serine Flipping.
- The flipping is known as the signal given to the Macrophage. One major defect of flipping is Scott Syndrome.
- The Macrophage engulfs the apoptotic bodies, which is called Efferocytosis.

Defects in Efferosome

- SLE
- COPD
- Bronchiectasis
- Cystic fibrosis

Important Information

Survival of Cancer cells:
- Cancer cells express CD47 to Macrophages.
- Cd47 is a "Do not Eat me" Signal.

Update Robbins IOc- 01:43:55

- Pro apoptotic molecules [BAC, BAX, BCLXS]: BH 1 - 3
- Anti-apoptotic molecules [BCL2, MCLI, BCLXL]: BH 1 - 4
- Stress sensors [BIM, BID, BAD, NOXA, PUMA]: BH 3 only
- SMAC / DIABLO : Proapoptotic
- Glucocorticoids: Proapoptotic
- Sex Steroids: Antiapoptotic

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Identification of Apoptosis

01:47:57

- Marker: Annexin V (marker for testing PS flipping)
- Molecular Marker: CD95/ Fas
- Microscopic Examination: Nuclear Chromatin Condensation. (The cytoplasm will be pink and Nucleus will be blue, along with cellular shrinkage)
- Stain: Tunel Stain (TdT dUTP Nick End Labelling)
  - Positive: Apoptosis
  - Negative: Necrosis
- Gel Electrophoresis: Step Ladder Apoptosis

Important Information

Smearing:

- Smearing is seen only in Necrosis.
- Step Ladder is seen both in Apoptosis and Necrosis.

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PREVIOUS YEAR QUESTIONS

Q. What is the first change in the cell injury?
Mitochondrial Dysfunction

Q. Which is the first morphological change in the cell injury?
The cellular swelling or Hydropic Change.

Q. What is the composition of Myelin Figures?
Primarily made up of phospholipids and a minor presence of calcium.

Q. What is the composition of Amorphous Flocculent Densities?
Calcium.

Q. The injury with which the Amorphous Flocculent Densities are associated?
Irreversible Cell Injury.

Q. The three stages of nuclear change in the cell injury?
Pyknosis
Karyorrhexis
Karyolysis

Q. What is the most common type of Necrosis?
Coagulative Necrosis

Q. Which are the most common organs affected by Coagulative Necrosis?
Heart

Q. Which necrosis happens inside the pancreas?
Liquefactive Necrosis

Q. Which necrosis happens around the pancreas?
Fat Necrosis

Q. Zenker's Degeneration be seen in which skeletal muscles?
Rectus Abdominis
Diaphragmatic Muscle

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2 NEWER CELL DEATHS

Newer Cell Deaths

- Necroptosis
- Pyroptosis
- Ferroptosis
- Anoikis

Necroptosis

00:00:22

Necrosis + Apoptosis makes Necroptosis

Definition: Caspase independent programmed cell death

Necrosis :

- Morphology
- Inflammation

Apoptosis :

- Programmed cell death

Necroptosis:

- Physiological
  - Growth Plate Formation in Human Body
- Pathological
  - Steatohepatitis (fatty liver)
  - Pancreatitis
  - Reperfusion Injury (MI)

Updates: **Robbins 10th Edition**

- Programmed Necrosis (New terminology to necroptosis)

CMV

- It does not undergo apoptosis due to the presence of caspase inhibitors.
- It undergoes necroptosis (caspase independent)

Mechanism of Necroptosis

- **1-2-3 death**
  - **Step 1 :** TNF comes and bind to TNF receptor
  - **Step 2: due to binding trio occurs** -RIPK 1, RIPK 3 (RECEPTOR INTERACTING PROTEIN KINASE,) PROCASPASE 8
  - **Step 3:** MLKL phosphorylation which results in Cell Death

Pyroptosis

Pyro means Fever + tosis means cell death.

- Associated with microorganisms.

Examples:

- Initially studied for Shigella and salmonella

Bacteria
↓ enters the body
Binds to NOD Receptors (gest activated)
↓
Inflammasomes
↓
activates CASPASE 1 → activates IL-1 → fever

4, 5, 11 — Cell death

Anoikis

00:12:27

Type of apoptosis - why is it different?

- Lack of natural environment
- Example:
  - Cells kept in an unnatural environment - cell dies.

Q Choose the incorrect statement about Necroptosis ?

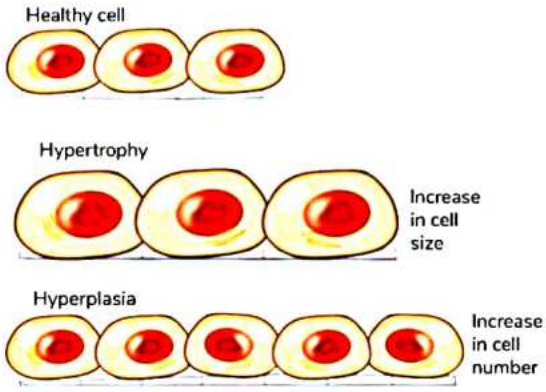
a. Is it a caspase independent cell death
b. RIP 1&3 is formed
c. Caspase 8 is required
d. Growth plate formation follow necroptosis

Q Choose the incorrect statement about pyroptosis ?

a. Seen in response to shigella
b. TLR is used
c. Caspase 1 is required
d. IL1 activated

Cell Injury - Cellular Adaptations

- Hypertrophy
- Hyperplasia
- Atrophy
- Metaplasia
- Dysplasia - PRECANCER



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Hypertrophy
- When the size of cells is increasing but the number remains same

Hyperplasia
- Number of cells increases, size remains same

Differentiation between Hypertrophy and Hyperplasia

Hypertrophy	Hyperplasia
Basic Difference	
Increase in Size of cells	Increase the number of cells
Mechanism	
Increase in -Transcription Factors • GATA 4 • NFAT • MEF 2 Are being elevated. • More RNA • More Protein Hence the size increases. • DNA $\xrightarrow[\text{Transcription}]{}$ RNA → Protein	• Happens by division/mitosis Increases the number of cells

Common Examples are:
Common examples of hypertrophy and hyperplasia are
1. Gravid uterus shows hypertrophy (more commonly) as well as hyperplasia
2. Breast development during puberty and pregnancy shows both hypertrophy as well as hyperplasia (more commonly)

Examples of Hypertrophy (only)
1. Bodybuilders - Skeletal Muscle Hypertrophy
2. Obstruction - Proximal to Obstruction

Q. How will the body adapt to obstruction?
Answer: The body is going to adapt to hypertrophy.
Example: Aortic stenosis → left ventricular hypertrophy

Examples of Hyperplasia (only condition)
For Females
- Endometrial Hyperplasia
  - Increased Estrogen is responsible.

Q. Name one condition where estrogen increases?
Ovarian Tumors - Granulosa Cell Tumor
Produces estrogen → it will cause endometrial hyperplasia → Risk of Endometrial cancer type 1.

For Males
- BENIGN PROSTATIC HYPERPLASIA
  - Now called Nodular Hyperplasia of the Prostate
  - Testosterone $\xrightarrow[\text{5 alpha reductase}]{}$ DHT - Dihydrotestosterone (metabolite) → hyperplasia
    ↑ inhibits
    Finasteride

Atrophy
- Cell size decreases
- Cell number decreases

Mechanism of action
UPP - UBIQUITIN PROTEASOME PATHWAY

Examples:
- Disuse Atrophy – Fracture → cast for six weeks → muscle atrophy
- Denervation Atrophy
  - Polio
- Ischemic Atrophy
  - Senile → Alzheimer's Disease
- Malnutrition
- Endometrial atrophy
  - Less of estrogen

More estrogen → endometrial hyperplasia	Less estrogen →endometrial atrophy
• Endometrial Cancer Type 1	• Endometrial Cancer Type 2 • Worst prognosis

Metaplasia
All cellular adaptations are Reversible.
Toughest to revert is Atrophy.

Q. What metaplasia is
- 100% reversible
- One tissue ⇌ another tissue
  - Epithelial ⇌ epithelial
  - Mesenchymal ⇌ mesenchymal

Mechanism of action
- Reprogramming of the Stem Cells.

Examples:
- Epithelial to epithelial

Q. Most common metaplasia
1) **Squamous metaplasia - most common condition**
Pseudostratified ciliated columnar epithelium
↓ Chronic Smoker
↓ Vitamin A (deficiency/excess)
squamous (end result) epithelium

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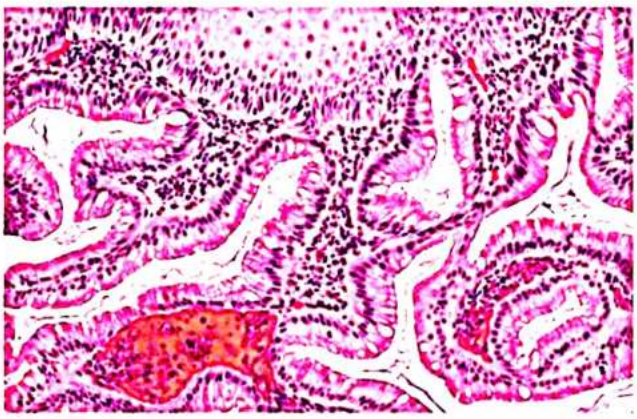
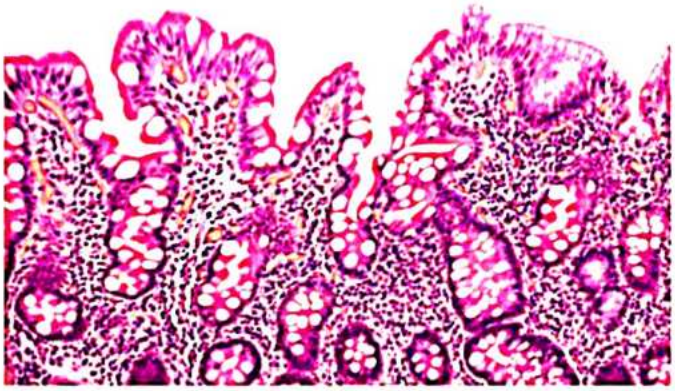
2) **Barrett's Esophagus** - Precancerous adenocarcinoma of the esophagus (cancer)

Squamous epithelium $\xrightarrow[\text{GERD}]{}$ intestinal columnar epithelium
↓
Contains goblet cells
↓
Acidic mucin
Alcian blue
positive (special stain)



Q. Hallmark of Barrett Esophagus

- Goblet cells



Q. Where do you see Mesenchymal metaplasia

- Myositis(muscle) + ossificans(calcium/bone)
- Muscle changes to bone→occurs due to trauma

Breast is one such organ where all the adaptation happens.

- In Puberty & Pregnancy Hypertrophy and Hyperplasia both occurs, M/C is hyperplasia.
- Atrophy occurs in postmenopausal occurs → old age ladies
- Metaplasia -
  - Squamous Metaplasia of the lactiferous Ducts (SMOLD)
  - Most commonly seen in chronic smokers female

MCQs 00:14:11

Q. All are true for metaplasia except?

a. Slow growth
b. Reversible with treatment
c. Irreversible
d. Can be precancerous

Q. Histopathological difference between Barrett's epithelium and gastric mucosa?

a. Barrett's mucosa is acidic and stains alcian blue positive
b. Barrett's is alkaline and stains prussian blue positive
c. Barrett's alcian blue negative because its neutral
d. Gastric mucosa is alkaline and stains alcian blue positive

Q. A 47-year old man visits an outpatient clinic with complaints of heartburn and chest pain for the past 6 months. His pain is retrosternal and was initially only associated with intake of solid foods, but it now occurs with liquid as well. Antacids don't relieve his pain anymore. He is worried about the pain as it is getting worse.physical examination including abdominal examination is normal. He has lost 2.7 kg(6 lbs).

Laboratory investigation reveals. :
Hgb - 10 gm
Platelet count - 168 * 10.9/L.
Esophagogastroduodenoscopy reveals an exophytic mass in the lower third of the esophagus. Which of the following is the most likely diagnosis in the patient?

a. Squamous Cell Carcinoma
b. Leiomyoma
c. Gastric Ulcers
d. Adenocarcinoma

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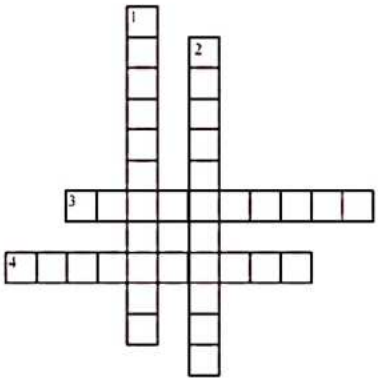
CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Initially studied for Shigella and salmonella
4. All cellular adaptations are REVERSIBLE.

Down

1. When the size of cells is increasing but the number remains same
2. Number of cells increases ,size remains same

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3 INTRACELLULAR ACCUMULATIONS

Pigment

Melanin

00:00:55

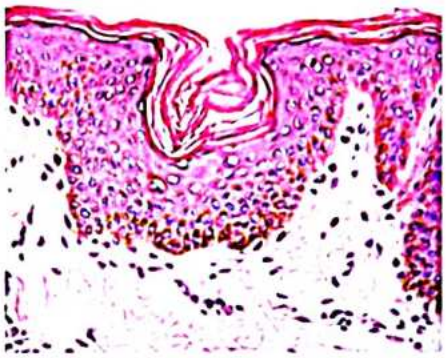
- Present in, skin, hair & eyes.
- In the brain, melanin is present in the substantia nigra.

Important Information

- In Parkinson's disease, there is a decrease in dopaminergic neurons, which causes a decrease in dopamine and melanin, causing the appearance of a pale substantia nigra.

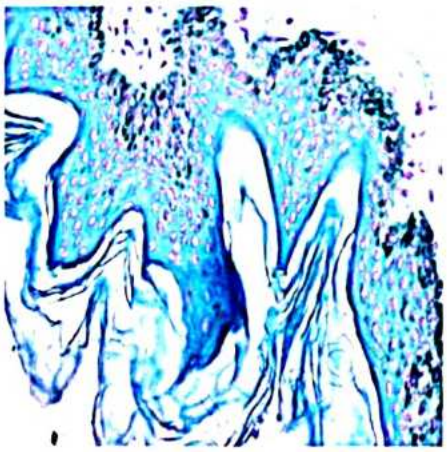
Melanin Stain

- In the skin, there is a brownish stain around the basale layer, which is called stratum basale.



- The blackish color image representation of the melanin stain is known as the Masson Fontana Silver stain.

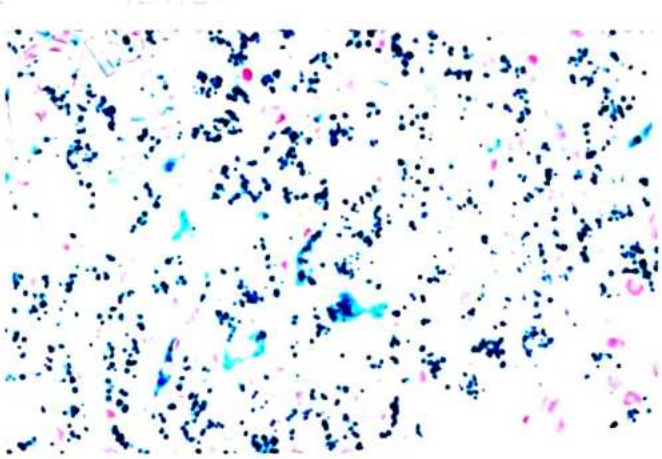
- The bluish color stain is known as the Schmorl stain.



- The best stain that we have for melanin is the Dopa Oxidase. It is also known as the enzyme histochemical stain.
- Immunohistochemistry - HMB 45 and S100.

Hemosiderin:

00:06:20



- It consists of any existence of hemorrhage or hematoma.
- Yellow. Brown, refractile (Shiny)
- Perl's stain or Prussian Blue stain can be seen.
- It consists of Fe^{3+}
- In the case of any iron overload, Hemosiderin will be appearing.

Important Information

- Lillie's stain will done for Fe^{2+}

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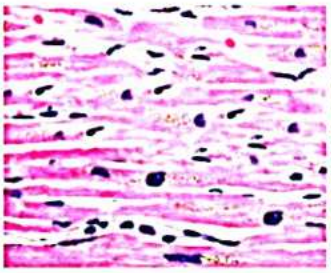
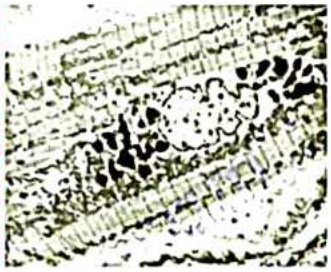
Lipofuscin or lipochrome: 00:08:50

- It is also defined as wear and tear pigment:
  - Senile Atrophy
  - Brown atrophy
- A telltale sign of free radical injury (more in old age)
- If a free radical injury increases, lipofuscin will increase because free radicals do lipid peroxidation, which causes the formation of lipofuscin.
- As it is present around the nucleus, it is also called perinuclear.





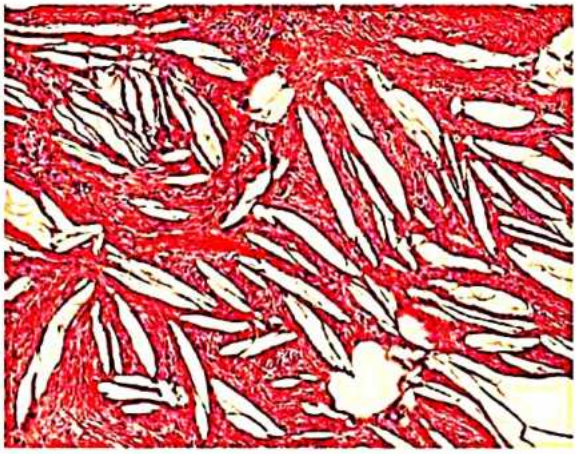
Q. What are the special stains?
Oil red O, ZN stain (Acid Fast)

Certain criteria regarding pigments:

- **Copper:**
  - Increased copper will cause Wilson's disease.
  - The special stain that is related to copper is Rhodanine and Rubeanic acid.
  - However, Rhodamine is related to mycobacterium TB (fluorescent)
  - The copper-associated protein is ceruloplasmin. Orcein stain is related to ceruloplasmin. It is the stain for the HBsAg and Elastin.
- **Hematin:**
  - It also shows Hamazoin, which comes up in the disease of malaria.
- **Anthracotic pigment:**
  - This pigment consists of carbon.
  - Therefore, individuals that smoke a lot show these varieties of pigment.
- **Homogentisic acid:**
  - It is also a black-colored pigment that gets deposited in the disease of Alkaptonuria.

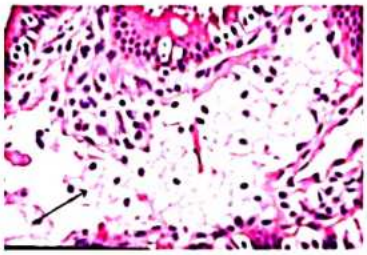
Lipids:

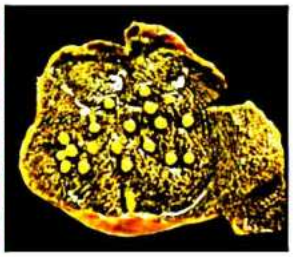
- The most common organ where the fat will get deposited is the fatty liver/steatosis.
- When the fat gets deposited in the heart, it is called the tigered effect or tabby cat.
- Some conditions for the deposition of fat in the heart are:
  - Cell injury
  - ARVCM (Arrhythmogenic right ventricular cardiomyopathy)
  - Diphtherial Myocarditis
- In the case of atherosclerosis, some empty spaces can be seen in the image. These empty spaces are called cholesterol clefts.



In H& E stain:

- Due to the processing with alcohol, the fat is washed off, which causes the formation of Cholesterol Clefts.

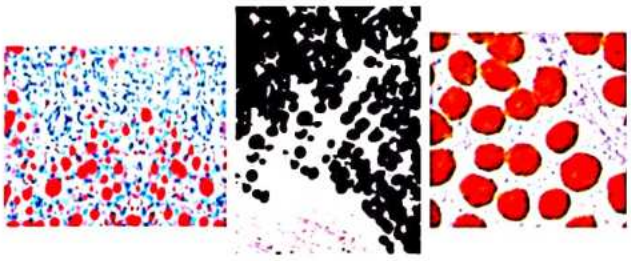




- The tiny yellow color dots are called Cholesterolosis of the gallbladder. It is also called the strawberry gallbladder.
- It also shows foamy macrophages.
- Therefore, the representation where mϕ has engulfed all the cholesterol.

Telegram - @nextprepladdernotes

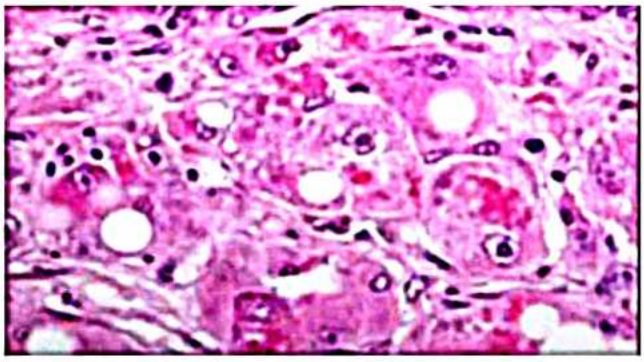
Stains for lipids:



- The red color stain is called the oil red O
- The black color stain is called Sudan Black B
- The orange color stain is called Sudan 4.
- One of the other stains is known as the osmium tetroxide.
- Cryostat or frozen section machine. This machine is used for the identification of the oil red O.

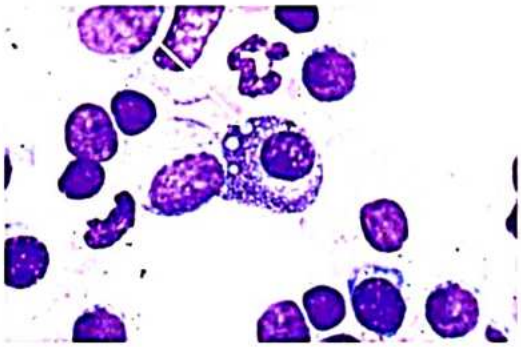
Protein: 00:25:47

- There are two conditions of protein accumulation:
  - Either there will be an accumulation of too much protein.
  - However, there must be another condition which is called the misfolding protein.
- Some examples are:
  - **Mallory hyaline/denk bodies:**



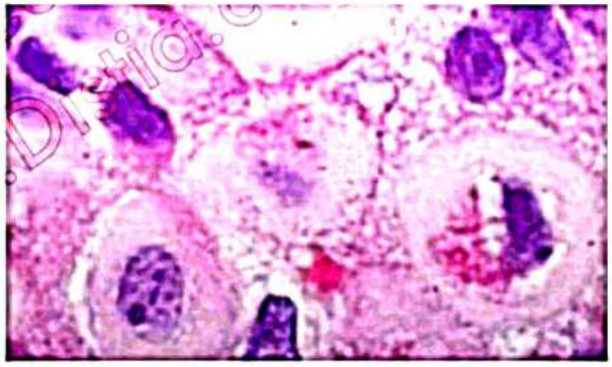
→ They are mostly seen in alcoholic liver disease.
→ It is composed of cytokeratin 8/18.

- **Multiple myeloma:**



→ It is also known as the tumor of plasma cells.
→ It is caused due to the increased production of antibodies.
→ Russell bodies are intracytoplasmic collections of immunoglobulins
→ Dutcher bodies are intranuclear collections of immunoglobulins

- **Crooke Hyaline Change:**



→ It is seen in the pituitary gland.
→ It is caused due to Cushing disease
→ It is composed of cytokeratin.

Deficiency.	Endoplasmic reticulum Stress (Misfolding protein.)	Both
LDL Rc (Hypercholestrolemia	Alzheimer's disease Abeta	AATD (Alpha 1 Deficiency)
CFTR (Mutation.)	Prion disease PrP protein	
Tay Sachs Disease (Hexosaminidase deficiency.)	Retinitis pigmentosa	

Telegram - @nextpreladdernotes

Important Information

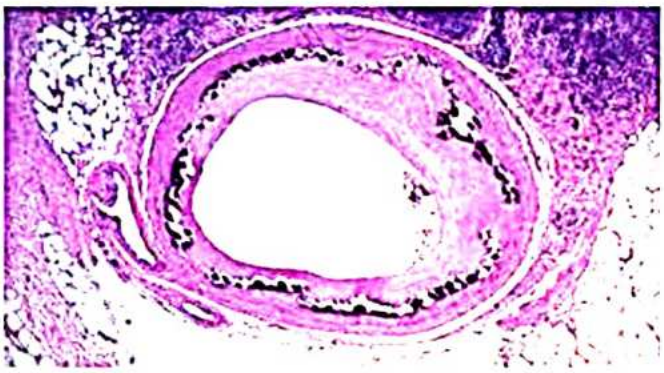
- The alpha-1 deficiency causes panacinar emphysema in the lungs (Caused due to the deficiency of the protein.) and cirrhosis in the liver (Caused due to the misfolding protein).

Glycogen: 00:35:27

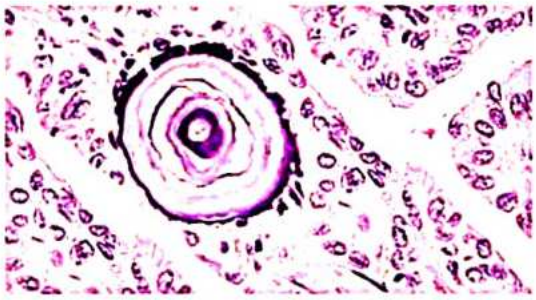
- Glycogen will be accumulated only in the case of
  - Glycogen storage disease.
  - Diabetes Mellitus. (Diabetic nephropathy.)
    - → Glycogen goes into the PCT (Proximal convoluted Tubule), then it is called an Armani Ebstein Lesion.
  - Some special stains are:
    - → PAS (Per Iodic acid Schiff)
    - → It will be pink in color.
    - → PAS + Diastase Sensitive shows that if you add the diastase, the glycogen will vanish. Hence, the pink color will disappear.

Calcification:

- Types of calcification:
  - **Dystrophic calcification:**
    - → Dead or degenerating tissues are seen.
    - → There will be a normal blood calcium levels.
    - → Some examples:
      - Dead Parasite
      - TB
      - Atheroma
      - Monckeberg Sclerosis:



  - It is also called calcific medial degeneration.
  - It happens in the tunica media.
  - Calcium occurs blue in color.
  - It occurs only as an old age phenomenon and there is no clinical significance.
  - Psammoma Bodies:

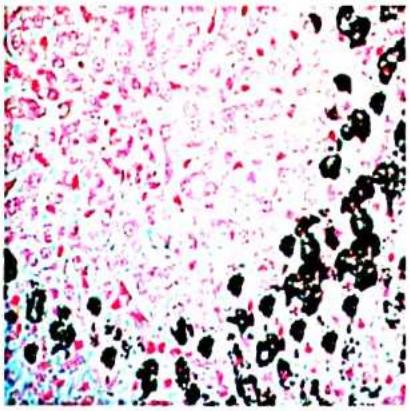


  - It is present as a concentric lamellations.
  - It shows an onion peel appearance.
  - The psammoma bodies are seen in
    - → Papillary Carcinoma
    - → Prolactinoma
    - → SomatoStatinoma
    - → Serous Ovarian Tumor
    - → Meningioma
    - → Mesothelioma
  - **Metastatic calcification.**
    - → Seen in normal tissues.
    - → The blood calcium level is elevated
    - → The most common organ affected due to metastatic calcification is the lungs

    Some Examples:
    - Hyperparathyroidism: It occurs due to the increase of the parathyroid hormone, Caused by the increase in the calcium
    - Cancer: RCC and Breast cancer shows an increase of calcium.
    - Vitamin D intoxication
    - Milk alkali syndrome.
    - Multiple myeloma: It shows bony lytic causing the increase of calcium level in the blood.
    - Sarcoidosis: It shows non-caseating granuloma, causing the increase of vitamin D3. Henceforth, increase in the calcium level.

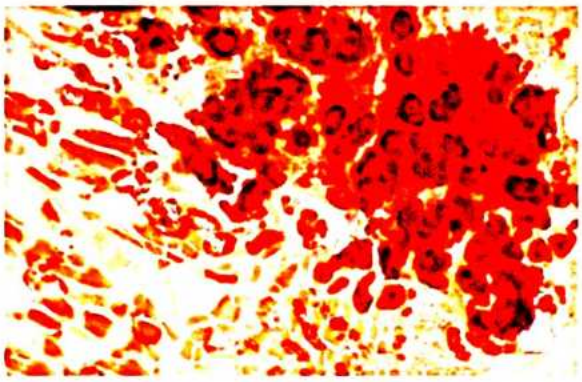
Stains for calcium: 00:51:50

- Von Kossa: It will give us a black color.



Telegram - @nextpreladdernotes

- Alizarin Red S:



  - It will give us a red color.
  - It is used to represent when the minute amount of calcium is present.
- Calcein
- AZAN stains:
  - It is used for differentiating between the mineralized bone and osteoid.

One-liner:

Q. What is the first place of deposition of the calcium?

Ans. Mitochondria
(However, in the history of kidney deposition, the calcium goes into the basement membrane)

MCQs:

Q. Elderly female patient presented to the outpatient department with the presence of a lump in the right breast measuring 5x4cm. The lump was firm to hard in consistency. The right axillary group of lymph nodes is also palpable and shows the presence of tumor deposits. There is the deposition of an amorphous material noted which stains positive with von Kossa stain. Which of the following statements is incorrect regarding the same?

a. Grossly calcium appears chalky white in color
b. Von Kossa gives a black color to calcium
c. Stains for picking up minute quantities of calcium include alizarin red S
d. The first site of deposition of calcium is the endoplasmic reticulum

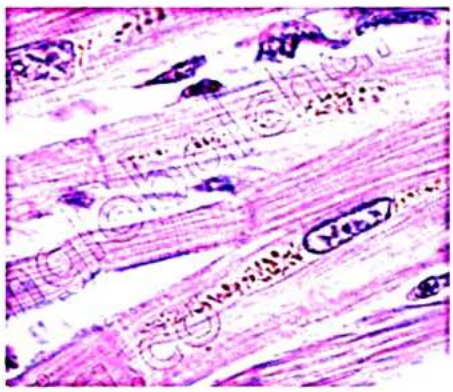
Ans: d. The first site of deposition of calcium is the endoplasmic reticulum

Q. A 54-year-old male patient presented with a pigmented lesion measuring 4x3cm on the right cheek. A biopsy of the lesion shows the presence of atypical cells with prominent nucleoli. Mitotic figures are noted. Histopathological diagnosis of malignant melanoma is made. Which of the following stains cannot be used for the diagnosis?

a. Masson Fontana
b. Schmorl's stain
c. HMB45
d. Masson trichrome

Ans: d. Masson trichrome

Q. Incorrect about the pigment shown.



a. Defined as yellow-brown in appearance
b. Formed due to lipid peroxidation
c. Can be positive for oil red O
d. Seen more commonly in infants

Ans: d. Seen more commonly in infants

Q. Which Of The Following Stains Is Best Suited For The Diagnosis Of Glycogen?

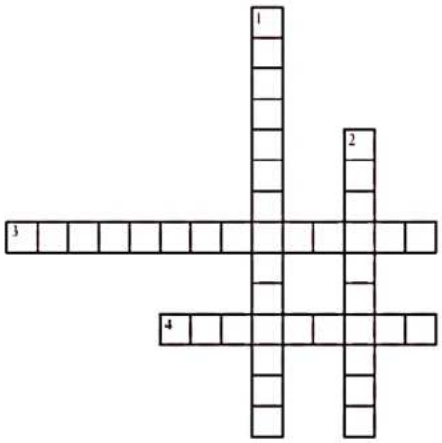
a. Oil red O
b. Perl's stain
c. PAS
d. Congo red

Ans: c. PAS

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. He is said to be the father of modern Pathology. His contributions are as follows:
4. This is caused by integrins in Leukocytes. Integrins

Down

1. Vasoconstriction (First event, Transient event lasting for few seconds, Reflex)
2. Leukocytes Mediated Injury: It is done by WBCs, and Leukocytes by releasing Enzymes.

Telegram - @nextpreladdernotes

4 AUTOPHAGY AND FREE RADICAL INJURY

- Auto = "Self" and Phagy = "Eating."
- Other name: Cell Cannibalism.
- A Japanese scientist named "Yoshinori Ohsumi",
  - Gave all the mechanisms of autophagy in 2016.
  - Received a Nobel prize for the discovery.

Examples of Autophagy 00:01:13

- Senile
- Malnutrition
- Cancers
- Neurodegenerative Conditions
  - Alzheimer's disease
  - Parkinson's disease

Types of Autophagy 00:02:24

Four types of mechanisms for autophagy:

1. Macro-Autophagy
2. Micro-Autophagy
3. Chaperone Mediated Autophagy (CMA)
4. Mitophagy

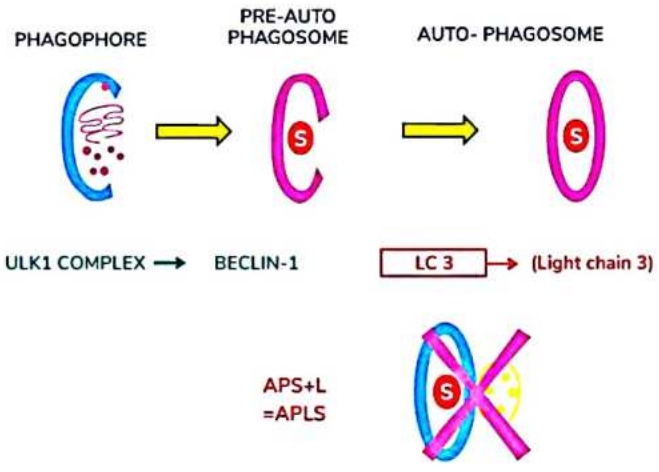
General outline of Macro-Autophagy

Refer Image 4.1

1. Macro-Autophagy 00:05:00

- Autophagosome formation occurs.

Q. Which is the autophagy in which the phagophore or autophagosome or the plate formation occurs?
Answer: Macro-Autophagy



- Eating of Endoplasmic Reticulum and Ribosomes occurs.
- ULK1 COMPLEX starts the formation of the plate called Phagophore.
- Plate will gradually elongate and become bigger.
- BECLIN-1 helps in elongation.
- LC3 (Light Chain 3) completes the full plate formation and now the plate is called Auto-Phagosome (APS).
- Auto-Phagosome fuse with the lysosome to form Autophagolysosome (APLS).
- Finally, all the substrates are broken inside the APLS.

Complexes Involving in Macro-Autophagy
ULK1 → BECLIN-1 → Lc3

Q. What is the marker of Autophagy?
Ans. LC3

- **Advanced Points**
  - mTOR decides the fate of autophagy.
  - At a well fed stage mTOR is activated and it will inhibit ULK1.
  - In malnutrition, mTOR is inhibited.
  - mTOR $\propto$ 1/Autophagy.

2. Micro Autophagy 00:10:00

- Simplest process.
- Direct uptake by lysosomes via endocytosis.

3. CMA 00:12:50

- CMA stands for Chaperone Mediated Autophagy.
- Chaperon corrects misfolded protein.

Examples of Chaperone

- HSP (Heat Shock Proteins)

Mechanism of CMA

- Chaperone binds with misfolded protein.
- The complex enters the lysosome via LAMP 2A (Lysosome Associated Membrane Protein 2A) for autophagy.

Q. LAMP 2A is used in which mechanism?
Ans. CMA

4. Mitophagy 00:16:36

- A type of Macro-Autophagy.
- Autophagosome formation.
- Only eating of mitochondria occurs.

Telegram - @nextpreladdernotes

- Old mitochondria are represented by PINK and PARKIN molecules on their surface.
- It confirms eating up of old mitochondria.

Important

Marker of autophagy	LC3 (Light Chain 3)
Most important gene for autophagy	ATG1
ATG5 gene mutation	Increased risk to get tuberculosis (TB)
ATG16L gene mutations	Increased risk of Crohn's Disease (Inflammatory Bowel Disease)

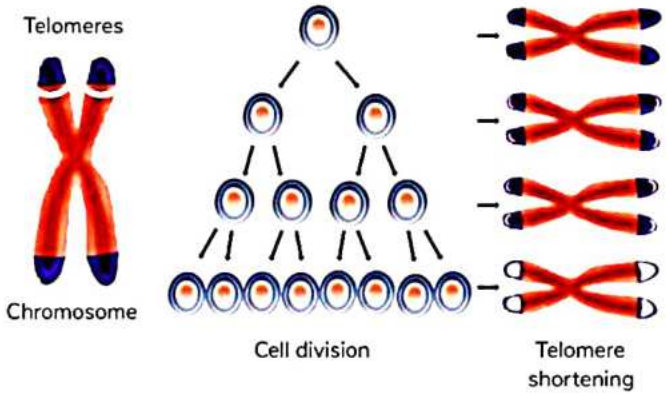
Cellular Ageing 00:21:53

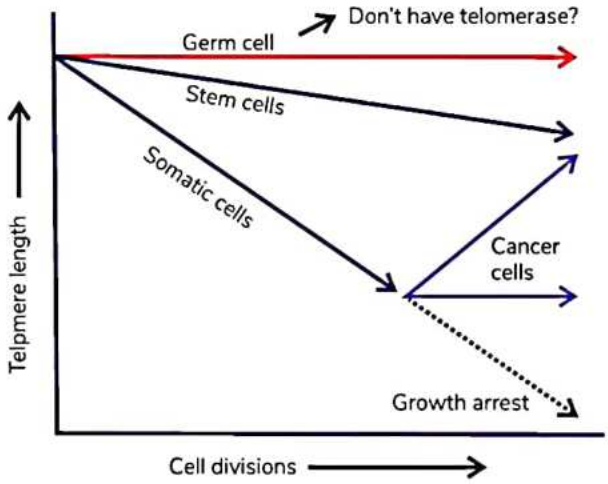
Causes of Cellular Ageing:

- Free radical injury (via chemicals, poisons and pollution) is the most common cause of ageing.
- Telomere shortening
- Insulin resistance
- DNA repair defects

Telomere Shortening 00:23:43

- Thousands of telomeres are present at the terminal end of chromosomes from human birth.
- One telomere sequence: TTAGGG.
- With each cell division, telomeres become shorter, thus ageing occurs.
- This can be prevented by "Telomerase".
- Telomerase is a RNA Dependent DNA Polymerase enzyme.
- Telomerase is very famously known as "Immortality Gene".





Type of Cell	Telomere Length
Somatic cell	Drops down
Stem cell	Gradual decrease
Germ cell	No change Maximum telomerase activity is seen
Cancer cell	Reactivate telomerase Survive longer

Hayflick Limit

- A cell undergoes 40-60 cell divisions before it gets old.
- In some books 60-70 cell divisions are the average cell divisions performed before ageing of cell.

How to Prolong Lifespan?

- SIRT Genes (SIRT 1-6) produces SIRTUIN proteins in the human body.
- SIRTUIN proteins are the histone deacetylases.
- High levels of SIRTUIN proteins can show anti-ageing properties.

Role of SIRTUINS

- Anti-ageing
- Promote cell repair, therefore, being used in cancer treatment
- Used in the treatment of diabetes as it increases insulin sensitivity

How to Increase SIRTUINS Levels

- Calorie deficit
- Red wine consumption

Telegram - @nextpreladdernotes

Premature Ageing

00:37:38

Progeroid Syndrome

Werner Syndrome or Adult Progeria Syndrome	Hutchinson Gilford Syndrome or Laminopathy	Cockayne Syndrome
Occurs in adulthood	 Cell nucleus of a healthy individual	Cachexia occurs in patients
	 Cell nucleus which has a dramatically mutated in a progeria patient	
		
Later onset disease	Early Childhood Onset	-
DNA Helicase defect	LMNA (Lamin A) gene defect where the nucleus is not developed properly	ERCC gene defect

Medical Therapy for Hutchinson Gilford Syndrome:
Lonafarnib - A farnesyl transferase inhibitor is under trial. It is supposed to correct the nucleus.

Note

Werner Syndrome	Wermer Syndrome
Adult progeria syndrome under premature ageing.	MEN (Multiple Endocrine Neoplasia) I Syndrome

Q. Werner Syndrome is defect in?
a. DNA Helicase
b. NER genes
c. MMR genes
d. All of the above

Q. Hutchinson-Gilford Progeria syndrome is due to the mutation in?
a. Keratin A
b. Keratin B
c. Lamin A
d. Lamin B

Free Radical Injury

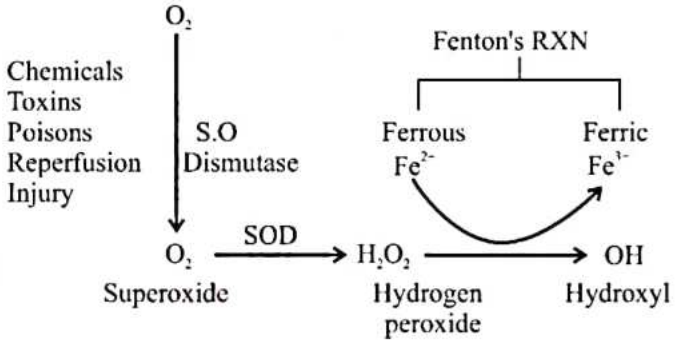
00:45:26

- Most common cause of ageing

Free Radical

- Also known as ROS (Reactive Oxygen Species).
- They are of three types:
  - Superoxide
  - Hydrogen peroxide
  - Hydroxyl (most potent free radical)
- All of them cause lipid peroxidation.
- Lipofuscin pigment is generated, a tell-tale sign of free radical injury.

Free Radical Formation

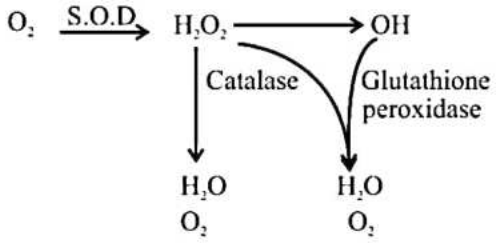


- **Oxygen → Superoxide** due to the environmental chemicals, toxins, poisons, and reperfusion injury.
- **Superoxide → Hydrogen Peroxide** by Superoxide Dismutase (SOD).
- **Hydrogen peroxide → Hydroxyl** (most potent) and the reaction is called Fenton's Reaction.
- In Fenton's Reaction the **ferrous ion → ferric ion.**

Free Radical Injury - Protection

- Diet should have antioxidants like vitamin A, C, and E.
- Transport proteins
  - Transferrin - Transports Fe
  - Ceruloplasmin - Transports Cu
- Protective Enzymes

Free radical injury – Protection?



Telegram - @nextprepladdernotes

- Superoxide Dismutase (SOD).
- Catalase acts on Hydrogen Peroxide → Water and oxygen.
- Glutathione peroxidase acts on both Hydrogen Peroxide and Hydroxyl → Water and oxygen.

Clinical Correlation

- Superoxide Dismutase (SOD) is of two types:
  - Cytoplasmic SOD (Cu-Zn SOD)
  - Mitochondrial SOD (Mn SOD)
- SOD 1 gene mutation can cause ALS (Amyotrophic Lateral Sclerosis - Microscope studies show Bunina Bodies).
- ALS is both upper and lower motor neuron disorder.
- Stephen Hawking had ALS.

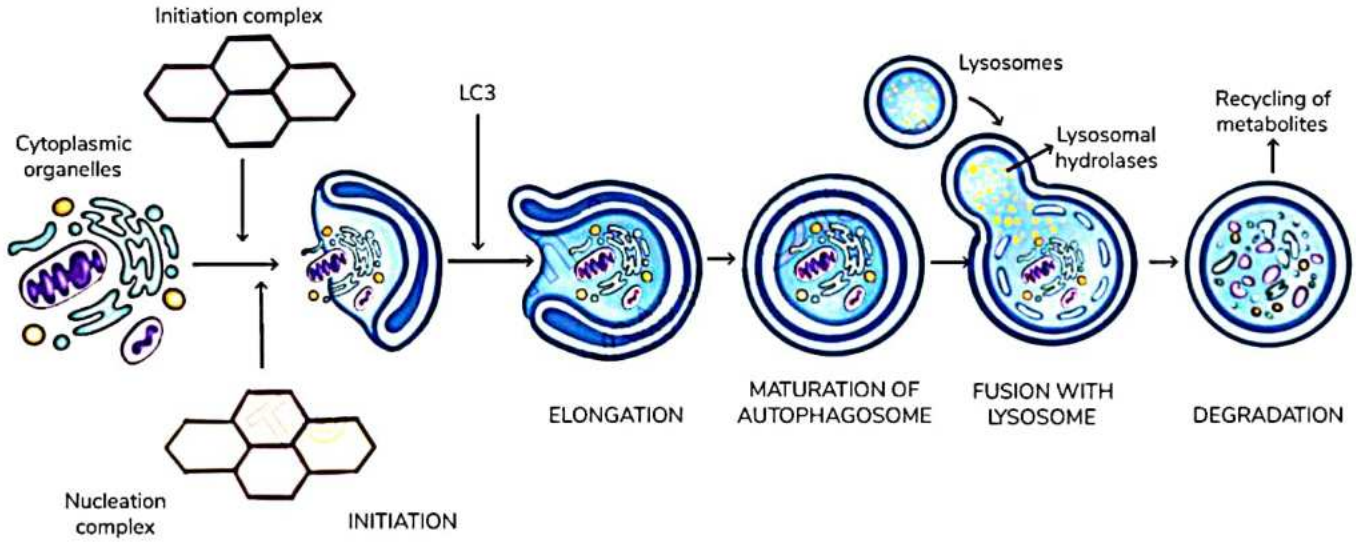
Ferroptosis 00:57:54

- Iron mediated cell death.
- Glutathione Peroxidase Type 4 is the master regulator.
- Mitochondria changes are very similar to necrosis:
  - Loss of cristae
  - Rupture of Membrane

Q. Which of the following pigments are involved in free radical injury?

a. Lipofuscin
b. Melanin
c. Bilirubin
d. Hematin

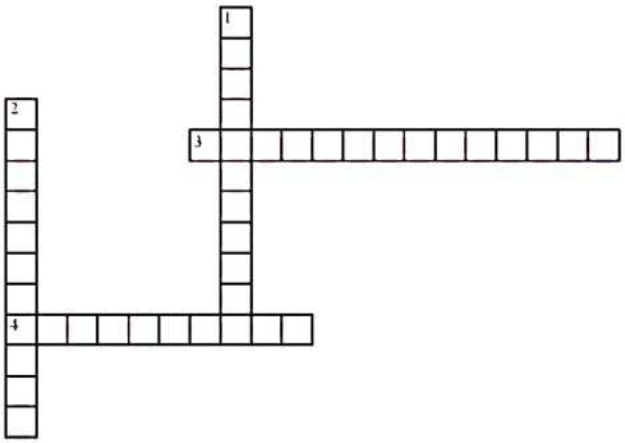
Image 4.1



Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Adult progeria syndrome under premature ageing
4. Reactivate telomerase

Down

1. Werner Syndrome is defect in
2. Drops down immediately

Telegram - @nextprepladdernotes

PREVIOUS YEAR QUESTIONS

Q. All are features of reversible cell injury EXCEPT? (AIIMS 2019)

A. Endoplasmic reticulum swelling
B. Dense deposition of mitochondria
C. Bleb formation
D. Detachment of ribosome

Q. A wedge shaped are in the adrenal gland is affected. On HPE nucleus is not seen but cellular outlines are intact. Which type of necrosis is being described? (JIPMER – Nov - 2018)

A. Coagulative
B. Liquefactive
C. Fibrinoid
D. Caseous

Q. BCL 2 protein is located in which of the following site? (JIPMER – May - 2018)

A. Cell membrane
B. Mitochondria
C. Nucleus
D. Cytosol

Q. APAF 1 is involved in the activation of which of the following caspases (AIIMS – June - 2020)

A. Caspase 8
B. Caspase 9
C. Caspase 3
D. Caspase 10

Q. Staining of lipids is best seen in which of the following conditions? (INICET Nov 2020)

A. Frozen section
B. Liquid paraffin
C. Formalin fixed
D. Karnovsky stain

Q. Dystrophic calcification seen in which of the following conditions? (AIIMS – May - 2019)

A. Myositis ossificans
B. Paget's disease
C. Metastasis
D. Sarcoidosis

Telegram - @nextpreladdernotes

5 ACUTE INFLAMMATION

Cardinal signs of Inflammation 00:00:37

Scientist Celsus gives 4 Cardinal signs of inflammation:

a) RUBOR = Redness
b) CALOR = Increase in temperature
c) DOLOR = Pain increased
d) TUMOR = Swelling

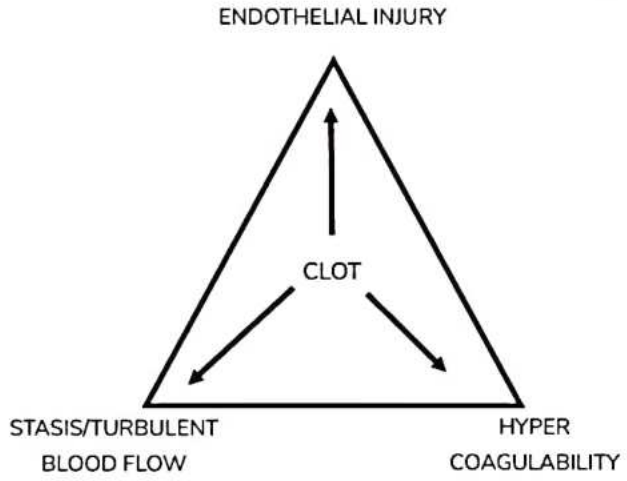
Fifth cardinal sign which was given by Sir Rudolf Virchow is:
(a) FUNCTIO LAESA = Loss of Function

Sir Rudolf Virchow

He is said to be the father of Modern Pathology. His contributions are as follows:

- Functio Laesa (5th Cardinal sign)
- Virchow Space (Brain)
- Virchow Node, (Left Supraclavicular Lymph node = Gastric Carcinoma)
- Virchow cell (Leprosy)
- Virchow Triad (Thrombosis)
  - When a combination of Endothelial Injury, Stasis/Turbulent Blood Flow and Hypercoagulability occurs, the patient gets a clot or thrombus.

Virchow Triad [Thrombosis]



Classification of Inflammation 00:06:44

1. Based on Fluid Type

a. **Serous Inflammation**: Watery
b. **Fibrinous inflammation**: Thready
   - In the case of Sero-Fibrinous Pericarditis (RHD), popularly known as Bread & Butter appearance (Watery and Thready fluid (Serous and Fibrinous inf) is visible).
c. **Purulent Inflammation**: Pus e.g., Abscess
d. **Catarrhal Inflammation**: Mucus & water e.g., Common cold

2. Based on Duration

a. Acute
   - Onset: Immediate
   - Duration: Short-lived
   - All the acute inflammation shows Neutrophils/Polymorphonuclear cells (PMC)
   - All of them show Neutrophils except for Typhoid (it shows Relative Lymphocytosis)

b. Chronic
   - Onset: Delayed
   - Duration: Long-lived
   - All the chronic inflammation shows Lymphocytes, macrophages and plasma cells
   - All of them show Lymphocytes, macrophages and plasma cells except for Chronic Pseudomonas (it shows Neutrophils)

Events of Acute Inflammation 00:13:34

Vascular events

The vessels which show this are arterioles, capillaries and venules.

I. Vasoconstriction
   - First event
   - Transient event (lasting for few seconds)
   - Reflex

ii. Vasodilation
   - Increased Redness (Rubor)
   - Increased Temperature (Calor)
   - Mediators of the event are Histamine & Serotonin

iii. Increased Vascular Permeability
   - Every blood vessel is lined by endothelial cells. They are positive for CD 34. The fluids (Plasma, plasma proteins, WBCs) exudate, which results in Swelling (Tumor)

iv. Stasis
   - When plasma comes out from blood vessels, the RBCs become concentrated. In other words, Viscosity is increased
   - This results in sluggish blood flow

Hallmark event of Acute Inflammation 00:20:45

Increased Vascular Permeability (most commonly seen in Post Capillary Venules)

Telegram - @nextprepladdernotes

Mechanisms:

i. Endothelial Gap Formation (Most common Mechanism): There is endothelial cell contraction and retraction. Immediate transient reaction . The mediators are Histamine and Serotonin.

ii. Direct Endothelial Injury: Delayed Prolonged Response . Second, it can be immediate and have a sustained response. Example: Delayed Sunburn (Delayed prolonged response) or Sepsis (Immediate, Sustained Response).

iii. Leukocyte Mediated Injury: WBCs damage the endothelial cells, and it occurs by releasing enzymes.

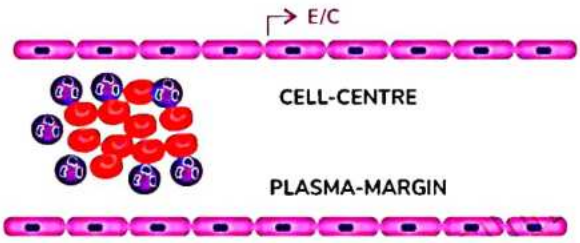
iv. Transcytosis: It goes through the cell by a tunnel formation of V-V Organelle (Vesiculo Vacuolar Organ). Mediators are Histamine & VEGF.

v. Angiogenesis: Immature new blood vessels are formed that are known as Leaky Channels. Mediators are Histamine & VEGF.

Cellular events

Diagram 00:33:50

7 events that can be remembered in sequence by the mnemonic "MRATCOP".



Normal Laminar Flow (Cell in center & Plasma at Margin) in the blood vessel. During inflammation, the Plasma is out, and WBCs occupy the margin space. The Cellular events start with:

i. **Margination:** During this process, cells are going to the margin to occupy the margin space.

ii. **Rolling & weak adhesion**: This is carried out by molecules called Selectins, also known as CD 62. There are three types of Selectins:
- P Selectin (Platelet, Endothelial cell): It binds to Sialyl Lewis X. It is present in Weibel Palade Bodies and vWf. The redistribution of P Selectin is done due to Histamine & Thrombin.
- E Selectin (Endothelial Cell): It also binds to Sialyl Lewis X.
- L Selectin (Leukocytes): It binds to Glycam/Madcam/CD34.

iii. **Adhesion (Strong):** This is caused by integrins in Leukocytes. Integrins are of 2 types:
- β_1 or VLA. It binds to VCAM.
- Beta2 or MAC/LFA or CD11/CD18 binds to ICAM

Note:

Q) ICAM is used which other organisms for binding?

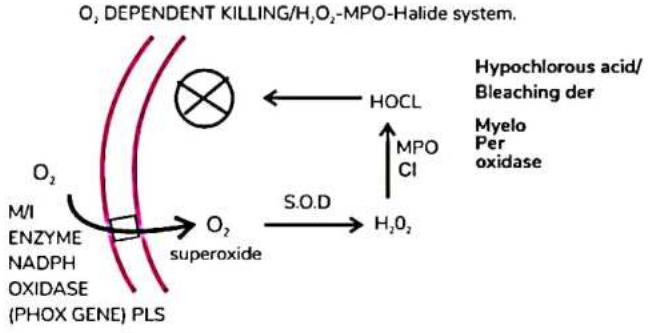
A) Rhinovirus anf Plasmodium falciparum

iv. **Transmigration/Diapedesis** (Most commonly seen in Post Capillary Venules): The cell wants to come out by breaking the basement membrane of the cell by means of MMP (Matrix Metallo Proteinase, (Zn)). PECAM/CD31 mediates this process.

v. **Chemotaxis**: It is the unidirectional targeted movement of the WBCs towards the bacteria. The chemotactic agents for chemotaxis are:
- Exogenous: Bacterial Products
- Endogenous: L - LTB^4
  I - Il^8
  C - C_{5a}

vi. **Opsonization** (Covering with Opsonin): It is done to make bacteria tastier. It is not a mandatory process. The added or covering substance is Opsonin. Opsonin is divided into 3 Categories:
- IgG (Most important opsonin)
- Complement proteins (C_{1b}, C_{4b}, C_{5b}): The most important complement opsonin C_{3b}.
- Plasma Protein (E.g., Fibrinogen)

vii. **Phagocytosis**: Given by scientist Elie Metchnikoff. Three steps are included in this:
- Recognition and Attachment: Macrophage/Neutrophil can have a receptor for IgG or Mannose or Scavenger.
- Engulfment: Formation of a structure called Pseudopodia from which the bacteria will be coming inside with a covering membrane called Phagosome. Phagosome will fuse with Lysosome, resulting in Phagolysosome.
- Killing: There are two types of killing:
  → Oxygen Dependent killing (More Potent): It is also known as H_2O_2 - MPO - Halide system. NADPH oxidase (Made by Phox Gene) is the most important enzyme for phagocytosis.



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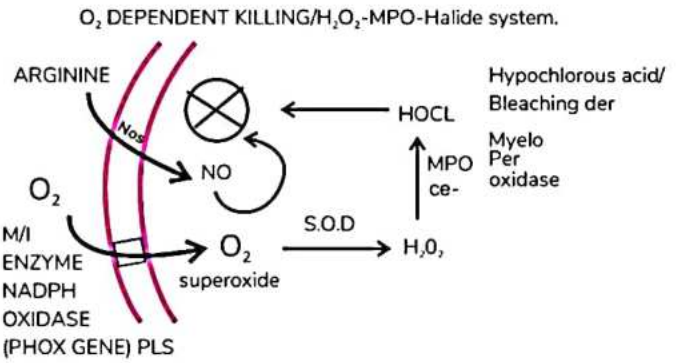
Another path is by mediator Arginine with Nitric Oxide Synthase, which results in Nitric Oxide (NO). Then, NO joins superoxide to kill the bacteria.



→ Oxygen Independent Killing: This consists of:

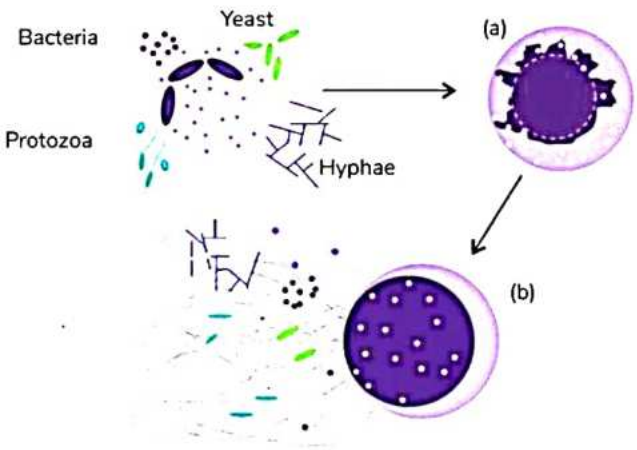
a. Lysozyme
b. Lactoferrin
c. Defensin
d. Major Basic Protein (MBP- Produced by Eosinophil to fight Parasitic infections)
e. Cathelicidins - In Vitamin D_3 deficiency, Cathelicidins are absent, and the risk of Tuberculosis increases.

Miscellaneous definitions 01:13:15

Frustrated Phagocytosis

- Macrophages encounter immune complexes, but macrophages cannot engulf these because they are adherent to the basement membrane. As Macrophage cannot engulf, it tries to kill immune complexes by releasing lots of lysosomal enzymes.

NETS (Neutrophil Extracellular Traps)/Beneficial Suicide



- It is a type of killing. This is seen in severe infections such as Sepsis. It occurs when Phagocytosis alone is not efficient. The nuclear chromatin is sent out of the cell with the nucleus, forming a trap for killing the bacteria. As the cell cannot survive without nucleus, in the end, the cell dies.
- The amino acid which helps in NETS is Arginine.
- Antinuclear antibodies are formed. This may result in SLE.
- NETS are extracellular, and Phagocytosis is intercellular.

Emperipolesis

It is a cell within a cell phenomenon. There is no killing. The big cell is a macrophage cell. This cell within the cell phenomenon is seen in the condition known as MARC:

M - MDS/MPN
A – AIH
R - Rosai Dorfman Disease
C - CLL

Entosis (Non-macrophage cell):

- It is also a cell within the cell phenomenon and there is no killing. The big cell is not a macrophage cell.

Q. Basement Membrane degradation is caused by:
(a) Matrix Metallo protein
(b) Oxidases
(c) Elastases
(d) Hydroxylase

Q. Main feature of Chemotaxis is:
(a) Random movement of Neutrophils
(b) Adhesiveness to blood vessels
(c) Phagocytosis
(d) Unidirectional locomotion of Neutrophils

Inflammation - Clinical Correlation 01:26:51

a) LAD (Leukocyte Adhesion Defect): All of them are autosomal recessive defects. Repeated infection are noted in all of them.

i. **LAD 1-** Integrin (β_2 integrin defect). They show delayed separation of umbilical cord stumps.
ii. **LAD 2-** Sialyl Lewis X defect. The patient's blood group is the Bombay Blood group.
iii. **LAD 3-** FermT3 Gene defect. The patient shows a lot of bleeding.

b) Bruton's Hypogammaglobulinemia

It is most commonly seen in boys. It is a B cell defect. The genetic defect is in BTK Gene. Moreover, there will be defective Opsonization.

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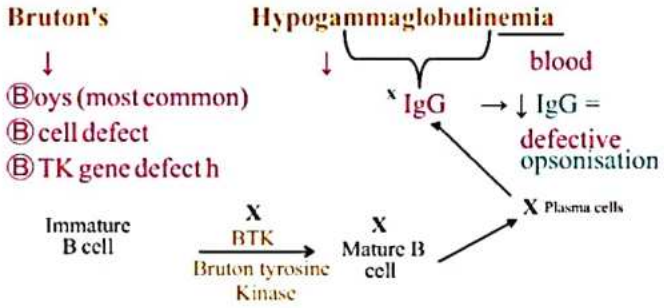
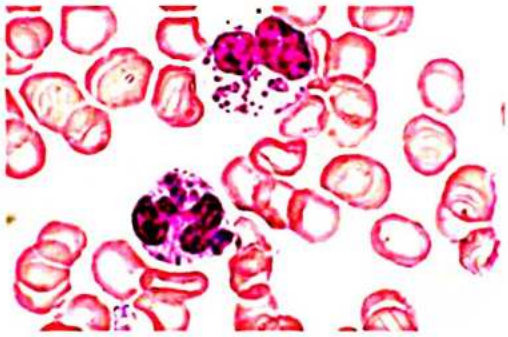


- Immature B cells (by BTK Gene) — Mature B cells — Plasma cells — IgG, this process stops because of defective BTK genes.

c) Chediak Higashi Syndrome

LYST gene defect (Lysosomal trafficking gene defect)
C - CNS/neurological abnormalities
HE - Hemorrhage (Platelets are affected)
DI - Decreased Immunity
A - Albinism (oculocutaneous albinism)
K – Coarse cytoplasmic granules are noted



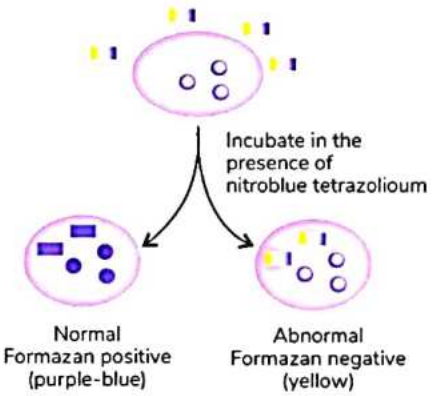
d) Chronic Granulomatous Disease

Defect: NADPH oxidase deficiency. It is a Phox Gene Defect.
There are two defects in Phox Gene:

- XLR: gp91 PHOX
- AR: gp41 PHOX, gp 61 PHOX.

The patient suffers a lot of infections. Diagnosis includes:

- Screening = NBT (Nitroblue Tetrazolium)

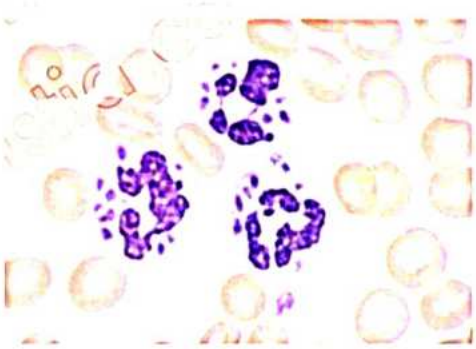


- Confirmatory/Diagnostic = DHR (Dihydrorhodamine done by flow cytometry)

Q. Defect in LAM is associated with?
a. Delayed closure of umbilical cord
b. Defect in Chemotaxis
c. Defect in Opsonization
d. Neutropenia

Q. A 19-month-old child presents with recurrent infections. Advanced studies show a defect in neutrophil adhesion. Further, the mother gives a history of delayed umbilical cord stump shedding after birth. The child's defect is most commonly attached to?
a. LAD 1
b. LAD 2
c. BRUTON'S Hypogammaglobulinemia
d. SCID

Q. 10 year old child present with repeated bleeding episodes, delayed milestones as well as hypopigmentation. Peripheral smear shows the following findings. What is your likely diagnosis?



a. Defect in selections
b. defect in phagocytosis
c. Defect in opsonization
d. Defect in integrins

Q. Peripheral smears show the following findings. What is your likely diagnosis?
a. Defect in Selectin
b. Defect in Phagocytosis
c. Defect in Opsonization
d. Defect in integrins

Q. DHR is a test for?
a. Rolling
b. Chemotaxis
c. Opsonization
d. Phagocytosis

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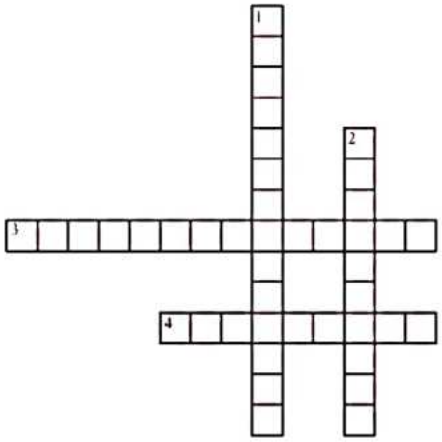
CROSS WORD PUZZLES

Crossword Puzzle



Across

3. He is said to be the father of modern Pathology. His contributions are as follows:
4. This is caused by integrins in Leukocytes. Integrins

Down

1. Vasoconstriction (First event, Transient event lasting for few seconds, Reflex)
2. Leukocytes Mediated Injury: It is done by WBCs, and Leukocytes by releasing Enzymes.

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6 INFLAMMATION-MEDIATORS

Classification

Classified into two categories:

1. Cell Derived

- Preformed
  - Histamine
  - Serotonin
  - Lysosomal enzymes
- Newly Synthesized
  - Nitric oxide
  - Platelets activating factors (PAF)
  - Chemokines
  - Cytokines
  - AA Metabolites

2. Plasma Derived

- Source will be Liver.
- They will have all the "K" sounding items, such as:
  - Complement system
  - Coagulation/clotting
  - Kinin pathway

Cell - Derived:

Preformed Mediators 00:02:47

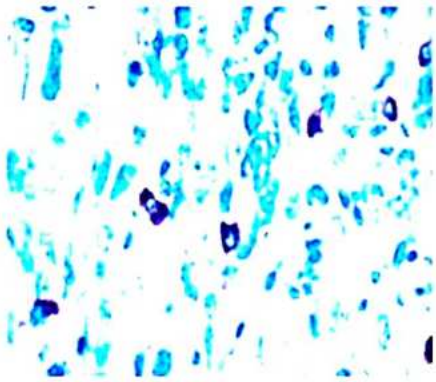
(Always present in our body)

- Given by Thomas Lewis

Histamine And Serotonin

Basis	Histamine	Serotonin (5HT)
Source	Mast cells (richest source) Basophils Platelets	Enterochromaffin cells (EC cells) (richest source) Platelets
Precursor	Histidine	Tryptophan
Function	The functions of Histamine And Serotonin are the same. They are: • Helps in vasodilation • Increased vascular permeability • Bronchoconstriction	

- Toluidine Blue is toluidine blue is a metachromatic stain for mast cells.
- It has a different colour (Blue) ultimately gives a different colour (Purple).



Lysosomal Enzymes

- These enzymes come from granules in neutrophils.
- There are three granules.
  - Primary/ Azurophilic granules
    - → Acid Hydrolase
    - → Bacteria killing Myeloperoxidase (MPO)
    - → Collagenase, Cathepsin
    - → Defensin
    - → Elastase
  - Secondary/ Specific granules
    - → Specific Types of collagenase
    - → Lactoferrin
  - Tertiary/ C-particle
    - → Gelatinase
- Lysozyme is released from primary and secondary granules.

Newly Synthesized Mediators 00:14:40

- Nitric oxide (NO)
- Platelet activating factors (PAF)
- Chemokines
- Cytokines
- Arachidonic acid metabolites (AA)

Nitric Oxide

Formation:

L-Arginine $\xrightarrow{\text{Nitric Oxide Synthases (NOS)}}$ nitric oxide and L-citrulline.

Three types of NOS:-

- eNOS - endothelial NOS
- nNOS - Neuronal NOS
- iNOS - Inducible NOS (inflammation)

Functions of Nitric Oxide (NO)

- Vasodilation
- Microbial killing
- Anti aggregation of platelets

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Platelet-activating factors (PAF) 00:18:13

- It is thousand times more potent than Histamine.
- Blood vessels will undergo vasoconstriction.
- Platelet aggregation.
- Bronchoconstriction
- At very low concentration, PAF can also cause vasodilation.

Chemokines

- These are chemo-attractants.
- Some of the basic terminology:
- C = Cysteine
- X = Any amino acid

Types of Chemokines

- CX3C
- CXC - α
- CC - β
- CC - γ

Refer Table 6.1

CXCR4
CCR5
↓
These are chemokine Receptors.
They are co-receptors for HIV.

Cytokines

Three broad categories of Cytokines.

1. IL (Interleukins)
2. TNF α
3. Interferons

TNF α

- Decrease in appetite
- Cancer cachexia
- Shock

To clear the confusion, differentiate on this basis:

TNF α = No appetite
TGF α = Gastric disease (Menetrier's disease)
TGF β = Most fibrogenic cytokine

Interleukins (IL)

Question	Answer
Fever	IL 1, 6, TNF a
TNF alpha	Fever, decrease in appetite
Pro & anti inflammatory	IL 4,6
Anti inflammatory	IL 10, TGF
T cell maturation	IL 7
Eosinophils activation	IL 5
Neutrophil recruitment	IL 8
Regulates APR (Acute Phase Reactants)	IL 6

Positive APR (Things that increases by more than 25% of normal)	Negative APR (Things that decreases by 25% of normal) (TTTAAN)
CRP	Transferrin
Ferritin	Transcortin
Hepcidin	Transthyretin
Haptoglobin	Albumin
Fibrinogen	Antithrombin
Procalcitonin	
SAA (Serum amyloid associate)	

CRP - Carbohydrate/ capsular polysaccharide of pneumococcus.

1. Interferons

	IFNα	IFNβ	IFNγ
Sources	Leukocyte (Macrophages)	Fibroblast	T_{h1}
Function	Antiviral properties	Treatment of Brain disease such as: Multiple sclerosis	1. Granuloma formation 2. Treatment of Chronic granulomatous disease:

AA Metabolites

- AA - Arachidonic Acid
- It is 20 carbon compounds.

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- It comes from Phospholipids.

Phospholipids
↓ Phospholipase A_2 & C
Arachidonic acid
↓
4 Pathways

COX **LOX** **Cyt P450 epoxygenase** **ISO Eicosanoid pathway**

Phospholipids —(Phospholipase A2 and C)→ AA —(COX 1, COX 2)→ PG

↑ Inhibits Steroids — ↑ Inhibits NSAIDS (Eg Aspirin)

Side effect of aspirin is Peptic ulcer disease.
Therefore, selective COX2 inhibitors are preferred.
Eg: Celecoxib

1. COX

- Cyclo-oxygenase
  - COX 1 = Gastroprotective enzyme (good)
  - COX 2 = Selective COX2 inhibitors eg. Celecoxibs.
  - NSAIDS (Aspirin) can shut off both COX1 & COX2.
- Prostaglandin (PGG2) will form due to the COX pathway.
- PGG2 will give rise to PGH2.
- PGH2 will form PGI2 (Prostacyclin) and TXA2 (Thromboxane A2)
- Now, PGH2 will give rise to PGD2, E2, F2α
- PGD2 causes Vasodilation.
- E2 causes pain.
- F2 α is carboprost.

Q. Which of these will aggregate the platelets?
Ans. Thromboxane A2 (TXA2)

Q. Who will inhibit platelet aggregation?
Ans. PGI2 (Prostacyclin)
Causes vasodilatation

2. LOX

00:55:15

- Lypo-oxygenase
  - 12 LOX
  - 5 LOX

5 LOX

- 5 LOX produces LTA4 (Leukotrienes)
- LTA4 further will form LTB4 (Chemotaxis), LTC4, LTD4, & LTE4.
- LTC4, D4, E4 also known as SRS-A (slow reacting substance - anaphylaxis)
- LTC4, D4, E4 causes bronchoconstriction (asthma)
- LT are more effective in causing Bronchoconstriction than Histamine.

12 LOX

- 12 LOX forms Lipoxin XA4 & Lipoxin Xb4
- LXA4 & LXB4 are anti-inflammatory.

$$AA \xrightarrow[12\ LOX]{5\ LOX} LT\ C4, D4, E4$$

Q. Function of Zileuton, why is it not good to use?
Ans. It inhibits 5 LOX and stops all its processes. So, it is better to use drugs like LTRc antagonists known as Montelukast zafirlukast.

1. Cytochrome P450 Epoxygenase

- It forms EET & HETE
- EET is anti HTN.
- HETE causes Renal vasoconstriction → hypertension (HTN)

2. Iso Eicosanoid

- Non enzymatic pathway.
- It results in formations of Isoprostane.
- Isoprostane causes vasoconstriction.

Plasma Derived Mediators

Refer Image 6.1

Complement System

- They have pathways - Classical, MBL (Mannose Binding Lectin) & Alternate pathway.
- Classical pathway is activated by Immune complex (antigen-antibodies reaction) (Igm, G)
- Alternate pathway is activated by IgA
- MBL is activated by Microbial organisms
- Most critical step = C3

Classical Pathway 01:10:05

- It activates molecule C1q.
- C1q will activate C4b C2a.
- C4bC2a together is called C3 convertase.
- It will then activate C3 - C3a3b.
- C4b 2a3b together = C5 convertase. They will activate C5 - C5a & C5b.
- C5 will combine together with C5b6789 and will be referred to as a membrane attack complex (MAC).
- It will break the bacteria and will cause bacterial lysis.

Alternate Pathway

- It will start from C3.
- C3 will be broken into C3a and C3b.

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- C3b will combine with factor B, D & properdin, will form C3bBb.
- C3bBb when attack C3 convertase.
- They will form C3bBb3b.
- C3bBb3b will again attack the C5 convertase.
- Will again form a membrane attack complex.

MBL (Mannose Binding Lectin)
- It will start with C2 & C4.
- And will directly target the C3 convertase.

Q. What is the composition of the membrane attack complex?
Ans. C5b to C9

Q. Which molecules are responsible for Chemoattraction?
Ans. C5a

Q. Which molecules are responsible for Opsonins?
Ans. C3b, C4b, C5b all the b molecules are responsible for Opsonins.

Q. Which molecules are responsible for Anaphylaxis?
Ans. C3a, C5a all the molecules are responsible for Anaphylaxis. C5a is the most potent attractant.

Deficiency	Disease
Most common complement deficiency	C2
C1,2,4 deficiency	SLE
MAC deficiency (C5b – C9)	Capsulated organism Neisseria infection
C1 inhibitor deficiency ↓ Controls activation of C1	HAE Hereditary angioEdema

Edema is around the lips and periorbital. If it happens in the neck, i.e, Laryngeal Edema, this means a person has entered into respiratory distress. It becomes a medical emergency.

Controllers of complement system.

Deficiency	Disease
CD55 (break C3 conv)	Paroxysmal nocturnal hemoglobinuria
CD59 (break C5 conv)	
Factor H	Atypical HUS (Hemolytic uremic syndrome)
Factor I	
CD46	
Factor H defect in ophthalmology	ARMD (Age related macular degeneration)

Q. What is MPGN II
Ans. It is Complementopathy, which means there is a problem in complement system. It is a defect in the kidney.

Clotting System
- There are total Factor I to XIII
- There does not exist any Factor VI.
- Factor IV is Calcium.

Q. What is the definition of blood clot?
Ans. It is the last product form, i.e, Factor I - Fibrin from Factor Ia.

- Factor XIII is known as the Fibrin stabalising factor/ Laki Lorand Factor.
- Factor XIII makes your blood clot very strong.
- Follows two pathways.
- Intrinsic and Extrinsic pathway.
- Both will merge at factor X, and will be known as a common pathway.

Intrinsic pathway

T = XII gets activated
↓
E = XI will get activated
↓
N = then, IX gets activated.
↓
Factor VIII Activated
↓
Now, Factor X will get activated.

Extrinsic pathway
- Factor VII will get activated first.
- Then, Factor III will get activated.

↓
Factor X (7+3) will get activated.

Common pathway [Intrinsic + Extrinsic]
- Factor X in presence of Factor V will activate Factor II.
- Factor II (prothrombin) will form IIa (thrombin).
- Then, Factor Ia (fibrinogen) will activate Factor I (fibrin)
- Fibrin is the clotting.

Tests For Clotting Factor:
- Two pathways
- Intrinsic pathway and extrinsic pathway.

↓ APTT test ↓ PT test

Telegram - @nextpreladdernotes

Kinin System

When Factor XII gets activated, there is a molecule called Prekallikrein which gets converted into Kallikrein. And this Kallikrein converts Kiniogen into Bradykinin.

- Factor XII activates this Kinin system.
- Bradykinin is famous for two things:
  - Vasodilation
  - Pain

Pain cause by:

- PGE2
- Bradykinin

MCQS

Q. Gelatinase is released by?

a. Primary granules
b. Specific granules
c. C particle
d. Azurophilic granules

C particles (Tertiary granules)

Q. Most FIBROGENIC mediator:

a. TNF alpha
b. TGF beta
c. Cytokine
d. PG

TGF beta.
[TNF alpha - (No appetite)]

Q. C-C includes which of the following chemokines?

a. IL-8
b. Eotaxin
c. Fractalkine
d. Lymphotactin

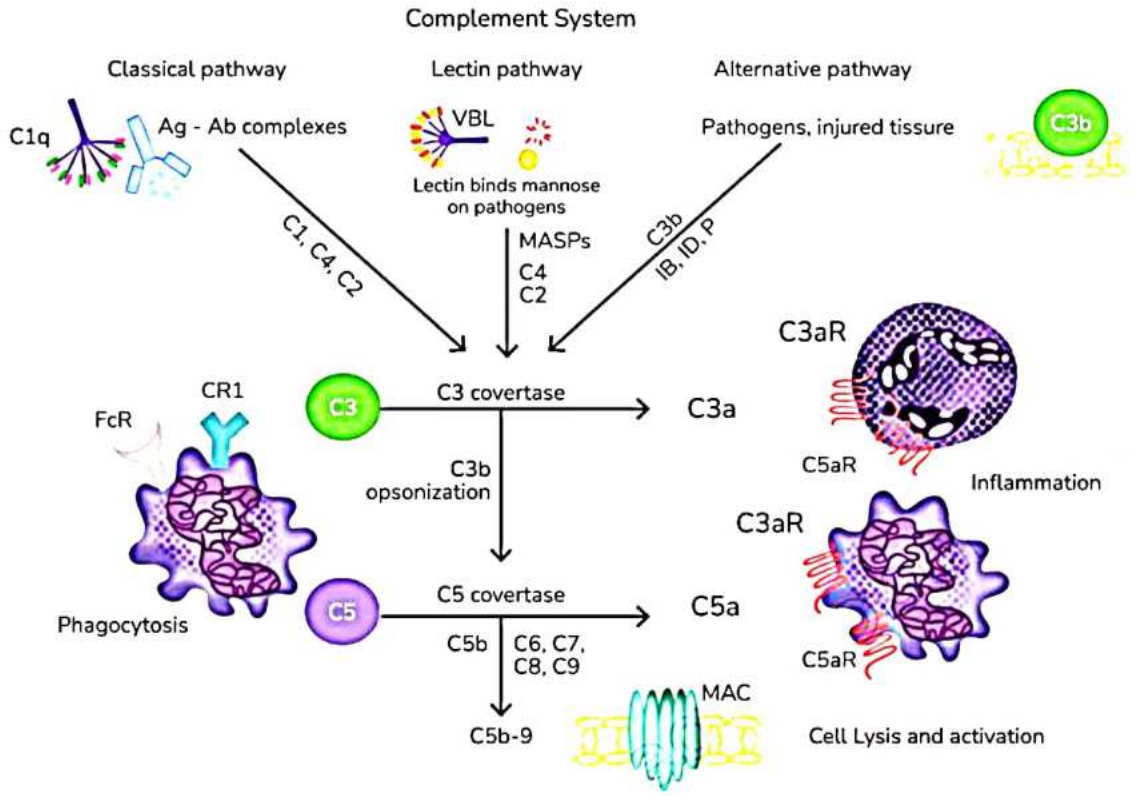
Eotaxin - it attracts Eosinophil.

Table 6.1

Basis	CX3C	CXC (a)	CC (b)	C (g)
Examples	Fractalkine	Interleukin 8 (IL8)	Eotaxin	Lymphotactin
Attracts	T-cells	Neutrophils	Eosino	Lymphocytes
	Monocytes		Rantes (T-cells)	
			MCP	
			Macrophages	

Telegram - @nextpreladdernotes

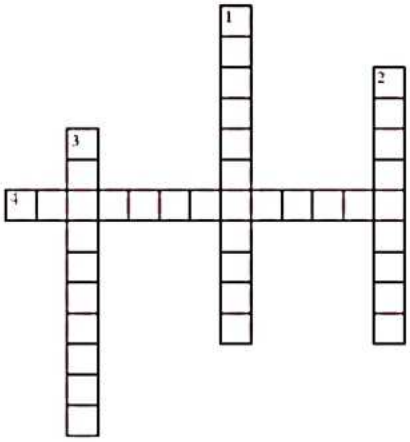
Image 6.1



Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

4. To break Phospholipids, you need Phospholipase A2 and C.

Down

1. L-Arginine is metabolized by nitric oxide synthases (NOS) to nitric oxide and L-citrulline.
2. These enzymes come from granules in neutrophils.
3. They have pathways - Classical, MBL (Mannose Binding Lectin) & Alternate pathway.

Telegram - @nextpreladdernotes

7 CHRONIC INFLAMMATION GRANULOMA AND WOUND HEALING

Chronic inflammation 00:00:25

Types of cells

- Lymphocytes
- Macrophages (MΦ)
- Plasma cells

Note - The most important of these cells are Macrophages.

Macrophages 00:01:09

Types -

- M1
  - Classically activated Macrophages
  - Activation occurs by Interferon-gamma (IFN-γ)
  - Releases Interleukin-2 (IL-2)
  - Proinflammatory kind of Macrophage
- M2
  - Alternatively activated Macrophages
  - Activation occurs from Interleukin 4 and 5
  - Releases Interleukin 10 and Transforming Growth Factor-Beta (TGF-β)
  - Anti-inflammatory Macrophages

Names of macrophages in different organs

Organ	Name
Brain	Microglia
Lungs	Dust cells / Alveolar Macrophages
Liver	Kupffer cells
Kidney	Mesangial cell
Spleen	Littoral cells
Placenta	Hoffbeaur cells
Bone	Osteoclast
Synovium	Type A Synoviocyte

Note -

- Histiocyte can be used for Macrophages in all the organs as a general term.
- Macrophages in the tissue can also be called histiocytes.

Granulomatous Inflammation

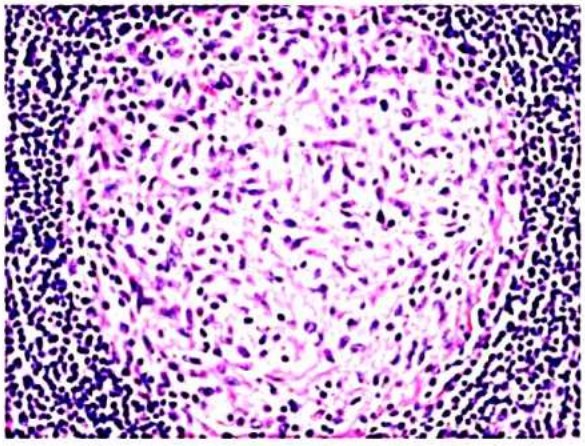
- T_{H1} releases Interferon-gamma (IFN-γ), which makes granuloma

Components of Granuloma

- The center will have something pinkish called Caseous necrosis.
  - It may or may not be present in a granuloma
- The cells surrounding caseous necrosis cells have different nucleus
  - Cells with a round nucleus - Macrophages
  - Cells with a slipper-shaped nucleus - Modified macrophages or Epithelioid cell (long cells)
- Giant cells with lots of nuclei are referred to as MNGC or multinucleated giant cells.
  - It is a fusion of MΦ.
- Dark blue collar around granuloma - Lymphocytic collar
  - T_{H1} lymphocytes are present in this lymphocytic collar.

Q. What is the most important cell of chronic inflammation?
Ans. Macrophages

Q. What is the most important cell of granuloma?
Ans. Modified macrophage or epithelioid cell.



Q. Which lymphocyte is present in the lymphocyte collar?
Ans. T_{H1} lymphocytes → releases IFN - g

Q. Which is the mediator responsible for granuloma formation?
Ans. Interferon-gamma (IFN-γ)

Q. Is there any condition where the lymphocytic collar is not seen?
Ans. Naked granuloma (a classical feature of Sarcoidosis)

Telegram - @nextpreladdernotes

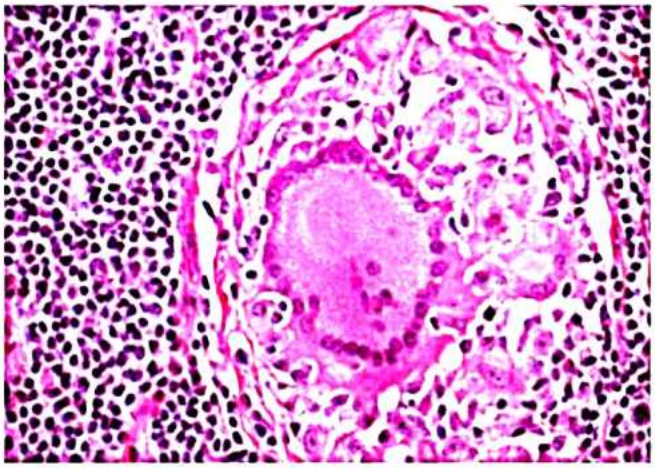
Types of Giant cells 00:15:25

Physiological giant cell

a. Osteoclasts (Present in Bone)
b. Megakaryocyte (Present in Bone marrow) - forms platelets
c. Syncytiotrophoblasts (Present in the placenta)

Pathological giant cell

1. Langhans giant cells

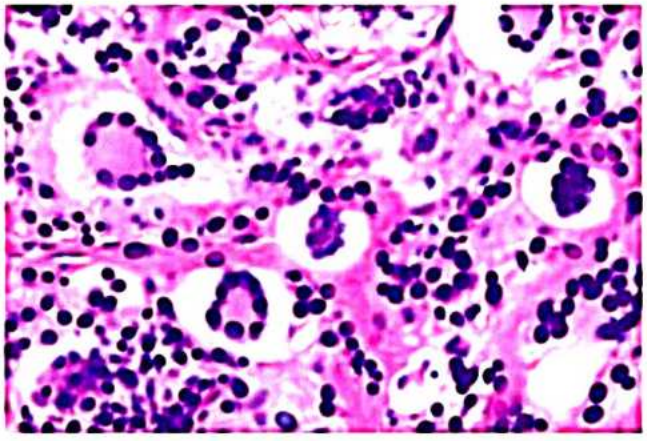


- HorseShoe arrangement of Nuclei
- Seen in tuberculosis

Note -

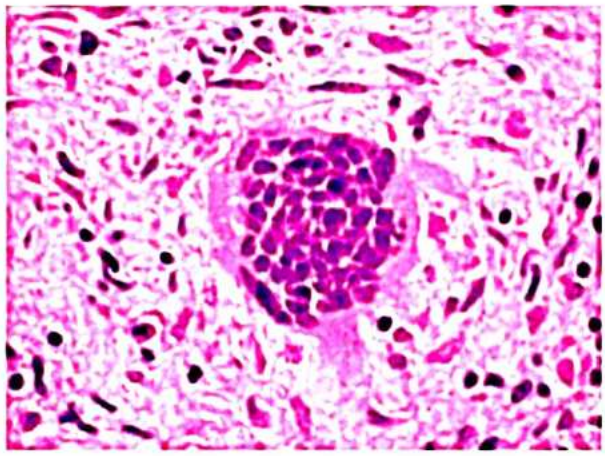
- Langans GC is present in TB and is different from Langerhans, Which is an antigen presenting cell in the skin

2. Touton giant cells



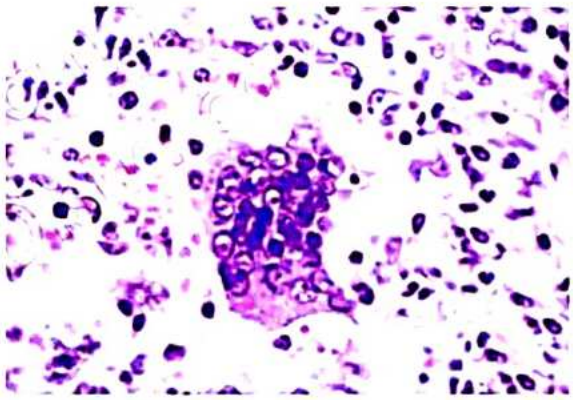
- Wreath-like arrangement of Nuclei
- Vacuolated cytoplasm containing fat
- Seen in Xanthoma or lipid disorder
- Oil Red O positive

3. Foreign body giant cell



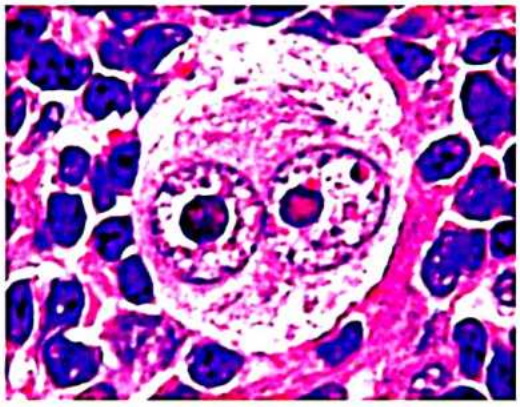
- Haphazard arrangement of nuclei
- Seen in Suture, Talcum powder
- Foreign body is seen on a polarising microscope.

4. Warthin Finkeldey giant cell



- Intranuclear and Intracytoplasmic inclusions are seen
- Seen in measles virus

5. Reed Sternberg cell (RS)



Telegram - @nextprepladdernotes

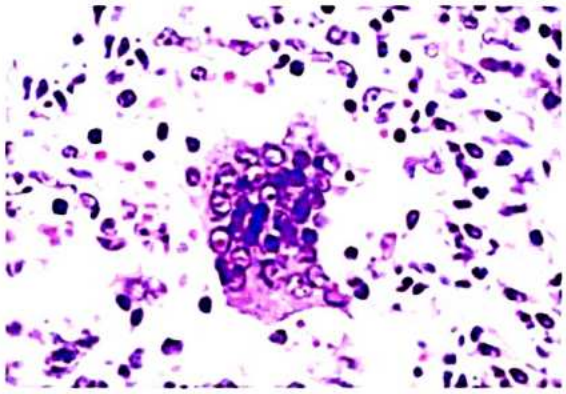
- Found in Hodgkin's lymphoma
- Two mirror image nuclei which have prominent pink inclusion (Nucleoli)
- Also known as Owl eye appearance (colour of eye - Pink)

Note -

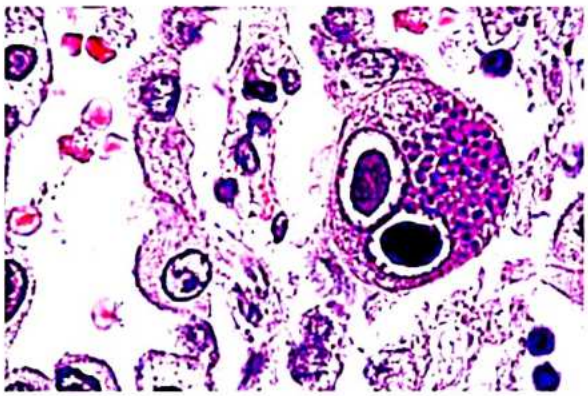
- The colour of the eye in the RS cell is pink, and in CMV (Cytomegalovirus) the eye is blue.

Intranuclear and Intracytoplasmic

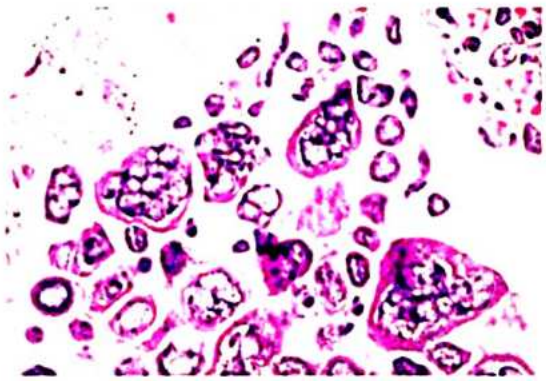
- Measles



- CMV



6. Herpes Simplex Virus (HSV)

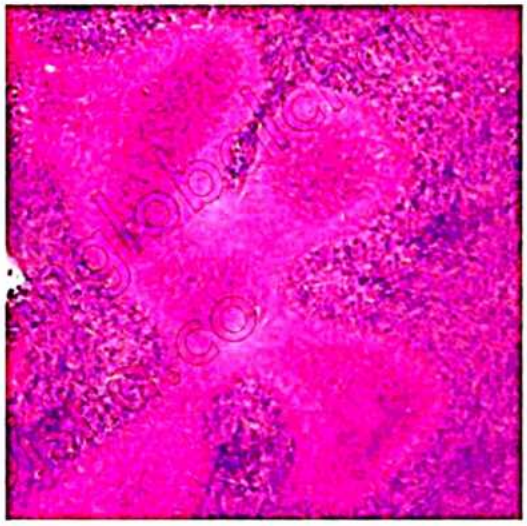


- Three M technique of HSV giant cell
  - → Multinucleated
  - → Moulding of nuclei
  - → Margination of the nuclear chromatin

Causes of granulomas 00:31:37

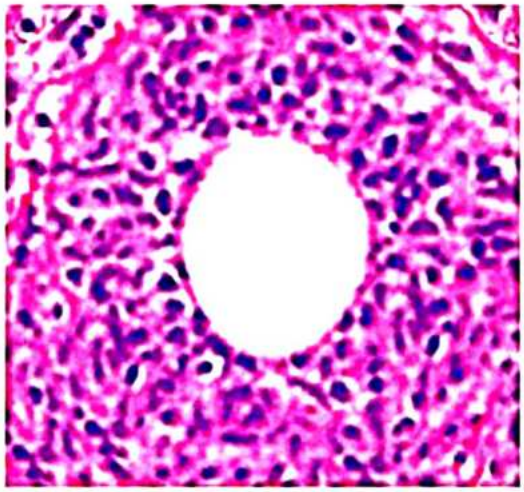
Associated with infections

- TB
  - Contains caseating and non-caseating granuloma
- Leprosy
  - Tuberculoid leprosy contains granuloma
- Syphilis
  - Formation of gummas containing granuloma
  - Plasma cells present in granuloma
- Stellate Granuloma



  - Seen in
    - → Cat scratch disease - caused by Bartonella henselae
    - → LGV lymphogranuloma venereum - caused by Chlamydia trachomatis

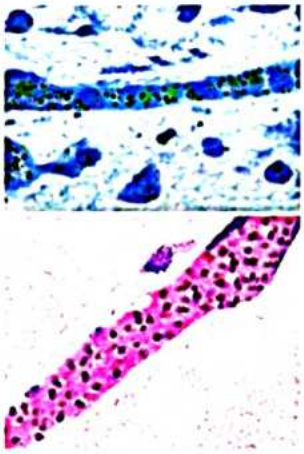
- Doughnut granuloma / Fibrin ring granuloma



Telegram - @nextpreladdernotes

- Seen in Q fever - caused by Coxiella
- Seen with Allopurinol

- Durck granuloma



- Seen in Cerebral malaria caused by Plasmodium falciparum / PF → by field stain
- Parallel lines containing PF trophozoites → by H & E stain

Non-Infectious

1. Sarcoidosis
2. Vasculitis
3. Crohn's disease

- Sarcoidosis
  - Shows naked granuloma (lymphocytic collar absent)

Q. Sarcoidosis will show caseating or non-caseating granuloma?

Ans. Both, and the most common variety is non-caseating granuloma

Regeneration and Repair

Regeneration

- Eg. Liver transplant
- Lost cells are replaced by normal cells of the organ

Repair

- Injury or lost cells are replaced by scar

Wound Healing

Types

- Primary intention
  - Eg - Surgical incision
    → Clean, regular margins
- Secondary intention
  - Eg - Road traffic accident
    → Dirty, irregular margins
  - More scar formation

Q. Which cell is responsible for wound contraction?

Ans. Wound contraction is mediated by a cell called Myofibroblast (Myo - contraction + Fibroblast - lays down collagen)

Types of cell

- Permanent cell
  - Do not divide
  - Eg - Neurons, cardiac and skeletal muscles
  - These cells go through hypertrophy
- Stable cell
  - Divide when required
  - Eg - Liver, PCT, DCT, endothelial cells and osteoblasts
- Labile cell
  - Continuously divides
  - Eg - bone marrow, skin epithelium, and GIT

Events of wound healing

00:47:46

- Learn the topic in terms of days - 0, 1, 2, 3, 5, 21 days (3weeks)

Day	Event
Day 0	• Platelets and Fibrin forms Blood clot
Day 1	• Blood clot remains • Neutrophils (acute inflammation)
Day 2	• Blood clot remains • Neutrophils (acute inflammation) • Thin epithelial layer forms
Day 3	• Chronic inflammation starts • Macrophages will come • Granulation tissue begins (blood vessel formation starts) • Beginning of collagen (Type III) formation ○ At the margins
Day 5	• Maximum granulation tissue (Maximum neovascularization) • Collagen bridging starts
Day 21 (Week 3)	• Maximum collagen (Type I)

Granulation Tissue

- Hallmark of repair
- Goal is the scar formation
- Made up of
  - Proliferating blood vessels
    → Makes it look extremely red in colour
  - Fibroblasts
  - Inflammatory cells

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Note -

- Initially, the collagen formed was Type III, and then at the end, it becomes Type I.
- Collagen Type I is the strongest.
- In the end, the Collagen I/Collagen III ratio = 4/1. {PYQ}

Factors promoting wound healing

- Vitamin C
- Zinc
  - Needed by MMPs (Matrix Metallo Proteinase)
  - Needed for remodeling (MMPs will break the scar, and TIMP will make the tissue scar)

Factors delaying wound healing

- Vitamin C deficiency
- Zinc deficiency
- Malnutrition
- Diabetes Mellitus
- Steroids
- Infections

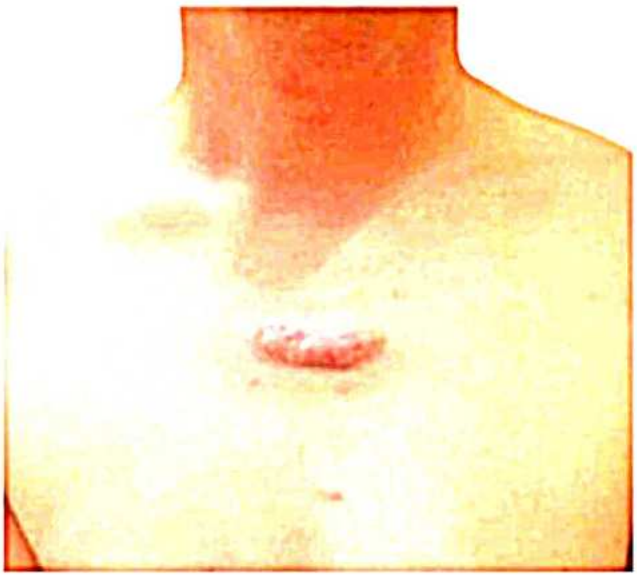
Note -

- Most common cause of delayed wound healing is infection

Excessive wound healing 01:01:53

1. **Too much granulation tissue formation**
   - Red colour tissue
   - Also known as Proud flesh (PYQ)
   - Treatment by cautery
2. **Too much scar formation**
   - Either keloid or hypertrophic scar

- Keloid

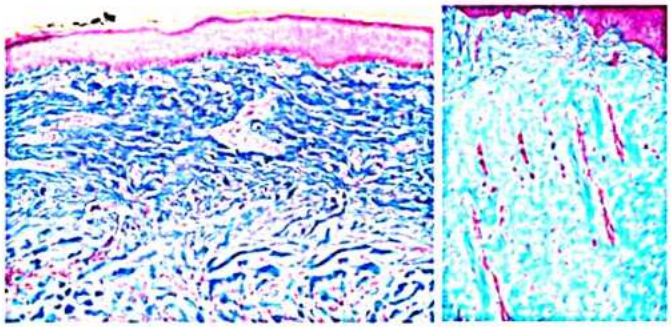


  - Formed due to trauma
  - Cross wound margins
  - Most common site
    → Sternum
  - Treatment
    → Initial treatment by Intralesional injection of steroid (triamcinolone)

- Hypertrophic scar

  - Formed due to trauma
  - Within the wound margins
  - Do not need a treatment

Masson trichrome stain



- Trichrome
  - Nucleus - Blue black
  - Muscle - Red
  - Collagen - Blue
- Checks whether wound has muscle or collagen
- Blue color is prominent - indicates collagen (scar)
- Collagen of keloid
  - Thick haphazard bundles of collagen

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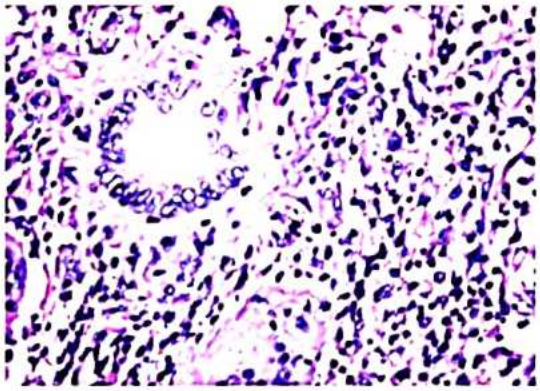
- Collagen of Hypertrophic scar
  - Thin orderly arranged collagen fibers

Summary

- **Types of collagen**
  - Type I - Bone, tendons
  - Type II - Cartilage - {Car2lage}
  - Type III - Blood vessel (granulation tissue)
  - Type IV - Basement membrane {basement floor -4}
- **Hallmarks**
  - Acute inflammation - Increased vascular permeability
  - Chronic inflammation - Tissue destruction
  - Repair - granulation tissues

MCQ

Q. Structure marked by the arrow in the given histopathological image is derived from?



a. B cell
b. T cell
c. Macrophages.
d. Fusion of epithelial

Explanation - The cell is Langhans giant cell fusion of macrophages

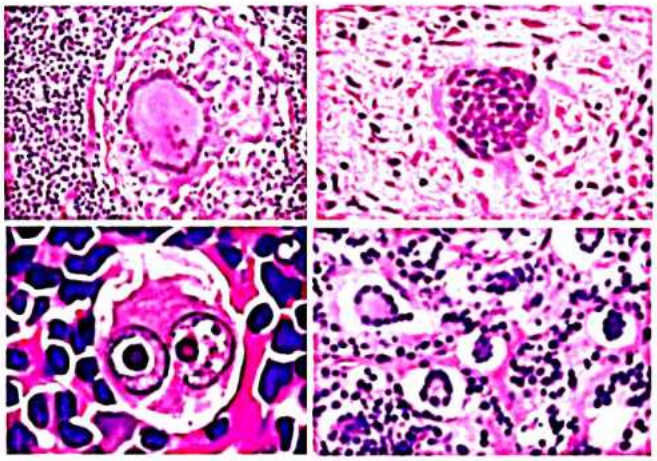
Q. Most important cell of a granuloma?
a. Giant cell
b. Macrophage
c. Lymphocytes
d. Epithelioid cells

Explanation - Epithelioid or modified macrophage

Q. Lymphocytic collar of a granuloma is primarily composed of which cells?
a. B cell
b. T helper cell (T_{h1})
c. T helper cell (T_{h2})
d. CD8 T cells

Explanation - T_{H1} releases Interferon-gamma (IFN-γ), making granuloma

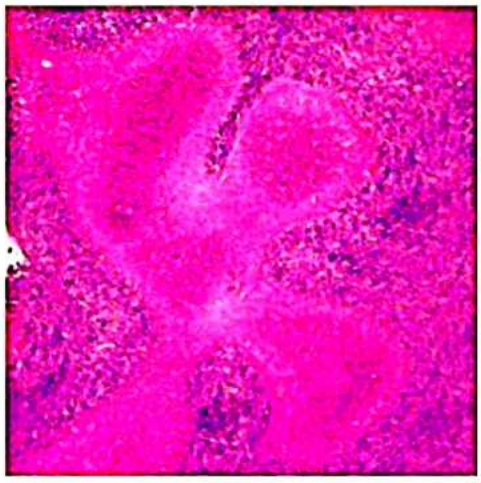
Q. Find the correct match?



a. Langhans-2 touton-1 foreign body-3 RS cell-4
b. Langhans-1 touton-3 foreign body-2 RS cell-4
c. Langerhans-1 touton-4 foreign body-2 RS cell-3
b. Langhans-1 touton-4 foreign body-2 RS cell-3

Explanation - Giant cell is langhans and not langerhans

Q. The granuloma shown below is associated with which of the following diseases?

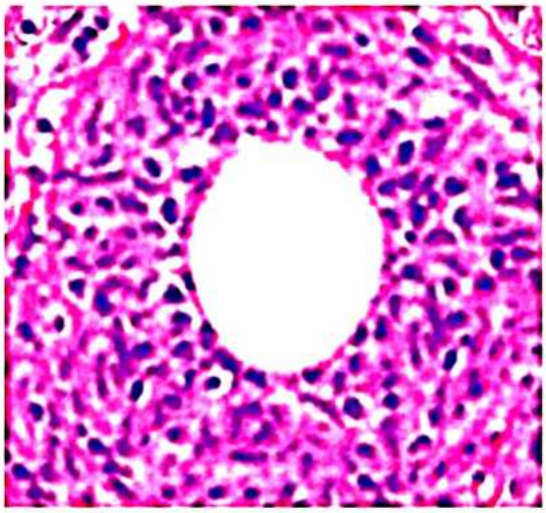


a. Cat scratch disease
b. LGV
c. Both a and b
d. None

Explanation - Star-shaped/stellate granuloma causes Cat scratch disease and LGV.

Telegram - @nextprepladdernotes

Q. The granuloma shown below is associated with which of the following diseases?



a. Coxiella burnetii
b. Q fever
c. Allopurinol
b. All of the above

Explanation - Doughnut granuloma is shown in the image and is associated with Q fever caused by Coxiella and Allopurinol.

Telegram - @nextprepladdernotes

PREVIOUS YEAR QUESTIONS

Q. Granulomatous inflammation is a subtype of which inflammation?
Ans. It is a subtype of Chronic inflammation.

Q. When does everything come at 100 percent normal?
Ans. Never

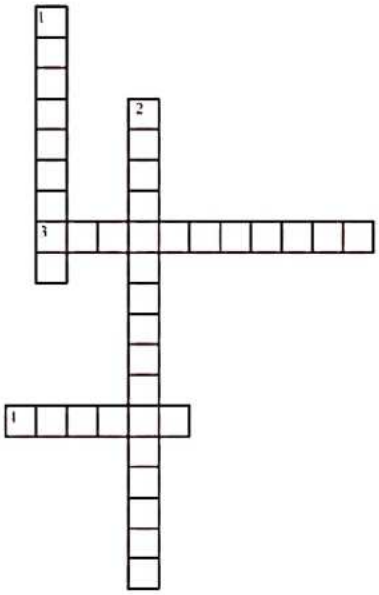
Q. What do you get at the end of one week?
Ans. 10 percent of strength comes back

Q. What do you get at the end of 12 weeks or three months?
Ans. 70 percent of strength comes back

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Activation occurs by Interferon-gamma (IFN-γ)
4. Formed due to trauma

Down

1. It is a category of chronic inflammation
2. Formed due to trauma

Telegram - @nextpreladdernotes

8 GENETICS INTRODUCTION AND MENDELIAN DISORDERS

Basics

- Number of Genes present: 20000+
- Part of a Gene: Exon (Coding region), Intron (Non-coding region). Intron comprises 98.5% of the Genome & Exons are roughly 1.5% of the Genome.

EXON INTRON EXON INTRON

- Locus: It is defined as the location of a gene on a Chromosome. Every chromosome has a short arm (p) and a long arm (q). Eg, P^{53} Gene is present in Chromosome 17p (short arm) 13.1 (RBS- Region, Band & Sub-band).
- Allele: every gene has two alternative forms, and these alternative forms are known as Alleles.
  - Dominant Allele: The capital form A
  - Recessive Allele: The small form a
- Same alleles (AA, aa) are known as Homozygous, while Different alleles (Aa) are known as Heterozygous.

Important Information

- Dominant Allele Expresses in Homozygous & Heterozygous conditions
- Recessive Allele Expresses in Homozygous conditions

- Codominance: It means both the dominant alleles will be expressed. Eg, AB Blood Group & HLA Gene. We have A (Dominant), B (Dominant), and o(recessive) in our blood.

Genetic composition and Blood Group

Gene	Blood Group
AA / Ao	A (Where there is a Dominant, it is expressed)
BB / Bo	B (where there is a Dominant, it is expressed)
AB	AB (Both dominating alleles are expressed) - Codominance
oo	O (As it is a recessive Allele and in a homozygous situation, it is expressed)

- Pseudodominance
  Example:
  1. Haemophilia (it is an X- linked recessive condition) → If there is Xx, then X is dominant, and there is a disease in x, but the dominant X will be expressed, so the patient will not show this disease. There is no disease manifestation. However, if the recessive Allele starts expressing, the patient will have the disease (the small suddenly starts showing dominating features).
  2. Red green color blindness
- Genotype: Genotype refers to genetic makeup (gene received from mother and father is genetic makeup). If the mother shows brown hair and the father has black, you have genes of both, but only one will be expressed (final expression).
- Phenotype: The expression of the gene is known as the Phenotype. E.g., you and your siblings might not have similar hair color or other things, despite having the same gene from the same set of parents.
- Pleiotropism: There is a single gene, but it has different effects.
  Example:
  1. In Sickle cell anemia (mutation at beta6 position), glutamine goes, and valine comes. Also, there is occlusion of blood vessels.
  2. Phenylketonuria; there is a different smell of urine and skin pigmentation. So, a single problem but multiple effects are there in Pleiotropism.
- Antagonistic Pleiotropism: There is a single gene with different effects; some effects would be positive, while others would be negative. Eg, P^{53} gene, the good effect of it won't let tumors happen, but the negative effect is that it also stops a lot of cells from dividing.
- Mutations: It is a permanent change (irreversible) in the DNA.
- It has two types:
  1. Point Mutation
  2. Frameshift Mutation.

1. Point Mutation

- There is a DNA sequence (CGCGGC GCGGCCGC), and suddenly there is a problem at one point, say (G), and it is replaced by another nucleotide (C), so it is happening at only one point. Therefore, Point Mutation is the replacement of one nucleotide by another.
- **Types of point mutation:**
  - Silent Mutation: Mutation happens, but there are no signs or symptoms.
  - Missense Mutation: It means the sense has changed. For example, a mutation has occurred in sickle cell anemia, and adult hemoglobin (normal) is changed to sickled hemoglobin. The sense has changed.

Telegram - @nextpreladdernotes

- Nonsense Mutation: It is seen in a case of Beta Thalassemia. Thalassemia means the beta chain production has stopped. The production stops due to UAA, UAG, and UGA- stop signal.

2. Frameshift Mutation

- CAGCGGCGGCGACAGCGG The body is reading them as Trinucleotides Frames. Now, by chance, one nucleotide is deleted or inserted, there is a shift in the trinucleotide frame as one gets deleted from between. This is known as Frameshift Mutation.
  Eg: Beta Thalassemia

Genetic Disorders

00:32:25

Classification

Mendelian disorders	Non-mendelian disorders	Chromosomal disorder	Multi factorial

Multifactorial

- Multiple factors are involved, so it is not just genetic defects, but many environmental factors are involved too.
- Eg: Diabetes Mellitus, Hypertension. Cleft Lip, Cleft Palate, and Congenital Heart Disease.

Mendelian Disorders

- The Mendelian Disorders include: AD, AR, XLR, XLD, and Y-Linked / Holandric

Difference between Autosomal Dominant & Autosomal Recessive:

AD (Autosomal Disorder)	Feature	Autosomal Recessive (AR)
Adult / Adolescence Onset (symptoms don't start in childhood).	Age	Childhood Onset
Incomplete Penetrance (Defective gene exists, but the disease does not occur).	Penetrance	Complete Penetrance
Variable: Same Disease, different expression Eg: NF (if there are three people with this disease, all should show the same symptoms, but all of them show different problems).	Expressibility (Eg: If some information is given to 100 students, all of them will not be able to answer the same number of questions.)	100 % (if there is a disease, all people will show the same phenomenon) Eg: Alkaptonuria (black urine), if this is where all people will show the same problem and symptoms.

1. AD (Autosomal Dominant)

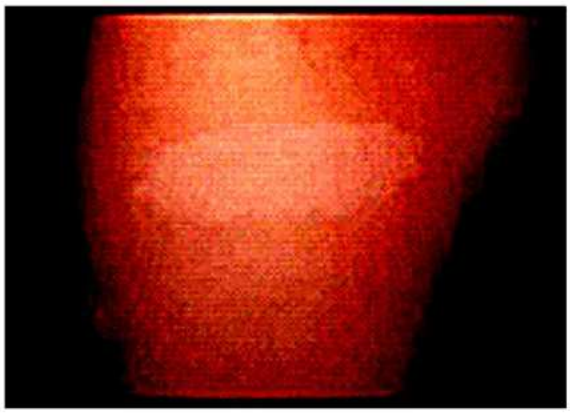
- It is the most common mode of inheritance. In most of the examples of Autosomal Dominant, there is a structural gene problem (structure of bone, RBCs, cartilage)

Examples

- He- Hereditary Spherocytosis Hereditary Telangiectasia, Hereditary Non-Polyposis Colon Cancer (HNPCC / Lynch Syndrome)
- Has- Huntington Disease, Hypertrophic Cardiomyopathy
- A- Autosomal Dominant Polycystic Kidney Disease (AD PKD)
- Very- Von Willebrand Disease (VWD), Von Hippel Lindau Syndrome (VHL Syndrome- chromosome 3p deleted)
- DOMINANT - Muscle dysTonia, Osteogenesis imperfecta, Marfan's Syndrome, Intermittent Porphyria, NF 1 & NF 2, Achondroplasia, Noonan Syndrome, Tuberous Sclerosis.
- Powerful- Pseudohypoparathyroidism
- Father - Familial Adenomatous Polyposis (FAP), Li Fraumeni Syndrome (mutation in p53), Familial hypercholesterolemia.

Tuberous Sclerosis/ Bourneville Syndrome

- It occurs because of the TSC Gene. There is TSC 1 (Chromosome 9) & TSC 2 (Chromosome 16) gene.
- Protein Made by TSC 1 is Hamartin & Protein made by TSC 2 is Tuberin. There is Multiple Hamartoma.
- Hypopigmentation regions known as Ash Leaf Macules are visible.



Marfan's Syndrome

- Chromosome 15 & Fibrillin 1 Gene (FBN 1) is affected, Fibrillin 1 Protein is defective.

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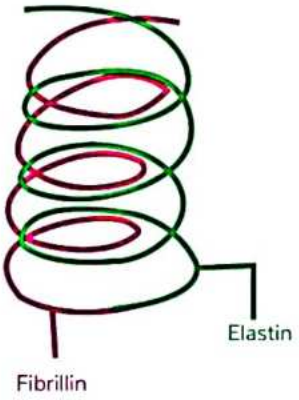


- One is Fibrillin, and the other closely bound is Elastin. As Fibrillin is defective, elastin will get negatively affected. And this concept is known as Dominant Negative.
- Fibrillin controls TGF Beta. And when Fibrillin 1 is defective → TGF Beta is overactivated, → Resulting in signs & symptoms.
- For the treatment of Marfan's, we have a Drug against TGF Beta, which is Losartan.

Diagnosis:

Revised Ghent's Criteria

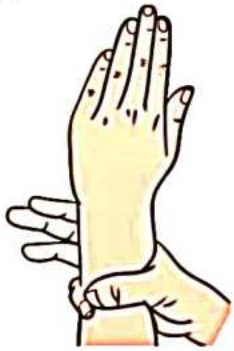
1. Family history (as it's Autosomal Dominant)
2. Clinical features
3. Fibrillin 1 Gene analysis.

Clinical Features:

- Mitral Valve Prolapse (MVP- most common Cardiac abnormality)
- Aortic Dissection (a most common cause of death)
- Retinal Detachment
- Freely moveable Joint (most common Thumb joint)
- Arachnodactyly (long fingers, spider fingers)

- High Arched Palate
- Nine Feet Tall,
- Pigeon chest (defect in sternum)
- Dislocation of the lens (Ectopia Lentis- Direction of dislocation is Supero Temporal; the opposite of this is Inferior Nasal whose disease is Homocystinuria)
- The most common Clinical Manifestation (overall) is Skeletal Abnormalities.

The Steinberg Sign	The Walker Sign
This test is used for the clinical evaluation of Marfan Patients.	The test is used for the evaluation of patients with Marfan Syndrome.
Fold your thumb into the closed fist. This test is positive if the thumb tip extends from the palm.	Grip your wrist with your opposite hand. If the thumb and fifth finger of the hand overlap with each other, this represents a positive Walker sign.



Pathology

- Microscopically:
  - Cystic Medial Degeneration of the vessel wall.
  - There is a loss of elastic fibers (Stain: Verhoeff Van Gieson- VVG). All Marfan problems were due to Fibrillin 1 Gene.
  - The problem due to Fibrillin 2 Gene defect includes: → Congenital Contractual Arachnodactyly

Neurofibromatosis (NF)

- These are of two types: NF 1 & NF 2.

Neurofibromatosis 1/ Von Recklinghausen syndrome		Neurofibromatosis 2/ Acoustic Schwannoma
NF1	**Gene defect**	NF2
Chr. 17	**Chromosome**	Chr. 22
Neurofibromin	**Protein**	Merlin

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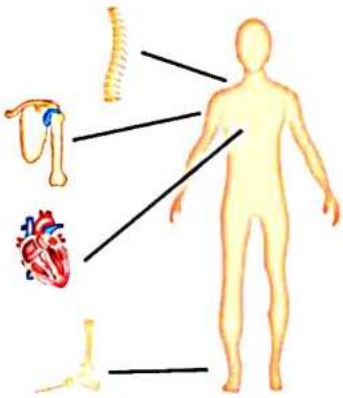
Neurofibroma	**Clinical Manifestations**	Schwannoma
Cafe Au Lit spots 		**M**-Multiple **I**-Inherited **S**-Schwannoma **N**-Meningioma **E**-Ependymoma
Lisch nodules in eyes (Pigmented iris hamartoma)		
Optic nerve glioma		
Juvenile myelomonocytic Leukemia (M/C leukemia)		

2. AR (Autosomal recessive) 01:21:18

- It is the most common mode of inheritance for the inborn errors of Metabolism (Lysosomal storage, Glycogen Storage disease). In examples of Autosomal Recessive, there is a lot of enzyme deficiency and metabolic disorders.

Examples:

- P- Phenylketonuria (PhenylAlanine Hydroxylase deficiency/Mousy/Musty odor of urine)
- A- Alkaptonuria (Homogentisic acid Oxidase deficiency: Black Color urine)
  Homogentisic Acid deposited in cartilage, nails, and urine chnages colour on standing.
- S- Sickle Cell Anemia
- Ta- Thalassemia
- Fried: Friedreich's Ataxia- Ataxia means there is some involvement of neurological elements.



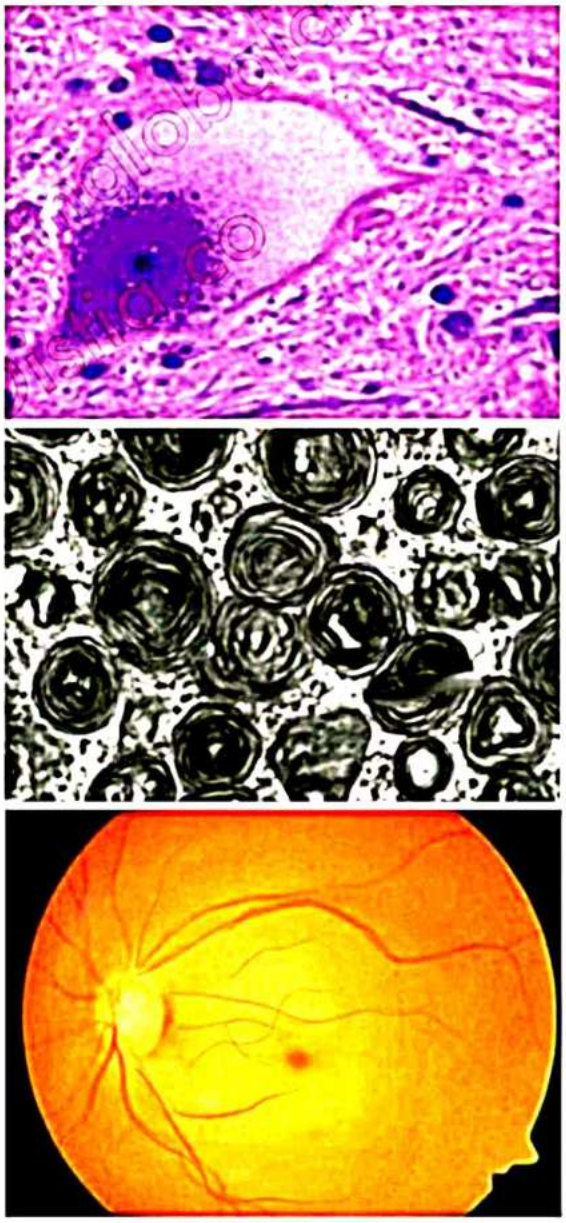
Scoliosis, ataxia, CVS, PES Cavus

- C- Cystic Fibrosis
- H- Haemophilia C, Homocystinuria, Hemochromatosis
- A- Alpha 1 Antitrypsin Deficiency
- W- Wilson Disease
- A- Albinism, Congenital Adrenal Hyperplasia
- L- Lysosomal Storage Disease, Glycogen Storage Disease
- Big: Bloom Syndrome
- Fa: Fanconi Anemia, Ataxia Telangiectasia
- X- Xeroderma Pigmentosum

Lysosomal storage diseases

Tay Sachs Disease

- They are Autosomal Recessive.
- childhood Onset
- Hexosaminidase A Deficiency
- This will occur in the Ashkenazi Jews.



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- Cherry red spot seen in the Macula of the eye.
- There are balloon neurons (these are Oil red O positive).
- On Electron microscopy onion skinning is seen, present in the Lysosome.

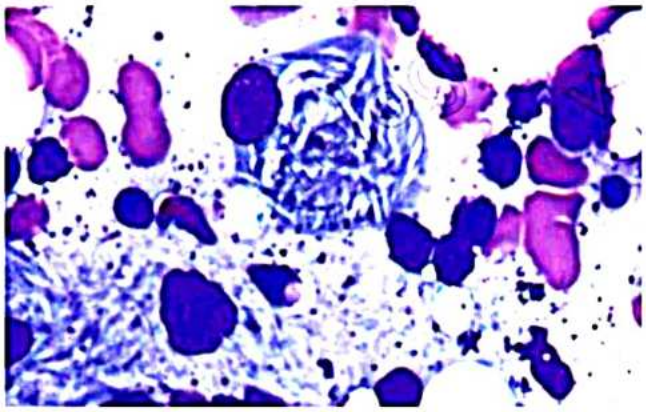
Niemann Pick Disease 01:37:05

- It is a sphingomyelinase deficiency.
- Everything else is the same as Tay Sachs Disease, including recessive, childhood Onset, etc.

- Light microscope: foamy cells
- Electrone microscope: zebra bodies

Gaucher's Disease

- These are patients who are going to present with bone pain bone pain.
- There is a glucocerebrosidase deficiency.



- This is famously known as a crumpled tissue paper appearance.
- It has two types:

1. Type 1 (no CNS involvement)
2. Type 2 (CNS involvement- most severe).

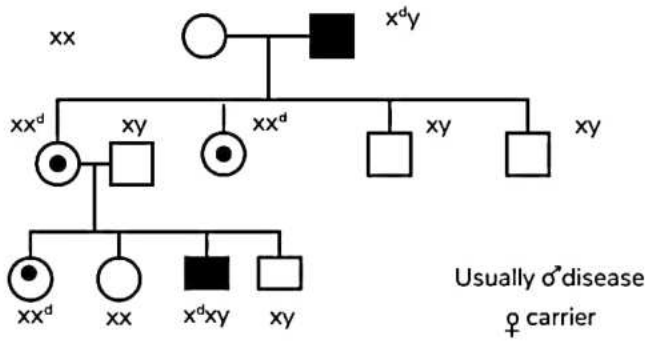
Pseudo-Gaucher Cell

- This cell is seen in leukemia called CML.
- It has the same appearance as Gaucher's Disease, which is a crumpled tissue paper appearance.
- It is differentiated by Iron Stain (Prussian blue stain +). Real Gaucher cells will have iron stains which pseudo gauchers will not have.

Enzyme Deficiency from Biochemistry

Disease (All Lysosomal Storage Disorders are Autosomal Recessive except for Fabry and Hunter Disease, which are x-linked)	Deficiency
Tay Sachs Disease	Alpha Hexosaminidase
Niemann Pick Disease (Spleen)	Sphingomyelinase
Gaucher Disease (Bone Pain)	Beta Glucocerebrosidase
Fabry Disease	Alpha Galactosidase
Krabbe Disease	Beta Galactosidase
Metachromatic Leukodystrophy	Aryl Sulfatase (MLA)
Hurler Disease	alpha-l-iduronidase
Hunter Disease	Iduronate-2-sulfatase

3. XLR 01:48:28



- **Turner's Syndrome Exceptions:** It is 45x0, which means this female has only one X chromosome and whatever may be in this (x) Chromosome will be shown up. So, these females can show disease.
- **Lyon's Hypothesis**: It is 46xx. It says even if females have two x Chromosomes, only one (x) Chromosome will be working, and one will sleep off. The female will be diseased if normal x is asleep and the other is diseased.
- If a diseased male ever gets married to a carrier female, the combination would include (x, Y), (x, x), which shows the female would be diseased in the second situation.

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Diseases Under XLR:

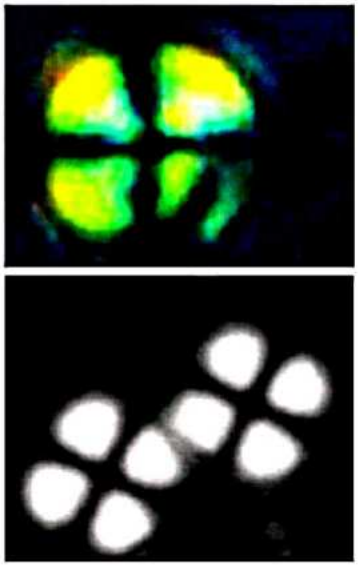
- Wise- Wiskott Aldrich Syndrome
- AB- Haemophilia AB (Haemophilia c is Autosomal Recessive)
- B- Bruton's Hypogammaglobulinemia
- C- Chronic Granulomatous Disease (NADPH Oxidase deficiency), Red Green Color Blindness, Ocular Albinism (Albinism particularly is Autosomal Recessive)
- D- DMD (Duchenne muscle dystrophy), BMD (Becker's Muscular Dystrophy): Dystrophy Gene is on X chromosome
- E- Lesch Nyhan Syndrome (HGPRT deficiency) 01:58:48

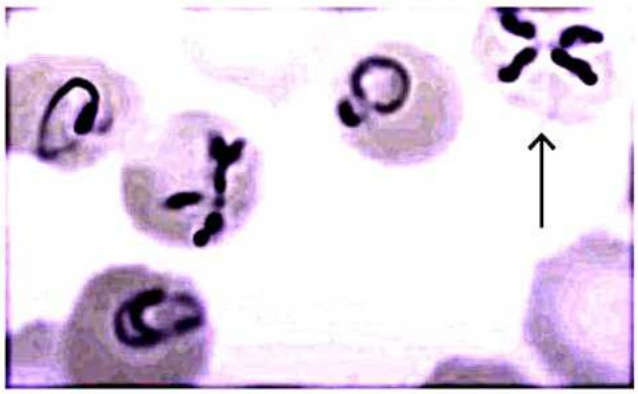
It is a picture of the syndrome because it shows a person who is biting his skin and nails.

- F- Fabry Disease, Fragile X Syndrome (XLR >> XLD)
- G- G6PD Deficiency
- H- Hunter Disease

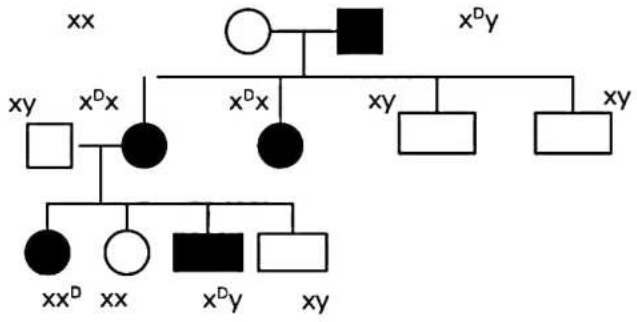
Fabry's Syndrome

- In this, we see a Maltese Cross Appearance because lipids coming out into the urine.
- Similarly, Nephrotic Syndrome is the same, and lipid comes into the urine.
- The Maltese Cross Appearance is seen in Microbiology. There is something like a cross known as Babesia.





4. XLD 02:03:35



Diseases in XLD

- R- Rett's Syndrome
- A- Alport Syndrome: It shows AD, AR, XLR, and XLD, all the disorders, but the most common is XLD.
- V- Vit. D Resistant Rickett (X linked Hypophosphatemia)
- I- Incontinentia Pigmenti

5. Y-linked / Holandric

- All the male members of the family chart will have these disorders. E.g., Hypertrichosis (Hairy Ears).

EDS (Ehlers-Danlos Syndrome)

- It can show all disorders AD, AR, and XLR.
- It is a collagen defect. Collagen is present in many parts, such as the skin→ the skin is very thin (described as Cigarette paper).
- Collagen is also present in blood vessels (with this defect, blood vessel breakdown happens and bleeding occurs)
- Joints (means joints are gone, patients have Hypermobile Joints- 360° movement).

Type	Aka	Inheritance	Defect
I	Classical EDS	AD	Collagen 5
II	Classical EDS	AD	Collagen 5
III	Hypermobile EDS	AD	Free Joints

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IV	Vascular type EDS (Flood Blood)	AD	Collagen 3
V	Same as II with different inheritance	X linked	Collagen 5
VI	Kyphoscoliosis Type	AR	LYSYL Hydroxylase
VII A, B	Arthrochalasia Type	AD (Autosomal Dominant)	Collagen 1
VII C	Dermatosparaxis Type	AR (Autosomal Recessive)	Collagen Peptidase

MCQs

Q. Sickle Cell Anemia is:
A. AD
B. AR
C. XLR
D. XLD

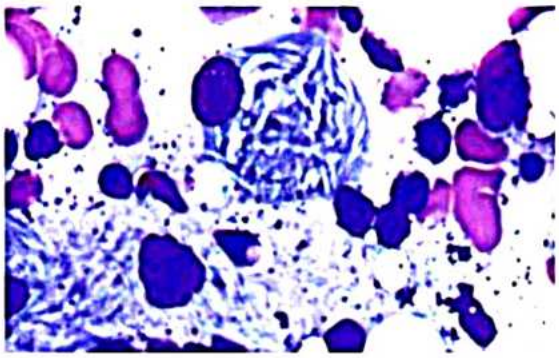
Q. Duchenne Muscular Dystrophy is:
A. AD
B. AR
C. XLR
D. XLD

Q. Haemophilia C is:
A. AD
B. AR
C. XLR
D. XLD

Q. Hypertrichosis is:
A. AD
B. AR
C. Holandric Inheritance
D. Mitochondrial Inheritance

Q. A 22-year-old man features an arm span greater than height, subluxated lenses, flattened and dilated aortic bulb. Which of the following genes is most likely to be mutated?
A. FBN 1
B. FBN 2
C. FBN 3
D. FBN R

Q. Crumpled tissue paper appearance is seen in?



A. Tay Sachs Disease
B. Gaucher's Disease
C. Niemann pick disease
D. Fabry Disease

Q. A 32-year-old pregnant female visits the ANC clinic for the first visit after she conceives. On the blood report, the Mentzer index is 11, and the doctor suggests Hb Electrophoresis. Her HbA2 levels are found to be 3.9%. Her husband also suggested the same test, and his levels were found to be 4%. What are the chances that the child will be suffering from this disease?
A. 0%
B. 25%
C. 50%
D. 75%

Q. Father has XLD disorder. How many percent of daughters will be affected by the disease further?
A. 0%
B. 50%
C. 75%
D. 100%

Q. A 12-year-old boy brought in by his parents in the medical outpatient department with bony aches and pains from the last 2 months. They have also noticed bending of his lower extremities. Family history was significant for similar complaints in her mother. His vitals are heart rate 90/min, blood pressure 120/80 mmHg, respiratory rate 14/min, and Temperature 98.6°F. Physical examination revealed an enlarged wrist and knee bowing of both legs. Investigation revealed Hypophosphatemia and normal calcitriol level. Considering the possible Diagnosis, what is the mode of inheritance of this condition according to the given family pedigree?

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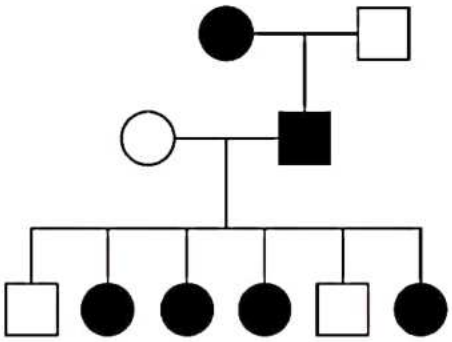

A. Autosomal Recessive Type
B. Autosomal Dominant Type
C. X linked Dominant type
D. Y linked recessive type

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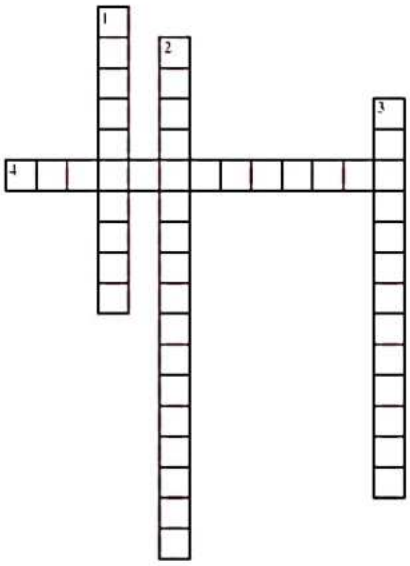
CROSS WORD PUZZLES

Crossword Puzzle



Across

4. Maltese Cross Appearance because lipids coming out into the urine

Down

1. The test is used for the evaluation of patients with Marfan Syndrome.
2. It is the most common mode of inheritance
3. This test is used for the clinical evaluation of Marfan Patients.

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9 NON MENDELIAN AND CHROMOSOMAL DISORDERS

Non Mendelian Disorders

The Non-Mendelian disorders are those which do not follow the Mendelian rules. There are four categories or types of Non-Mendelian disorders, which include:

1. **Mitochondrial inheritance**
2. **Trinucleotide repeat disorders**
3. **Genomic imprinting**
4. **Gonadal mosaicism**

1. Mitochondrial Inheritance

- **How are mitochondria related here?**
  Although most of the DNA is in the nucleus, the Mitochondria also contain small amounts of DNA, known as Mitochondrial DNAs (mtDNA). This mtDNA is from maternal inheritance.

- **How do defects affect the offspring in these disorders?**
  If maternal mtDNA is having a defect, it would be inherited by all the offspring (Mother to all)
  If paternal mtDNA is having a defect, none of the offspring would be affected (Father to none)

Example of Common Mitochondrial diseases

MELAS (Mitochondrial Encephalopathy, Lactic Acidosis, Stroke-like episodes)

- It is the most common overall Mitochondrial disease.

CPEO (Chronic Progressive External Ophthalmo-Plegia)

- Ophthalmo-Plagia is the muscle of the eye.
- It is the most common Mitochondrial muscle-related disease.

LHON (Leber's Hereditary Optic Neuropathy)
NARP (Neurogenic Ataxia, Retinitis Pigmentosa)

Rare case Mitochondrial diseases:

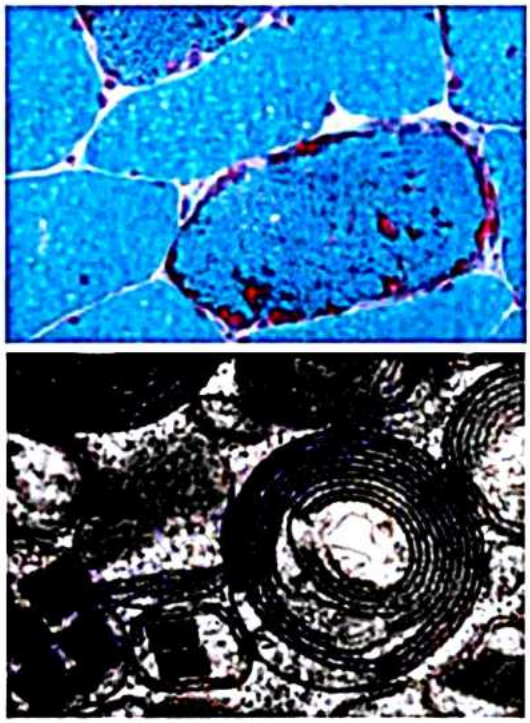
Leigh's Syndrome

Pearson Syndrome: 00:07:00

- Pancreatic Exocrine insufficiency or problem
- An Anemia with ring sideroblast
- Erythroid cells vacuolization
- It is usually seen in kids, if the patient survives to teenage the disease transforms into KSS (Kearn's Sayre Syndrome)

Kearn's Sayre Syndrome

MERRF (Myoclonus Epilepsy Red Ragged Fibers)
Mitochondrial diseases commonly affect the organs like the brain and eye.



- The image taken is a Muscle Biopsy (caused because of muscle involvement)
- The red & Blue stain is Gomori's Trichrome stain.
- Red ragged fibres are the abnormal mitochondria accumulated over muscles.
- Phonographic record & parking lot appearance in the electron microscope.

Heteroplasmy 00:12:25

- In this, there are two different DNAs in the same cell, one is normal DNA, while the other is mutated DNA.
- When normal DNA is more than mutated DNA, then the person does not carry any disease.
- When mutated DNA is more than normal DNA, the case of disease manifestation arises, and either all kids are not affected, or one is affected, and others are not with the disease.

2. Trinucleotide Repeat Disorders

In these, there is an excessive repetition of trinucleotides. The regions where the disorder occurs:

i. Intron or non-coding region

a. FRAGILE X SYNDROME (CGG repeats)
b. myoTonic dysTrophy (CTG repeats)
c. Fredreich's AtaAxiA (GAA repeats)

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ii. Exon or coding region

(Kennedy is hunting in a CAGE)

a. Huntington disease (CAG repeats)

b. Kennedy (CAG repeats)

a. FRAGILE X SYNDROME 00:18:52

- It is related to the chromosome Xq.
- The problem is caused due to the FMR 1 gene (Familial Mental Retardation Gene).
- CGG repeats.
- It is called fragile because of its appearance in a folate-deficient media as an unstained area.
  - → If CGG repeat is around < 55 = normal.
  - → If CGG repeat is between 55-200 = Permutation. It results in Fragile X Tremors and Ataxia.
  - → If CGG repeats are between 200-4000 = Mutation. This is when it results in FRAGILE X SYNDROME.
- Sherman's Paradox /Anticipation says that with each generation, the number of repeats and severity of disease increases.
- Occurs mainly in males and females are carriers.
- The carriers female is shown to have Premature Ovarian Failure (POF) (<40yrs)

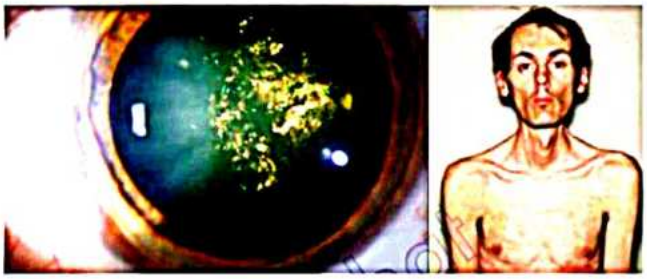
Clinical Features:

- MR (IQ= 20-60)
- Long face
- Large ears
- Large Jaw/mandible
- The most characteristic feature is Macro-orchidism (large testicular size)

Diagnosis:

- Diagnosis of choice → PCR (if fails) → southern blotting
- Southern (Dosa) = DNA detection
- Northern (Roti) = RNA detection
- Western (Pizza) = Protein detection
- Eastern = Post-translational modifications

b. Myotonic dystrophy (CTG repeats)



- The patient has a strong grip. They will not terminate the grip.
- Patient have "Hatchet Facies"
- C= Christmas tree Cataract
- T= Testicular Atrophy
- G= Hypogammaglobulinemia

3. Genomic Imprinting (Gene Silencing) 00:33:40

- Normal Phenomenon
- Since everything is in pairs.
- One chromosome of the set comes from the mother and the other chromosome of the set comes from the father from the mother and one from the father. So, switching one gene off is known as silencing.
- It is done through DNA methylation.
- DNA methylation silences, and DNA acetylation activates.

For instance, in chromosome 15:

The first mechanism for PRADER WILLI and ANGELMANN SYNDROME:

There are two genes. One is the Snorp gene, and the second is the UBE3A gene.

- If in the Snorp gene, i.e., maternal imprinting is done, and paternal deletion happens, then PRADER WILLI SYNDROME is caused.
- If in the UBE3A gene, i.e., paternal imprinting is done, and maternal deletion happens, then ANGELMANN SYNDROME is caused.

The second mechanism for PRADER WILLI and ANGELMANN SYNDROME:

- Known as UPD (Uni Parental Disomy). Both the chromosomes in the gene come from the same parent. If a

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similar case is taken in the first mechanism, maternal disomy 15, it means that both chromosomes from the mother and father are missing.

- The conclusion is if the paternal region is not there, it is PRADER WILLI SYNDROME. And if the maternal region is not there, it is ANGELMANN SYNDROME

Clinical Features:

a. PRADER WILLI

- MR (Maternal Retardation)
- Short Stature
- Obesity / hyperphagia (because of an increase in the hormone called Ghrelin)

b. ANGELMANN SYNDROME (known as "Happy Puppets")

- Seizures
- Ataxia
- Mental Retardation (MR)
- Inappropriate laughter

Loss of maternal imprinting

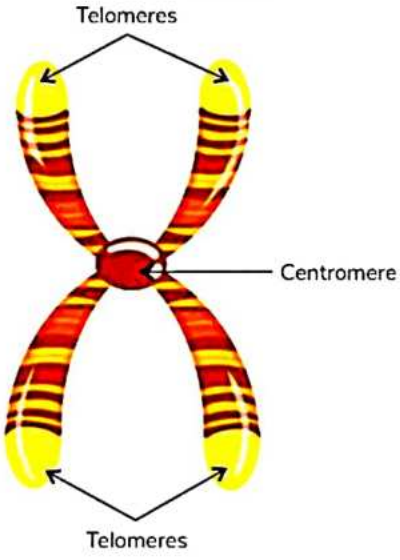
- Beckwith wiedmann syndrome→ causes wilms tumor

4. Gonadal mosaicism / Germline Mosaicism: 00:51:10

If there is a case of osteogenesis imperfecta in child and if no one in the family is affected, then it is said to be Gonadal mosaicism.

- The parents are normal and have normal sperm and ova.
- It is caused due to Post Zygotic Mutation in germ cells.

Chromosomes



- Every chromosome has a central part known as the centromere.
- The terminal part is the telomere

Types of Chromosome

Types	Location of Centromere	Example
Metacentric	Centromere at centre P=q	Chromosome 1 in humans
Submetacentric	Centromere away from centre P<q	Chromosome X in humans
Acrocentric	Centromere almost at tip P<<<q	Chromosome 13,14,15, 21,22,23(Y) in humans
Telocentric	Centromere at tip	Not present in humans

There are two categories of Chromosomal Disorders:

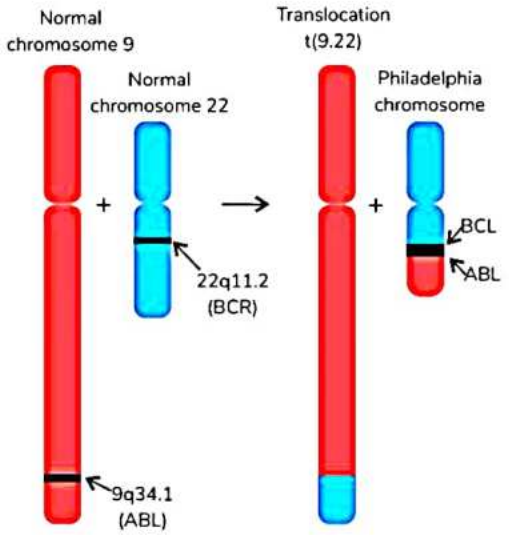
1. Structural disorders
2. Numerical disorders

i. Structural Disorders

a. Deletion: If a certain part of a chromosome or whole chromosome is deleted.
→ E.g. VHL syndrome.

b. Inversion: It is like a 180° flip of a chromosome.
→ If it involves the centromere or around the centromere, then it's known as Pericentric inversion.
→ If it's away from the centromere, then it's known as Paracentric inversion.
→ Eg. AML M_4 shows inversion 16

c. Translocation:



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Example- t(9:22)
This is a balanced reciprocal translocation. The normal chromosome 9 has the AbL gene. The normal chromosome 22 has the Bcr gene.

→ The AbL of chr. 9 moves to Bcr of cr. 22 and a Bcr-AbL fusion occurs.
→ This is translocation resulting CML.
→ Philadelphia is where the fusion occurs, i.e. on chromosome 22.

d. Robertsonian Translocation:
This occurs between Acrocentric Chromosomes 13,14,15,21,22,23 (Y).
Example: t(14:21)
→ After the translocation, one abnormal long chromosome is formed

e. (Ring Chromosome:
The ends of a chromosome are damaged Chromosome ends stick together.
→ This property converts the chromosome into the form of a ring.
→ It is seen in TURNER SYNDROME, in which either there is a deletion of an X chromosome or X chromosome is functionally weird.

f. Isochromosome
It's a chromosome with two similar arms. It means that this chromosome will have either small arms or big arms. (2p or 2q)
→ This happens when there is a transverse division of chromosomes.
→ The most common Isochromosome is = ixq (iso, chr. X & two q arms)
→ The most common Isochromosome associated with cancers = I 17q
→ The most common Isochromosome particularly associated with Testicular cancers = i 12 p

g. Chromothrypsis:
Chromo → Chromosome
Thrypsis → Fragmentation
It means the chromosome had shattered into multiple pieces and these pieces would combine again to form a chromosome, but it is done randomly.
→ These result in cancers like bone and blood cancers.

ii. Numerical Disorders

a. Trisomy
→ The most common Trisomy is T16. But this is not compatible with life.
→ The most common Trisomy compatible with life is T21 (DOWN'S SYNDROME).

b. Monosomy
→ The most common Monosomy compatible with life is TURNER'S SYNDROME = 45 x 0

Down's Syndrome (Trisomy 21) 01:28:38

It is caused by the mother. Three ways by which one can get DOWN'S SYNDROME:

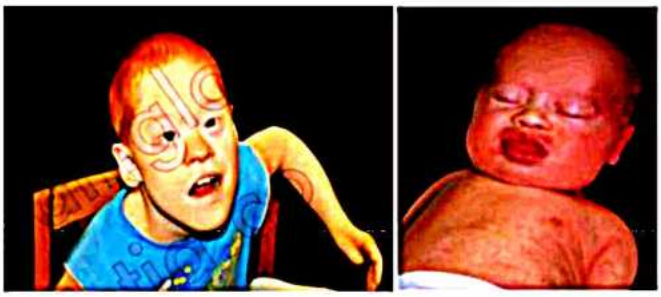
Pathogenesis-

i. Meiotic Non-disjunction of chr. 21 in Ova (almost 95% of DOWN'S is because of the meiotic problems).
- This is associated with advanced maternal age.

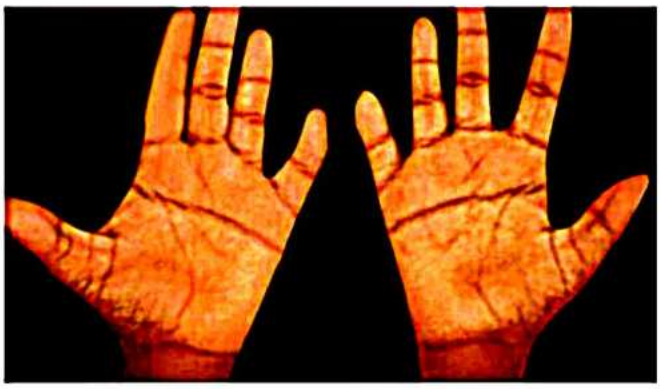
ii. Robertsonian Translocation (4%)
- This is not associated with the age of the mother.

iii. Mitotic Non Disjunction (1%)

Clinical features:



- Presents with mental retardation
- Mongoloid Facies (Oblique Palpebral Fissure, Flat nasal Bridge/ nasal bone hypoplasia)
- Open mouth
- Micrognathia (Small chin)
- Macroglossia
- Brushfield spots (iris)
- Simian Crease (Single Palmar Crease)



- Single crease (Little finger)
- Sandal Toe (gap b/w bigger and smaller toes)

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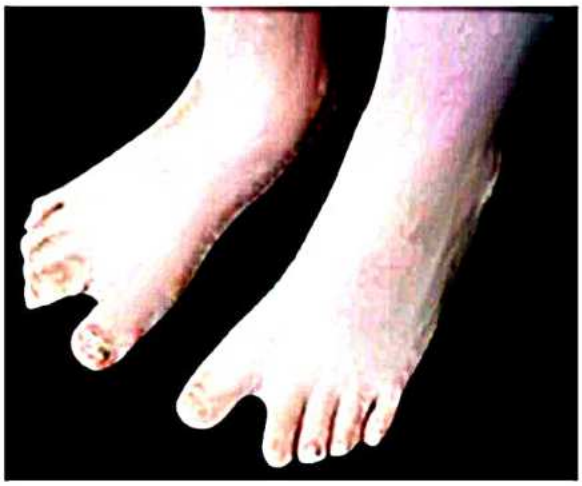


Complications of down's syndrome:

- Leukemia: The most common Leukemia overall is "ALL".
- Most common Leukemia in children less than <3 yrs of age is "AMLM7" (Acute Megakaryoblastic Leukemia)
- CNS: They have Presenile Alzheimer's disease (in 30's)
  - MCC of early deaths and down syndrome
- CVS: Endocardial Cushion defects >> vsd.
- GIT: Duodenal Atresia, Pancreas annular, HIRSCHSPRUNG disease
- Endocrine: A.I. Thyroiditis (Hypo)
- The most common cause of early death in DOWN'S SYNDROME is "CVS" (Cardiovascular).

Testing of Down's Syndrome:

Screening

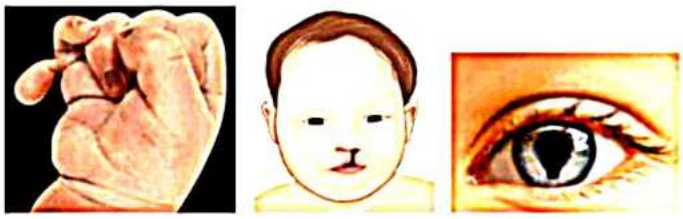
- USG: (Nasal Bone Hypoplasia, Nuchal Translucency)
- Dual Marker Test (First Trimester): Beta-HCG & PAPP-A.
- β-HCG is high and PAPP-A is low, then alert signal.
- Triple Marker Test (2nd Trimester): Tested for β-HCG, AFP, Estriol.
- If β-HCG is high, AFP and Estriol are low; it is an alert signal.
- Quadruple Marker Test (3rd Trimester): β HCG, AFP, Estriol + inhibin A. All are decreased except for β-HCG and Inhibin A.
- Confirmatory test → Karyotyping

Patau Syndrome: Trisomy 13

01:40:57

- Age → 13
- Maternal origin
- Mental Retardation
- Heart: PDA (Patent Ductus Arteriosus)
- Rocker bottom feet

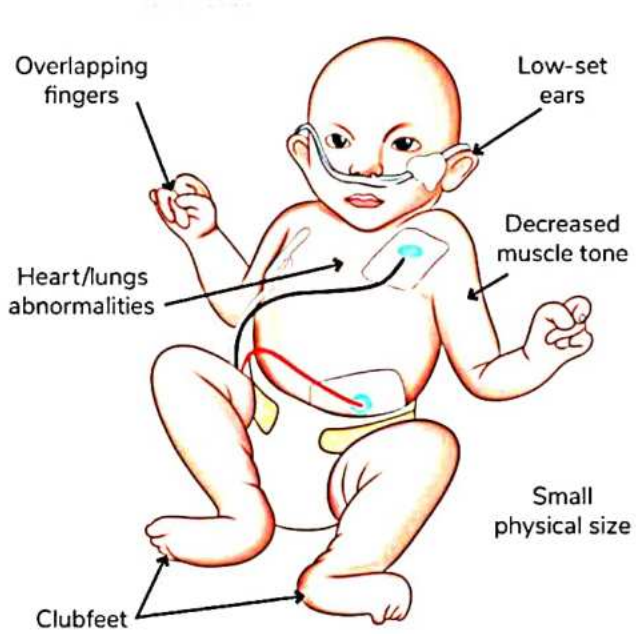
Symptoms:
Polydactyly (finger), cleft lip and cleft palate, Keyhole/ Coloboma.



Edward's Syndrome: Trisomy 18

- Age → 18
- Maternal origin
- Mental retardation
- Heart: PDA (Patent Ductus Arteriosus)
- Rocker bottom feet

Symptoms: Strawberry Skull (means small chin & Prominent Occiput), Horse-Shoe kidney, overlapping fingers



Cat Eye vs Cat Cry

- Cat eye means Multiple vertical colobomas (Trisomy 22).
- Cat cry is also known as CRI DU CHAT syndrome (partial deletion 5p).

Klinefelter Syndrome

01:46:43

- Male Patient With Extra X Chromosome
- The total number is 47 XXY if only one X chromosome is extra.
- More the number of X xhromosomes, more is the mental retardation

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- It could be either from maternal or paternal origin. But most common from maternal origin.

Clinical features

- Mental retardation.
- Decrease in secondary sexual characteristics
- Hypgonadism
- Tall individuals

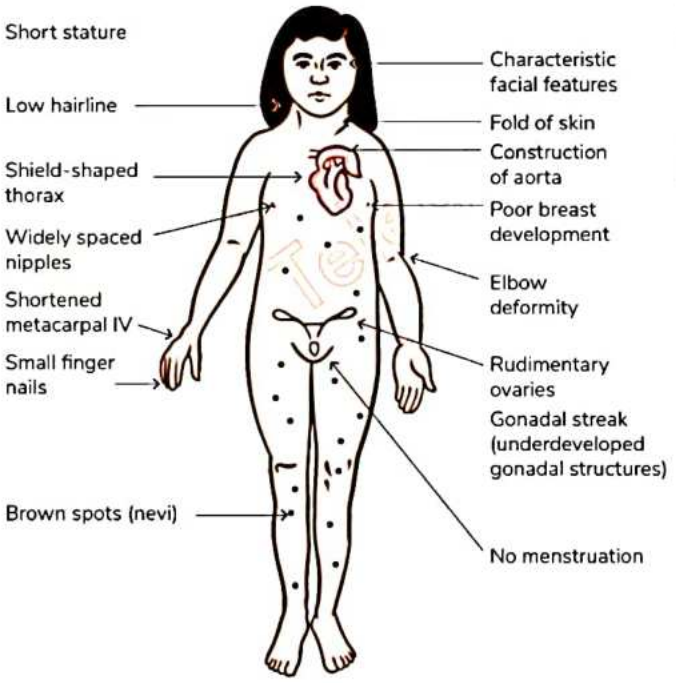
Complications

- Cancers: Most common is Male Breast Cancer, Extra Gonadal Germ cell tumor (Teratoma, Seminoma) tumors
- Mitral Valve Prolapse (MVP)
- Associated with Autoimmune Disorders (SLE)
- Hormones: Decreased Testosterone Levels, LH & FSH are increased.

Turner's Syndrome (Monosomy)

- **Pathogenesis:** Either 45 x 0 (deleted) or 46 x r(X) (Ring Chromosome)
- In Turner's Syndrome, the IQ is usually normal.
- Patients with webbing of the neck have higher chances of Cardiac symptoms.

Clinical Features



- Short Stature
- Low hairline
- Webbing of Neck
- Shows Cystic Hygroma- Lymphangioma
- Most common is Bicuspid Aortic valve >>> COA
- Widely spaced Nipples
- Rudimentary Ovaries (Ovaries are underdeveloped)
- No Menstruation (most common cause of Primary Amenorrhea)

Noonan Syndrome

- It is male counterpart of Turner.
- Chromosome 12 problem associated with PTPN gene.
- The IQ can be low in this case.

MCQs

02:00:55

Q. Find the incorrect match with regard to disease and trinucleotide repeat:

a. Fragile x Syndrome CGG
b. Myotonic Dystrophy CTG
c. Huntington disease CAG
d. Friedreich's Ataxia GAA

Q. Incorrect for Fragile X Syndrome:

a. Shows CGG premutation
b. Associated with loss of function of FMR gene
c. Repeats can be detected by PCR
d. Females can show POF

Q. Which one of the following is incorrect about Prader Willi syndrome?

a. Associated with Paternal imprinting
b. Associated with Paternal Deletion
c. Associated with Maternal imprinting
d. Can be due to maternal Disomy

Q. A 4-year-old boy diagnosed with osteogenesis imperfecta comes to the clinic with his parents. On pedigree analysis, none of the family members has the disorders. Which of the following mechanisms do you attribute to the condition of this boy?

a. Autosomal recessive inheritance
b. Gonadal mosaicism
c. Genomic imprinting
d. Mitochondrial DNA Mutation

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10 DIAGNOSIS OF GENETIC DISORDERS

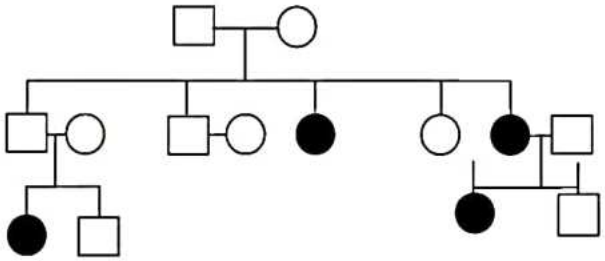
Pedigree analysis

00:00:25

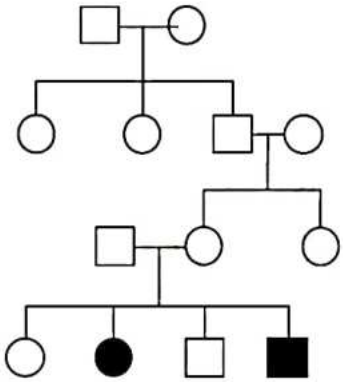
Refer Table 10.1

Rules

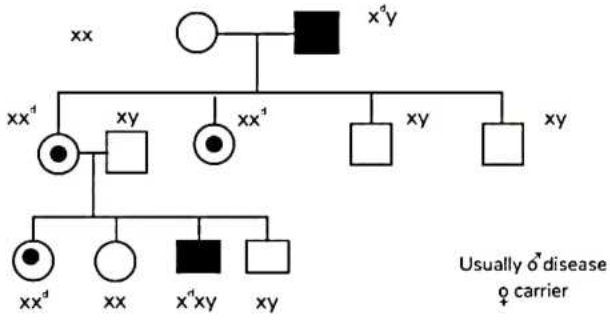
1. **Autosomal dominant:** No gender bias; all the generations are affected



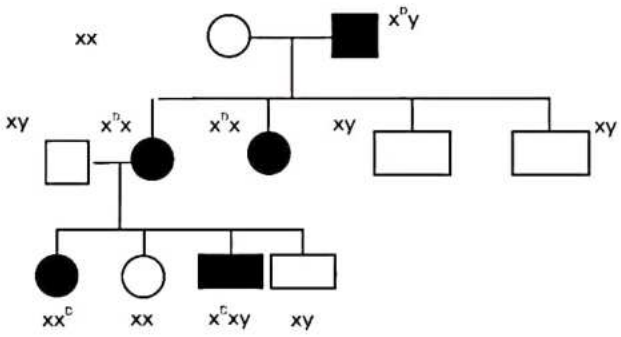
2. **Autosomal recessive:** No gender bias, skipping of generation



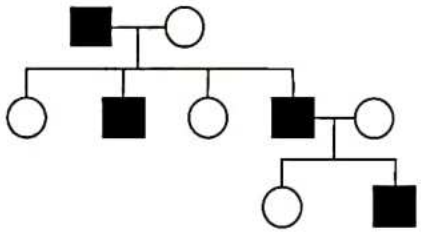
3. **X-linked recessive:** Males are affected, and females are carriers



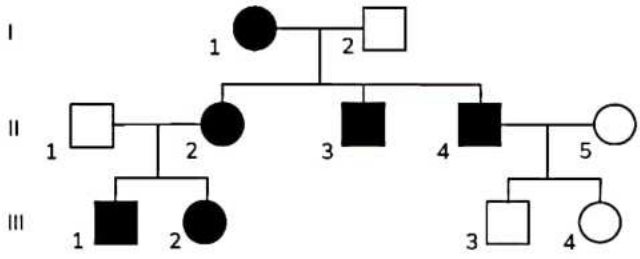
4. **X-linked dominant** (DDD concepts): From Dad to all Daughter. From the Daughter(mother) to 50% of their offspring.



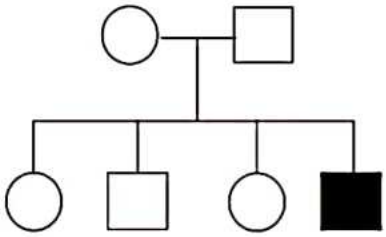
5. **Y linked:** only Male members are diseased(father to son)



6. **Mitochondrial inheritance:** Mother to all, father to none.



7. **Gonadal mosaicism:** Only one child in the family is diseased



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2G rule:

1. Check for gender discrimination.
2. Check for generation involvement

Karyotyping

00:14:35

- Study of chromosomes

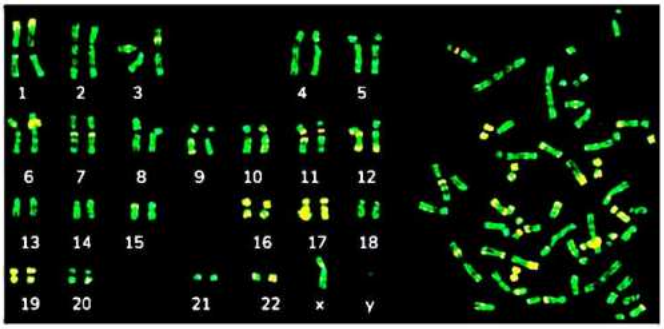
Steps involved:

1. Sample collection:

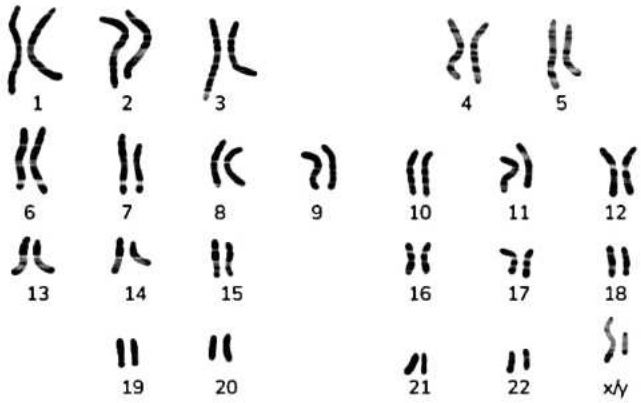
Patient type	Sample type	Reason
Pregnant woman	Amniocentesis	14-16 weeks
	Chorionic Villous Sampling	Early detection (6-8 weeks)
Otherwise	Lymphocyte Blood sample	Preferable one
	Fibroblast skin sample	

2. Arrest: It is carried out in metaphase using chemical colchicine.
3. Fixative: Using Carnoy fixative (Methanol: Glacial acetic acid:: 3:1)
4. Staining/ Banding: Chromosome has a dark(AT nucleotides rich) and light (\CG nucleotides rich)bands.
   a. Q banding: Using Quinacrine stain and Fluorescent microscope (fluorescent pigmentation)

Q- banding



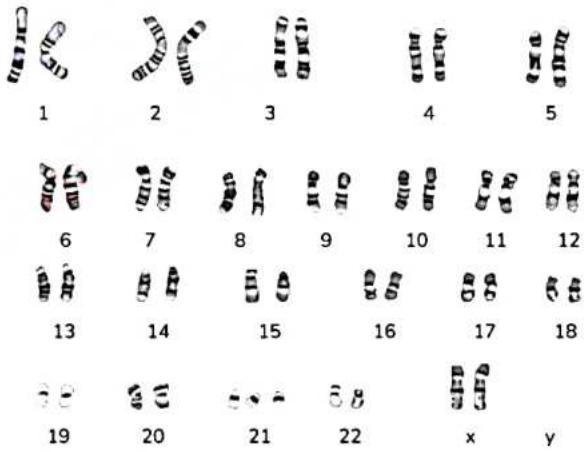
   b. G banding: Giemsa stain with a light microscope (most common)
   c. R banding: Reverse banding with a light microscope
   d. T/c banding: T- Telomere/terminal, C- Centromere part of chromosome with a light microscope.



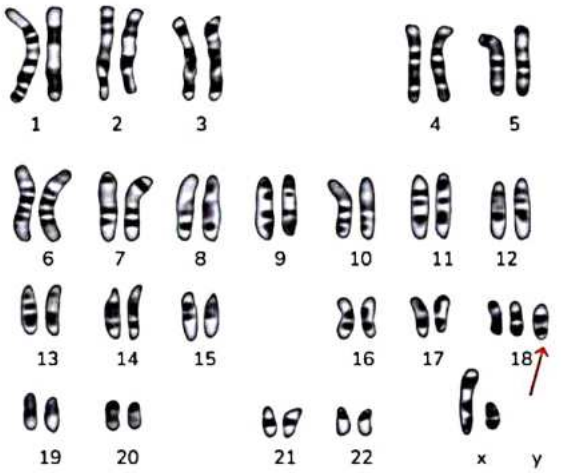
Normal Karyotype

5. Arranging of chromosomes: According to length in descending order.
   a. Shortest chromosome is chromosome 21.

6. Interpretations:
   - Trisomy 21(Down's syndrome): 3 of chromosome 21



   - Trisomy 18 (Edward's syndrome): 3 of chromosome 18



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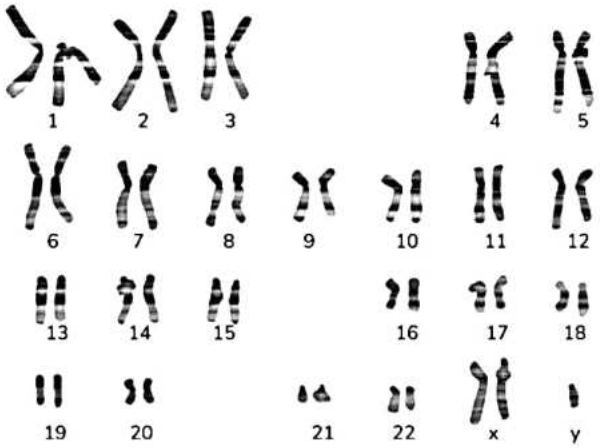
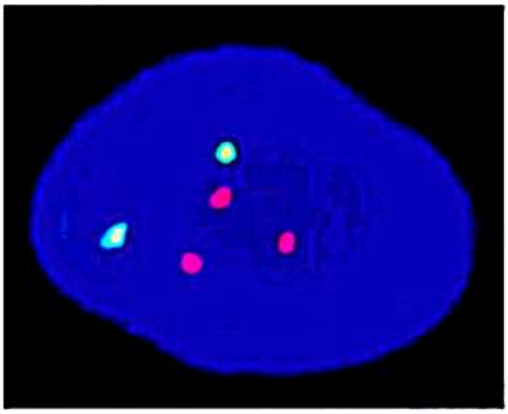
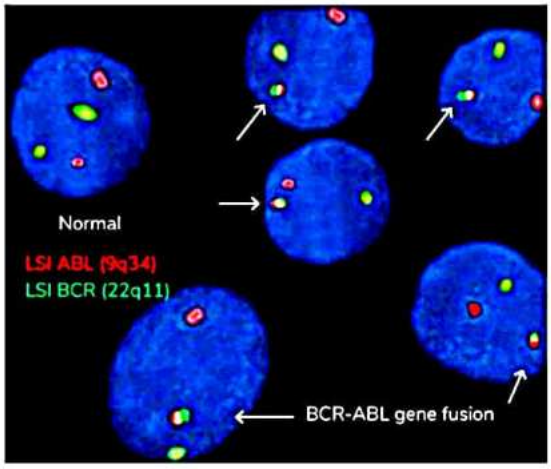
- XXY- Klinefelter syndrome



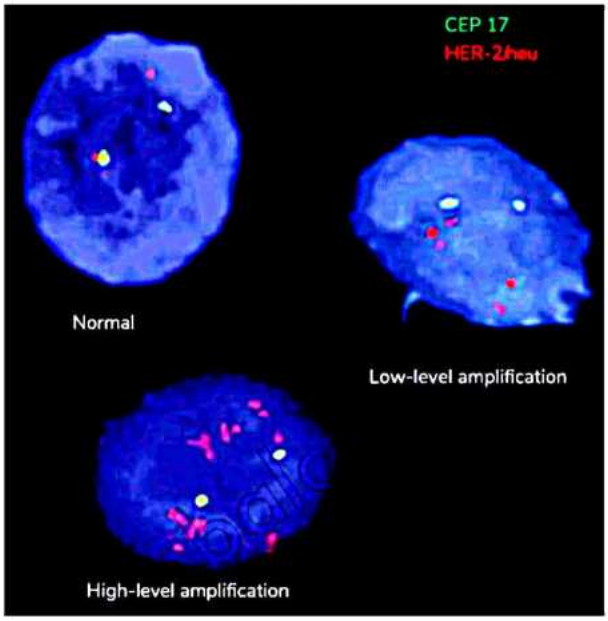
FISH (Fluorescent Insitu Hybridisation) 00:24:52



- Cells used are kept at an interface.
- Helps in identifying chromosomal abnormality.
- For a 2-color FISH, the chromosome that doesn't have a pair is identified as abnormal.
- Fusion of 2 different signal(color) can help in identifying translocation in Philadelphia chromosome.
- Translocation in Philadelphia chromosome



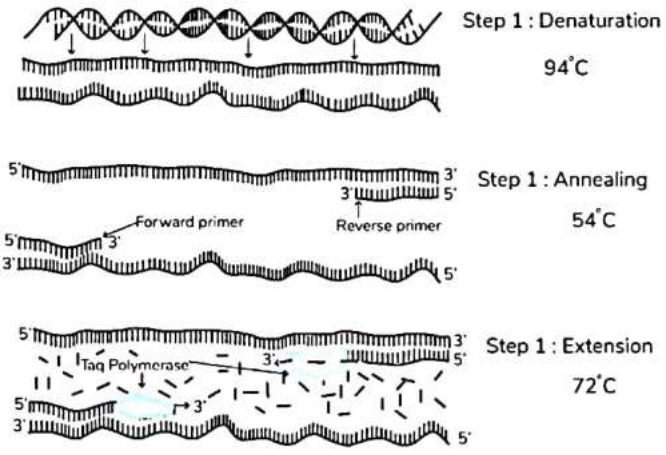
- HER 2 NEU amplification: when CEP17 (chromosome 17) ratio is greater than 2.2 then it is classified as breast cancer.
- When HER 2 NEU genes are amplification (more than 2.2 ratios), it helps identify breast cancer.



- Multicolor FISH is called Spectral Karyotyping (SKY)
  Ratio = Her2/Cep17>2.2→ Breast cancer

PCR 00:32:43

PCR: Polymerase Chain Reaction



- Found by the scientist named Karry B Mullis.

Steps:

1. Denaturation of DNA: Split double-stranded DNA into Single stranded ones. At 94° C.
2. Annealing of PRIMER: At 54° C.

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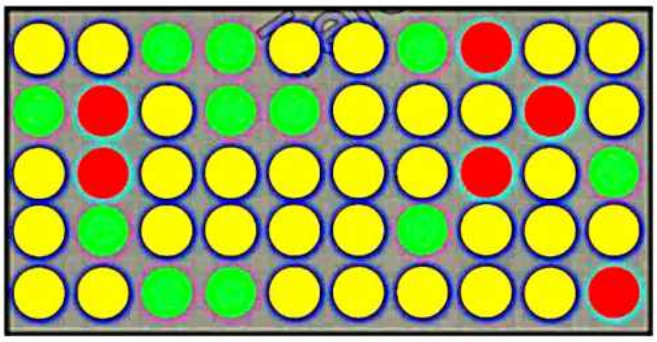
3. Extension: Converting single-stranded DNA to Double-Stranded DNA at 72°C.
4. STOP the cycle: By Adding DDNTP(Dideoxy Nucleotide proteins).

Types of PCR:

1. **Sanger sequencing:** Gold standard.
2. **SBPE:** Single base primer extension- For Known locus
   - Hairy cell leukemia patients have a mutation called BRAFV600.
   - Single area to target as the locus is known.
3. **RFLP:** Restriction fragment length polymorphism - For unknown locus
4. **Amplicon Length analysis:** When the genome is lengthy. (CGGGGGGGGG……..)
   - For fragile X syndrome
5. **Reverse transcription PCR:** For RNA virus
6. **qPCR-** Quantitative / Real-time PCR: Quantitative (How many)
   - For COVID test
   - Cancer CML
7. **GWAS -** genome-wide association study: Research using huge data of people.
   - E.g. Diabetes, Hypertension
8. **Pyrosequencing-** When dealing with a contaminant sample (low tumor yield).
   - When only 10% of the sample has targetted cells(cancer) and 90% is miscellaneous.
   - Better technique: Next-generation sequencing

CGH (Comparative Genomic Hybridization)

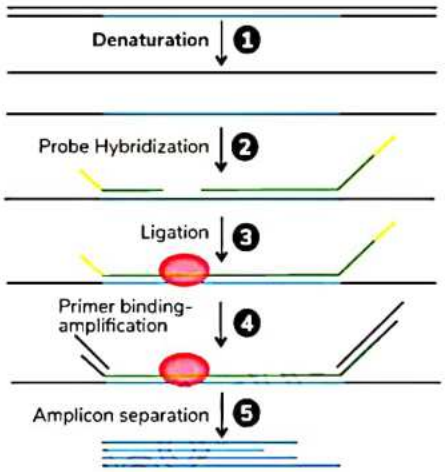


- When it is carried out on many patients- Microaaray
- Fusion of patient genes with a normal gene to understand if the patient is diseased or not.
- For example, red→patient gene + green → normal gene, results in
  - Red → Gain of function
  - Yellow →Normal person
  - Green → Loss of function

MLPA (Multiplex Ligation dependent Probe Amplification)

00:49:07

- Mutation too large for PCR and too small for FISH are detected by MLPA
- For multiple mutations detection
  - E.g., CFTR gene (cystic fibrosis)

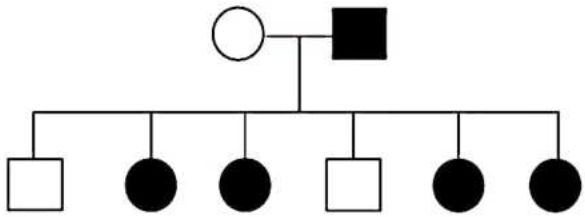


Steps:

- Denaturation
- Annealing- using multiple probes (Probe amplification).
- Ligation- Joining 2 probes
- Amplification

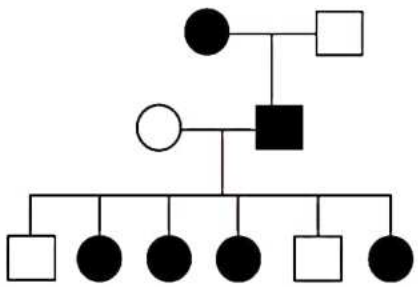
MCQ

Q. Identify the inheritance shown in the Pedigree chart below.



a. AD
b. AR
c. XLR
d. XLD

Q. Identify the inheritance shown in the Pedigree chart below.



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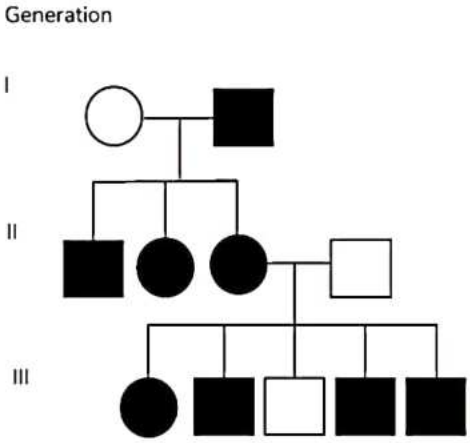
a. AD
b. AR
c. XLR
d. XLD

Q. Identify the inheritance shown in the Pedigree chart below.



a. AD
b. AR
c. XLR
d. XLD

Q. Which of the following is associated with karyotyping?
a. Carnay fixative
b. Carnoy Fixative
c. Sacchomano fixative
d. Formalin

Q. Karyotyping is done using a fluorescence microscope with which of the following technique?
a. G banding
b. T banding
c. C banding
d. Q banding

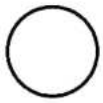
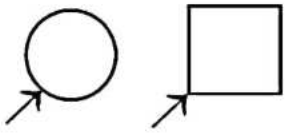
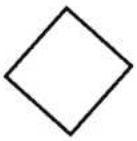
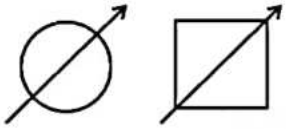
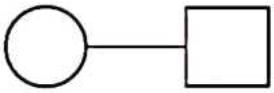
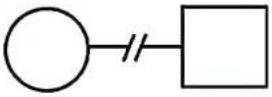
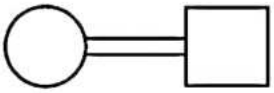
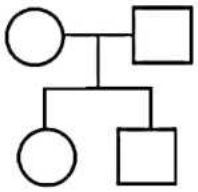
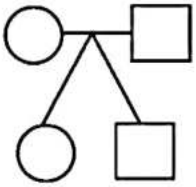
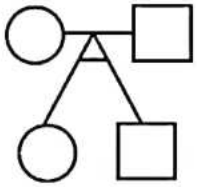
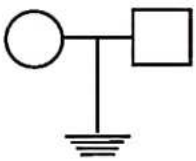
Q. Which of the following is considered as the gold standard PCR utilizing chain termination sequence?
a. Pyrosequencing
b. Sanger Sequencing
c. SBPE
d. GWAS

Q. 54-year-old female has presented with a breast lump measuring 9X7 cm in the upper outer quadrant. The lump is firm to hard inconsistency and seems to be fixed to the underlying muscle. Trucut biopsy is done, which reveals infiltrating ductal carcinoma Further that tumor cells are sent for genetic testing. However, the number of tumor cells constitutes only 8% of biopsy. The rest is compromised on stromal fat and ducts. Which of the following techniques is best suited?
a. Sanger sequencing
b. SBPE
c. Pyrosequencing
d. Amplicon length Analysis

Q. 29-year-old male patient presents with red plethoric face along with episodes of dizziness. The patient is found to be hypertensive on examination. Further, he also gives a history of intense burning sensation in hands and feet along with itching following a hot water bath. On CBC analysis his Hb is 21 gm/dl. He is advised EPO level testing along with JAK2 testing. EPO levels are found to be sub-optimal. JAK2V617F mutation is found in this patient. Which of the following methodology of testing must be used for testing of the same?
a. Sanger sequencing
b. SBPE
c. RFLP
d. GWAS

Telegram - @nextprepladdernotes

Table 10.1

SYMBOL	MEANING	SYMBOL	MEANING
	Female		Carrier
	Male		Index case (Proband)
	Gender unknown		Death due to disease
	Diseased		X-Linked Carrier
	Marriage		Divorced
	Consanguinous marriage (marriage between relatives) Increased risk of Autosomal Recessive disorder.		Off-springs
	Dizygotic twins		Monozygotic twins
	Infertility/No kids		

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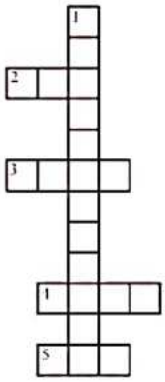
CROSS WORD PUZZLES

Crossword Puzzle



Across

2. Found by the scientist named Karry B Mullis.
3. For multiple mutations detection
4. Cells used are kept at an interface.
5. When it is carried out on many patients- Microaaray

Down

1. Study of chromosomes

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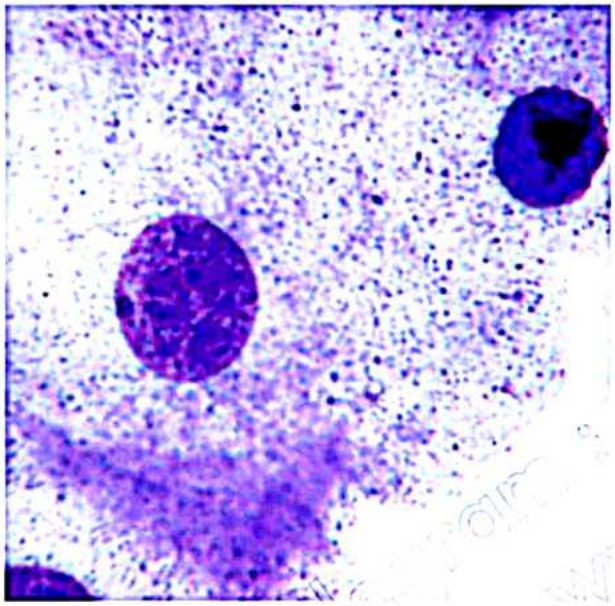
11 MISCELLANEOUS CONCEPTS IN GENETICS

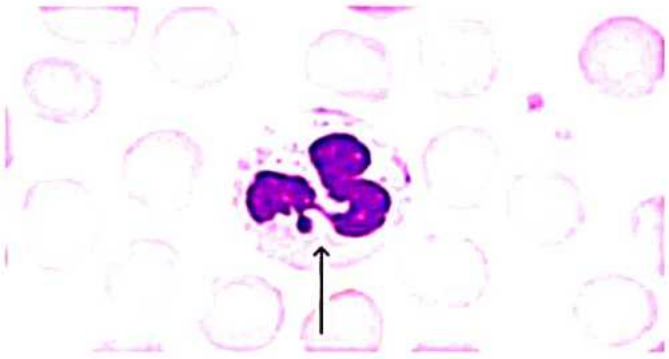
Lyon Hypothesis

It is a hypothesis proposed to decide which of the two X chromosomes to be kept inactive in a female child.

- Random inactivation: Maternal or paternal chromosome inactivation (3-30% activity).
- It takes place on day 5.5 of the embryo stage.
- It is carried out using the process of DNA Methylation.
- The XIST gene regulates this.
- The inactive X chromosome is called the Barr body or Davidson body.



- Barr body is a dot-like structure at the nucleus's periphery in the buccal mucosa sample.
- Davidson body is the drumstick-like structure present in the peripheral smear sample.



Formula:

No. of Barr body = No. Of X chromosome-1

- For Normal females (46XX) → 1
- For Normal males (46XY) → 0
- For Klinefelter's male (47XXY) → 1
- For Klinefelter (48XXXY) → 2
- For tuner's female (45XO) → 0

Knock Down and Know Out Technology 00:08:00

Knock Down Technology

Non coding RNA

DNA	DNA
↓	↓
RNA	RNA
↓ Coding RNA	↓x Non coding RNA
Protein	Protein

- Micro RNA is made up of 22 nucleotides (small size).

Formation of Micro RNA

- In the nucleus, out of multiple hairpin-like structures(Primary Micro RNA) created by DNA.
- A single-looped precursor microRNA is cut out using the scissors Drosha and DGCR8.
- The precursor microRNA enters the Cytoplasm using a channel called Exportin5.
- In the cytoplasm, the precursor microRNA's head is diced out from the loop using DICER.
- The design results in a two-strands structure called a duplex.
- The duplex is divided into two single strands, known as Single stranded mRNA.
- This RNA gets incorporated into the RISC (RNA-Induced Silencing Complex) molecule, stopping protein synthesis.

Types of Micro RNA

Nature	Name	Count	Helps
Good	Tumor Suppressor mRNA	mRNA 15-16	Prevents cancer
Bad	ONCOmirs	155-200	Causes Cancer

- In leukemia CLL, 13q is deleted, good mRNA 15,16 present in that also gets deleted. Thus, CLL leads to cancer.
- ONCOmirs lead to Hematological malignancies.

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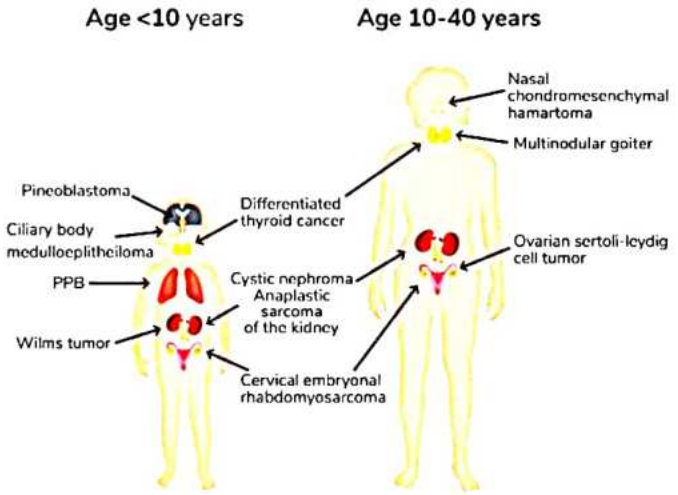
Dicer Mutation

00:20:07

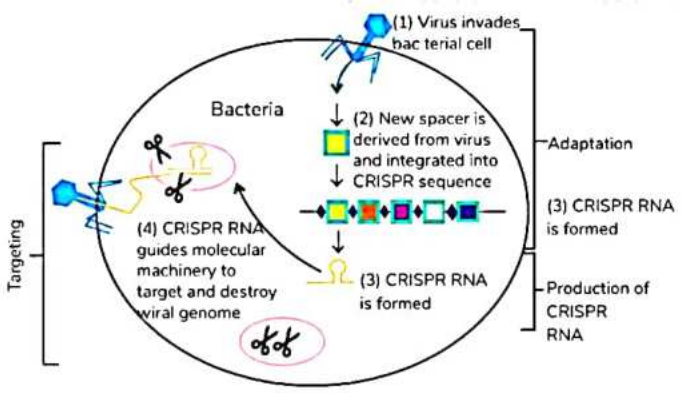


- It is present in children under 10 in
  - Pinealoblastoma
  - Pluero pulmonary blastoma (PPB)
  - Wilms Tumor nephroblastoma.
- And for ages 10-45, it is present in
  - Multinodular goiter
  - Sex cord-stromal tumors of the ovary
  - Ovarian seritoli Leydig cell tumor

Knock Out Technology

Editing DNA

CRISPR (Clustered Regularly Interspaced Palindromic Repeats) Case9



- Jennifer and Charpentier was given a Nobel prize for detailed study of CRISPR Cas9.
- It is a micro-bacterial defense against viruses.
- The spaces between the palindromic sequence take a snap of the virus that tries to enter the bacteria.
- This mechanism helps in remembering the particular virus and kills it the next time enters.
- Once the bacteria has collected the screenshots of viruses, the CISPR is formed.
- Cas9 acts as a scissor and cuts the segment of the virus and throws it out of the cell.
- Presently, CIPR Cas9 is used for genome editing in humans for protection against viruses
- The technology has helped in treating HIV, and muscular dystrophy
- Feluda Test of COVID-19 is based on the CISPR Cas9.

Epigenetics (above genetics):

00:28:42

1. Also known as imprinting.
2. Techniques used for analysis of Epigenetics:
   - bisulfate sequencing,
   - CHIP technique(Chromatin Immuno Precipitation), and
   - DNA methylation studies.
3. These techniques help to identify if there is any muting or activation in the genome.

MCQ

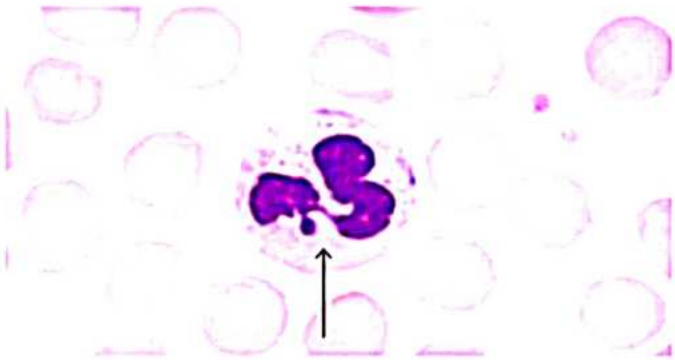
Q. What does RISC combine with?

a. Pre miRNA
b. Pri miRNA
c. miRNA-miRAN duplex
d. Ss miRNA

Q. During the process of microRNA production, which of the following molecule is responsible for removing the loop in the hairpin loop configuration?

a. DROSHA
b. DICER
c. DNA POLYMERASE
d. REVERSE TRANSCRIPTASE

Q. Which of the following statement is incorrect about the phenomena shown below?



a. Inactivation of X chromosomes is seen.
b. 100% genes on X chromosomes are inactivated.
c. Inactivation occurs in embryo.
d. molecular basis of lyonisation involved in the XIST gene.

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PREVIOUS YEAR QUESTIONS

Q. All are seen in fragile X syndrome except. (JIPMER 2018)
A. Testicular enlargement
B. Mental retardation
C. Trinucleotide repeats
D. Genomic imprinting

Q. Which of the following is not a part of quadruple test? (AIIMS 2018)
A. AFP
B. Estradiol
C. βHCG
D. Inhibin B

Q. Which of the following is a manifestation of 22q11 mutation syndrome? (AIIMS 2018)
A. Hypercalcemia
B. Conotruncal abnormalities
C. Thymic hyperplasia
D. Dysmorphogenesis of 1st & 2nd pharyngeal pouches

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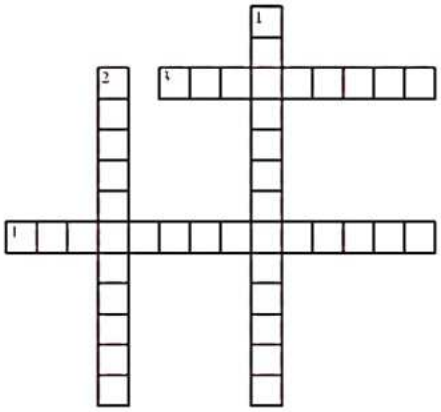
CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Editing RNA into Micro RNA during the post-transcription phase.
4. It is a hypothesis proposed to decide which of the two X chromosomes to be kept inactive in a female child.

Down

1. It is present in children under 10 in Pinealoblastoma
2. These techniques help to identify if there is any immuting or activation in the genome.

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12 CLASSIFICATION OF NEOPLASMS

- Neoplasia refers to the new growth of tumours.
- When tumour cells are growing, host cells produce fibrous stroma, creating a condition known as Desmoplasia, resulting in a hard consistency to tumour cells
- On Palaption:
  - Lump is soft, firm, it indicates a benign tumour;
  - If it is a hard lump, it is malignant tumour.
  - The more aggressive the tumour, the more desmoplasia (formation of fibrous stroma) and the hard consistency of the tumour.
- Parts of a tumor can be divided into Tumor cells and Fibrous Stroma
- Maximum Dysplasia is seen in:
  Cholangiocarcinoma (Cancer of the bile duct) and Pancreatic Adenocarcinoma

Classification of Neoplasms 00:05:06

- Benign tumours
- Malignant tumours
- Teratoma
- Mixed tumours

Benign Tumors (OMA suffix used)

- Oma is usually referred to as benign mesenchymal tumours
- Eg: Lipoma (benign tumour of fat), Fibroma (benign tumour of Fibroblast), Osteoma (Benign tumour of bone), Adenoma (benign tumour of glands)
- Papilloma is often confused with Polyp as both have finger-like projections: however, the basic difference indicates that Papilloma indicates surface whereas Polyp indicates internal organs; for example, Intestinal Polyp, Skin Papilloma.

The Rule And The Exception

- Usually, OMA refers to benign tumour types; however, there are a few exceptions to the case
- Lymph"oma": Lymph cancer
- Melan"oma": Skin cancer
- Semin"oma": Testicular Cancer
- Terat"oma": Cancer of ovaries (+/-)
- Chloroma: Blood cancer (AML) occurring in soft tissue (M/C in Orbit resulting in Proptosis)

Malignant Tumors (Cancer)

- Carcinoma (originating from epithelial tissue) Eg: Squamous Cell Carcinoma, Transitional Cell Carcinoma, Adenocarcinoma.
- Sarcoma (Originating from mesenchymal tissue) Eg: Osteosarcoma, Liposarcoma, and Chondrosarcoma.

Myosarcoma Muscle Cancer

- Leiomyosarcoma (smooth muscle cancer)
- Rhabdomyosarcoma (skeletal muscle cancer)

Teratoma

- Definition: ⩾ 2 germ layer (Ectoderm, mesoderm, endoderm)
- Teratoma can be benign (mature teratoma) or malignant (immature teratoma)
- Dermoid Cyst (Benign Cystic Teratoma)

- Tuft of hair present, hollow cavity and Rokitansky Protuberance present.
- Cutting through Rokitansky Protuberance will show whether cancerous changes are present or not, indicating whether the teratoma has malignant tendencies.

Mixed Tumor

- Example:
  - Pleomorphic Adenoma/ Benign Mixed Tumor [tumor of salivary gland (M/C parotid gland)]
  - It is known as Benign Mixed Tumor because it contains both epithelial (adenoma) and mesenchymal (chondromyxoid stroma) components
  - Wilms Tumor/ Nephroblastoma [Contains Epithelial, Mesenchymal, and Blastemal components]
  - Also Known as Triphasic Tumor
  - Occurs in kidneys of kids.

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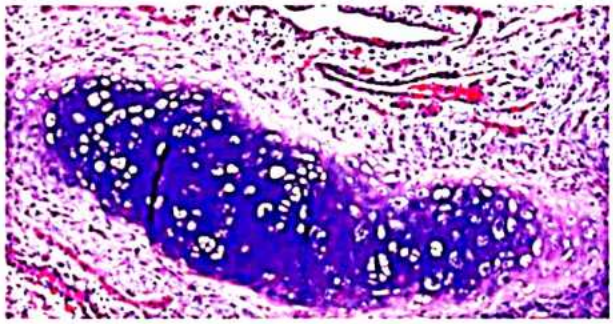
Hamartoma 00:25:48



- Benign Tumor caused due to abnormality in Chromosome 6.
- Haphazard Proliferation of native/ indigenous tissue.
- E.g., Pulmonary Hamartoma, AKA Chondroma.
- Radiology Shows Coin Lesion And Popcorn Calcification

Choristoma

- Not a tumour, it is an ectopic tissue proliferation.
- Eg. pancreatic tissue present on stomach wall

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13 FEATURES OF NEOPLASIA

These are some of the features that are common across all types of cancer.

- Anaplasia
- Rate of growth
- Local invasion
- Metastasis

One Liners

- Anaplasia - Hallmark of neoplasia
- Rate of growth
- Local invasion - second most reliable sign
- Metastasis - a sure or reliable sign of malignancy

Anaplasia 00:01:16

- Loss of differentiation is known as anaplasia.
- Differentiation is the structural and functional similarity of a tumor to parent organs.

Hallmarks:

1. Hallmark of apoptosis - Nuclear chromatin condensation (NCC)
2. Hallmark of Neuronal apoptosis - apoptosis-inducing factor (AIF)
3. Hallmark of Barrett's esophagus - Goblet cell
4. Hallmark of Acute inflammation - Increase vascular permeability (IVP)
5. The hallmark of chronic inflammation - Tissue destruction
6. Hallmark of repair - Granulation tissue

Dysplasia and Anaplasia

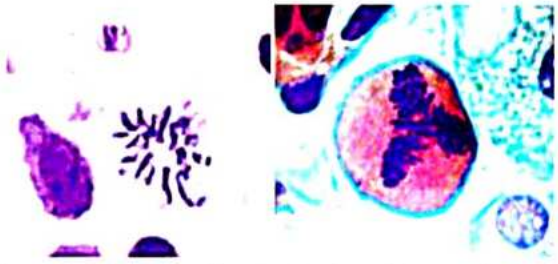
- Dysplasia is precancer, and Anaplasia is cancer.

Common features:

1. Loss of polarity - Random arrangement of cells
2. Pleomorphism - variation in size and shape
3. Increase N: C ratio

a. Normal cell: Nucleus: cytoplasm ratio is 1:4 - 1:6
b. Cancer cell: Increased Nucleus: cytoplasm ratio is 1:1

4. Hyperchromasia - More of blue color due to high chromatin
5. Mitosis - signifies the division of cells.

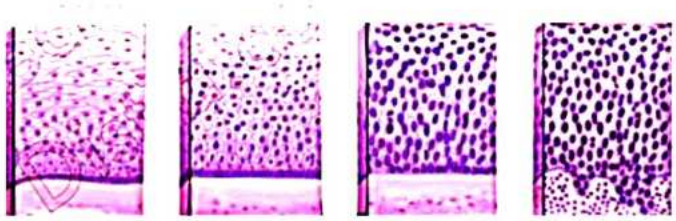


Showing mitosis → sunburst mitosis and mercedes benz sign

Dysplasia	Anaplasia
1. As long as the basement membrane is intact → precancer stage	1. As soon as the Basement membrane breached → cancer stage

Metaplasia	Dysplasia	Anaplasia
1. This is one tissue changing into another tissue.	1. Precancer	1. Loss of differentiation/ cancer
2. Reversible	2. Partially reversible	1. Irreversible

Precancerous - Dysplasia / CIN (Cervical Intraepithelial Noplasia)



CIN I CINII CINIII(CIS) Cancer

CIN-I - lower 1/3rd of the epithelium shows dysplasia.
CIN II - lower 2/3rd of the epithelium shows dysplasia.
CIN III / CIS - almost full thickness
CANCER the basement membrane is broken. This is anaplasia (cancer)

Rate of Growth 00:21:05

- Rate of growth for benign Tx is less, while a rating of growth is more for malignant tx.

Q. Minimum weight of tumor for clinical detection - 1 gram = 10^9 cells

Q. Maximum weight of tumor compatible with cell - 1 kg = 10^{12} cell

Local invasion and metastasis

- All cancers are going to show metastasis except
  - BCC - Basal cell carcinoma / Rodent's ulcer
  - Brain Tumor/Glioma
- These above show loca invasion more commonly.

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Pathways of metastasis

- Lymphatic spread
  - All carcinomas, except;
    - → RCC - (Renal cell Carcinoma)
    - → HCC - (Hepatocellular Carcinoma)
    - → Follicular carcinoma thyroid
  - The above ones are going to show hematogenous spread.

Lymphatic Spread -Sentinel LN 00:32:33

- First lymph node to receive lymphatic drainage of an organ.
- Axillary LN is sentinel LN for the breast.
- If sentinel LN is skipped and another LN is involved - Skip Metastasis
- Why does this happen? There is radiotherapy-induced fibrosis that is causing a blockage.
- Sentinel LN is used in the breast, colon, vulval and melanoma.
- Hematogenous spread
  - Arteries have thick walls. Veins have thin walls. So, veins are easily affected.
  - All sarcomas, except;
    - → Rhabdomyosarcoma
    - → Synovial Sarcoma

    This will go into lymphatic channel.
- Direct seeding of body cavities
  - Body cavity
    - → Largest body cavity around GI organs - Peritoneal cavity
  - Example:
    - → **Pseudomyxoma Paritonei**- Mucin secreting carcinoma from appendix or ovary shows spread to peritoneal cavity
    - → Most common cause of pseudo myxoma peritonei - Appendix carcinoma
  - Example:
    - → Distal end of peritoneal cavity -> pouch of Douglas
    - → Gastric carcinoma can spread to pouch of Douglas, which is known as the Blummer shelf.
    - → **Drop Metastasis:** Spread of medulloblastoma to CSF

- Transcoelomic spread 00:43:55
  - Coelomic means body cavity.
  - Examples:
    - → It goes via the body cavity to the ovary & gets deposited in the ovary, known as Krukenberg Tumor.
    - → Breast cancer
    - → Stomach cancer
    - → Colon cancer
    - → Pancreas cancer
- Tumors showing Bony metastasis
  - P - Prostate cancer
    - → goes to the Lumbosacral bone via Batson's venous plexus.
    - → It will make a new bone that is osteoblastic.
  - U - x
  - B - Breast cancer
    - → It can be osteoblastic or It can be osteoblastic or osteolytic.
  - L - Lung cancer
    - → It can be osteoblastic, or It can be osteoblastic or osteolytic.
  - I - Intestinal cancer
    - → It can be osteolytic.
  - K - Kidney cancer
    - → It can be osteolytic.
  - Toilet - Thyroid cancer
    - → It can be osteolytic.

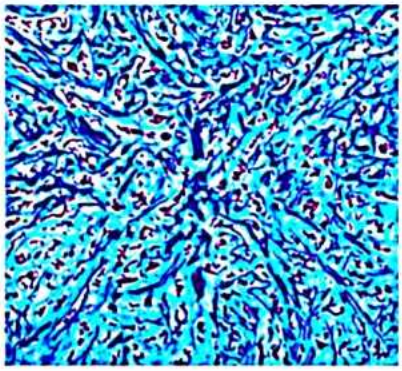
Q. A 63-year-old woman with chronic bronchitis presents with shortness of breath. A chest x-ray reveals a 2-cm "coin lesion" in the upper lobe of the left lung. A CT-guided lung biopsy is obtained. Which of the following describes the histologic features of this lesion if the diagnosis is hamartoma?

a. Mass of epithelial tissue
b. Disorganized normal tissue
c. Ectopic islands of normal tissue
d. Granulomatous inflammation

HAMARA

- Native tissue
- Coin lesion
- Popcorn calcification in fibroadenoma is common

Q. A 50-year-old woman presents with a lump in her breast. A 4 cm firm and fixed mass are noted on breast examination. Excisional biopsy reveals malignant cells that form gland-like structures and solid nests surrounded by a dense collagenous stroma. A connective tissue stain (trichrome) of the biopsy is shown in the image. Which of the following descriptive terms best describes the blue areas observed in this specimen?



a. Mucinous carcinoma
b. Tubular carcinoma
c. Desmoplastic change
d. Inflammatory carcinoma

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- Muscle means red
- Collagen means blue
- Every tumor cell has two parts - tumor cells and collagen made of fibrous tissue

Q. A 20-year-old woman has an ovarian tumor removed. The surgical specimen is 10 cm in diameter and cystic. The cystic cavity is found to contain black hair and sebaceous material. Histologic examination of the cyst wall reveals a variety of benign differentiated tissues, including skin, cartilage, brain, and mucinous glandular epithelium. What is the diagnosis?

a. Adenoma
b. Chondroma
c. Hamartoma
d. Teratoma

Q. A 45-year-old woman presents with abdominal pain and vaginal bleeding. A hysterectomy is performed and shows a benign tumor of the uterus derived from a smooth muscle cell. What is the appropriate diagnosis?

a. Leiomyoma
b. Leiomyosarcoma
c. Myxoma
d. Rhabdomyoma

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14 FUNDAMENTALS OF NEOPLASIA PART-1

Self-sufficiency of growth signals 00:01:00

Factors needed for growth

1. A growth factor is required for the growth of any cell.
2. The growth factor binds with the Receptor to make the Growth factor reception (GFRc).
3. The GFRc sends a signal from outside the cell body to nuclear transcription factors (NTF) inside the cell, called signal transduction protein.
4. The NTF using the signal, activates the cell cycle.

Growth Factor Mutations 00:04:47

1. PDGF beta: β → Brain tumor
2. TGF Alpha: α → Astrocytoma.
3. FGF gene: 2 forms
   a. HST- osteosarcoma
   b. FGF3- Urinary bladder and gastric cancer
4. HGF (Hepatocyte GF)- Hepatocyte cellular carcinoma (liver tumor)
   a. Also known as the Scatter factor

Growth Factor Receptor Mutations

Point mutation

Amplification - Increase in activity

- ERBB2/EGFR2/HER 2 neu : Breast cancer

1. ERBB1/EGFR1: Adenocarcinoma of lungs
   ERBB2/ HER2 NEU- Breast carcinoma
2. FLT3: Leukaemia
3. ALK:
   - Adrenal gland (Child)-Neuroblastoma
   - Adenocarcinoma lungs
   - IMFT(Inflammatory myofibroblastic tumor)
4. RET gene: (gain of function mutation)
   - Present in chromosome 10
   - Causes medullary carcinoma thyroid
   - Associated with Men 2a, Men 2b syndrome.

   } Gain of function
   - If there is a Loss of function, mutation in the RET gene leads to the loss of Ganglion cells, which causes Hischsprung disease.
5. KIT(CKIT/CD117):
   - Gastrointestinal stromal tumor (GIST)
   - AML leukemia
   - Mastocytosis
   - Melanoma
   - Seminoma
6. PDGFR beta: Brain tumors

Signal Transduction Protein Mutations 00:18:22

1. **RAS-** Most common oncogene to a mutation in human tumors

Working of RAS:

- Normal cells have receptors called Farnesyl anchors, which anchor on RAS.
- RAS is activated by changing GDP into GTP.
- RAS activation leads to BRAF and PI3K pathway activation.
- To restrict the working of RAS, GTP is broken using GAP(GTPase activation proteins).
- The NF1 gene is a GAP.

Type of RAS

A. KRAS- MNEMONIC: KAPIL
   - Colon cancer
   - Pancreatic cancer
   - Lung

B. HRAS:
   - Renal cell carcinoma
   - Urinary bladder cancer
   - Pituitary carcinoma

C. NRAS:
   - AML
   - Melanoma

D. GNAQ- Uveal melanoma(eye tumor)

E. GNAS- Somatotroph adenoma

2. **NOTCH- T Cell leukemia**
3. **JAK- Janus kinase** (seen in polycythemia vera)
4. **ABL**
   - Non-receptor Tyrosine kinase activity.
   - BCR and ABL fusion translocation(9:22) increases Tyrosine kinase activity.
   - Different leukemia is caused according to the weight t(9:22) BCR-ABL fusion:
     → 190 kd - ALL
     → 210 kd- cause CML
     → 230 kd- cause chronic neutrophilic leukemia (CNL).
     → Less than the weight of protein fusion, then Tyrosine kinase activity increases.

5. **BRAF(BRAFV600E):**
   - Single BRAF mutation can cause:

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→ HCL (Hairy cell leukemia)
→ LCH (Langerhans cell histiocytosis)
→ Papillary carcinoma thyroid
→ Pilocytic astrocytoma (Brain)
→ Melanoma
→ Colon cancer

Nuclear Transcription Factors Mutation 00:34:05

1. **Myc:**
   Types:
   - C Myc- Burkitt Lymphoma (children)
   - L Myc- small cell lung cancer
   - N Myc: Neuroblastoma
2. **FOS**
3. **JUN**
4. **REL:** associated with Hodgkin's lymphoma.

Cell Cycle Phases

1. G0- Inactive
2. G1- Enters Cell Cycle
3. S- Synthesis of DNA → Point of no return in a cell cycle → 6 hrs
4. G2 → 4 hrs
5. M → Mitosis (2 hrs)
   - G0 and G1 have a variable time frame.
   - G2 and M are the most radiosensitive phases. (M>>G2)
   - The least radiosensitive phase is the S phase.
   - Most radiosensitive tumor - Ewing's sarcoma.
   - Most radiosensitive testicular tumors- seminoma.
   - Most radiosensitive cells- Lymphocyte
   - Least radiosensitive cell- Platelet

2 checkpoints in the cell cycle at which the p53 gene (policeman) is present:
- G1S phase (p53, RB gene)
- G2M phase (p53, BRCA gene)

The RB gene (governor of the genome) and is present at the G1S phase

Cell Cycle Progression (Smooth phase-to-phase transmission)

- Cyclins
- CDK (Cyclins dependent Kinases)
  → Cyclins and CDK work in combinations:

Cyclins	CDK	Purpose
D	4,6	Triggers cells to move G0→ G1→S phase
E	2	Prepares cell for DNA replication in S phase
A	2	Activates DNA replication inside the nucleus in S phase
B	1	Promotes assembly of mitotic spindles and prepares the cytoplasm for mitosis

Cell cycle inhibition: 00:47:52

CDK Inhibitor families stop the cell cycle.

- Cip/Kip family: p21,p27,p57
  - P21 is induced by p53
- INK4/ARF family-p16,p14
  - Deletion/ mutation of P16(CDKN2A) causes PSM tumors
    → Pancreatic cancers
    → Squamous cell carcinoma
    → Melanoma

Cell Cycle Mutation

1. Cyclin D1- causes mantle cell lymphoma
2. CDK4 - causes glioblastoma (Grade 4 tumor)

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15 FUNDAMENTALS OF NEOPLASIA PART-2

- Self-sufficiency of growth signals
- Inactivation of tumor suppressor genes
- Limitless replicative potential
- Evasion from apoptosis
- Angiogenesis
- Invasion and metastasis
- DNA repair defects
- Warburg effect
- Immune surveillance escape

Tumor Suppressor Genes

00:00:20

Gene	Chromosomes
P53	Chr 17p13
RB	Chr 13q14
NF1	Chr 17
NF2	Chr 22
BRCA1	Chr 17
BRCA2	Chr 13
WT1	Chr 11p
WT2	Chr 11p
APC	Chr 5q21
PTEN	Chr TEN 10
CDHI	E-cadherin 16 Chr

p53:

- Chromosomes:
  - Chr 17p13
- Aka:
  - Policeman of genome
  - Guardian of genome
  - Gatekeeper of genome
- p63
  - It is needed for Squamous Epithelial Differentiation.
- p73.
  - It is the big brother of p53.
  - It is Pro-apoptotic.

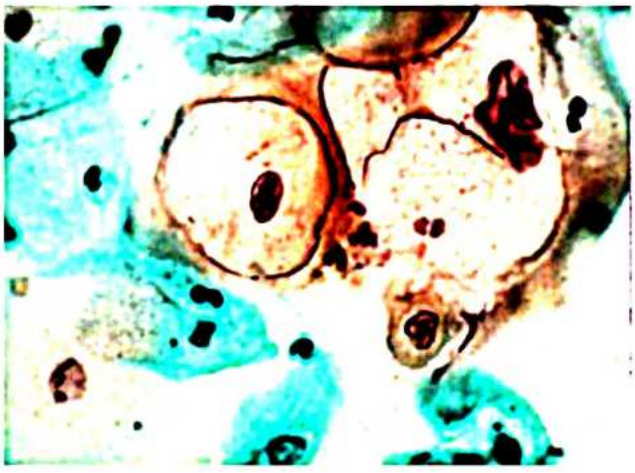
Normal Functions:

- Whenever there is DNA damage, p53 gets activated.
- It gives a stop cell cycle sign at the initial stage by activating the p21.
- This plays a major role in repairing the cell by GADD45.
- If the cell repair fails.
  - p53 activates apoptosis.
    - → Initially, it will activate the miRNA 34.
    - → Arrest cell cycle p21
    - → Repair GAAD 45
    - → If this fails, then MiRNa 34 will activate proptosis.
- p53 will be regulated by MDM2. They work in opposition, i.e., it is also denoted as a negative regulation.

p53 Disease:

- **Germline Mutation:**
  - If there is a germline mutation. This will result in a syndrome, which is called the Li-Fraumeni syndrome(AD).
  - This syndrome can be identified by:
    - → Breast cancer
    - → Brain cancer
    - → Bone cancer
    - → Blood cancer
  - Chompret criteria are associated with Li-Fraumeni syndrome.

- **Human papillomavirus:**



  - It causes cervical cancer.
  - HPV consists of 2 proteins, E6 and E7.
  - The protein E6 will turn off the p53, and the E7 will affect the RB.
  - Koilocytes are the cells that are associated with HPV.

RB Gene:

- Chromosome:
  - Chr 13q14

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- Aka:
  - Governor of Genome.
- Normal form:
  - RB stands for retinoblastoma gene.
  - The normal form of the RB gene is hypophosphorylated
  - It is always associated with E2F.
  - If phosphorylation occurs, the RB will combine with Po4.
  - The E2F molecule, Will enter the cell cycle and causes Proliferation.

RB Gene Mutation:

- It will cause retinoblastoma.
- The hypothesis which is associated with the mutation is Knudson'sTwo hit hypothesis, which is also known as (LOH) loss of heterozygosity.
- There are 2 types of RB:
  - **Familial RB:**
    - → The first hit will occur before birth.
    - → 2nd hit after birth, leads to retinoblastoma
    - → Bilateral tumor
    - → There must be risk of other cancers as well, which include all osteosarcoma and pinealoblastoma.
  - **Sporadic RB:**
    - → Here, when the person is born, they are born normally without any hit.
    - → Therefore in the lifetime, when they get 2 hits, Only then retinoblastoma occurs.
    - → The most common variety of retinoblastoma is the sporadic RB.
    - → Unilateral tumors
- **Trilateral RB:**
  - When a person has bilateral RB and occurs with Pinealoblastoma, then they are said to have trilateral RB.
  - It is a form of familial RB.
- Microscopically
  - Homer Wright PseudoRosette.
  - Flexner wintersteiner Rosette.

APC Gene:

- If an APC mutation occurs, it causes Familial Adenomatous polyposis.
- The polyposis has cancer potential to cause colon cancer
- Therefore, the APC gene is also called a protector against colon cancer. It is also known as the gatekeeper of colonic neoplasia.
- Chromosomes:
  - Chr 5q21

PTEN Gene: 00:30:25

- Chromosome:
  - Chr 10
- Mutation:
  - CowDen Syndrome Shows:
    - → Endometrial carcinoma
    - → Thyroid cancer
    - → Trichilemmomas
    - → Polyps of intestine
    - → Breast carcninoma

CDH1 Gene:

- It forms of e-cadherin.
- Acts as cell adhesion molecule
- Loss of E-cadherin leads to
  - Diffuse gastric cancer
  - Invasive lobular carcinoma.

Limitless replicative potential: 00:35:30

- Associated with telomerase which is limitless and keeps on replicating itself.

Evasion from apoptosis:

- Under this fundamental, the anti-apoptotic factor will increase.
- For example:
  - Follicular lymphoma: In this, the Bcl2 increases due to t(14:18).

Angiogenesis: 00:38:07

- In this case, the cancer is associated with the fact that they will form their own blood vessels.
- There will be 2 factors related to angiogenesis:
  - Promoters:
    - → If there is any growth factor, it will promote angiogenesis.
    - → VEGF
  - Inhibitors:
    - → If there is any Angiostatin, Endostatin, or Vasculostatin, it plays the role of an inhibitor.
    - → Thrombospondin is also an inhibitor.

Important Information

- Thrombospondin Inhibits angiogenesis
- Thrombostenin is the Contractile protein of platelets.

Invasion and metastasis:

- Purpose: to spread
- Initially, the detachment of the tumor cell takes place through the decrease in E-cadherin.
- Then degradation of the extracellular matrix occurs by MMP2, 9. (Matrix Metalloproteinases).
- Meanwhile, Tumor cells attach to Extracellular matrix by Integrins.

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- Lastly, the invasion into the blood vessels happens.
- After the invasion, the tumor cells become tumor emboli when the tumor cells get attached to the WBCs and platelets.

Important Information

- CD44
  - Some of the cancer cells express a marker which is called CD 44.
  - The CD44 will take it to the high endothelial venules of the lymph nodes.
- Epithelial-Mesenchymal Transition:
  - Epithelial cells will start looking like Mesenchymal cells.
  - They start looking at this because it is easier to metastasize.
  - The two molecules associated with metastasis are:
    - → SNAIL
    - → TWIST

Driver mutation VS passenger mutation:

- When E-cadherin mutation results in cancers like diffuse gastric cancer and invasive lobular carcinoma it is known as DRIVER mutation.
  However, when E-cadherin is lost as a secondary change in other tumors for the purpose of invasion it is known as PASSNGER mutation.

DNA repair Defects:

- Defects of DNA are:
  - **NER defects:**
    - → It stands for nucleotide excision repair gene.
    - → Considering the fact that UV lights create pyrimidine dimers in our DNA. Therefore, the NER will excise these dimers to repair the DNA.
    - → In case of NER defects, the person will be affected by Xeroderma Pigmentosum. It will cause a lot of skin cancers:
      - SqCC
      - BCC
      - MM
  - **MMR defects:**
    - → It stands for mismatch repair
    - → In cases where the DNA mismatches with each other in the process of repairing, the MMR plays the role of correcting it.
    - → For example:
      - MLH1
      - MSH 2, 6
      - PMS 1, 2
    - → If there is an MMR defect, it causes Hereditary non-polyposis colon cancer / CEO has gone for LUNCH
      - Colon cancer
      - Endometrial cancer
      - Ovarian cancer.
  - **Homologous recombination defect:**
    - → The UV light causes the double-stranded DNA break.
    - → Therefore, the homologous recombination genes help in repairing these breaks.
    - → However, in the case of Homologous Recombination defects, it causes:
      - Bloom Syndrome
      - Fanconi anemia (Bone marrow failure.)
      - Ataxia telangiectasia.

Important Information

- All the DNA repair defects are Autosomal recessive except for the HNPCC, which is autosomal dominant.

Warburg Effect:

- It is named after the scientist Sir Otto Warburg.
- It is also called aerobic glycolysis and Glucose hunger.
- By the oxidative phosphorylase, in a normal cell, 36 ATP is obtained.
- If oxygen is not present, glycolysis needs to be done to get 2 ATP.
- However, in the case of cancer cells, they want to go under any aerobic glycolysis, even in the presence of oxygen.
- This leads to the production of intermediate metabolites.
- The radiology Correlation of Warburg effects:
  - 18FDG Pet Scan:
    - → This is used to know how many cancer cells have been spread within the body.
    - → The Fluoro Deoxy Glucose injection is given to the patient.
    - → Glucose will be uptaken by the cancer cells.
    - → This helps in identifying the location of the cancer cells.

Escape Immune Surveillance

- Antigen-negative variants of tumor cells
- Antigen masking by glycocalyx
- Decreased MHC I expression
- Immunosuppression by- CTLA4, PDL1 (tumor cell), TGF beta
- Treatment of cancer - antiCTLA4 drugs (ipilizumab)

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MCQs:

Q. Which of the following is an incorrect match
a. JAK2-Point Mutation - PV
b. PGDFR B-Amplification - Glioma
c. RET-POINT MUTATION - Medullary Carcinoma
d. ABL-POINT MUTATION - CML

Ans. d. ABL-POINT MUTATION - CML

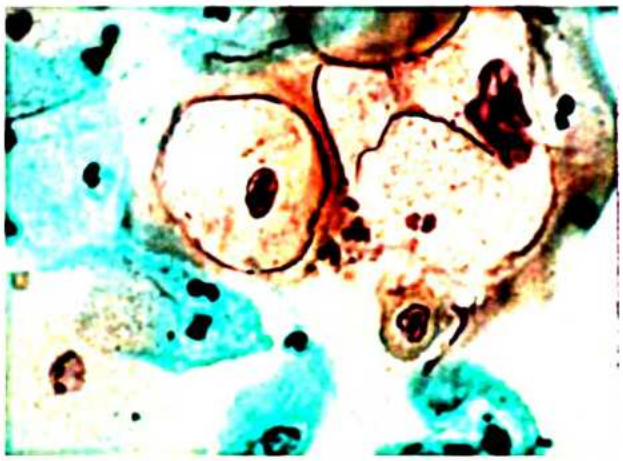
Q. GNAQ mutation is associated with?
a. Small cell lung carcinoma
b. Adenocarcinoma lung
c. Uveal melanoma
d. Pituitary adenoma

Ans. c. Uveal melanoma

Q. Which of the following describes the correct order of cyclins in cell cycle progression?
a. A/D/E/B
b. D/E/A/B
c. B/E/A/D
d. D/E/B/A

Ans. b. D/E/A/B

Q. A 25-year-old woman presents for a gynecologic examination. The cervical Pap smear shows "koilocytic atypia" characterized by perinuclear halos and wrinkled nuclei (shown in the image). A cervical biopsy reveals invasive squamous cell carcinoma. Molecular tests for human papillomavirus (HPV) in the tumor cells are positive. Which of the following mechanisms of disease best explains the role of HPV in the pathogenesis of neoplasia in this patient?



a. Activation of cellular oncogenes b.
b. Enhanced transcription of telomerase gene
c. Inactivation of tumor suppressor proteins
d. insertional mutagenesis

Ans. c. Inactivation of tumor suppressor proteins

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16 ETIOLOGY OF NEOPLASIA

Etiology and Diagnosis of Neoplasia

Q. Why do cancers occur?

- Radiation
- Chemicals
- Microorganisms

Radiation Carcinogenesis

00:03:25

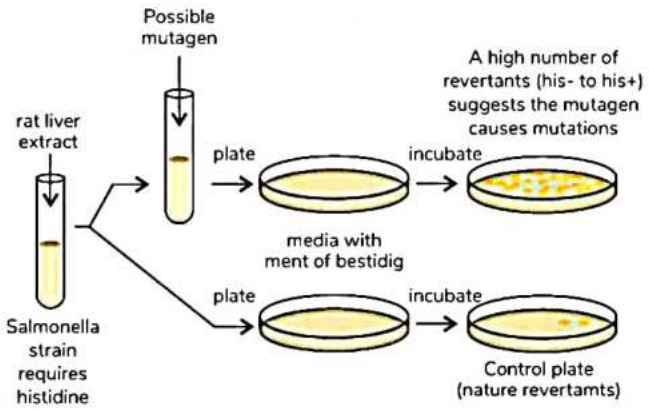
Ultraviolet radiation	Ionizing radiation
UV A	Q1.Most common cancer - Acute myeloid leukaemia —- AML
UV B - Bad UV - Skin cancer —- BCC,SQCC	Q2. Most common thyroid cancer- Papillary carcinoma thyroid
UV C	Q3 . Leukaemia Never associated with IR. Radiation —-CLL

Chemical Carcinogenesis

History

Sir Percival Pott : scrotal cancer occuring in chimney workers

AMES Test - used to know the mutagenic potential of a chemical



Important chemical-causing Cancers

- Alcohol — Hepatocellular carcinoma, Oropharynx, larynx
- Asbestos — it can cause lung tumour
  - Most common cancer— adenocarcinoma lung
  - Specific cancer —- mesothelioma lung
  - Head and neck tumour
- Benzene — AML
- Benzidine — urinary bladder cancer
- DES (DiEthyl Stillbestrol a hormone preparation) → given to pregnant mother→ to help in normal vaginal delivery → if girl born she has clear cell tumor of genital tract
  - DES daughters
- Estrogen → endometrial cancer
- OCP → cervical cancer
- Tobacco → oral, lung, RCC

Pesticides Sons

If pregnant ladies exposed to pesticides → then sons are born with testicular dysgenesis

Metals

- Cadmium– prostate cancer– pyq
- Chromium– lung cancer
- Nickel- Lung cancer
- Cobalt-Lung cancer
- Radon- Lung cancer
- V– Vinyl chrolide (PVC plastic)
- Arsenic (pesticides)
- T- Thorotrast (thorium - contrast dye)
- VAT directly impacts LIVER → Angiosarcoma liver

Microbial carcinogenesis

Bacteria — Helicobacter pylori in stomach

- Gastritis
- PUD
- Cancers
  - Gastric Adenocarcinoma
  - Gastric Maltoma

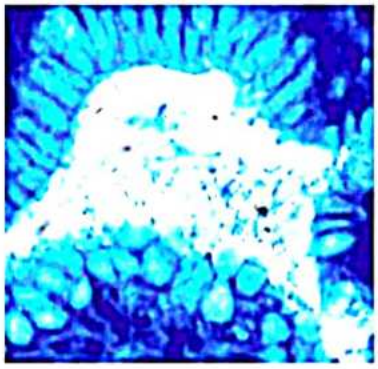
How does helicobacter pylori cause cancer?

Carcinogenesis is caused by:

- CAGA
- VACA

Stains

Telegram - @nextprepladdernotes



Warthin starry silver stain– black colour
Steiner stain
Modified Giemsa stain

Fungal 00:20:00

Aspergillus flavus → AFLA toxin– these contaminate peanut /groundnut
Causes mutation at CODON 249 of p53 gene– HCC

CODON 249

$P^{9}\ 5^{4}3^{2}$

Parasitic

S - S: Schistosoma Hemotobium - causes squamous cell carcinoma of urinary bladder

- Which microorganisms that cause carcinogenesis by metaplasia?
- Schistosoma

C - C: Clonorchis /Opisthorchis, it will cause cholangiocarcinoma

Viral

Refer Table 16.1

Cancer in EBV

Carcinoma	Nasopharyngeal carcinoma Gastric carcinoma– most common
Lymphoma	HL - HODGKIN'S – mixed cellularity NHL - non hodghkins • Burkitt's lymphoma • DLBCL • Post transplant lymphoma
Sarcoma	LMs leiomyosarcoma – smooth muscle cancer

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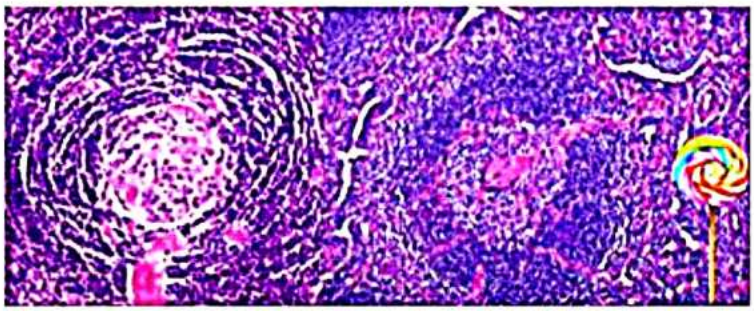
Table 16.1

HBV Hepato cellular carcinoma
HCV By HbX gene

HHV8 Human Herpesvirus, 8 causes

- Kaposi sarcoma –vascular tumour
- Primary effusion lymphoma
- Castleman's disease: Lypmphadenopathy:
  - Fever
  - Weight loss
  - LAP
  - Night sweats

Microscopically:

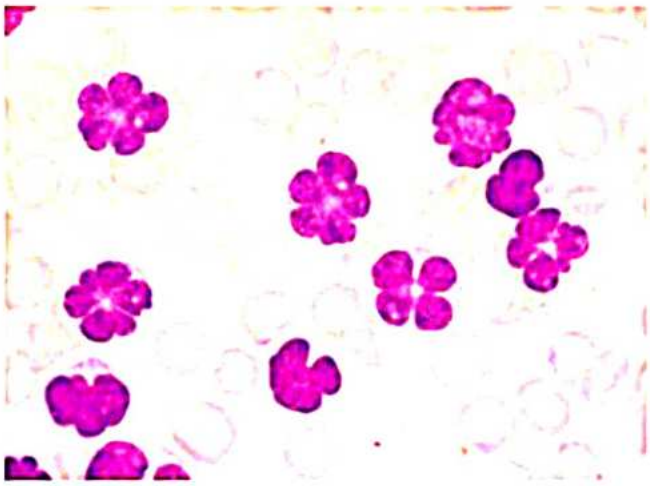


Types of Castleman's disease

- Hypervascular type— Onion skinning cells of lymphoid (mantle zone) & lollipop follicle (blood vessel)
- Plasma cell type

HTLV Human T Cell Leukaemia lymphoma virus, it causes:

- ATLL- Adult T cell Leukaemia Lymphoma
- Tropical spastic paraparesis
- Via tax gene cause cancer
- Clover leaf cell



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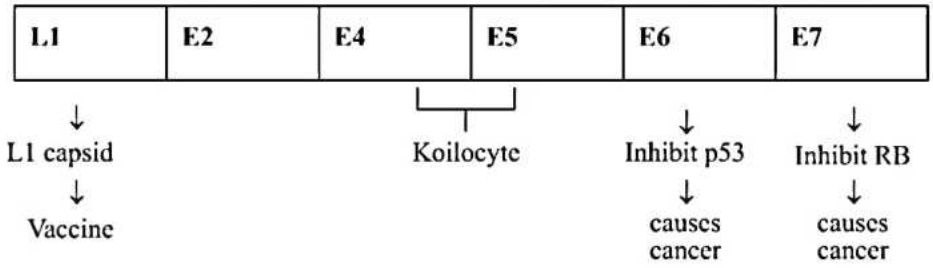
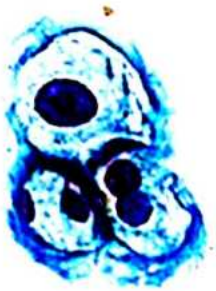
HPV Human papillomavirus

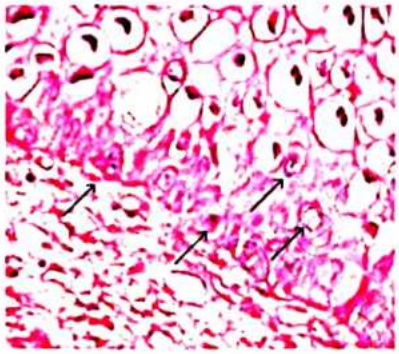
- Low Risk HPV
  - HPV 6, 11
  - they cause warts- condyloma accuminatum
- High-Risk HPV
  - HPV 16, 18
  - Squamous Cell Carcinoma –

[cervix penile ,anal , oesophagus, Larynx]

HPV Structure







Koliocyte

- Raisanoid nucleus is very dark in colour
- Around the nucleus is white, not pink
- Perinuclear Halo white space around the nucleus

EBV Ebstine Barr Virus

Q. Enters which cell of the body?
Ans. Adapt the B cell

Q. How does it enter the B cell?
Ans. CD 21 & CR 2 are the receptors

B cell

LMP 1

Act as CD 40 ligand

EBNA 2

- Increase in Cyclin D cell cycle starts working
- Increase in SRC Proto-oncogene

Virus IL10

- Interleukin 10 it is an(Ten)ti inflammatory

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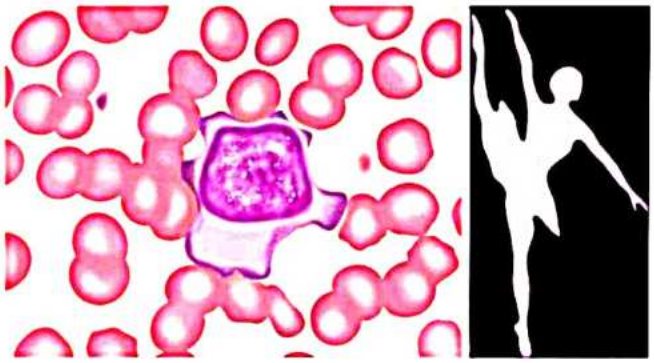
EBV



B cell is surrounded by EBV activates CD_8T CELL — known as ballerina skirt appearance (DOWNEY CELL).

Infections: **Infectious mononucleosis→ kissing disease → grandular fever →** enlarged glands, lymph nodes, salivary glands are enlarged.

Kissing disease – caused by close contacts

Diagnosis – Paul Bennell test
Monospot test

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17 DIAGNOSIS OF TUMORS AND PARANEOPLASTIC SYNDROME

Diagnosis of Tumors include:
- Tumor Markers
- FNAC
- Biopsy
- IHC

Tumor Markers 00:00:20

- Helps in
  - Diagnosis of cancer
  - Response to treatment
  - Follow up
- **Sample:** Blood (mostly)
- **Other samples:** Stool, Urine, Sputum.

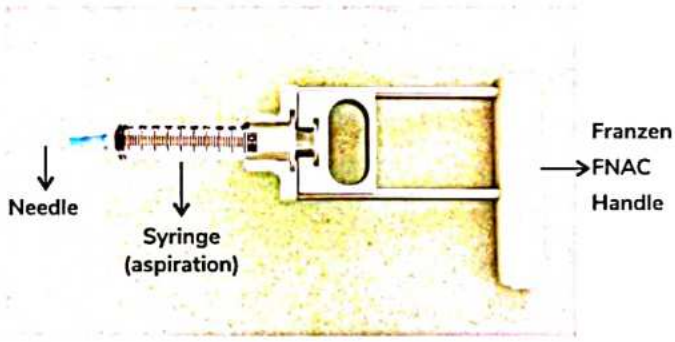
Why is only Beta HCG tested and not Alpha HCG?

- ALPHA SUBUNIT is common to other hormones such as TSH, FSH, LH and hence is non specific

Table of Tumor Markers 00:02:50

Category	Examples	Indicating Cancer
Hormones	• Beta HCG	• Choriocarcinoma, Non-seminomatous germ cell tumors.
	• Calcitonin	• Medullary carcinoma, Thyroid.
	• Catecholamines	• Pheochromocytoma.
	• 5HIAA (5 hydroxy indole acetic acid)	• Carcinoid tumor.
Oncofetal Proteins	• Alpha fetoprotein (AFP)	• Yolk Sac Tumor, Hepato cellular carcinoma (HCC), Hepatoblastoma.
	• Carcino embryonic antigen	• Colon cancer, Pancreatic cancer, Lung cancer.
Lineage Specific	• Prostate specific antigen (PSA)	• Prostate cancer.
	• Prostate acid phosphatase (PAP)	• Prostate cancer.
	• Immunoglobulins (IG)	• Multiple myeloma.
	• Neurons specific enolase (NSE)	• Neuroblastoma, Neuroendocrine tumors.
CA markers	• CA 125	• Surface epithelial Ovarian tumors.
	• CA19-9	• Pancreatic cancer.
	• CA15-3	• Breast cancer.
	• CA27.29	• Breast cancer.
	• CA72-4	• Gastric carcinoma.
Others	• NMP22	• Recurrent bladder cancer.

FNAC: Fine Needle Aspiration Cytology



- A 25-26 gauge needle is used.
- Contains following parts:
  - **Needle** - for insertion.
  - **Syringe** - for aspiration.
  - **Franzen FNAC Handle** - for support.

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Procedure for FNAC

- Swelling is visible - Direct FNAC.
- Swelling is not visible - Ultrasound-guided FNAC.

FNNAC: Fine Needle Non Aspiration Cytology
- Needle is used for FNAC without syringe/ aspiration.

Sample collected using FNAC or ultrasound-guided FNAC, or FNNAC
↓
Kept on a slide
↓
Air dry the sample and put Romanowsky stain
(or)
Dip into 95% ethanol - fixative of PAP stain (gives better nuclear details).

Contraindications of FNAC
- Bleeding defects.
- Hydatid cyst - the risk of anaphylaxis if FNAC is performed.
- Testicular cancer - risk of spread of cancer.

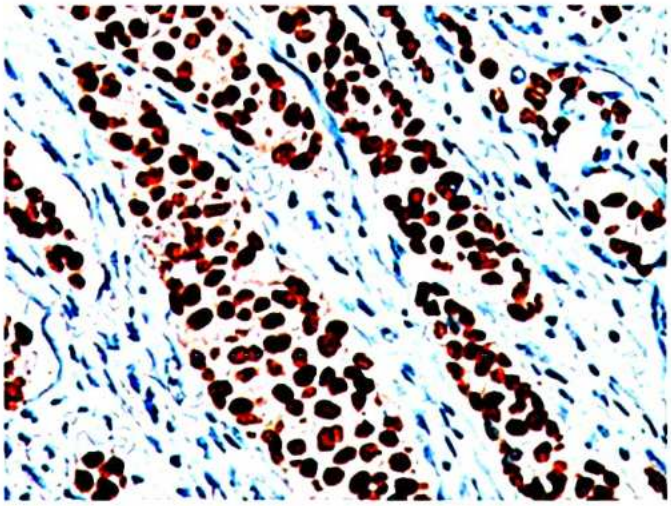
FNAC Pitfall
- Cannot differentiate between benign and malignant thyroid
  - Capsular and vascular invasion is seen with follicular carcinoma.
  - not seen with follicular adenoma

Biopsy
- Gold standard test for tumor diagnosis.
- **Tru Cut Biopsy:** Only a core of the tissue is collected and examined.
- **Excision Biopsy:** Entire lump is excised.

How is the sample sent to the pathology lab?
- **Electron microscopy:**
  - Use 2-2.5% glutaraldehyde as fixative.
- **Light microscopy:**
  - Use 10% Neutral Buffered Formalin (NBF) as fixative.
  - **Stains used:** Eosin (pink color), Hematoxylin (blue color).

History of Hematoxylin
- Originated from Heartwood of the Logwood tree (Hematoxylin campechianum).
- Previously used to dye clothes.

Immunohistochemistry
- Special staining technique.
- DAB (Diaminobenzidine) is used.
- Gives brown color.



Immunohistochemistry Markers 00:28:57

Marker	Tumor
Cytokeratin	Carcinoma
Vimentin	Sarcoma
Leukocytes common Ag (LCA) or CD45	Lymphoma
HMB45	Melanoma
S100	• Melanoma • Neurogenic tumors • Cartilage tumors • Langerhan cell histiocytosis

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IHC for Sarcoma 00:32:47

Leiomyosarcoma	Rhabdomyosarcoma
Smooth muscle sarcoma	Skeletal muscle sarcoma
Vimentin positive	Vimentin positive
Smooth muscle actin positive	• Desmin positive • MyoD1 positive

IHC for Carcinoma: CK7 and Ck20 00:34:43

Marker	Tuamor
CK7+ and CK20+	• Bladder cancer • Ovarian mucinous tumors **Mnemonic:** Both positive.
CK7+ and CK20-	• Breast cancer • Endometrial cancer • Ovarian tumors • Cervical cancer • Salivary gland tumors • Lung adenocarcinoma • Pancreatic cancer **Mnemonic:** Above colon and predominant in females.
CK7- and CK20+	• ColoRectal cancer • Merkel cell cancer **Mnemonic:** Below colon and CM20
CK7- and CK20-	• Hepatocellular carcinoma (HCC) • Renal cell carcinoma (RCC) • Squamous cell carcinoma of lungs (SqCC) • Small cell carcinoma of lungs (SmCC) **Mnemonic:** CC.

Paraneoplastic Syndrome - PNS 00:40:39

- Signs and symptoms of tumors but not due to metastasis.
- Most common PNS:
  - Hypercalcemia

Most common

- Endocrinopathy Cushing's disease
- Most common tumor associated with PNS - Small Cell Carcinoma of lungs.

PNS Table

PNS	Tumor
Hypercalcemia	• Lung cancer (SqCC) • Breast cancer • Renal cell carcinoma
Cushing's syndrome	• Lung cancer • Renal cell carcinoma
SIADH	• Lung cancer • Intracranial tumors
Hypoglycemia	Fibrosarcoma
Hyperglycaemia	Bone tumors
Acanthosis nigricans	• Lung cancer • Gastric cancer
Lambert Eaton syndrome	Lung cancer
Myasthenia gravis	Thymoma
Pure red cell aplasia	Thymoma
Migratory thrombophlebitis	• Lung cancer (adenoma) • Pancreatic cancer
Polycythemia (new update)	• Cerebellar Hemangioblastoma • RCC, HCC • Adrenal cortical tumors • Pheochromocytoma • Leiomyoma
Osteomalacia (new update)	Phosphaturic mesenchymal tumor (PGF 23 gene mutation).

00:51:05

Q. A 60-year-old man presents with a 4-month history of increasing weight loss, wheezing, and shortness of breath. He has smoked two packs of cigarettes a day for 40 years. His past medical history is significant for emphysema and chronic bronchitis. A chest X-ray shows a 10-cm mass in the left lung. Bronchoscopy discloses obstruction of the left main stem bronchus. A biopsy is obtained (shown in the image). Immunohistochemical studies of this biopsy specimen would most likely show strong expression of which of the following tumor markers?

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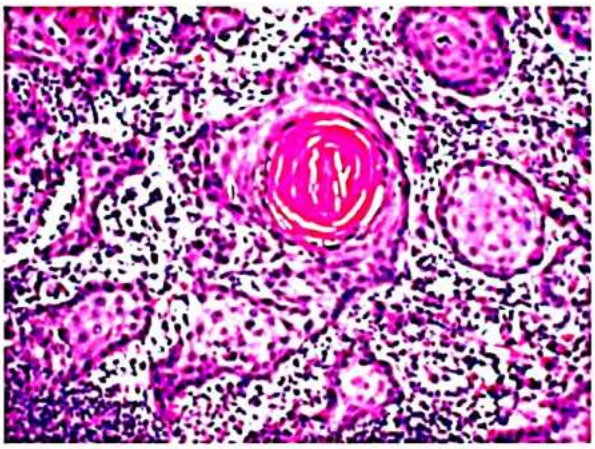

a. Alpha-fetoprotein
b. Calretinin
c. Carcinoembryonic antigen
d. Cytokeratins

Q. CA 19-9 is a tumor marker of?
a. Stomach cancer
b. Pancreatic cancer
c. Colon cancer
d. Lung cancer

Q. A 65-year-old man complains of muscle weakness and a dry cough for 4 months. He has smoked two packs of cigarettes daily for 45 years. A chest X- ray shows a 4-cm central, left lung mass. Laboratory studies reveal hyperglycemia and hypertension. A transbronchial biopsy is diagnosed as small cell carcinoma. Metastases to the liver are detected by CT scan. Which of the following might account for the development of hyperglycemia and hypertension in this patient?
a. Diabetes mellitus
b. Paraneoplastic syndrome
c. Pituitary adenoma
d. Pituitary metastases

Q. Find the incorrect match about tumor markers given below?
a. AFP - Yolk sac tumor
b. CA125 - Ovarian tumors
c. CA19-9 - Pancreatic cancer
d. Prostate alkaline phosphatase - prostate cancer

Miscellaneous Topics with New Updates 00:55:16

Onco Metabolism 00:55:24

- Cancer caused due to defects in metabolic enzymes.
- Enzyme mutations included:
  - Isocitrate dehydrogenase (IDH) mutation.
  - Succinate dehydrogenase (SDH) mutation.
  - Fumarate hydratase (FH) mutation.

Enzyme Mutation	Associated Tumor
MNEMONIC- I have EGO IDH mutation	• Oligodendroglioma • Glioblastoma • Enchroma
SDH mutation	Pediatric GIST
FH mutation	Papillary RCC

Spontaneous Regression

- Spontaneous vanishing of a tumor.
- Seen with: Neuroblastoma.

Liquid Biopsy 00:59:38

- Tissues are not the samples.
- body fluids like blood are taken as samples.
- Helpful to identify the tumor cells in blood.
- Samples found are:
  - Circulating tumor cells
  - Cell free DNA (cancer cell or normal cell).
  - Circulating tumor DNA.

Chimeric Antigen T cell - CART 01:03:59

- Named as Live Drug Kymriah.

Blood samples are taken
↓
T cells are separated
↓
Receptors are added to the T cell (based on the tumor cell type)
↓
Return the prepared T cells into the body
↓
Extracellular part of T cell receptor binds to Ag on tumor cell
↓
Intracellular part of the T cell releases mediators
↓
Destroys the tumor cell

CART therapy- live drug KYMRIAH

Epigenetics in Cancer 01:08:28

- Functional changes in the genes.
- Qualitative defect.

Refer Table 17.1

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Table 17.1

Epigenetic Changes in Genes	Tumor
DNMT3A	Acute myeloid leukemia
MLL1	Acute leukemias
MLL2	Follicular lymphoma
CREBBP	DLBCL
ARID1A	• Ovarian tumors • Endometrial tumors
SNF5	Malignant Rhabdoid tumor
PBRM1	Rental malignancy
H3K27	Diffuse midline glioma

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18 TYPES OF IMMUNITY

Types of Immunity

Acquired Immunity/ Adaptive immunity	Native/ Naive/ Natural/ Innate Immunity
• It is acquired after exposure to particular bacteria, virus or any infection. • It has memory • It has specificity • Macrophages present	• Since birth • No memory • It has no specificity • Macrophages present • Alternate complement system

Components of Innate Immunity

- **Epithelial barriers**: Skin, Mucosa
- **Cells**: Neutrophils, Macrophages (They help in phagocytosis)

- **Complement system**
  - **Other Name:** Alternate complement system (God has given)

- Innate lymphoid cells/ NK cells
- Pattern recognition receptors

Natural Killer Cells (NK Cells) 00:04:15

Other Names

- Innate lymphoid cells (ILC)
- Large granular lymphocytes
- MHC unrestricted cells
- Non B non T cells
- Null cells

NK Cells: Markers

- CD 16 (Receptor for Fc IgG)
- CD 56
- CD 94 (New marker)

NK Cells Towards Self Cells

- One arm is called activating arm(NKG2d)
- The other arm is inhibitory arm(CD94)
- Each self cell of our body contains MHC I
- When MHC1 cells are found, NK cells recognise and put the inhibitory arm forward and it doesn't kill it.

NK Cells towards Foreign Cells

- Viruses & cancer decrease the expression of MHC1.
- The activating arm kills the cell.

NK Cells

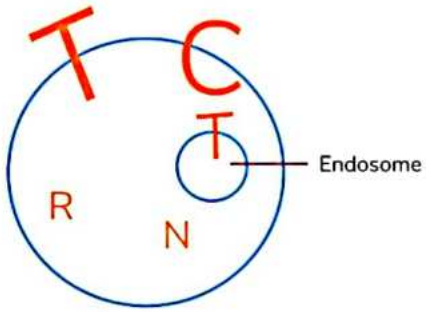
- Stimulation of NK cells:
  - Il2
  - Il15
- Activates killing of target cells
  - IL 12

Antibody Dependent Cellular Cytotoxicity (ADCC)

- Neutrophils
- Macrophages
- Eosinophils
- NK cells
- If any cell gets infected with virus/cancer, it gets coated by opsonin, IgG.
- To kill this virus IgG is picked up by these cells.
- For eg. NK cells are supposed to be attached with the FC portion of IgG.
- CD16 is the receptor for the FC portion of IgG.
- This is known as Antibody Dependent Cellular Cytotoxicity.

Pattern Recognition Receptors

- Toll like receptors
- C type lectin receptors
- RIG receptors
- NOD receptors
- These are the receptors which will recognise the pattern of bacteria, virus or any parasite.



PRR

- There are two receptors which are present on the membrane of the cell. These are membranous;
  - Toll like receptors
  - C type lectin receptors
- These are also present on membranes of organelles like endosomes.
- RIG and NOD receptors are cytoplasmic

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Functions:

- Toll-like receptors are going to identify a bacteria.
- C type lectin receptors will identify fungal organisms.
- RIG receptors will identify viral organisms.
- NOD receptors will identify;
  - **N:** Necrotic or dead cells
  - **O:** organisms
  - **D:** associated with Diseases like Diabetes mellitus

  NOD2 mutation in inflammatory bowel disease.
- Cell death associated with NOD2 receptors - Pyroptosis

TLR (Toll like receptors) (activate the NF Kappa B pathway).	Binds To
2	Gram positive(teichoic acid), TB
3	dsRNA
4	Gram negative
5	Flagellin
6	Mycoplasma (fried egg colonies)
7, 8	Ssrna (M/C)
9	CpG DNA

Update – Interferonopathies

- RIG Receptor is Retinoic Acid Inducible Gene
- It activates STING - Stimulator of Interferon Gene
- It increases IFN Alpha that results in a group of diseases known as Interferonopathies.

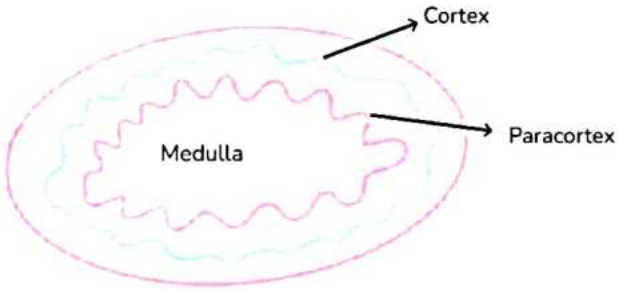
Acquired Immunity

- Acquired Immunity is two types
  - Cellular immunity: B and T lymphocytes
  - Humoral immunity: Immunoglobulins

Cellular Immunity- B and T Cells 00:27:20

Characteristic	T cells	B cells
Percentage	60-70%	20-30%
Formation	Bone marrow	Bone marrow
Maturation	Thymus	Bone marrow
Sites		
Lymph node	Paracortex	Cortex
Spleen	White pulp (PALS)	White pulp
GIT	IEL	MALT/PEYER'S patches

Lymph Node 00:30:10



- Cortex have B cells
- Paracortex have T cells
- Medulla and it has Macrophages (M for M).

T Cells

- Every T cell has T cell receptor (TCR)
- 95% cases are alpha beta receptors.
- In 5% cases it is the gamma, delta receptors

Gamma Delta T cells

(Recent PYQ)

- These are present only 5-10%.
- These are present in GIT, genitourinary tract.
- They don't require any MHC.
- They process lipids, peptides.

CD Markers of T Cells

- T cells have: CD 1, 2, 3, 4, 5, 6, 7, 8, 28, 40L.
- The Pan T cell marker is Cd3
- CD3 is going to take signal transduction molecules.
- Helper T cell is CD4 (TH)
- Cytotoxic T cell is CD8. They participate in killing the antigen
- CD4:CD8 ratio is 2:1

To Remember

In entire immunology, there is a Rule of 8 to study.

- CD4:CD8 = 2:1
- Rule of 8
- CD4:CD8 ratio reduces → CD4 reduces → HIV
- CD4:CD8 ratio increases → CD4 increases → sarcoidosis

Q1. Who is the master regulator of the immune system?
Answer: CD4 cells or TH cells are considered as the master regulators of the immune system.

Relation of the T-cells in Cancer

- T- cells has a PD receptor
- The cancer cell will code itself with PDL1
- When this receptor and ligand bind together, suppression of immunity (Immunosuppressive response)

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Treatment:

- Drugs like anti-PD-1 (Pembrolizumab) will block the PD receptors to prevent immunosuppression.

B Cells

- These cells have the B cell receptor with IgM and IgD antibodies.
- CD markers of B-cells
  - CD 10 (CALLA)
    - → C: Common
    - → ALLA: ALL Antigen
  - CD 19
  - CD 20, 21, 22, 23
  - CD 40
  - CD 79a, 79b
- **Pan B cell marker:** CD 19
- **Signal transduction molecule:** CD 79ab

Q2. How does the Epstein Barr virus enter the body?
Answer: Through B cells

Q3. How does it enter through B cells?
Answer: CD 21/ CR2 marker

Interaction between B and T Cells

- B cells have IgM and IgD antibodies
- IgM and IgD are converted into IgG, IgA, IgM, IgD, and IgE by a process called Isotype switching (Change)
- Mnemonic: **Ch**ange
  - **CH**aalis: 40
  - **Ch**aar: 4
- B cell will have CD 40 receptor
- T cell will have CD 40 ligand
- When a foreign body (Antigen) enters into the body then it will get attached to IgM antibody
- Then this B cell give the antigen to T Cell
- T Cell further converts them into the Il4
- The IL4 will convert the IgM and IgD into IgG, IgA, IgM, IgD, and IgE

Q4. Which interleukin is needed for Isotype switching?
Answer: Il4

Q5. Which CD marker is needed for Isotype switching?
Answer: CD 40

Humoral Immunity 00:51:41

- Formation of antibodies is generally considered as humoral immunity
- IgG (maximum concentration)
- IgA
- IgM
- IgD
- IgE (minimum concentration)

Types of APCs	Description
Professional APCs	• These are further divided into o Dendritic cells o B cells o Macrophages • These dendritic cells are present in two regions o Skin: They are called as Langerhans cells o Lymph node: Follicular dendritic cells → Reservoirs for HIV (Langhans cells are not antigen presenting cells, these are the giant cells generally found in the TB condition)
Non - professional APCs	It includes • Thymic epithelial cells • Endothelial cells • Fibroblasts

- First this antigen will be presented on the MHC and then it will be given to T cells

Major Histocompatibility Complex 00:57:40

- It was originated from the HLA gene that is presented on the chromosome 6p (Short arm)

Chromosome 6

The genes present in the chromosome 6 includes

- HLA gene
- HFE gene (Gene for hemochromatosis)
- Autosomal recessive polycystic kidney disease

Structure of HLA Gene

- It has many parts
  - HLA gene I
  - HLA gene III
  - HLA gene II
- HLA gene I has HLA IA, HLA IB, HLA IC, HLA IE, and HLA IG
- HLA gene II has DP, DQ, and DR
- HLA gene III has no subdivisions
- HLA E will be the self recognition target for Natural Killer cells
- HLA G (Gestationalis) responsible for the feto-maternal tolerance
- HLA I gene → MHC I
- HLA II gene → MHC II
- HLA III:
  - Complementary proteins like C2, C4
  - Properdin
  - Tumor necrosis factor alpha
  - Heat shock proteins (HSP)

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Difference Between MHC I and MHC II

Characteristic	MHC I	MHC II
Structure	• It has arms o Alpha 1 o Alpha 2 o Alpha 3 o Beta2 microglobulin	• It has o Alpha 1 o Alpha 2 o Beta 1 o Beta 2
Antigen binding cleft	At the junction of alpha 1 and alpha 2	At the junction of Alpha 1 and the Beta 1
Present on cells	• All nucleated cells in the body • Platelets	Present in all the antigen presenting cells
Not present	• RBC • Sperms	
Present to cells	CD 8 cells	CD 4 cells
Diagnosis	Allo antisera testing	Mixed lymphocyte reaction testing

Important Information

- As RBC doesn't have the MHC I, to survive they will adsorb the MHC I from the surroundings

Rule of 8
MHC I: CD 8 (1 ×8 = 8)
MHC II: CD 4 (4 ×2 = 8)

Rule of 8 is also studied in
CD4 /CD8 = 2 /1
MHC I = CD8
MHC II = CD4

Types of T Cells

It is of two types

- CD4 cells
- CD8 cells
- It is further divided into
  - TH 1
  - TH 2
  - TH 17

TH 1	TH 2	TH 17
• It releases IL 2 • IL 2 will activate TH1	• It releases IL4, IL5, IL13 (Responsible for asthma)	• It releases IL17 and Il22
• It has an association with IL12 and TNF gamma (Controlled) • INF gamma will help in the formation of granuloma	• IL4: Helps in isotype switching • IL5: It results in eosinophilic activation • IL13: Helps in mucous production	• They are responsible for neutrophils and monocytes

To Remember: All the asthma allergies are hypersensitivity type 1

02. CD8 T cells

- These are cytotoxic T cells

Mechanism of Cytotoxic cells

- It has two mechanisms

Mechanism of Cytotoxic Cells	
Mechanism I	**Mechanism II**
CD95 and CD95L will help in Apoptosis ↓ An Apoptic cell will have CD95 ↓ Now a T cell will reach there with CD95L ↓ Hence they help in apoptosis	**Name: Perforin Granzyme Pathway** CD8 T cells have perforin and granzyme ↓ When the foreign body enters then perforin will enter the foreign body and then it will make some perforation ↓ Then granzymes will enter through the perforations ↓ Hence it kills the foreign body (Bacteria)

Q6. True statements about MHC are all except?
A. Chromosome 6 harbors gene for MHC
B. Gene encoding complements are adjacent to MHC
C. Monocytes have MHC class II molecules on their surfaces
D. Class III MHC does not encode complement

Answer: Class III MHC does not encode complement

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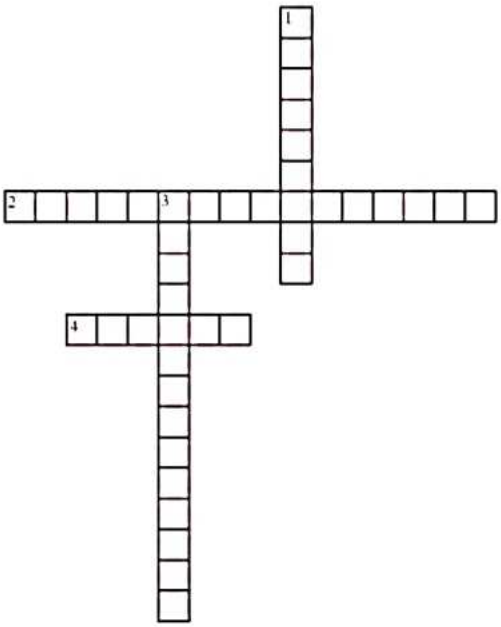
CROSS WORD PUZZLES

Crossword Puzzle



Across

2. Immunity acquired after exposure to particular bacteria, virus or any infection
4. The outermost area of the Lymph Node is called

Down

1. These are also present on membranes of organelles
3. God gifted immunity

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19 TYPES OF HYPERSENSITIVITY REACTIONS

Immunity - Hypersensitivity Reactions

Gell & coomb classification

- Type I- Anaphylaxis
- Type II- Cytotoxic
- Type III- Immune Complex

} Antibody mediated

- Type IV- Delayed Type → Cell mediated

Type I Hypersensitivity Mechanism 00:02:18

- The first exposure: allergen.
- Antigen-presenting cells pick up the allergen & going to keep the allergen on MHC and present it to the T cell.
- T cells differentiates into TH2 cells.
- TH2 cells produce interleukin 4, interleukin 5, and interleukin 13.
- Interleukin 4: isotype switching → IgE production
- Interleukin 5: eosinophils activation happens.
- Interleukin 13: mucus production will occur.
- IgE coats the mast cells leading to sensitization.
- When the second exposure happens, it will bridge the gap in the mast cell & undergoes polymerization. This will cause degranulation of the mast cell.
- As a result, the mast cell bursts, will release histamine as the mast cell is a rich source of histamine.

Q. What is the special stain for mast cells and basophils?
Ans - Toluidine blue

Type I Hypersensitivity Early Phase

- Occurs within 2 hours
- Preformed mediators - release of histamine & Tryptase.
  - Patients with allergies have a rise in serum tryptase.
- Newly formed mediators
  - PG
  - Leukotrine
  - Platelet Activating Factor
- All of these above mediators cause bronchoconstriction.
- Histamine causes vasodilation in the blood, and it causes bronchoconstriction in the lungs.

Type I Hypersensitivity Delayed Phase

- Occurs after 2 to 24 hours.
- Due to eosinophils
- Interleukin 5 is associated with it.
- Eosinophils release MBP (Major Basic Protein)

Difference between Anaphylaxis and Anaphylactoid

- Anaphylaxis: IgE is involved here
- Anaphylactoid reaction: IgE is not involved

Type I Hypersensitivity Examples

a. Allergies (allergic dermatitis, rhinitis, conjunctivitis.)
- Other names of allergic conjunctivitis - VKC (Vernal KeratoConjunctivitis)
- Drug of choice for VKC - Olapatadine
- Olapatadine is a mast cell stabilizer. It also has antihistamine properties.
- ATOPY - Allergy with a genetic determinant.
  - Associated with chromosome 5.

b. Bronchial Asthma

c. Casoni Test
- The test is done for Echinococcus Granulosus.

d. Drugs (Penicillin - Test dose)
- Some other hypersensitivity reaction examples include
  - PK reaction
  - Theobald smith phenomenon

Type II Hypersensitivity Mechanisms 00:17:33

Mechanisms	
Opsonization and Phagocytosis	Antibody and antigen reactions IgG will opsonize it, and phagocytosis will occur.
ADCC	Once the antigen is covered with IgG, it will be identified by NK cells and macrophage and all resulting in ADCC (Antibody dependent cellular cytotoxicity.)
Complement activation and inflammation	Antigen and antibody reactions activate the complement system.

Type II Hypersensitivity Examples

- My blood group is Rh+ve (mnemonic)
  - My: Myasthenia Gravis
  - Blood: blood transfusion reaction
  - Group: Good pasture syndrome and Graves disease
  - Is: Immune hemolytic anemia (IHA) → Immune thrombocytopenic purpura (ITP)
  - Rh: Rh incompatibility, RHD
  - Positive: Pemphigus (Bullous) and Pernicious Anemia
- Myasthenia Gravis and Graves disease are now classified as Type V hypersensitivity.
  - In Myasthenia Gravis, Antibody is against acetylcholin receptor
  - In Graves disease, the antibody is against the TSH receptor
  - Antibodies against cellular receptor → type V hypersensitivity

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Type III Hypersensitivity - Phases 00:27:10

- Immune complex
- Occurs in 3 phases:
  - **Immune complex formation** - Antigen and antibody both are mobile and combine to make an immune complex
  - **Immune complex Deposition** - These deposit in tissues (joints, skin & serosa)
  - **Complement Activation** - leading to signs and symptoms
  - Duration - 10 to 14 days
- Difference with Type II
  - In Type II, the antigen is fixed.
  - In Type III, both antibodies and antigens are mobile

Type III Hypersensitivity – Examples

- **S** - SLE
- Serum sickness (anti-tetanus / anti-rabies serum)
- Schick test C. Diphtheriae)
- **H** - Henoch Schonlein Purpura (IgA Vasculitis in children)
- **A** - Arthus reaction (localized immune complex disease)
- **R** - Raji Assay, Reactive Arthritis
- **P** - PSGN (Poststreptococcal Glomerulonephritis) cola color urine
- PAN,
- Parasitic diseases (eg, malaria → nephrotic syndrome → affecting kidneys)

Type IV Hypersensitivity 00:36:20

- Delayed type hypersensitivity
- Only cell-mediated
- It has CD4 T cells and CD8 T cells
- T cell to form TH1 cells.
- TH1 releases interferon-gamma, forms granuloma.
- CD8 T cells work as well in apoptosis and perform granzyme pathways.

Type IV Hypersensitivity – Examples

- Granuloma
- Skin test
  - Mantoux test
  - Lepromin test
- IBD (Inflammatory Bowel Disease)
- Multiple sclerosis
- Contact dermatitis
  - Most common cause worldwide- poison ivy
  - Most common cause in india- detergent usage and artifical jewellery

Type V Hypersensitivity – Examples 00:41:45

- Myasthenia Gravis and Graves disease.
- There are antibodies against cellular receptors.

Controversial Hypersensitivity

- Rheumatoid arthritis. - Type 3 and Type 4
- Hypersensitivity pneumonitis - Type 3 and Type 4
- Transplant rejection
  - Hyperacute - Type 2
  - Acute - Type 2 and Type 4
  - Chronic - Type 4
- SLE: combination of type 2 <<< type 3 hypersensitivity.

Q. A 30-year-old HBsAg positive female complaints of feeling generally ill and fatigued, having fever, and loss of appetite and weight loss. Occasional episodes of muscle and or joint pains are reported. On GPE, the skin sores are noted. There is no cardiopulmonary discomfort noted. Her BP is raised. On arteriography, irregular narrowing and dilation of the blood vessels is noted. Biopsy of the vessels show fibrinoid necrosis. Which of the following hypersensitivity reactions play a role in this disease?

a. Type I
b. Type II
c. Type III
d. Type IV

Ans - Type III

Q. A 42-year-old patient presents with cough and sputum. He also complains of weight loss, fever, and night sweats. On sputum examination, there are multinucleated giant cells along with lymphocytes , and macrophages. Numerous scattered cells with slipped shaped nuclei are noted. Which of the following hypersensitivity reactions play a role in this disease?

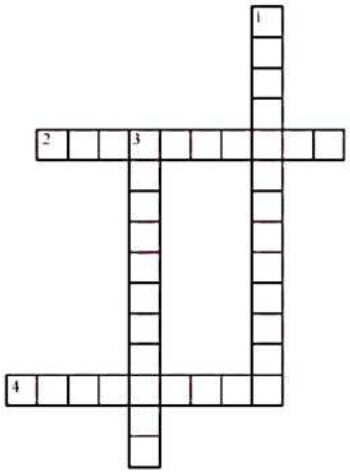
a. Type I
b. Type II
c. Type III
d. Type IV

Ans- Type IV

Telegram - @nextpreladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. The test is done for Echinococcus Granulosus
4. The special stain you have for mast cells and basophils

Down

1. This is a classical hyperthyroidism
3. Drug of choice for VKC

Telegram - @nextpreladdernotes

20 TOLERANCE AND AUTOIMMUNE DISORDERS

- Tolerance to self-antigen
- Two types of tolerance:
  - Central tolerance (Bone marrow, Thymus)
  - Peripheral tolerance

Central Tolerance 00:01:12

Two mechanisms:

1. Deletion/negative selection

- All the self-reactive T Cells are going to be deleted.
- The regulator of this entire process: Autoimmune regulator (AIRE) gene.
- Defect in the AIRE gene: Autoimmune Polyendocrinopathy (AIPE) (diabetes Mellitus, Addison's disease, etc.)

2. Receptor editing

- B cells (self-reactive)

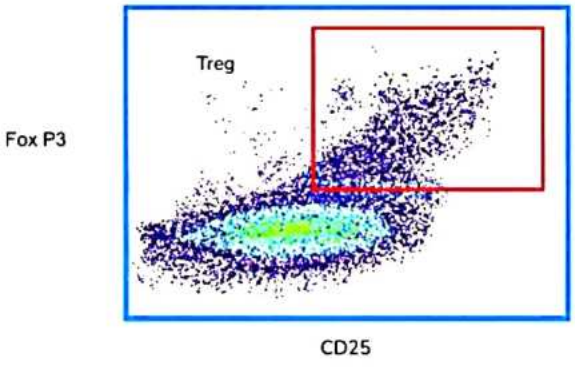
Peripheral Tolerance 00:06:06

- If central tolerance fails, peripheral tolerance will work.

1. T regulatory cells

- In peripheral tolerance, T regulatory cells are present.
- This is a Type of T cell that decreases immunity. It is called immunosuppression.
- They are a type of CD4 T cells that are CD25+/IL2Rc.
- FOXP3 genes regulate them.

Flow Cytometry



- CD25+ & FOXP3+ = T regulatory cells
- T regulatory cells are regulated by an increase in CTLA4 and PD1 and this causes immunosuppression
- Interleukin 2 receptor mutation:
  - Inflammatory bowel disease
  - Multiple sclerosis.
- FOXP3 gene mutation: there will be IPEX.
- Two genes: FOXP3 gene and FOXL2 gene.
- FOXP3 - causes IPEX Syndrome.
  - I- Immune dysregulation
  - P- Polyendocrinopathy
  - E- Enteropathy
  - X- X-linked disorder
- FOXL2
  - Granulosa cell tumours of the ovary.

2. Killing of self reactive cells

- These cells will be killed.
- Killing happens by Apoptosis.
- Apoptosis is mediated by Cd95
- If there is CD95 or Fas mutation: Autoimmune lymphoproliferative syndrome (ALPS)

3. Anergy 00:13:44

- It is applicable to T and B cells.
- **For T cells:**
  - When an antigen comes into a cell, the antigen-presenting cell is going to present it via an MHC to T cells.
  - T Cell receptor is alpha and beta.
  - Signal 1: When APC and MHC is going to give antigen to T cell (T Cell receptor)
  - But T cells need one more signal.
  - APC increases molecules on it's surface, and these are known as B7.1 (CD80) and B7.2 (CD86)
  - They will go and bind to Cd28.
  - This will be Signal 2, known as the costimulatory signal.
  - Now, the T cell will get activated.
  - CD28 is a costimulatory signal and will activate the T cell.
  - So, when there is a foreign antigen, signal 1 and 2 signal are needed. If there is self-antigen, then no signal 2.
  - Not having signal 2 is known as Anergy.
- **For B cells**
  - The B cells have IgM and IgD.
  - They will pick & give antigens to the T cell this is Signal 1.
  - Signal 2: B cell has CD40 receptor, and T cell has CD40 ligand.
  - Binding between these two occur & are known as costimulatory signals.
  - It activates T cells and releases Interleukin 4 and this will change IgM and IgD into IgGAMDE.
  - When there is self-antigen, signal two will not be there and this will be known as Anergy.

Telegram - @nextpreladdernotes

Cryptic/Hidden Antigen

Sites where antigens are not shown:

- B - Brain antigens except for area postrema,
  - Hidden by a blood-brain barrier.
- E - Eyes except for optic Nerve
  - Hidden by a blood-ocular barrier.
  - Eg. Trauma eye → B-O-B breaks → Traumatic uveitis
- T - Tests except for epididymis,
  - hidden by a blood testicular barrier.

Autoimmune Disorders 00:25:10

Systemic Lupus Erythematosus

- It is a combination of type 2 and 3 hypersensitivity reactions.
- It is associated with early complement deficiency = C1, C2, C4.
- More common in females.
- Also associated with Klinefelter syndrome.
- There is also a cell death associated with NETs.

SLE - Criteria

There are a total of 11 Criteria; 4 are needed for the diagnosis.
Mnemonic: **MD SOAP BRAIN**

- **M** - Malar Rash
  - Butterfly-shaped rash on face
  - Spares nasolabial fold.

- **D** - Discoid Rash (Round like a discoid)

- **S** - Serositis
- **O** - Oral Ulcer (Aphthous; painless)
- **A** - Arthritis (non-erosive arthritis)
- **P** - Photosensitivity
- **B** - Blood
  - Pancytopenia (low Haemoglobin, low WBC, low platelets)
  - LE cells
- **R** - Renal
  - Lupus Nephritis
    - → Has total of 6 classes, from Class I & VI
    - → Microscopically: Most characteristic lesion - Wire loop lesion - Class III, IV & V
    - → Maximum wire loop lesion - Class IV
- **A** - ANA (antinuclear antibody)
- **I** - Immunological (antibody)
- **N** - Neurological (Psychosis)

Antibodies

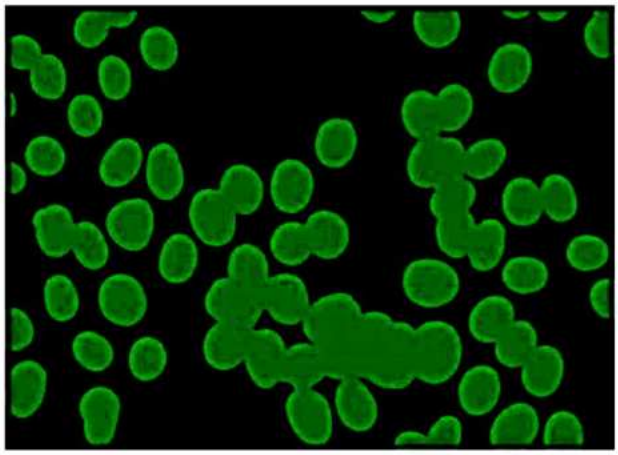
- Most sensitive: ANA (Antinuclear antibody)
- Most specific: ASA (anti-Smith antibody)
- An antibody that is both sensitive and specific: Anti-double-stranded DNA antibody (anti-dsDNA antibody)
- SLE resulting in psychosis - Anti ribosomal P antibody
- SLE has happened in children - Neonatal Lupus - anti-Ro antibody

Q. How do you detect antinuclear antibodies?
Ans: Immunofluorescence

Patterns of ANA

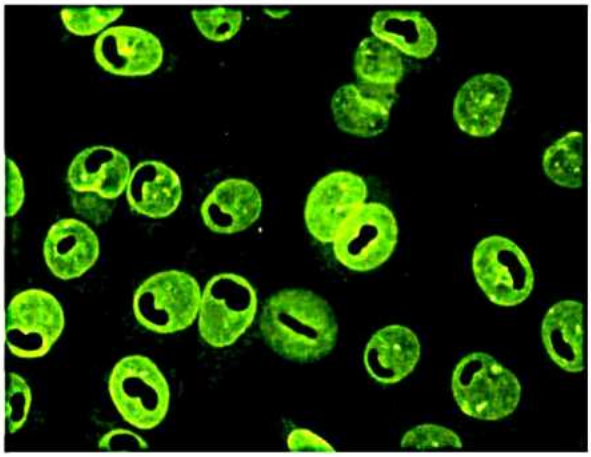
Mnemonic - **HAPPENS (HPNSC)**

H - **H**omogenous technique



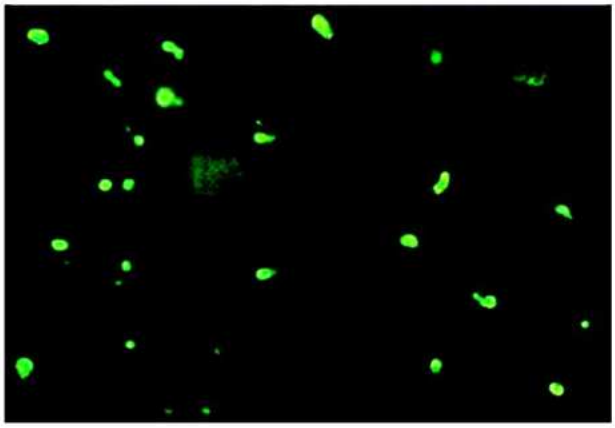
P - Peripheral or rim pattern

Telegram - @nextpreladdernotes



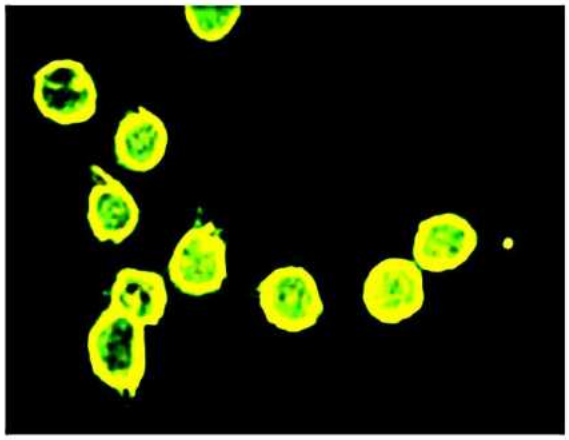
- It happens when antibodies are only against anti dsDNA

N - Nucleolar



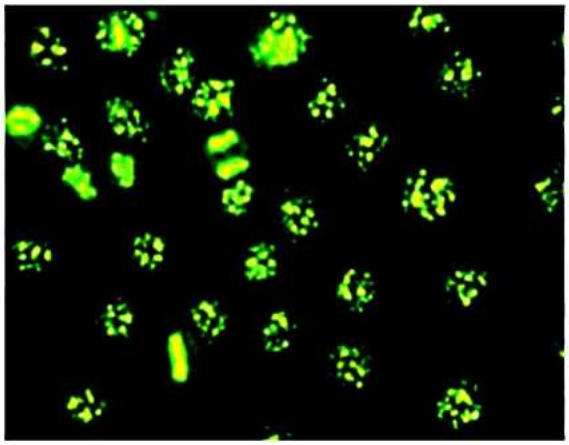
- It happens when antibodies are against RNA.

S - Speckled pattern



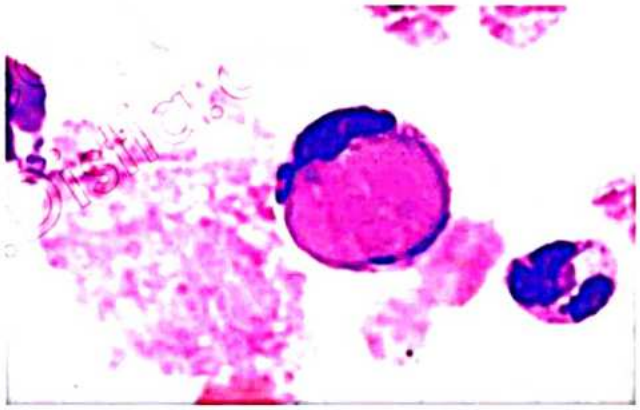
- Most common patterns in autoimmune disorder
- Ab Against
  - Anti-Smith: most common for SLE
  - Anti RO and Anti LA: related to Sjogren syndrome
- Least specific pattern

C - Centromeric pattern



- Related to CREST syndrome

LE cell



- Lupus erythematosus cell
- It is a neutrophil or a monocyte that has engulfed damaged nuclear material (Hematoxylin body).
- The damaged nuclear material left is called the Hematoxylin body (blue-colored body)
- These dead cells will then be engulfed with either a neutrophil or a monocyte.

LE Cell	Tart Cell
Seen in SLE Related to autoimmune disorder	Normal cell (macrophages, monocytes engulfed other cells)

Other Organs affected by SLE

- Heart- Libman Sacks Endocarditis
- Lungs- Patient shows shrinking lung
- Spleen- Onion skinning pattern around the arterioles

Telegram - @nextpreladdernotes

Drug-induced Lupus (DIL)

- Variant of SLE
  - SHIP Drugs
    - → S - Sulfonamides
    - → H - Hydralazine
    - → I - Isoniazid
    - → P - Procainamide
    - → D-Dapsone

SLE	DIL
• Antibody's: ANA, ASM, dsDNA • Organs - CNS, Renal • Treatment - steroids	• Antibody: Anti-histone antibody • Brain and kidney does not get affected • Treatment - Withdraw the drug

Sjogren syndrome

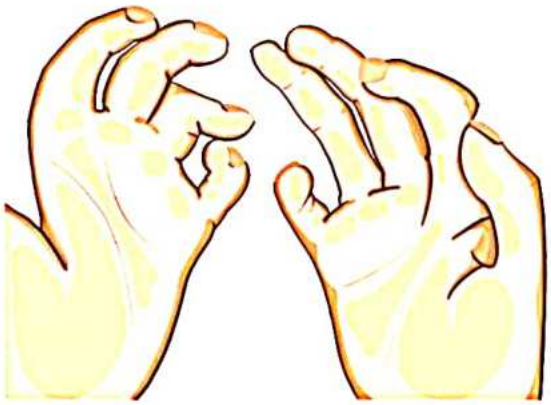
- Known as dry eyes and dry mouth
- Antibodies against salivary glands:
  - Dry mouth
  - Halitosis (Bad breath)
- Antibodies against lacrimal glands:
  - Dry eyes
  - Gritty sensation in the eyes
- Antibodies are:
  - Anti-SS-A (Referred to as Ro) - Most sensitive
  - Anti SS-B (Referred to as La) - Most specific
- **Diagnosis**
  - Serology to find the antibody
  - Lip biopsy: Lip has minor salivary glands
  - M/E: Lymphocytes
- Sjogren syndrome is also associated with Primary biliary cholangitis in the liver, marginal zone lymphomas.

Scleroderma

00:55:05

- Sclero: stiff or hard/thick
- Derma: Skin

Sclerodactyly



- Sclerosis of digits
  - Claw-shaped digits due to stiffness
- This can happen anywhere in the body
- Two forms of scleroderma

Limited

- Caused by anti-centromere antibody
- Limited involvement of skin
- A limited form of scleroderma is associated with CREST syndrome.

Diffused

- There is diffuse involvement of skin
- Caused by anti-Topoisomerase antibody / Anti SCL 70 (most specific)

CREST syndrome

- Associated with anti-centromere antibody
- **C** - Calcinosis cutis
- **R** - Raynaud's phenomenon
- **E** - Esophageal dysmotility
- **S** - Sclerodactyly
- **T** - Telangiectasis (problems in blood vessels)

Mixed Connective Tissue Disorder

- Mixed features of SLE, Sclerosis, Polymyositis
- Antibody: Anti U1 RNP (RIbonucleoprotein) antibody
- Treatment - Steroid

Myositis

- Three types of myositis

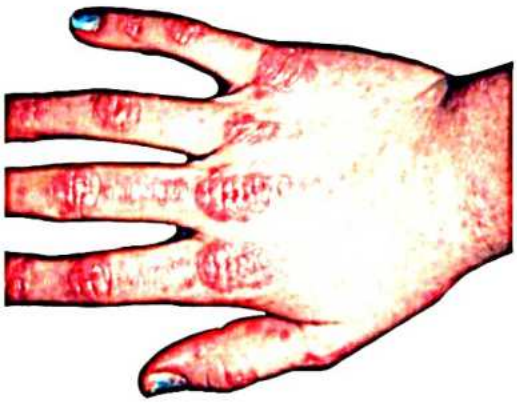
1. Dermatomyositis
2. Polymyositis
3. Inclusion body myositis

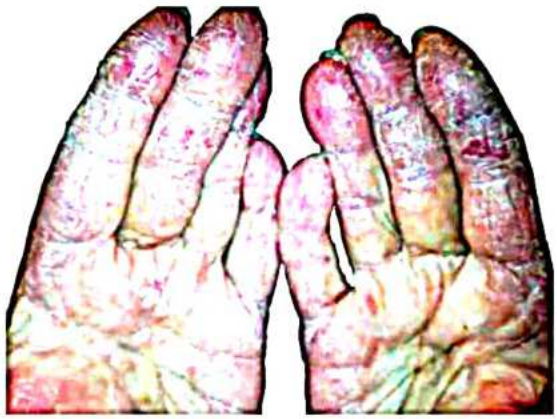
Dermatomyositis

- Myo - Proximal muscles will be involved
  - Causing weakness in patients
- Derma - Involvement of skin
  - Causing rash around the eyes
    - → Heliotrope rash

  - Papules present on the knuckles and phalanges
    - → Gottron papules

Telegram - @nextprepladdernotes

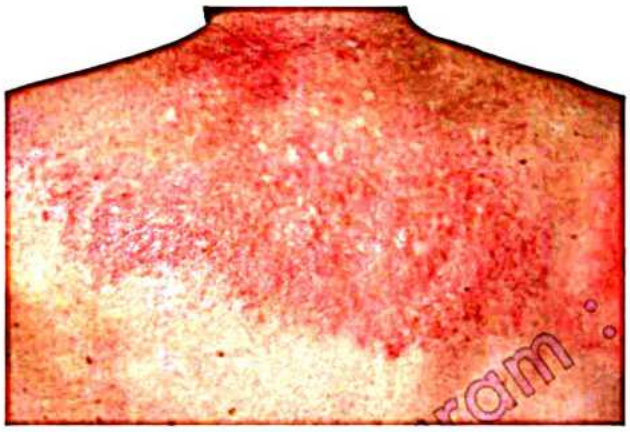


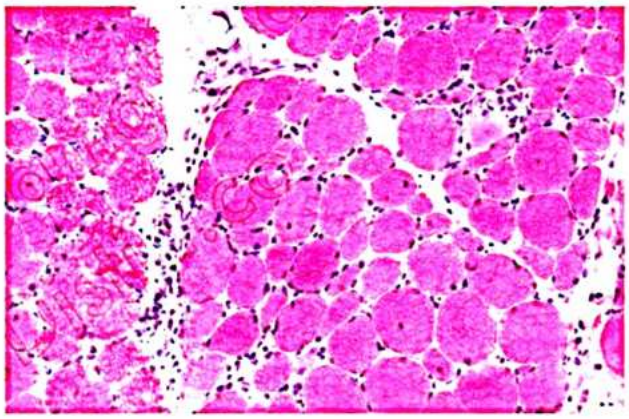


- Rash on the skin of the neck and back
  → Shawl sign

Muscle biopsy:

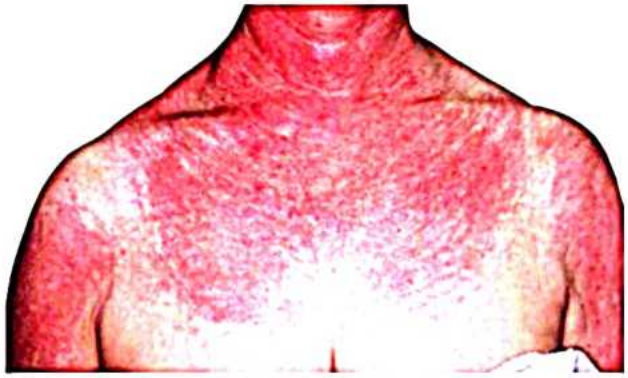
- All the inflammation is perifascicular (around the fascicle)

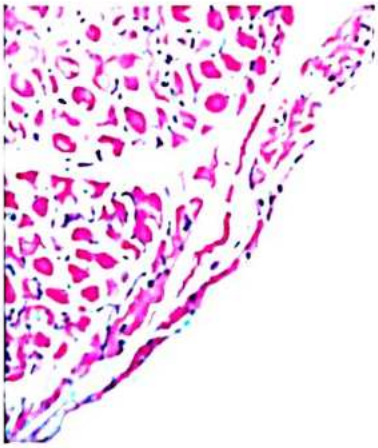




- V-shaped rash on neck
  → Necklace sign

- Perifascicular atrophy has also occurred





- Presence of cracked hands
  → Mechanic hands

- The muscle fibres in the centre are bigger, and at the periphery, the muscle fibres have become very small, called peri fascicular atrophy

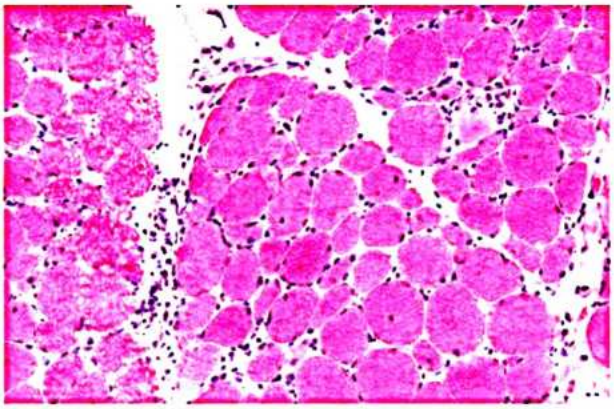
Telegram - @nextpreladdernotes

Antibodies:

- ANA+ve
- Anti Jo 1 Ab +ve: Mechanic hand
- Anti Mi2 Ab +ve: skin features
- Anti P 155 Ab +ve: Paraneoplastic syndromes Anti P
- 140 Ab +ve: Juvenile Dermatomyositis

Polymyositis

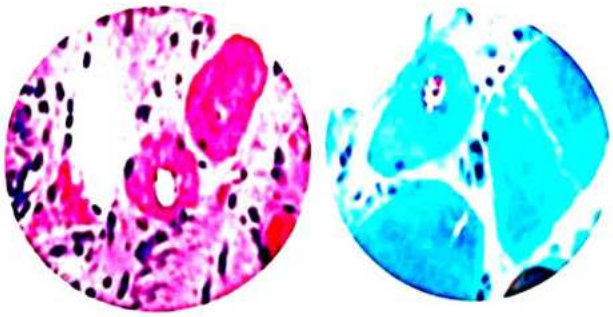
- Proximal muscle weakness.
- There is no derma problem.
- The inflammation here is Endomysial inflammation.



- In Dermatomyositis, there was perimysial atrophy.
- But in Polymyositis, there is Endomysial inflammation and no atrophy.

Inclusion Body Myositis

- IBM happens more in adults.
- Muscle biopsy shows rimmed vacuoles



- Stain: Gomori Trichrome stain also shows rimmed vacuoles that contain Amyloid like inclusions.
- Stain for Amyloid will be Congo red.

Q. Which of the following hypersensitivities can be noted in SLE?

A. I, III
B. II, III
C. III, IV
D. I, IV

Q. Which of the following inflammatory cells is more commonly seen in a lip biopsy of a patient of Sjogren syndrome?

A. Neutrophil
B. Lymphocyte
C. Monocyte
D. Basophil

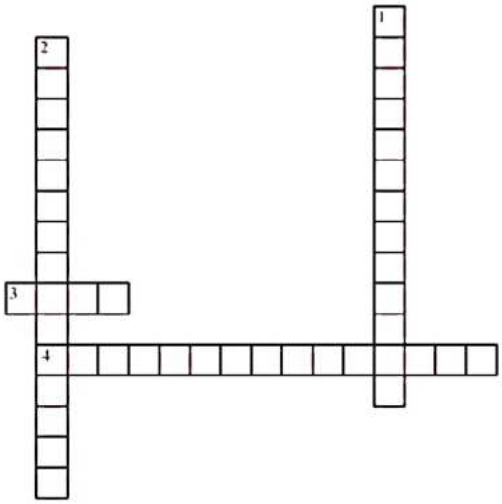
Q. LE cell is a_____ that has engulfed a_____?

A. Neutrophil, lymphocyte
B. Neutrophil, hematoxylin body
C. Macrophage, lymphocyte
D. Macrophage, neutrophil

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. (CD25+) – (FOXP3+)
4. Known as dry eyes and dry mouth

Down

1. Dot dot appearance inside the nucleus
2. There is a Type of T cell that decreases immunity

Telegram - @nextpreladdernotes

21 IMMUNODEFICIENCY DISORDERS

Types of immunodeficiency

- Primary immunodeficiency
- Secondary immunodeficiency

1. Secondary immunodeficiency
- Causes
  - Malnutrition (most common)
  - HIV/Aids
  - Cancer
  - Chemotherapy

Primary immunodeficiency 00:01:50

It is divided into 3 groups
1. Defect in Lymphocytes maturation
2. Defect in lymphocytic activation and function
3. Systemic disease

Defect in Lymphocytes maturation

1. SCID
2. Bruton's hypogammaglobulinemia
3. Bare lymphocyte syndrome
4. Digeorge syndrome

1. SCID (Severe Combined Immunodeficiency)

- B, T, NK cells are affected.
- It is of two types

XLR (X linked recessive variety)

- Most common problem in the gamma subunit of cytokine receptors: IL2, IL4, IL7, Il15).
- T and NK cell are affected. B cells are not affected

AR (Autosomal recessive variety)

- ADA (Adenosine deaminase) deficiency(most common) Involves all cells (B, T, NK)
- JAK3 defect:(T and NK cells are affected. B cells are normal)
- RAG1 and RAG2 (Recombinant activation gene) defect: B and T cell affected. NK cells normal
- If all the B, T, and NK cells are defective, then it will be ADA deficiency that is autosomal recessive.
- If B and T cells are defective and NK cells escape, it will be RAG 1,2.

Clinical features

- Morbilliform rash
  - Red rash on face of child
  - Maternal T cells attack featal skin
- Pneumonia
- Pneumocystis
- CMV

Treatment

First disease to be treated with gene therapy
- For Gamma subunit (XLR)
- T cell proliferation started (Leukemia)

Enzyme replacement used now
- ADA deficiency

2. Bruton's hypogammaglobulinemia

- Occurs most commonly in boys, XLR
- BTK gene defect
- B cell defect

$$\text{Immature B cells} \xrightarrow{\text{Bruton tyrosine kinase}} \text{mature B cells} \longrightarrow \text{plasma cells and antibodies}$$

- BTK is not there, so B cells won't mature, then plasma cells and antibodies will not be formed.
- Less antibodies will cause hypogammaglobulinemia.

Clinical features

- Enterovirus infections
- Polio
- ECHO
- Coxsackie virus infection.
- Live polio vaccine is always contraindicated.
- Reduced IgG → defective optimisation

3. Bare lymphocyte syndrome

- MHC II is missing

4. Digeorge syndrome- mnemonic- CATCH22 00:18:22

- Also known as Velo Cardio Facial syndrome.
- There is deletion 22q11 (TBX 1 gene is going to be deleted) → 3rd and 4th pharyngeal pouches are not developed. Thymus and parathyroid hormones will not form.
- Reduced parathyroid hormones, reduced calcium levels → hypocalcaemia.
- T cells mature in Thymus, if thymus won't mature and cause thymic aplasia and T cell defect will be there.
- also known as velo cardio facial syndrome.
- Tetralogy of fallot.
- Abnormal facies are there: Cleft in lip and cleft palate.

Diagnosis

- FISH: diagnosis of choice
- TBXgene: seen in digeorge syndrome and schizophrenia.

Telegram - @nextpreladdernotes

Defect in lymphocytic activation and function

1. **Common variable Immunodeficiency (CVID)**
- It is a B and T cell problem
- B cell problem is because of the BAFF gene defect and the T cell problem is because of ICOS gene defect.

Differences between Bruton's hypogammaglobulinemia and CVID. 00:24:24

Bruton's hypogammaglobulinemia	CVID
• BTK gene defect • Occurs in boys • B cell defect • Hypoplastic germinal centers	• BAFF gene defect • Occurs in boys & girls • B & T Cell defect • Hypergerminal centers

2. **Absence of CD ligand or CD receptor leading to hyper IgM**
- The patients of hyper IgM syndrome are at the risk of pneumocystis carinii.

3. **Hyper IgE Syndrome**
- Mutation: STAT 3 defect
- It is also known as job's disease.

4. **Isolated IgA Syndrome**
- IgA is a mucosal antibody present in mucosa (GIT, respiratory mucosa)
- when IgA goes down, mucosal immunity goes down that causes mucosal infection.
- GIT affected diarrhoea
- Respiratory mucosa affected respiratory infections
- In the patients of Isolated IgA deficiency, washed RBCs are given to patients.

5. **XLP disorder (X linked lymphoproliferative syndrome)**
- SAP defect (SLAM activating protein)
- SLAM: Signal Lymphocyte Activation Molecule
- Needed by B/T/NK cells
- Increased risk of EBV infections

Systemic disease

1. **Wiskott Aldrich Syndrome (WAS)**
- X linked recessive disorder and WASP gene defect.
- Patients have bleeding manifestations (because of platelet defects, small size platelets)
- Infections
- eczema.
- Ig profile: IgM decreased, IgA and IgE increased

2. **Ataxia Telangiectasia**
- It is associated with ATM gene
- Immunodeficiency is also there.

MCQ's

Q. A new born child presents with a morbilliform rash. Mother also gives a history of recurrent diaper rash. Examination reveals oral candidiasis and failure to thrive?

A. LAD 1
B. LAD 3
C. Bruton's agammaglobulinemia
D. SCID

Q. BAFF genetic defect associated with germinal centre hyperplasia leading to hyperplasia of peyer's patches and tonsils?

A. Bruton's agammaglobulinemia
B. SCID
C. CVID
D. CGD

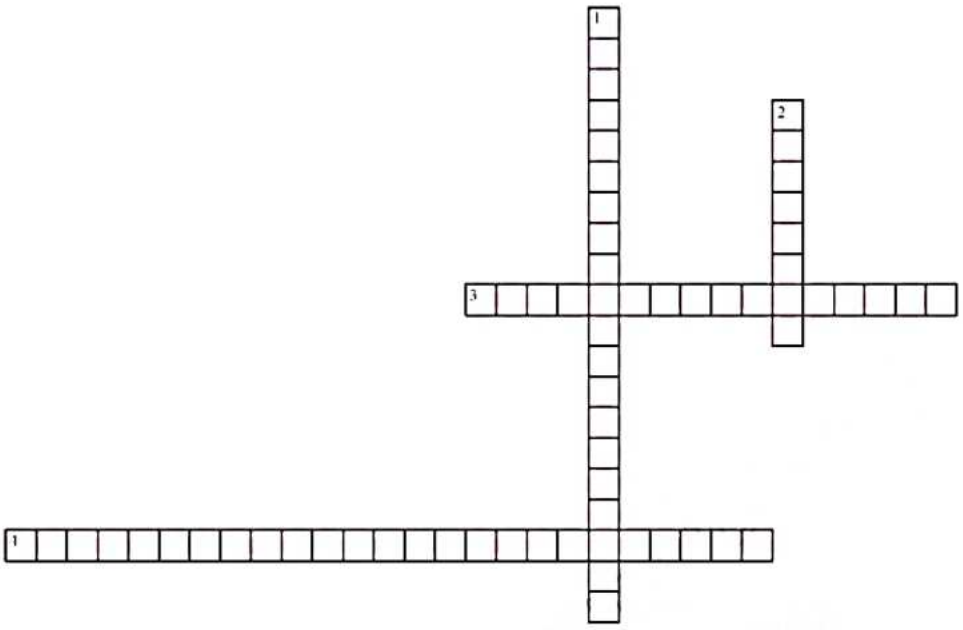
Q. Select the incorrect match?

A. Del 22p - DiGeorge syndrome
B. Baff - CVID
C. ADA deficiency - SCID
D. BTK - Bruton's hypogammaglobulinemia

Telegram - @nextpreladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. It is also known as velo cardio facial syndrome
4. Full form of AR

Down

1. This converts immature B cells into mature B cells
2. It makes plasma cells and antibodies from mature b cells

Telegram - @nextpreladdernotes

22 TRANSPLANT IMMUNOLOGY

Pre-Transplant

Types of Grafts

00:00:52

- Autograft - SELF
- Isograft - Identical twins
- Allograft - Grafting between the same species.
  - Kidney transplant or bone marrow transplant
  - Human to human
- Xenograft - Grafting done between different species
  - Example: Prosthetic heart valves, that are taken from different animals
  - Porcine, bovine different types of heart valve

Prior to Transplant

ABO Matching	Matching of blood group between Donor and Recipient
HLA Matching	Between Donor and Recipient

Criteria for HLA matching

Bone Marrow	100%
Kidney	50%
Cornea	0% - because it is a avascular structure
Heart/ liver / Lung	Alongwith HLA matching- viability test first priority • Vitality will be gone if waited

HLA matching 00:03:15

Adults

Ideal Matching
- Genes that are targeted: HLA, A, B, C, DQ and DR
- **Criteria:** Minimum 8 on 10 should be matched

Practical Matching
- Genes that are targeted: HLA, A, B, C & DR
- **Criteria**: minimum 6 out 8 should be matched

Children

- HLA A, B, DR genes to be considered
- **Criteria**: minimum 4 out of 6 should be matched

Identical Twins get a perfect 6/6 alleles matched.

Q. Which is the important HLA gene that has to be matched?
Ans: The most important matching is DR matching

Post-Transplant

Transplant Rejection & Graft v/s Host Disease (GVHD)

00:10:52

- Recipient reject donor graft
- Donor attacks recipient

Types of Transplant Rejection

- **Hyperacute Rejection**
  - Within first 48 hours
  - Type 2 Hypersensitivity reaction
- **Acute rejection**
  - Few days to weeks and rejection done after that
  - Type 2+4 Hypersensitivity reaction
- **Chronic Rejection** (most common)
  - Type 4 Hypersensitivity reaction

Hyperacute Transplant Rejection

- Donor graft has been rejected within 48 hours.
- Most of the time it happens on the operation table itself
- Reason: PREFORMED ANTIBODIES

For example: A kidney (donor graft) is placed and anastomoses the blood supply.
But the recipient rejected it.

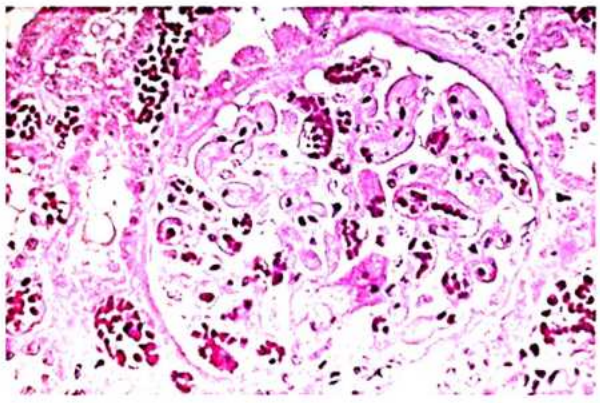
Q. Why does this happen?

- Due to Preformed antibodies

Q. Which types of patient will have pre-formed antibodies?

- Previous multiple blood transfusion
- Multiparous women
- **Blood vessels-** fibrinoid necrosis
- **Kidney-** coagulative necrosis

Microscopically:



Telegram - @nextpreladdernotes

1. Fibrinoid necrosis in the blood vessels
2. Intraluminal thrombi
3. Coagulative necrosis in the solid organs
4. Necrosis always show inflammation - neutrophilic infiltration

Q. How will the surgeon know about the rejection?

- Kidney becomes floppy and turns blue due to no supply of blood
- Few drops of blood in urine

Acute Transplant Rejection

Refer Table 22.1

Summary of Pathways

- **Direct Pathway:** Acute Cellular Rejection
  - Donor's antigens cells are creating the problem
- **Indirect Pathway:** Chronic Rejection
  - My own APC were having problems

Chronic Transplant Rejection

- Type 4 HS and is related to cells Rejection
- Self antigen presenting cells are going to activate CD 4 T cells
- CD4 cells differentiate into: T helper 1 cell
- IFN gamma- granuloma
- Epithelioid cells
- Type 4 hypersensitivity

Question: What is going to happen to the Kidney?

G- GBM duplication - Glomerular basement membrane duplicate
O- Obliteration/sclerosis of blood vessels
T- Tubular atrophy - Atrophied
I- Interstitial fibrosis

Hyperacute Rejection	Acute Rejection	Chronic Rejection
Within 48 hours	Within few days to weeks	Few weeks to months
HS 2	HS 2+4	HS 4
PREFORMED ANTIBODIES (Blood Transfusion or multiparous pregnancy)	• Humoral antibodies o CD 4 o Rejection vasculitis • Cellular Rejection o Donor APC - DIRECT PATHWAY o Tubulitis and Endotheliitis	Indirect Pathway Self APCs that create problems **G-**GBM duplication - glomerular basement membrane duplicate **O-** Obliteration /sclerosis of blood vessels **T-** Tubular atrophy - atrophied **I-** Interstitial fibrosis
Blue, floppy kidney Blood in urine		
M/E • Coagulative necrosis • Fibrinoid necrosis		

Graft Versus Host Disease 00:39:03

- Donor graft attacks recipient
- Eg. Kidney attacks the receiver
- Donor is immunocompetent
- Recipient is immunocompromised

Acute GVHD	Chronic GVHD
Within 100 days	After 100 days
Skin- Rash Intestinal - Diarrhea Liver- Jaundice	Skin - Fibrosis - nodular & sclerosis. Intestine - stricture Liver - Fibrosis

Should we suppress DONOR T CELL?

- NO
- Why?
- Engraftment (graft has to make a place for itself) is done by DONOR T CELL

In case of bone marrow transplant

- Leukemia: these T cells from outside are normal and helps in killing the leukemic blasts
- GVL - Graft vs Leukemia

Post-Transplant Complications

- Cytomegalo Virus infection
- BK virus: belongs to POLYOMAVIRUS DNA family
- Urine microscopy examination shows decoy cells
- Immunosuppressive state of patient:
  - Human papilloma virus - cervical cancer
  - EBV
  - HHV8

MCQs

Q. What is the graft between different members of the same species termed as?

A. Autograft
B. Isograft
C. Xenograft
D. Allograft

Telegram - @nextprepladdernotes

Q. Which of the following histological changes is suggestive of chronic transplant rejection

A. C4d positivity along peritubular capillaries

B. Presence of glomerular basement membrane double contours

C. Presence of inflammatory cells within glomerular capillaries

D. Presence of inflammatory cells within peritubular capillaries

Q. Which of the following organs is least likely to be affected in GVHD?

A. Liver

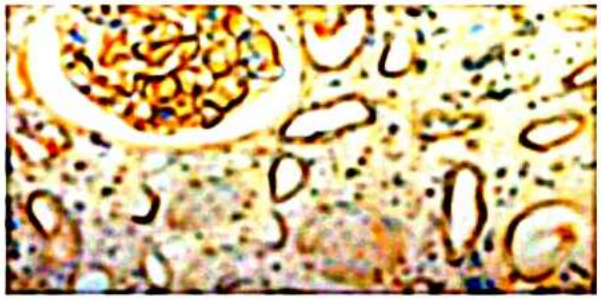
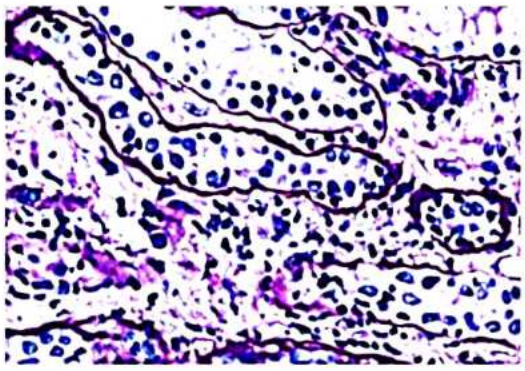
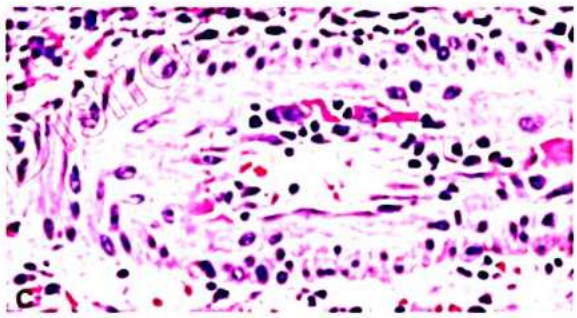
B. Skin

C. Intestine

D. Lung

Telegram - @nextpreladdernotes

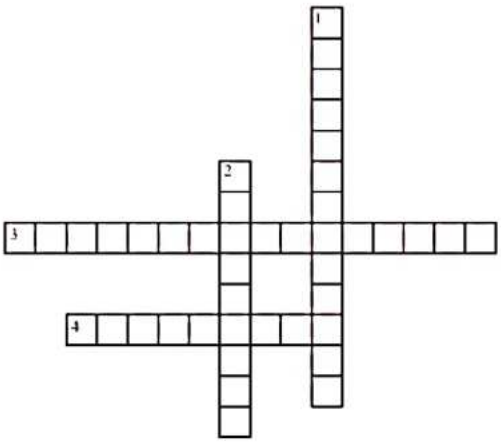
Table 22.1

Acute Humoral Rejection	Acute Cellular Rejection
• Hypersensitivity 2 reaction • Donor kidney carries its own MHC1 & 2 • These act as antigens for recipients to make antibodies • Immune complex is formed • Complement activation	• Hypersensitivity 4 reaction • Donor's antigens presenting cells to recipient • CD 4 & CD 8 T Cells • So much inflammation which results in:
IIIC:	Tubulitis
	
C4D around Vessels (Rejection Vasculitis)	Endotheliitis
	

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Big big cells
4. Grafting done between different species

Down

1. Dark blue color cells are called?
2. Grafting between the same species

Telegram - @nextpreladdernotes

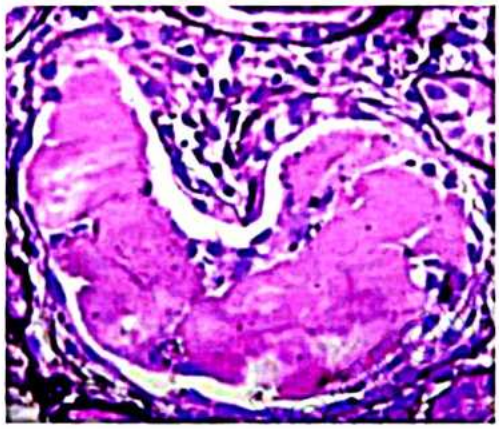
23 AMYLOIDOSIS

- It is defined as a proteoglycan.
- It is misfolded protein that is seen extracellular.
- It is of two types:
  1. Primary Amyloidosis (1°)
  2. Secondary Amyloidosis (2°)

1. Primary Amyloidosis 00:01:20

- Multiple myeloma (cancer of plasma cells)
- These antibodies have light chain excess that deposits as AL protein (amyloid light chain) in kidney known as myeloma kidney.
- The protein (pink protein) comes out in urine, known as BJP (Bence Jones Proteinuria)

Light Chain Cast



- Light chain cast in kidney is referred to as fractured cast

2. Secondary Amyloidosis 00:05:05

- It is also known as reactive amyloidosis.
- Most common cause is rheumatoid arthritis.

Disease	Amyloid
Chronic inflammation	AA associated with IL6
Cancers	AA associated with IL6
Familial Mediterranean Fever	AA associated with IL6
Medullary Carcinoma Thyroid	Acal (calcitonin)
Systemic Senile Amyloidosis	ATTR (increased Quantity)
Familial Amyloidotic Polyneuropathy	ATTR (Mutant)
Alzheimer's Diseases (brain)	A beta (Beta plaque)
Hemodialysis (kidney)	A beta2 ($\beta_2$microglobulin)
DM	AIAPP (Amyloid islet pancreatic polypeptide)
Multiple Myeloma	AL (light chain)

- All Chronic inflammation diseases produce amyloids except chronic bronchitis
- All cancers (mainly RCC, and HL)
- Familial mediterranean fever (pyrin gene defect) produce amyloids.
- **TTR:** A protein, Trans Thy Retin that transports thyroid hormones and retinoic acid (Vitamin A).
  - It has two problems:
    - → TTR Increased quantity (Quantitative): It results in systemic senile amyloidosis
    - → Mutant TTR (Qualitative): It results in familial amyloidotic polyneuropathy

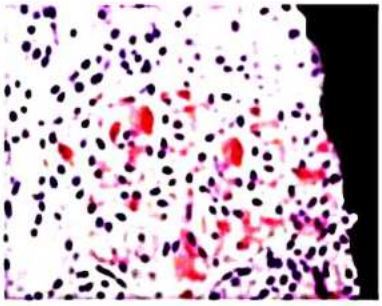
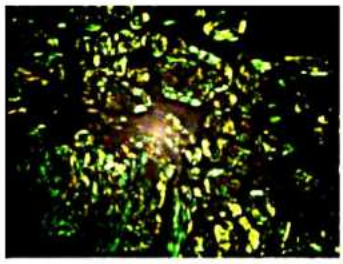
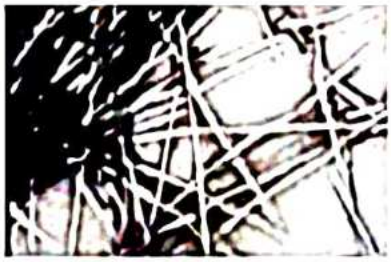
Organ	Site
Kidney (most common)	Mesangium (Nephrotic syndrome)
Heart (most common in old people)	Subendocardium (Restrictive cardiomyopathy)
Liver	Space of Disse
Spleen red pulp	Lardaceous spleen
Spleen white pulp	Sago spleen
Skin	Pinch purpura
GIT	Organomegaly
Joints (associated with Hemodialysis Patients)	CTS (Carpal Tunnel Syndrome)-Median Nerve
Blood Vessels	Sub-endothelial

- All organs in amyloidosis show organomegaly but kidney in late stage amyloidosis shows shrinkage.

Gross findings - Amyloidosis

Telegram - @nextpreladdernotes

- "Waxy kidney"
- Gross stain is Lugol's iodine that gives Mahogany brown colour. On adding H2SO4 into it, it changes into blue colour.

Detection	Feature
Gross stain	Lugol iodine (Mahogany brown)
Microscopic stains	
Congo red	Light microscopy: Salmon pink/Red stains
	
	Polarising microscopy: Apple Green birefringence
	
	Polarising microscopy due to Beta pleated sheets on X-ray crystallography
Thioflavin T	UV light
Methyl Violet	Amyloid is positive
Crystal Violet	Amyloid is positive
PAS	Amyloid is positive
Electron microscopy	
	Non-branching fibrils Indefinite length 7.5-10 nm diameter
X ray crystallography	Beta pleated sheets (responsible for apple green birefringence)

Sample collection: 00:29:22

- Abdominal fat aspiration is preferred over rectal biopsy.

Extent of Amyloidosis:

- Scintigraphy: It measures SAP

Integrate:

- Adsorption of Factor X on surface
- People who have amyloidosis are associated with Factor X deficiency. This person will result in bleeding manifestations.

MCQ 00:32:15

Q. Find the incorrect match with regard to type of amyloid?

A. Chronic inflammation - AA
B. Familial mediterranean fever- AA
C. Familial amyloidotic polyneuropathy- ATTR
D. Alzheimer's disease- A beta2

Q. Which of the following can be associated with AL amyloidosis?

A. Factor V deficiency
B. Factor IV deficiency
C. Factor X deficiency
D. Factor XII deficiency

Q. A 54-year old male is a patient of chronic renal failure and is currently on hemodialysis. Few months later he presents with wrist joint pain. Which of the following amyloid depositions best describes this phenomenon?

A. A beta
B. A beta2
C. AA
D. AL

Q. A 44-year old diabetic patient shows deposition of Abeta 2 amyloid deposition around the median nerve. Which of the following renal cancers is he at a risk of?

A. Clear cell RCC
B. Papillary RCC
C. Medullary RCC
D. Chromophobe RCC

Q. Which of the following stains uses UV light for examination?

A. Congo Red
B. Pas
C. Thioflavin T
D. Lugol's iodine

Telegram - @nextprepladdernotes

PREVIOUS YEAR QUESTIONS

Q. HLA is located on? (FMGE 2018)
A. Short arm chromosome 6
B. Long arm chromosome 6
C. Short arm chromosome 3
D. Long arm chromosome 3

Q. Immune privilege site is. (JIPMER 2019)
A. Optic nerve
B. Seminiferous Tubule
C. Area postrema
D. Spinal cord

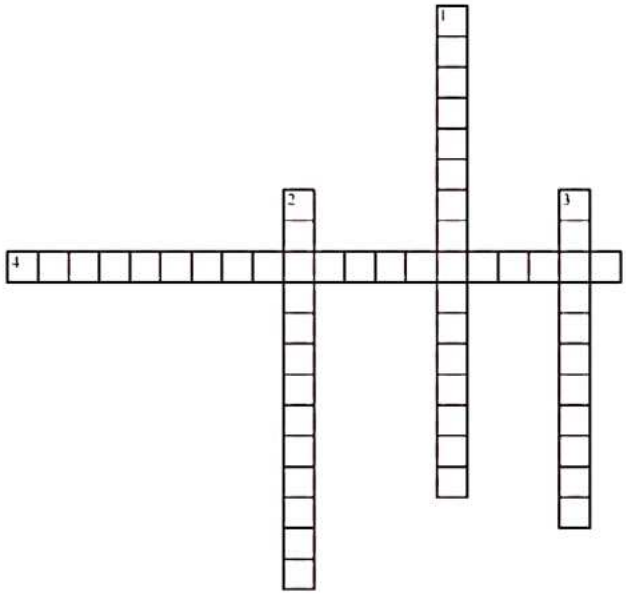
Q. Which of the following cannot be diagnosed with +ve ANA? (AIIMS 2018)
A. Drug induced lupus
B. SLE
C. Scleroderma
D. Sjorgen syndrome

Q. Co-stimulatory factor of T-cell include all except. (JIPMER 2018)
A. B7.1
B. B7.2
C. B7.3
D. CD40

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

4. It is also known as reactive amyloidosis

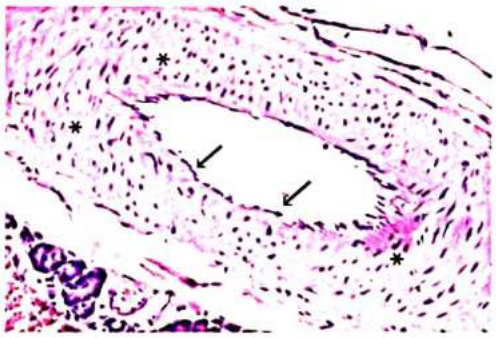
Down

1. It is referred to as a fractured cast because it will not be completely pink and it will have little cracks in between
2. TTR is a protein and stands for?
3. This stain uses UV light for examination

Telegram - @nextprepladdernotes

24 VASCULITIS

Appearance



- There are three layers of the Blood Vessels:
  - Tunica Intima (Endothelial Cells)
  - Tunica Media (Muscular Layer)
  - Tunica Adventitia
- At the junction of T. Intima and T. Medial: Internal Elastic Lamina is present.
- At the junction of T. Medial and T. Adventitia: External Elastic Lamina is present.

Important Information

Elastic Tissue:

- There is a stain used for elastic tissue, namely VVG. (Verhoeff Van Giesen.)

Weibel Palade Bodies 00:03:50

- The endothelial cell contains Weibel Palade Bodies.
- Each Weibel palade Body consists of Von Willebrand Factor and P Selectin.
- There are three markers related to endothelial cells:
  - Cd34+
  - Cd31+
  - F viii+
  - VWF+

Vasculitis 00:06:05

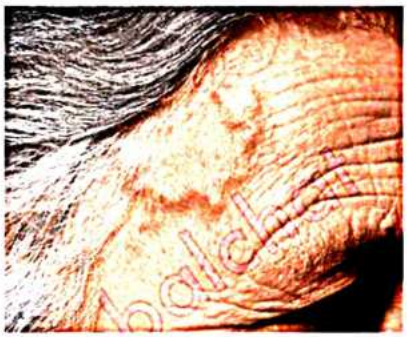
Chappel Hill Classification.

1. Large Vessel
2. Medium Vessel
3. Small Vessel

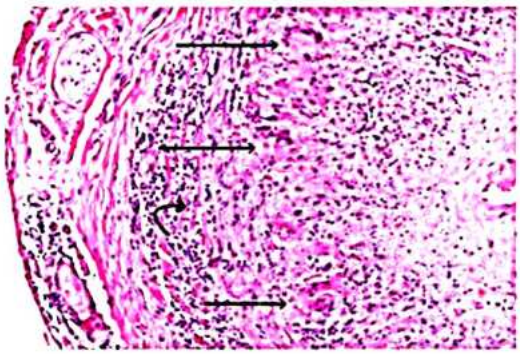
Large Vessel Vasculitis:

Temporal Arteritis

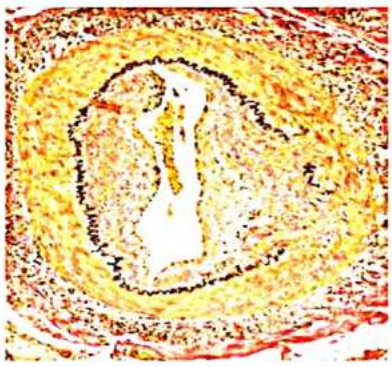
- AKA: Giant Cell Arteritis.
- Age: It is most commonly seen in adults, > 50 years of age
- The most Common Vessel Affected: Superficial Temporal Artery.
- Most Common Symptoms: unilateral throbbing headache.
- Most Specific Symptoms: Jaw Claudication.
- The most dangerous symptom: Loss of vision.
- Other Symptoms: Polymyalgia Rheumatica.



- In case of nodular swelling along the temporal Artery, a 3 to 5 cm biopsy is required.
- The number of nuclei will give the microscopic examination of the giant cell.



- Fragments of internal Elastic Lamina will be present.

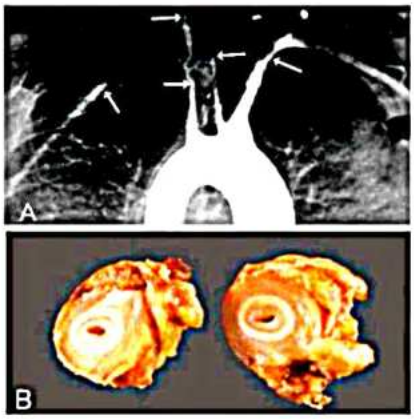


- Start the steroids immediately before the path results come up.

Telegram - @nextpreladdernotes

Takayasu Arteritis

- AKA: Aortic Arch Syndrome or Aorta Arteries or Pulse Disease.
- Age: It occurs in adults below 50 years of age.
- Most commonly affected- subclavian artery
- Least commonly affected- coronary artery



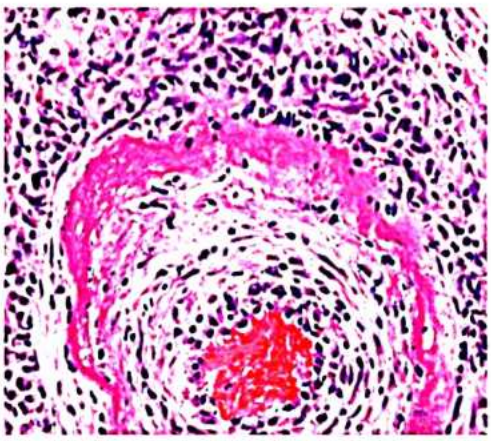
- Occurrence of narrow lumen slit.
- Presence of the Intimal Thickening
- Observation is carried out that the upper limb pulse will be weak, and the lower limb pulse will be normal.

Medium Vessel Vasculitis

PAN (Polyarteritis Nodosa) 00:18:52

- Vessels: Medium Vessels
- Most common vessel- Renal vessel
  However does not cause glomerulonephritis.
- Vessel never affected- pulmonary circulation
- Viral Markers: HBsAg (Hep B Surface Ag/ Australia Ag)

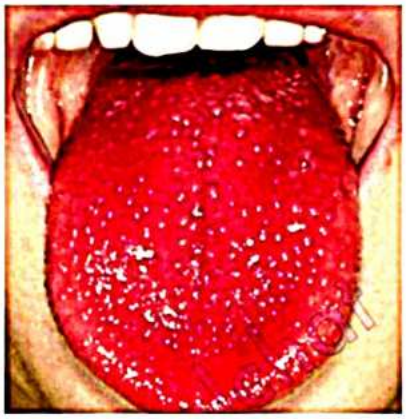
Microscopically: Fibrinoid Necrosis→ segmental, transmural



- All stages shall coexist.

Kawasaki Disease

- Also known as Mucocutaneous Lymph node Syndrome.
- Age: it usually occurs in <5 years old kids.
- Definite Criteria: Fever
- Criteria: Four of the five criteria need to be present.
  - Conjunctivitis
  - Rashes
  - Edema
  - Lymph Adenopathy(mostly unilateral & cervical region)
  - Mucosal Involvement (Strawberry Tongue)

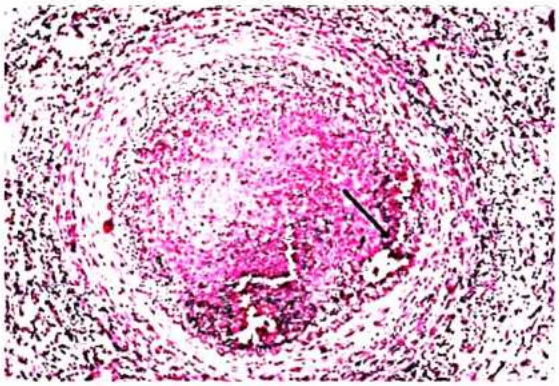


- Update: The Anti-endothelial cell antibodies are positive.
- Complications: The complication depends on which artery is affected → coronary artery(M/C)
  - Aneurysm
  - MI

Important Information

- The strawberry tongue can be seen in Kawasaki and Scarlet Fever.
- Blood test→ increased platelet count (thrombocytosis)

- **Buerger's Disease** 00:31:10
- Also called Thrombo Angiitis Obliterans
- Associated with: Smoking
- Involvement: Artery, Veins, and Nerves.
- Clinical Presentation: Intermittent Claudication-Pain at Exertion (arteries & vein involved) and Pain at Rest (nerves involved gradually)



- Lymphatic Channels are not affected.

Telegram - @nextpreladdernotes

Important Information

- Buerger's disease is vasculitis.
- Berger's disease is kidney-related. (IgA Nephropathy)
- Buerger's Disease is also caused in non-smokers due to the HLA A9 and B5 Polymorphism

Treatment: ceassation of smoking

Small Vessel Vasculitis 00:37:55

Immune Complex

It includes diseases like

- SLE
- HSP (Henoch Schonlein Purpura or Non-Thrombocytopenic Purpura):
  - IgA mediated
  - It mostly occurs in children.
  - Platelets count: normal or increased
  - Clinical features:
    MNEMONIC- PAAR pura
    → Purpuric Rash
    → Arthralgia
    → Abdominal Pain
    → Renal (Hematuria) (m/c)

Important Information

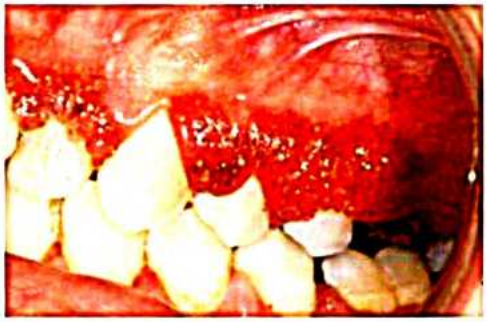
- The most common vasculitis in children is HSP.
- The most common vasculitis that leads to death in children is Kawasaki disease

Pauci Immune:

All of the diseases are caused due to the presence of ANCA Antibodies(present in blood & not in tissues)

It includes diseases like

- **Wegener's Granulomatosis:**
  - It is also called GPA
  - Two triads. The first triad involves granulomas in the
    → Kidney,
    → Blood Vessels,
    → Lungs.
  - The second triad involves MNEMONIC -ENT
    → Otitis Media,
    → Nasal Septal Perforation,
    → Strawberry Gums



Limited wegner's granulomatosis: involvement of only lungs

- **Microscopic Polyangiitis:(opposite of PAN)**
  - It is a small vessel disorder.
  - No hepatitis B involvement.
  - Pulmonary involvement is seen.
  - Glomerulonephritis is seen.
  - Granuloma is not seen. Therefore,

Microscopically: it will show Leukocytoclastic Vasculitis.

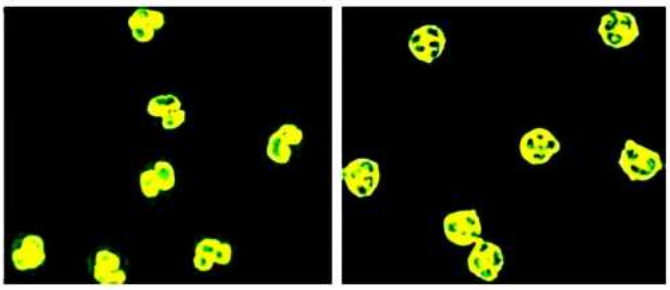
- Inflammatory cells(WBC's) are seen.
- Broken Cells are seen.
- All lesions will be at the same stages.

- **Churg Strauss Syndrome:**
  - It is also called Allergic Polyangiitis Granulomatosis.
  - Excess of eosinophils seen
  - It will cause cardiac complications. (common cause of death)

ANCA Association: 00:53:33

- Anti Neutrophil Cytoplasm Antibody.
- Here, the antibodies affect the cytoplasms.
- There are two types of ANCA,
  - Cytoplasmic ANCA
  - Proteinase ANCA
- In case only the area around the nucleus is targeted, that is P-ANCA. (Perinuclear ANCA) also called Myeloperoxidase ANCA.
- Also represented in immunofluorescence, where the PR3 ANCA and P-ANCA can be seen.

Cytoplasmic staining of granulocytes Perinuclear staining



C ANCA/PR3 ANCA	P ANCA/ MPO ANCA
Wegener's granulomatosis →	Wegener's granulomatosis, microscopic polyangitis, churg strauss syndrome, ulcerative colitis, primary sclerosing cholangitis

- If C/PR3-ANCA and P/MPO-ANCA occur together, they are called Atypical ANCA seen in - IBD, Rheumatoid artheritis
- Inicet information
  - Infections trigger the formation of the PR3-ANCA.
  - Drugs trigger the formation of the P-ANCA.

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Behcet's Disease:

Small & medium vessel disease

- Involvement of the HLA B51
- Triad: ulcers in mouth and genitals and uveitis
- Dural sinus thrombosis is the most common CNS manifestation.
- It can be diagnosed with a pathergy test.

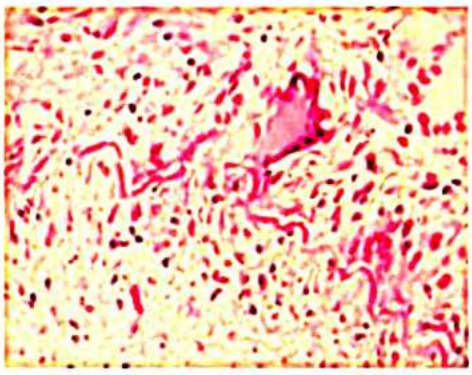
MCQ

01:05:37

Q. A 20-year-old woman complains of double vision, fainting spells, tingling in the fingers of her left hand, and numbness in the fingers of her right hand. Physical examination reveals the absence of a pulse in her right arm. Laboratory tests show elevated erythrocyte sedimentation rate and thrombocytosis. An Aortogram demonstrates narrowing and occlusion of branching arteries, including the right subclavian artery. The patient subsequently develops heart failure and dies of massive pulmonary edema. At autopsy, the aorta has a thickened wall and shows vasculitis and fragmentation of elastic fibers. Which of the following is the most likely diagnosis?

a. Buerger disease
b. Kawasaki disease
c. Polyarteritis nodosa
d. Takayasu arteritis

Q. A 70-year-old woman complains of a throbbing unilateral headache and vision problems. She reports weight loss and mandibular pain while eating. The patient also has a history of recurrent bouts of fever accompanied by malaise and muscle aches. Physical examination reveals nodular enlargement of the temporal artery with pain on palpation. A biopsy is obtained(shown in the image). What is the appropriate diagnosis?



a. Giant cell arteritis
b. Kawasaki disease
c. Polyarteritis nodosa
d. Wegener granulomatosis

Q. A 45-year-old man presents with pain in the legs upon exercise and the destruction of the tips of his fingers. He has an 80-pack-year history of smoking. Laboratory values include hemoglobin of 16 g/dL, WBC of 8,500, serum cholesterol of 220 mg/dL, fasting blood sugar of 90 mg/dL, and negative tests for antinuclear antibodies. A biopsy of the affected area (shown in the image) reveals intraluminal thrombi in medium-sized arteries and inflammation extending from arteries to neighboring veins and nerves. What is the appropriate diagnosis?

a. Buerger disease
b. Churg-Strauss disease
c. Kawasaki disease
d. Polyarteritis nodosa
e. Takayasu arteritis

Q. A 6-year-old girl presents with a 2-week history of a skin rash over her buttocks and legs and joint pain. The parents report seeing blood in the urine. Physical examination reveals palpable purpuric skin lesions and markedly swollen knees. The results of laboratory studies reveal an abnormally high erythrocyte sedimentation rate (30 mm/h), BUN of 25 mg/dL, and serum creatinine of 3 mg/dL. Urinalysis demonstrates BCs and BC casts. The stool guaiac test is positive. Biopsy of lesional skin reveals deposits of IgA in the walls of small blood vessels. Which of the following is the most likely diagnosis?

a. Henoch - schönlein purpura
b. Hypersensitivity vasculitis
c. Kawasaki disease
d. Poststreptococcal glomerulonephritis

Blood Vessels- Sclerosis

01:12:50

It causes the stiffening or thickening of the body areas. The classification can be done as

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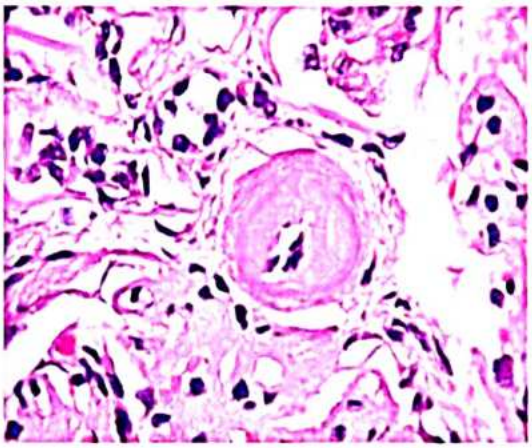
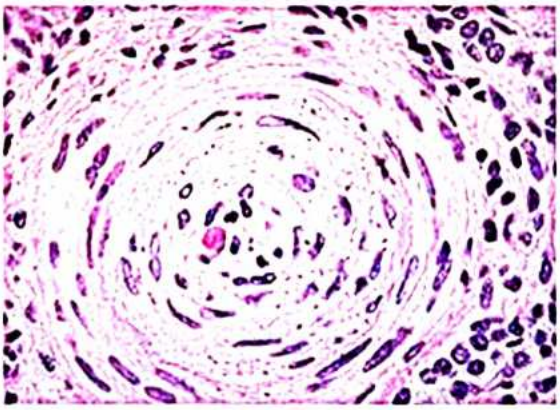
- **Hyaline Arteriolosclerosis:**



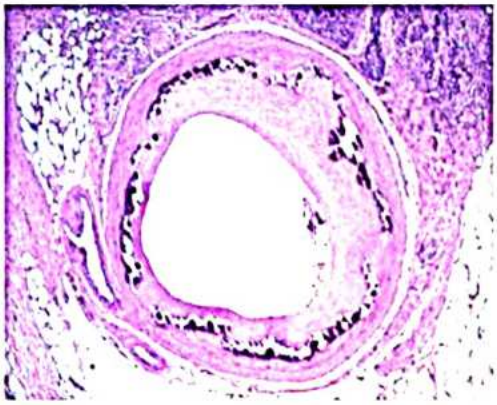
  - It shows a pink glassy appearance.
  - It is seen in Benign hypertension and Diabetes militis.

- **Hyperplastic Arteriolosclerosis:**



  - It shows the appearance of onion skinning.
  - It causes an increase in the smooth muscles and fibroblasts.
  - It is seen in Malignant HTN.

- **Monckeberg's Sclerosis:**



  - It is also called Calcific Medial Degeneration.
  - Calcific - Dystrophic Degeneration
  - Medial - Tunica Media
  - Degeneration - Old Age

- **Atherosclerosis-**

Risk Factors:

Non- Modifiable Risk Factor

  - Age
  - Male gender
  - Family history
  - Type A personality

Modifiable Risk Factors

  - Smoking
  - Diabetes mellitus
  - Obesity
  - Sedentary lifestyle
  - Lipoprotein increase
  - Hyperhomocysteinemia
  - Infections, including herpes, chlamydia, etc.

- **Atherosclerosis- Pathogenesis:**

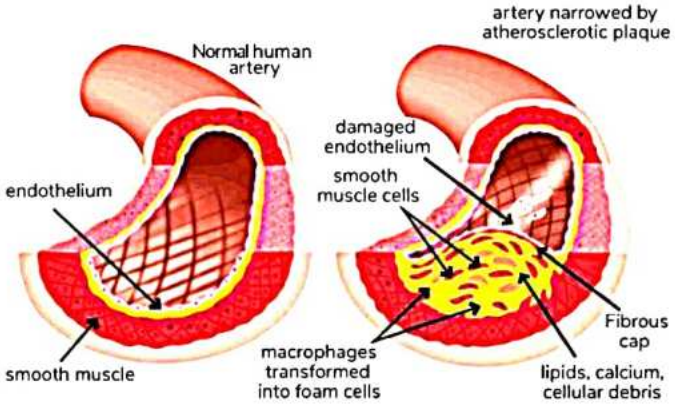
It responds to the injury hypothesis:

  - Hyperlipidemia
  - Inflammation
  - Hemodynamic disturbances

Important Information

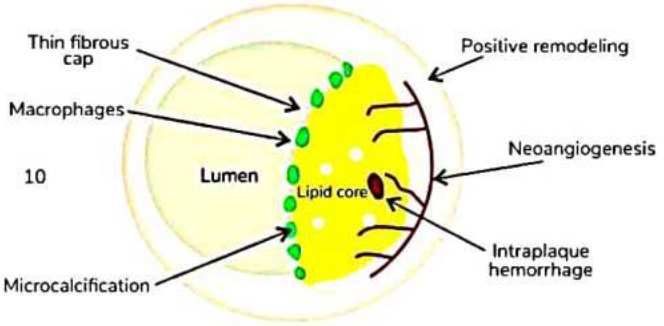
- Kruppel Like Factor 2 is now associated with atherosclerosis.

- **Atherosclerosis- Progress:** 01:22:12



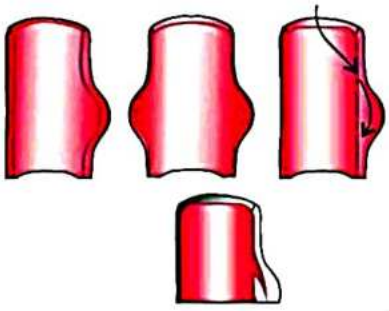
  - The first lesion - a fatty streak.
  - Leading to the atheromatous Plaque (Subintimal).
  - The plaque comprises Fibrous Cap and a core filled with lipid materials.

Telegram - @nextprepladdernotes



- Later, the Mϕ will engulf all the lipids, causing the foam cells.

- **Atherosclerosis- Complications:(A/C/U/T/E)** 01:27:05
  - **Aneurysm:**
  - **Calcification**
  - **Ulceration**
  - **Thrombosis**
  - **Embolism.**



→ It causes out pouching in blood vessels.
→ When all the three-layer experience outpouching, it is called a True aneurysm
→ A false aneurysm is seen when a blood collection occurs, showing an outpouching.
→ Saccular Aneurysm is seen when single side-out punching occurs.
→ If a tree bark aorta appears, it is called a Syphilitic Aneurysm
  - MARFAN SYNDROME- fibrillin defect
  - EDS- collagen defect
  - LOEYS DIETZ SYNDROME- TGF beta receptor defect leading to elastic defect
  - SCURVY- collagen cross linking defect

Important Information

Risk of rupture of aneurysm:
<=4CM= NO RISK
4-5CM= 1% PER YEAR
5-6 CM= 11% PER YEAR
>6CM= 25% PER YEAR

Mnemonic- ACP Delhi traffic is cute

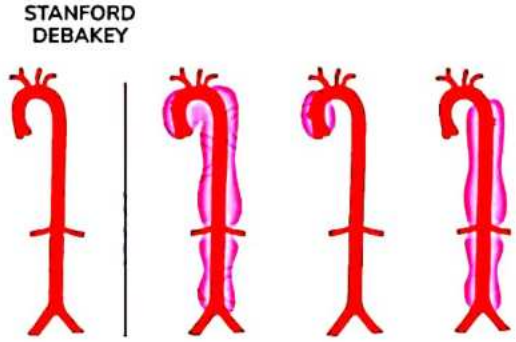
Vessels affected:

- Abdominal Aorta
- Coronary Artery
- Popliteal Artery
- Descending Thoracic Aorta
- Internal Carotid Artery.
- Circle of Willis.

Most common ↕ Least common

Aortic Dissection

- Tears in the intima.
- In case of chronic dissection, it causes Double Barrel Aorta.
- Most common cause of hypertension
- The Standford DeBakey, the affected areas can be categorized as

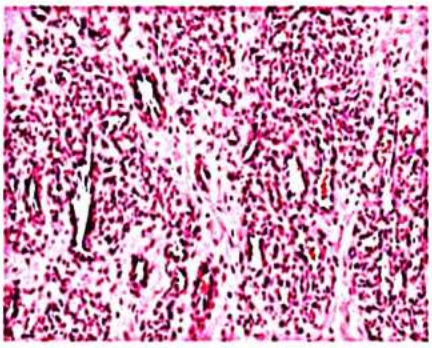


  - Stanford A/Debakey 1: Both ascending and descending aorta are affected
  - Stanford A/Debakey 2: Only the Ascending aorta is affected
  - Stanford B/Debakey 3: Only the descending Aorta is affected.

Vascular Tumors- Classification 01:42:20

Benign:

- **Hemangioma:**
  - Capillary Hemangioma:

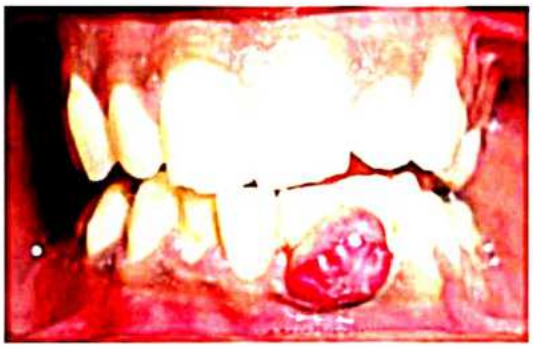


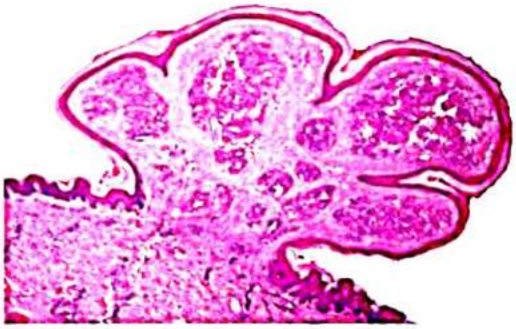
→ It has closely packed small thin blood vessels.
→ It occurs skin & superficial sites external sites.
→ Associated with Variant strawberry Hemangioma.

Telegram - @nextprepladdernotes

→ IN children: soon after birth, red lession(oral cavity) → 1-3 years size increases →size reduces slowly → after 7 years size reduces or tumor vanishes

- Cavernous Hemangioma:
  - → Dilated blood vessels are seen.
  - → It occurs on deep tissues.
  - → Most common tumor in liver
  - → A syndrome associated with cavernous Hemangioma is Von Hippel Syndrome.
- Lobular Capillary Hemangioma (pyogenic granuloma/granuloma gravidarum/pregnancy tumor)



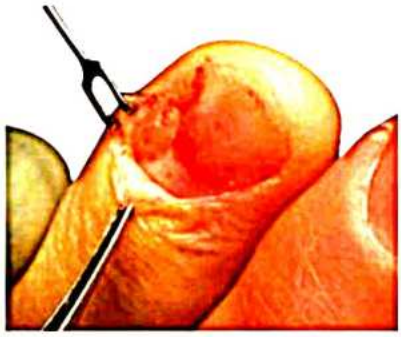


- → Small lobules of blood vessels are seen.
- → Usually seen in pregnancy tumors.
- → It can happen in the oral cavity, fingers, etc.
- → Regresses after pregnancy

- **Lymphangioma:**
  - Capillary Lymphangioma
  - Cavernous Lymphangioma
    - → When the cavernous Lymphangioma occurs in the neck, it is called Cystic Hygroma associated with turner's Syndrome

- **Glomus Tumor:**
  Origin: glomus body
  - Glomus body is arteriovenous anastomosis present on the finger tips for thermoregulation
  - It occurs at the subungual sites.
  - These tumors are excruciatingly painful.

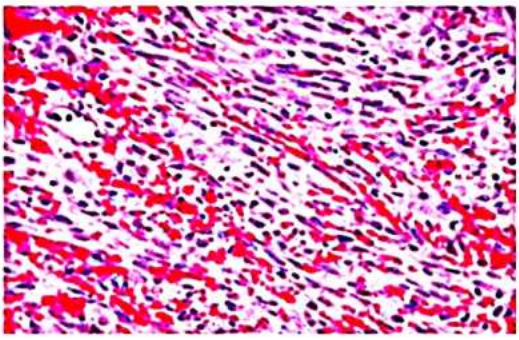
- **Bacillary Angiomatosis:**



  - It is associated with Bartonella henselae.
  - Positive for warthin starry silver stain

Intermediate:

- **Kaposi Sarcoma:**
  - It is caused by Human Herpesvirus 8
  - It is associated with HIV/immunodeficiency
  - Most common location is lower limbs(skin)
  - Second most common location is lymph nodes.
  - Categories:
    - → Chronic/classic/european- skin only
    - → Endemic/african- Skin +Lymph nodes
    - → Immunodeficiency associated- deep tissue + lymph node
    - → Transplant associated - deep tissue + lymph node



  - It will show a patch, plaque, and Nodules (showing a red-purple color). The blood vessel will be slit due to spindle cell proliferation.
  - The Latency Associated Nuclear Antigen, which is an Antigen for HHV8.

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Malignant:

- **Angiosarcoma:**
  - Angiosarcoma Liver is caused due to the Vinyl Chloride, Arsenic, and Thorotrast.
  - **Stewart Treves Syndrome:**
    → It occurs in long-standing Lymphedema.
    → It can stand upto 10 years.
- **Hemangiopericytoma:**
  - Pericytes or Rouget cells are cells around the blood vessels.
  - Microscopy shows staghorn or fishhook blood vessels

Important Information

- There are certain markers for differentiating the blood vessels to the lymphatic channels, i.e., the lymphatic channels will have D2 40 positive.
- However, they have common markers such as CD34, CD31, and F8.

MCQs:

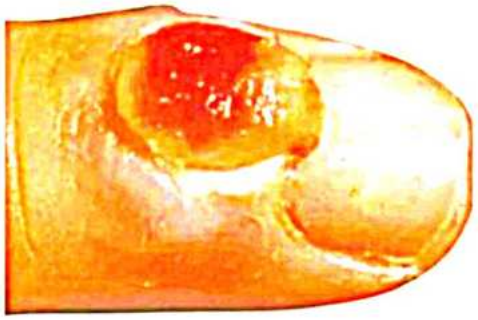
Q. A 35-year-old pregnant female presents with a soft reddish mass of 2 x 1 cm on the gums. The biopsy shows a lobular capillary hemangioma. Which of the following tumors is best suited for this clinical scenario?

a. Pyogenic granuloma
b. Glomus tumor
c. Hemangiopericytoma
d. Bacillary angiomatosis

Q. A 45-year-old male patient presents with a subungual mass of 1 x 1 cm. The patient complained of excruciating pain. Diagnosis?

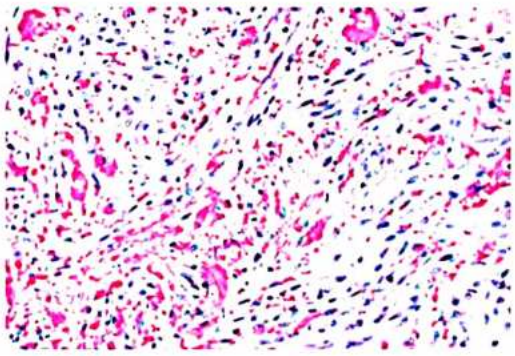
a. Pyogenic granuloma
b. Glomus tumor
c. Hemangiopericytoma
d. Bacillary angiomatosis

Q. A 35-year-old pregnant lady presents with a lesion over the finger, as shown condition with?



a. Cavernous hemangioma
b. Glomus tumor
c. Kaposi sarcoma
d. Lobular capillary hemangioma

Q. A 33-year-old man with AIDS presents with multiple purple-colored skin nodules on his hands and feet. The lesions vary in size from 1 mm to 1 cm in diameter. A biopsy of lesional skin is shown in the image. Which of the following viruses is implicated in the pathogenesis of this patient's skin neoplasm?



a. Cytomegalovirus
b. Human herpesvirus-6
c. Human herpesvirus-8
d. Human immunodeficiency virus

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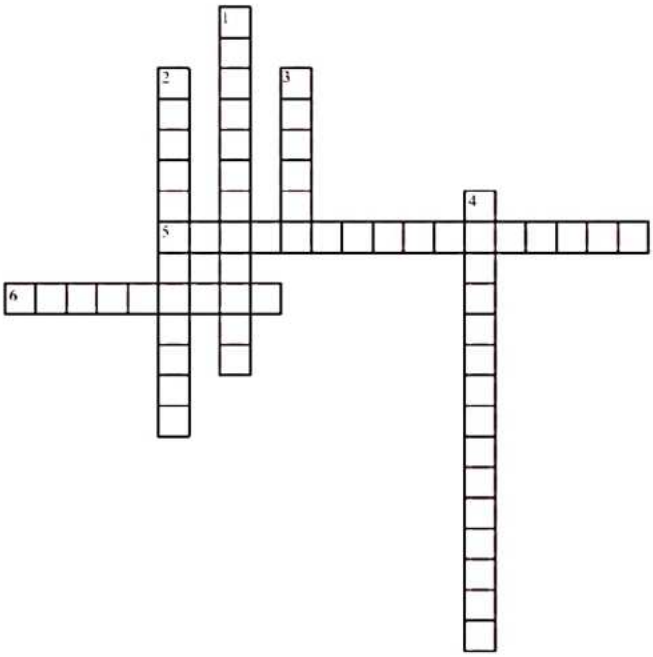
CROSS WORD PUZZLES

Crossword Puzzle



Across

5. In case of chronic dissection, it causes Double Barrel Aorta.
6. It has closely packed small thin blood vessels.

Down

1. Associated with the blood vessels, showing atypical cells and many mitoses.
2. It has closely packed small thin Lymphatic Channels.
3. It is called Human Herpesvirus 8
4. Causes ulcers in the mouth and genitals.

Telegram - @nextpreladdernotes

25 CARDIAC PATHOLOGY

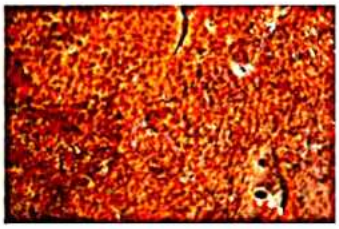
Various cardiac disorders:

- Heart failure
- Ischemic heart disease
- Endocarditis
- Cardiomyopathy
- Cardiac tumors

Heart Failure

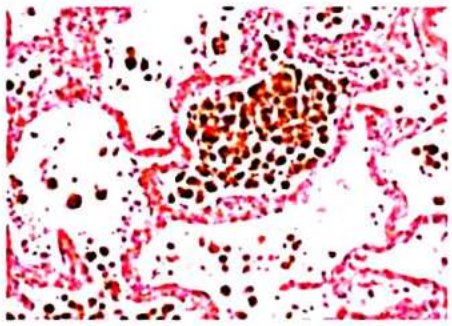
00:00:21

- Divided as
  - Left-sided Heart failure.
  - Right-sided Heart failure.

Left-Sided Heart Failure	Right-Sided Heart Failure
Left side of the Heart stops working.	The right side of the Heart stops working.
Leads to • Pulmonary congestion • Pulmonary edema	Leads to Chronic venous congestion in • Liver • Spleen
Gross Appearance: Wet and boggy.	• Liver - Nutmeg liver.
Microscopically Presence of hemosiderin pigment ↓ Engulfed by macrophages (called Heart failure cells)	 • Spleen - Gamma Gandy bodies. Composition of Gamma Gandy Bodies • Hemosiderin • Calcium • Fibrosis

Heart Failure Cells

- Observed due to Left-Sided Heart failure.
- Seen in lungs.
- Hemosiderin laden macrophages
- **Special stain used:**
  - Perl's stain/ Prussian blue stain



Ischemic Heart Disease

00:08:51

- Includes angina and myocardial infarction (MI).

Angina

00:09:20

- 3 types:
  - Stable Angina
  - Unstable Angina
  - Variant or Prinzmetal or Vasospastic Angina.

Stable Angina	Unstable Angina	Variant or Prinzmetal Angina
Has an uncomplicated stable atheromatous plaque.	Has a complicated plaque.	Vasospasm of coronary artery.
No serious complications.	Complications include • Aneurysm, • Calcification, • Ulceration, • Thrombosis, • Embolism **Mnemonic:** ACUTE	Vasodilators are used to reduce the spasm.
Pain on exertion.	Pain on a lesser degree of exertion/rarely at rest also	Pain at rest. Has the best response to vasodilators.

Myocardial Infarction

00:13:51

- Left-sided chest pain.
- Radiate to:
  - Jaw
  - Shoulder
  - Left arm
  - Epigastric region

Telegram - @nextpreladdernotes

- Other symptoms
  - Uneasiness
  - Nausea and vomiting
  - Diaphoresis (excessive sweating)

Diagnosis 00:15:21

ECG

- Gives different results depending on type of MI
- STEMI: ST-segment elevated MI
  - Seen in Transmural MI
- Non-STEMI: ST-segment depressed
  - Initially subendocardium is affected.

Cardiac Enzymes (16:51)

- Identified through blood samples.
- Cardiac Enzymes:
  - **My-** Myoglobin - non-specific marker.
  - **Time-** Troponin T
  - **To-** Troponin I - most important Marker.
  - **C-** CK-MB
  - **A-** AST
  - **LL-** LDH1

Cardiac enzyme	Start time	Peak time	Fall time
Myoglobin - Non specific - Early rise, early fall	2 hrs	-	24 hrs
Troponin T and I	2-4 hrs	48 hrs	7-10 days
CK-MB	2-4 hrs	24 hrs	48-72 hrs
AST/SGOT	<12 hrs	48 hrs	4-5 days
LDH1	24 hrs	4-5 days	After 10 days

Updates: Earlier Cardiac Markers

- Ischemia modified albumin (IMA)
- Heart fatty acid binding protein (HFABP)

Why is only LDH1 measured?

- Due to flipping of LDH ratio.
- In normal person:
  - LDH2 >> LDH1 in blood.
  - LDH1 >> LDH2 in Heart.
- In MI patient:
  - Heart injury occurs.
  - LDH1 levels increase in blood.
  - LDH1 >> LDH2 in blood.

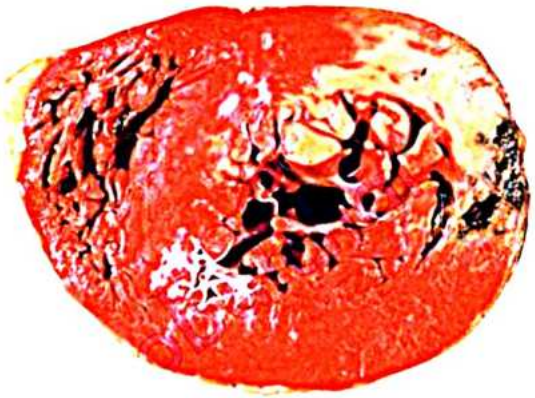
What is the marker for reinfarction?

- Troponin.
- Serial monitoring of troponin is done.
- Rise of 20% or more from baseline indicates reinfarction.

Autopsy Findings 00:28:07

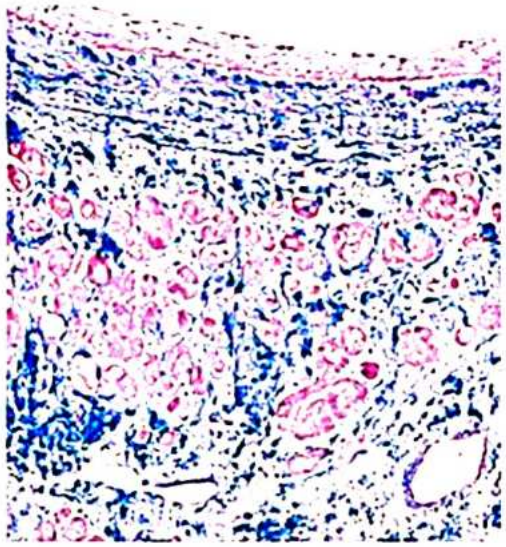
Refer Table 25.1

- For the gross appearance of heart within 4 hours of death:



  - Stain with TPTC (triphenyl tetrazolium chloride).
  - Stains LDH1.
  - Produces brick red color.
  - Normal heart: More LDH1 - more brick red color.
  - MI heart: Less LDH1 - more pale color.

Masson Trichrome Stain



- Used to distinguish muscle and collagen.
- Muscle - red color
- Collagen - blue color (indicates Scar formation).

Telegram - @nextprepladdernotes

Important Questions

Q. Which is the first electron microscopy change?
Ans. Relaxation of myofibrils and mitochondrial swelling.

Q. Which is the first light microscopy change?
Ans. Vacuolation of cells and waviness of cardiac fibers.

Q. Which is the first Gross change?
Ans. Occasional dark mottling.

If the patient survies after MI:

Reperfusion Injury 00:45:45

- Any clot in the heart's vessels.
- Deposition of calcium and free radicals near the clot
- Thrombolysis as a result of treatment.
- All the deposited Calcium and free radicals reach the heart and cause muscle contraction
- Formation of contraction bands occur on the heart.
- More severe cause: Free radicals.

Complications 00:48:26

Act Rapid

- Arrhythmia
  - **<1 hr** - Ventricular fibrillation
  - **>1 hr** - Supra ventricular tachycardia
- Contractile dysfunction
- Thrombus
- Rupture - risk maximum between 3 to 7 days
- Aneurysm
- Papillary muscle dysfunction
- Infarct extension
- Dressler syndrome
  - occurs around 3 weeks
  - Also called Autoimmune pericarditis

Multiple Choice Questions 00:51:34

Q. A 61-year-old female patient presents with left-sided chest pain, radiating to the left arm and jaw. The patient explains that the pain has increased severely over the past 40 minutes. She is immediately rushed to the hospital. Cardiac enzymes are elevated. The patient was admitted and started on thrombolytic therapy. However, on the 5th day of observation, she suddenly collapses and dies. Which necrosis are you most likely to find in this patient's heart?

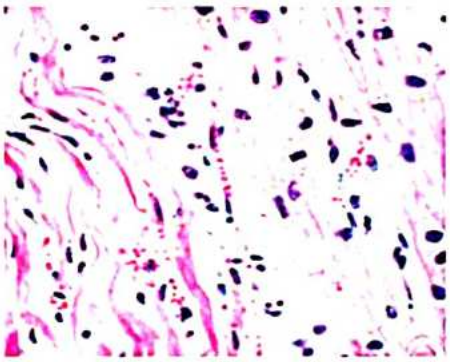
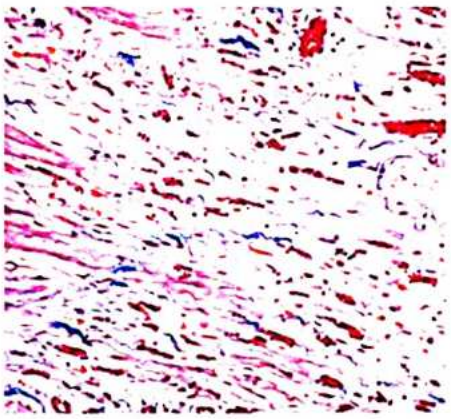
a. Liquefactive necrosis
b. Coagulative necrosis
c. Fat necrosis
d. Fibrinoid necrosis

Q. Autopsy diagnosis of myocardial infarction can be made by immersion of tissue slices in a solution of?

a. Triphenyl tetrazolium chloride
b. 100% alcohol
c. Orcein stain
d. Crystal violet

Telegram - @nextprepladdernotes

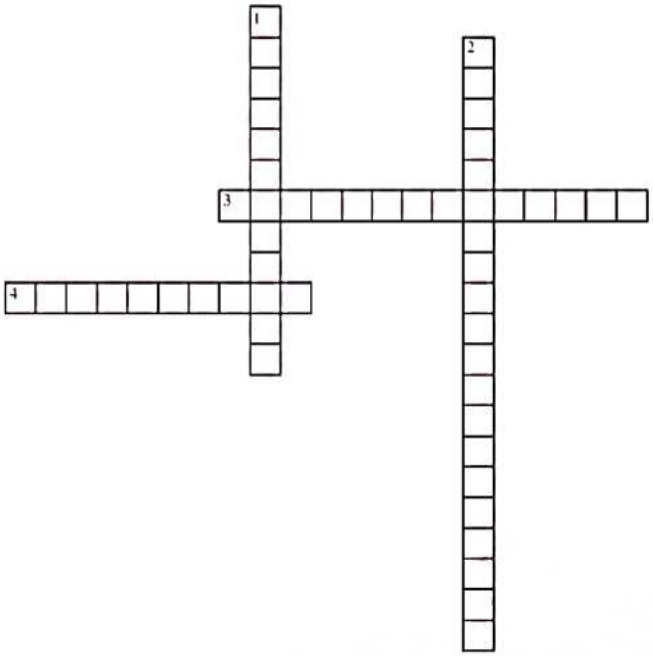
Table 25.1

Timings of death	Gross	Light microscopy	Electron microscopy
Reversible cell injury (20-40 minutes)	-	-	• Myofibrils relaxation • Mitochondrial swelling
Irreversible Cell Injury 30 mins to 4 hrs	-	• Vacuolation of cell • Waviness of cardiac fibers 	• Amorphous densities (Calcium in mitochondria)
4 hrs to 12 hrs	Occasional dark mottling	Coagulative necrosis	
12 hrs to 24 hrs	Dark mottling	• Coagulative necrosis • Neutrophils • Contraction band necrosis	
1 day to 3 days		Neutrophils (acute inflammation)	
3 days to 7 days		Macrophages (Chronic inflammation)	
7 days to 10 days		Early Granulation tissue 	
10 days to 14 days		• Granulation Tissue • Early collagen	
2 weeks to 2 months		High collagen	
>2 months		Scar formation	

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Identified through blood samples
4. Contractile dysfunction

Down

1. Has an uncomplicated plaque
2. Used to distinguish muscle and collagen.

Telegram - @nextprepladdernotes

26 CARDITIS AND CARDIAC TUMORS

- Divided based on the three layers of the heart:
  - Pericarditis
  - Myocarditis
  - Endocarditis

1. Pericarditis 00:00:26

- **Cause:** Bread and butter pericarditis or Sero(watery) fibrinous(thready/stringy) pericarditis
- Commonly seen in Rheumatic heart disease.
- **Pericardium have two layers:**
  - Outer parietal pericardium,
  - Inner visceral pericardium.
- The space between these two layers are filled with a fibrinous fluid.
- Hence, it is named as Bread and Butter Pericarditis

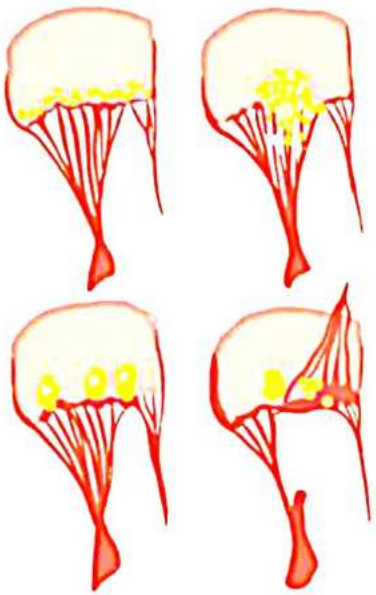
2. Myocarditis

Cause:

- **Viral organisms:** Coxsackie, Herpes
- **Parasitic:** Trichinella spirali

3. Endocarditis 00:03:24

- Shows vegetation or verruca
- It is classified into four types
  - Rheumatic Heart Disease (RHD)
  - Infective Endocarditis (IE)
  - Non Bacterial Thrombotic Endocarditis (NBTE) / marantic endocarditis
  - Libman-Sacks Endocarditis (LSE)



Rheumatiche art disease	Infective Endocarditis (IE)	Non Bacterial Thrombotic Endocarditis (NBTE)	Libman-Sacks Endocarditis (LSE)
• Vegetation: Small and warty Present along the lines of closures	• Vegetation: Large and bulky • It may or may not show embolisation	•Vegetation: Small and friable Shows embolisation	•Vegetation: on Both sides of the walls are effected More common in lower surface Seen in SLE
sterile	Non-sterile	strile	sterile

Questions	Answers
Which vegetation has the ability to show embolisation?	• Infective Endocarditis (May or may not) • Non Bacterial Thrombotic Endocarditis (Definitely cause embolization)
Where is infected vegetation present?	Infective Endocarditis (IE)

a. Rheumatic Heart Disease

- **Other Name:** Pancarditis
- **Cause:** Group-A beta hemolytic streptococcus(S. pyogenes)
- **Age:** 5 - 15 years
- **Incidence:** 3%

Mechanism 00:13:05

- **Other Name:** Molecular Mimicry

Streptococcus pyogenes has M proteins similar to glycoproteins (in human body)

↓

M proteins are entered into the human body

↓

Antibodies are produced as a defence mechanism

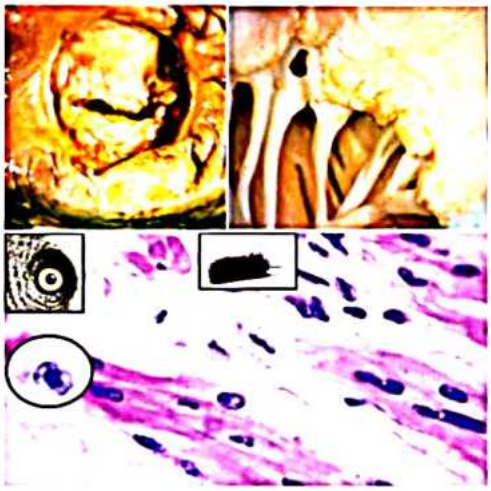
↓

They attack on both glycoproteins (present in the body) and M proteins

Telegram - @nextprepladdernotes

Affected Regions of the Heart 00:15:38

- **Most affected valve:** Mitral valve (Blood moves backward (mitral regurgitation) where it leads to the build-up of pressure in the left ventricle will develop posterior lesions in the posterior wall)
- Least affected valve: Pulmonary valve
- **Acute RHD:** Mitral valve regurgitation→ left atrium develops pressure→ irregular lession in posterior wall→ Mc Calluns patch
- **Chronic RHD:** Mitral valve stenosis → left atrium Fish mouth and buttonhole are seen



Most Characteristic Lesion in RHD 00:19:48

- Aschoff bodies

Questions	Answers
Where can you find Aschoff nodules?	Present in all heart layers: Pericardium, Myocardium (maximum), and Endocardium
What is included in Aschoff bodies?	• In the centre of the Aschoff nodules, fibrinoid necrosis is present • These are surrounded by Anitschkow cells • One cell has Caterpillar chromatin • Other cells have owl eye appearance

Revised Jones Criteria 00:23:55

1. Major criteria

Mnemonic: J O N E S Criteria

- **J O (Joint involvement):** Early manifestation: Migratory Polyarthritis
- **N (nodules):** Subcutaneous nodules (Painless)
- **E:** Erythema marginatum
- **S:** Sydenham's chorea (late manifestation)
- **C:** Carditis (Pancarditis)

2. Minor criteria

Two types:

- Clinical
  - → Fever
  - → Polyarthralgia
  - → Prolonged PR interval
- Lab
  - → Increased ESR
  - → Increased CRP

3. Supporting Evidence

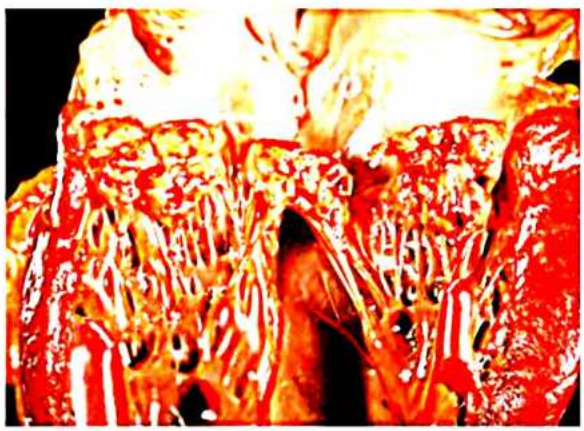
Supporting Evidence	Tests
• Antigen of bacteria	• Rapid antigen test
• Antibody of bacteria	• Antistreptolysin O
• Streptococcus organism	• Throat swab culture

Diagnosis 00:28:53

2 major
Or
2 minor
} + supporting evidence

b. Infective Endocarditis 00:29:32

- Depends on the native valve or prosthetic valve
- Native valve is further classified
  - → **Community-acquired infection**
    - Infants: Staphylococcus aureus
    - Adults: Streptococcus
  - → **Nosocomial:** Staph.aureus
- **Prosthetic valve infection**
  - → < 2 months: Staphylococcus epidermidis



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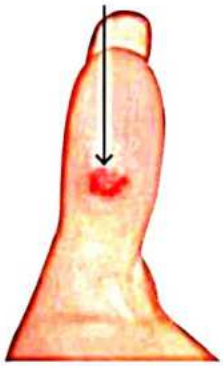
Modified Duke's Criteria

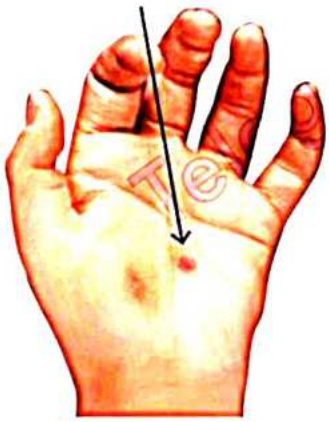
1. **Major criteria**
   - Blood culture positive (2 cultures > 12 hrs apart)
   - ECHO: Endocardial involvement
2. **Minor criteria**
   - Temperature (Fever)
   - **Immunological criteria:** Certain manifestations like osler nodes (Painful), Roth spots (Retinal emboli)

Osler Node



   - **Microbiological criteria:** Other organisms that leads to infections
   - **Vascular phenomenon:** Janeway lesion (Plams, soles)

Janeway Lesion



   - Predisposing factors/ iv drug abuser

- 2 major criteria or 1 major criteria and 3 minor criteria
- If major criteria is absent then 5 minor criteria should be present

Affected Regions of Heart 00:37:29

- **Most affected valve:** Mitral valve (Left side)
- **Most affected valve in I.V drug abuser:** Right sided heart valve

c. Non-Bacterial Thrombotic Endocarditis 00:38:28

- **Other Name:** Marantic Endocarditis
- **Example:** AML M3 (acute promyelocytic leukaemia)
- Formation of clots in the regions of heart

d. Libman-Sacks Endocarditis 00:39:23

- Both sides of the valve
- Mostly found in the lower surface.
- Seen in SLE

Cardiomyopathy 00:39:53

They are classified into five types,

- Dilated cardiomyopathy
- Takotsubo cardiomyopathy
- ARVCM
- Hypertrophic cardiomyopathy
- Restrictive cardiomyopathy

1. Dilated Cardiomyopathy 00:49:18

- Dilation of four chambers of heart. Globular structure.
- Ninja star nucleus (usually seen in the mutations of tit in gene)
- Hypertrophy of the cardiac fibres are also noted

Etiology

- Idiopathic causes
- **Genetic**
  - Titin (Largest protein) associated with AD
  - Dystrophin associated with X-Linked recessive
  - Mitochondrial associated with Childhood dilated cardiomyopathy
- **Others**
  - Alcohol(M/C)
  - Anthracycline
  - Cobalt
  - Catecholamine
  - Cocaine
  - Peripartum (increase level of prolactin)

    Peripartum may occur in;

    → Females of 30 years in the third trimester.

    → Increased levels of PRL (Prolactin) in females just after giving birth.

Questions	Answers
Is there DCM which can be associated with selenium?	It is found in certain countries like China, where selenium intake in diet is reduced leading to a Keshan disease same as DCM.

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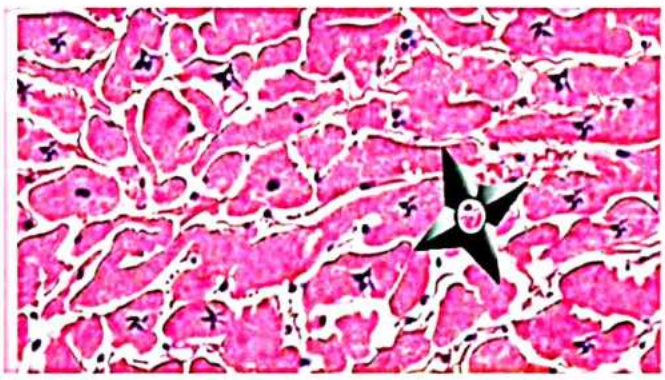
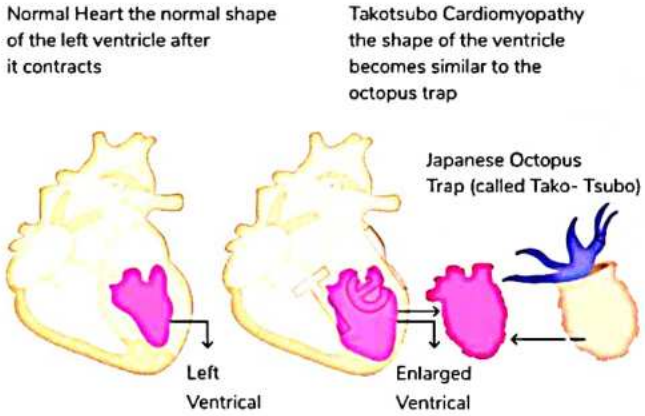


Ninja structure nucleus - usually associated with TITIN gene mutation DCM.

2. Takotsubo Cardiomyopathy 00:45:42

- **Other Name:** Broken Heart Syndrome
- Japanese Octopus trap (known as Tako-Tsubo)
- Variant of Dilated cardiomyopathy
- Increase of catecholamines due to emotional stress
- Finally, it dilates left ventricle(maximum receptor for catecholamines present in left ventricle)



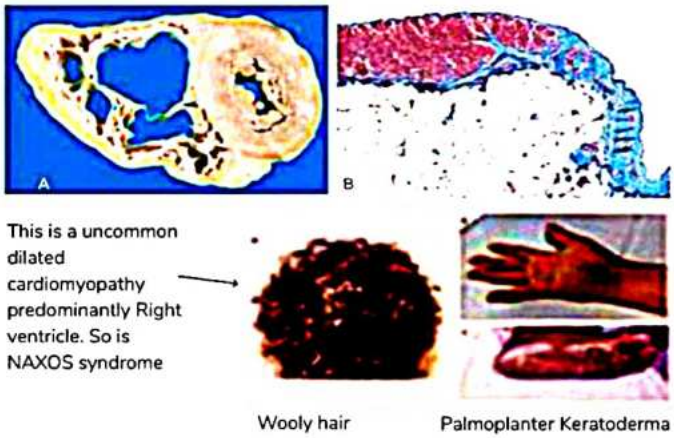
3. Arrhythmogenic Right Ventricular Cardiomyopathy

00:49:02

- Autosomal dominant disorders
- Damage to the desmosomes
- Infiltration of fats in the right ventricle
- **Associated with syndrome:** NAXOS syndrome (Defect of Plakoglobin)

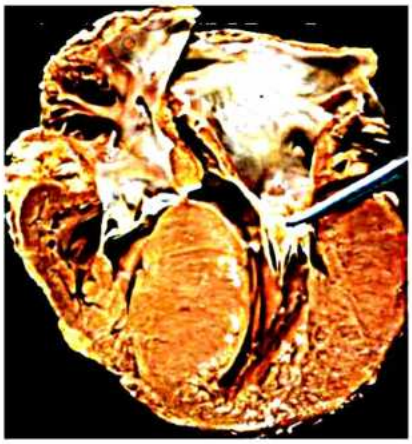
In case of NAXOS syndrome, patient experience or have a

- Arrhythmogenic Right Ventricular Cardiomyopathy
- Woolly hair
- Palmoplantar keratoderma

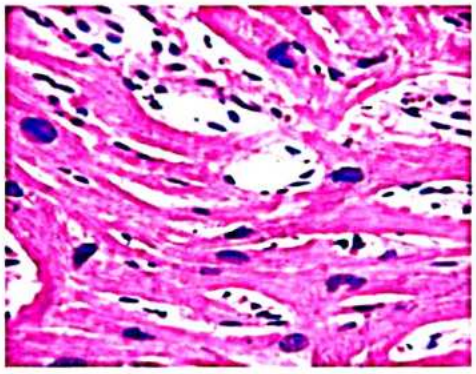


4. Hypertrophic Obstructive Cardiomyopathy 00:52:30

- Defect in the beta myosin heavy chain
- Autosomal dominant disorder
- **Indication:** Banana-shaped Heart
- Hypertrophy occurs in the interventricular septum.
- Only hypertrophy of IVS (Interventricular septum) occurs.
- In this condition the ratio of IVS/LV > 1.5 as a result;
  → Patients will experience obstruction to the blood flow, which ultimately leads to sudden death or collapse.



- **Under the microscope:** Helter Skelter Appearance
- **Appearance of Cardiac fibres:** Helter skelter, Haphazard, and Disarray of cardiac muscle fibers.



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5. Restrictive cardiomyopathy 00:57:26

- Diastolic dysfunction

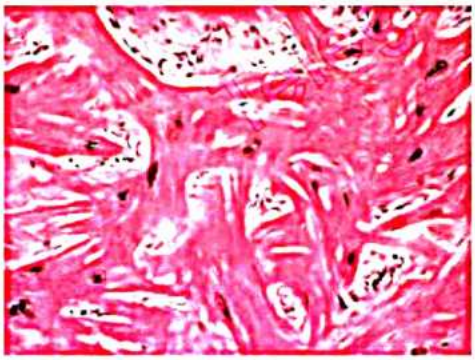
Etiology

- Amyloid(M/C)
- Metastasis (Cancer)
- Storage disease
- Loeffler's endomyocarditis (Increase in eosinophils)
- Fibroelastosis (Usually seen in Mumps or in Tafazzin mutation in children < 2 years)

Questions	Answers
Which type of cardiomyopathy cause iron overload (Hemochromatosis)?	DCM and RCM (if you have one option then DCM is preferred).

Multiple Choice Questions

Q. A 19-year-old college basketball player suddenly collapses on the court. A cardiac monitor shows ventricular tachycardia and then ventricular fibrillation. He is successfully resuscitated and hospitalised. The patient's history indicates that his father died suddenly at 38 years of age. The boy's echocardiogram reveals a thickened left ventricular wall, with a small slit-like chamber. The interventricular septum is also markedly thickened. Five years later the patient expires suddenly. Histologic examination of the heart muscle at autopsy is shown in the image. Which of the following is the most likely diagnosis?



a. Amyloidosis
b. Cardiac myxoma
c. Dilated cardiomyopathy
d. Hypertrophic cardiomyopathy

Q. 20-year-old previously healthy athlete suffers a sudden cardiac arrest while playing sport. On autopsy, there is massive myocardial hypertrophy. Microscopy shows myofiber disarray and interstitial fibrosis. What is the most likely diagnosis?

a. Alcohol-induced cardiomyopathy
b. Amyloidosis
c. Hypertrophic cardiomyopathy
d. Dilated cardiomyopathy

Q. A 60-year-old man presents with increasing girth and fatigue. Physical examination reveals peripheral edema, ascites, and hepatomegaly. Liver function tests are normal. An echocardiogram shows a remarkably enlarged right heart and no signs of valvular heart disease. Endomyocardial biopsy discloses interstitial, pink amorphous deposits between cardiac myocytes. Which of the following is the appropriate diagnosis?

a. Carcinoid heart disease
b. Cardiac amyloidosis
c. Dilated cardiomyopathy
d. Hypertrophic cardiomyopathy

Cardiac Tumours 01:05:35

- Common cardiac malignancy: Metastasis
- Cardiac valve tumour: Papillary FibroElastoma (Sea anemone like appearance)

Primary Cardiac Tumours		
Type of Tumour	Adults	Children
Benign tumours	Myxoma	Rhabdomyoma
Malignant tumours	Angiosarcoma	Rhabdomyosarcoma

Features of Myxoma and Rhabdomyoma 01:08:54

Features	Myxoma (Image 1:10:23)	Rhabdomyoma (Image 1:10:47)
Age	Adults	Children
Chamber	LA Tumour plop sound	LV
Cells	Lepidic cells	Spider cells
Syndrome	Carney complex (PRKAR and GNAS mutation)	Tuberous sclerosis (TSC)

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PREVIOUS YEAR QUESTIONS

Q. ANCA negative vasculitis amongst the following is? (JIPMER Nov 2017)

A. Wegner granulomatosis
B. Churg Strauss syndrome
C. Polyarteritis nodosa
D. Microscopic polyangiitis

Q. A 30-year-old male presented with severe dyspnea. His investigations showed mitral stenosis with left atrial enlargement. The histopathology report from his mitral valve is shown below. What is the likely diagnosis of these patients? (AIIMS Nov 2017)

A. Sarcoidosis
B. Fungal granuloma
C. Tuberculous
D. Rheumatic Heart disease

Q. Irregular, bulky and friable vegetation in cardiac valve is a finding of which of the following disorders? (NEET Jan 2020)

A. Infective endocarditis
B. Rheumatic endocarditis
C. Libman sack endocarditis
D. Nonbacterial endocarditis

Q. Most common tumor in a female diagnosed with tuberous sclerosis. (JIPMER 2018)

A. Rhabdomyosarcoma
B. Angiomyolipoma
C. Pulmonary lymphangio-leiomyomatosis
D. Optic Glioma

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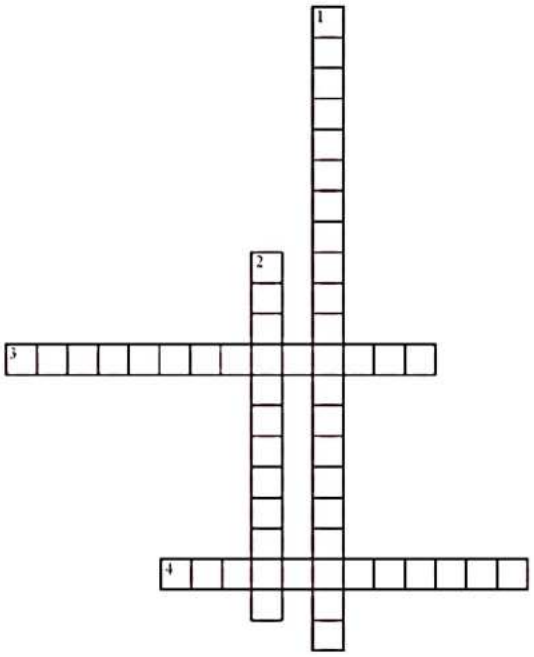
CROSS WORD PUZZLES

Crossword Puzzle



Across

3. There are classified into five types4.Bread and butter pericarditis

Down

1. Present along the lines of closures
2. Verruca

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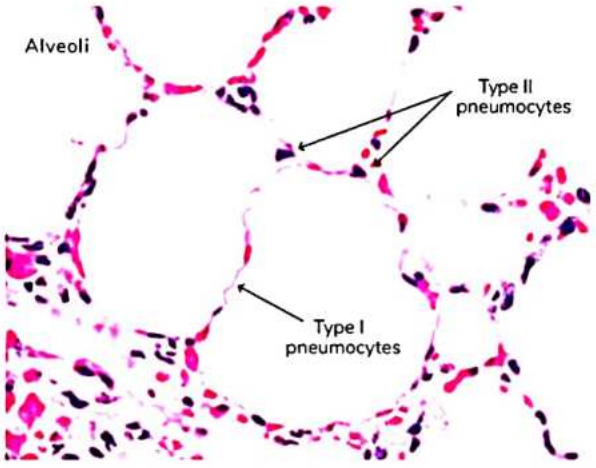
27 LUNG PATHOLOGY PART-1

Basic Anatomy of Lungs

Trachea
↓
Bronchi
↓
Bronchioles
↓
Terminal bronchioles →
respiratory bronchioles → alveolar duct → alveolar sac

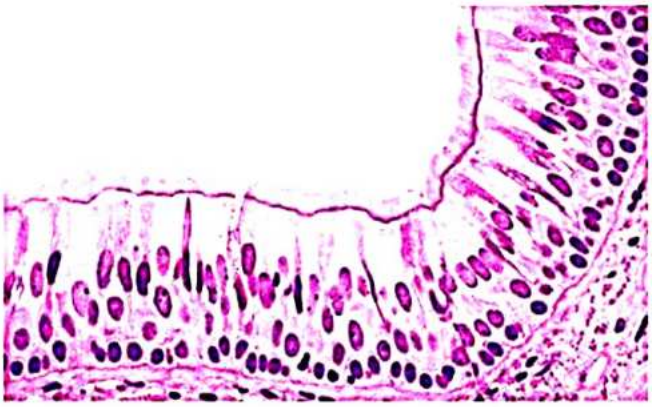
ACINUS



Alveoli

Histology of Lungs

00:01:52



- Lining of respiratory tract: pseudostratified ciliated columnar epithelium.
- Cilia has a dynein arm which is defected leads to kartagener's syndrome.

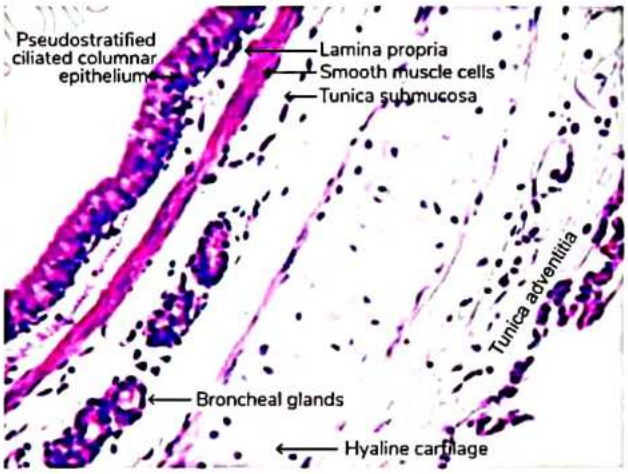
Ciliocytophthoria

- The ciliated cells get degenerated.
- **Ex:** In viral infections.

- All the structures of the respiratory tract are lined by ciliated epithelium except vocal cords, pneumocytes.
- Vocal cords are lined by Squamous epithelium but not ciliated epithelium.
- Pneumocytes - present in alveoli.
  - **Type 1:** Most predominant - 95%.
    → Helps in air exchange.
  - **Type 2:** Less predominant - 5%.
    → Involves in surfactant production and cell repair.

Communications

- Alveoli to alveoli - Pores of kohn.
- Bronchiole to bronchiole - Channel of Martin.
  - Mnemonic: BBM.
- Bronchiole to alveoli - Channel of Lambert.
  - Mnemonic: BAL.



Respiratory layers

- **Comprises of**
  - Mucosa
  - Submucosa
  - Muscle
  - Cartilage

- **Mucosa:**
  - Epithelium

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- Lamina propria
- Muscularis Mucosae

- **Submucosa:** Comprises mucosal glands.

Congenital Abnormalities

00:13:19

1. Bronchogenic Sequestration

- Extra tissue of the lung is present.
- Embryological abnormality.
- **2 types**
  - **Intralobar sequestration:** Extra lung tissue present within the lungs.
  - **Extralobar sequestration:** Extra lung tissue present outside the lungs.

> **Important Information**
>
> - Separate branches of the aorta supply the extra lung tissue.

2. Lung Abscess

- Abscess occurs due to an infection.
- **Causes:**
  - Foreign body
  - pneumonia
  - Septicemia
  - Malignancy
- **Location:** Right lower lobe.

- The Foreign body when aspirated enters into the right lower lobe of lung.
- This is because the right bronchus is more towards the midline.

- **Clinical presentations**
  - Fever
  - Foul smell
  - blood tinged sputum.
- Treat the underlying cause along with antibiotics.

Obstructive Pulmonary Disease

It includes 3 disease: Mnemonic- CAB

- COPD
  - Emphysema
  - Chronic bronchitis
- Asthma
- Bronchiectasis

1. COPD

- Includes emphysema and chronic bronchitis.
- Both are associated commonly with smoking.

A. Emphysema

- Irreversible damage to airways which are distal to the terminal bronchioles.
  - **Involves:** Respiratory bronchiole, alveolar ducts, alveolar sacs (RDS).

Pathogenesis of Emphysema

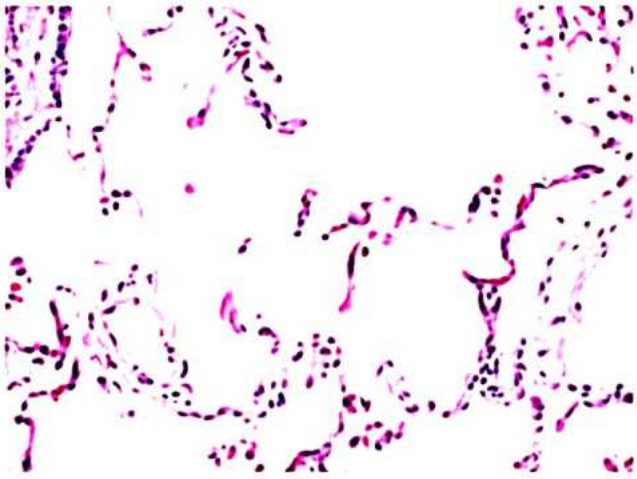
- Lung has 2 enzymes:
  - **Elastase** - Breaks elastase tissue.
  - **Anti-elastase** - Prevents breakage of elastase tissue.
    → Alpha1 antitrypsin.
  - Both enzymes are present in the same quantities.
- **Chronic smokers:** There is a high amount of elastase.
- **Alpha1 antitrypsin deficiency:** There is less amount of anti-elastase.
- In both situations, elastic tissue breaks - leading to emphysema.

Alpha1 Antitrypsin

- Alpha1 Antitrypsin gene is present on chromosome number 14.
- Normal amount of alpha1 antitrypsin genotype is called:
  - PiMM (pi = protease inhibitor).
- Diseased genotype called - PiZZ.
- Carrier genotype called - PiMZ
- Inheritance of carrier - Autosomal resistance disorder.
- **Affected organs:** Lungs and liver.
  - Liver is affected by cirrhosis.
  - Lungs are affected by panacinar emphysema.

Types of Emphysema

Refer Table 27.1



Emphysema microscopic picture

- Broken walls are seen - known as floating septa.

Clinical Features of Emphysema

- In emphysema elastic tissue is broken which results in elastic recoil.

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- Oxygen can easily enter the lungs.
- Difficult to eject carbon monoxide outside.

- **Symptoms include**
  - **Barrel shaped chest**
    → Flattened diaphragm.
    → Seen radiologically.
  - Pink puffers
    → In late cases it leads to cor pulmonale.

B. Chronic Bronchitis

- Productive cough (cough with sputum) for 3 months in at least 2 consecutive years.
- After all other causes are ruled out then the condition can be called chronic bronchitis.

Pathogenesis of Chronic Bronchitis

- Most common cause is smoking.

Smoking causes irritation in the lungs
↓
Increases mucous secretion
↓
Increases risk of infections and also increases Reid's index
↓
Infections
↓
Most common infections are Haemophilus influenzae and 2nd most common - moraxella
↓
Increase in Reid's index indicates the increase in submucosal glands

Reid's Index

RI = Thickness of submucosal gland layer ÷ overall thickness of the wall

- Let Submucosa is 'a' and the total layers is 'b' then,
  - RI = a ÷ b
- Normal Reid's index: 0.4

Clinical Presentations of Chronic Bronchitis

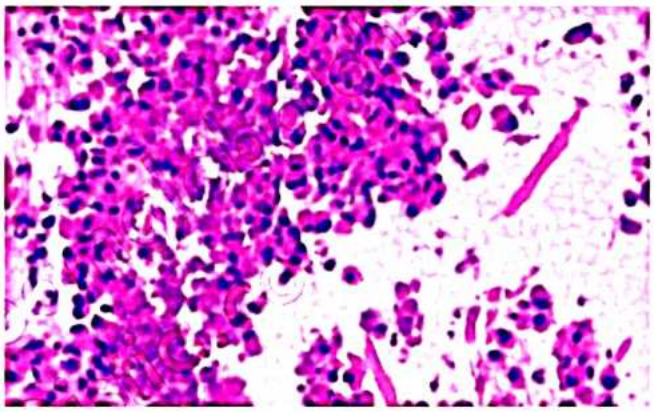
- As the alveoli is lined by more mucous oxygen doesn't diffuse out of the alveoli.
  - Decrease in oxygen saturation.
    → Called blue bloaters.
- Hypoxia

When patient has hypoxia
↓
All the blood vessels dilate to give more oxygen to the body - vasodilation
↓
While the pulmonary vessels constrict - pulmonary vasoconstriction
↓
Increases pulmonary resistance
↓
Leads to pulmonary hypertension - leading to right heart failure
↓
This condition is called cor pulmonale

- Cor pulmonale is commonly seen in chronic bronchitis.

2. Asthma 00:48:22

- It is a type of hypersensitivity type 1 reaction
- Cough with sputum.
  - Sputum is fixed with saccomanno fixative.
  - Microscopy shows 3Cs
    → Charcot Leyden Crystal

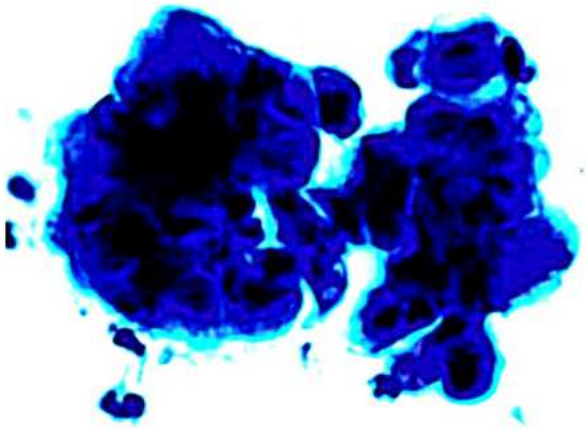


- Needle shaped crystals.
- Composed of Galectin 10.

Important Information

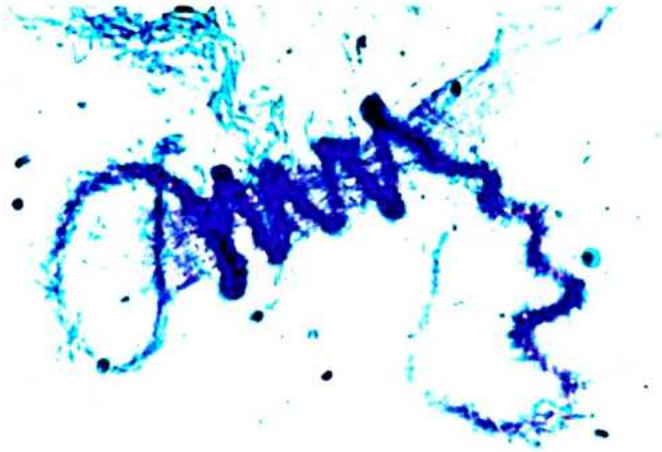
- Galectin 10 - Derived from eosinophils.

- Cereola bodies



Telegram - @nextprepladdernotes

- Made of epithelial cells.
- Crushmann spiral



- Made of mucus.
- Looks like spirals.
- Inside the lungs airway remodeling is seen.

Airway Remodeling

- Changes in airway pattern occur.
- No increase in Reid's index
  - There is an increase in mucous secretion but along with it all other cells are also increased.

Extra Edge

Asthma is also related to genetics.

- **Atopy gene:** Genetic determinant in allergy.
  - Associated with chromosome 5q.
- IL13 polymorphism.
- ADAM33 polymorphism.
- Increased YKL40 - correlated with severity.

3. Bronchiectasis 00:56:49

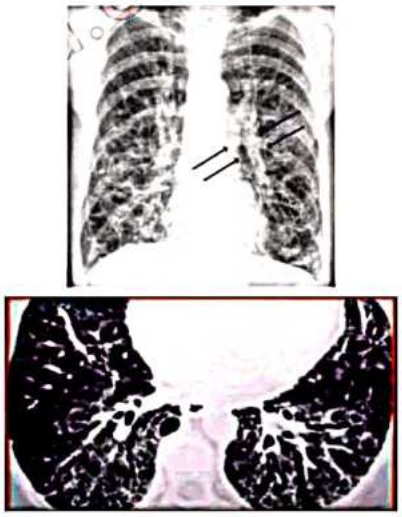
- It is the permanent dilation of bronchi or bronchioles.
- **Causes**
  - **Genetics**
    → Kartagener's syndrome
  - **Acquired**
    → Sepsis
    → Obstruction

Kartagener's syndrome

- Dynein arm defect in cilia.
- Patient has a triad of diseases - the BSS triad.
  - Bronchiectasis
  - Sinusitis
  - Situs Inversus (dextrocardia)
- Also leads to infertility in both males and females.

- **Clinical Features:** Cough with sputum - more in the morning.

- **Complications**
  - Lung Abscess
  - Septicemia
  - Amyloidosis (in long term cases)
- **Investigation of choice:** HRCT
  - Tram track appearance is seen.
- **Most common affected lobe:** Left lower lobe.



X-ray and HRCT of asthma Lung

Restrictive Lung Diseases 01:02:33

- **Fibrosing diseases:** IPF, NSIP, BOOP/COP.
- **Granulomatous diseases:** Hypersensitivity pneumonitis, sarcoidosis.
- **Smoking related:** PLUM LCH
- PAP (pulmonary alveolar proteinosis)
- Pneumoconiosis.

1. Fibrosing Diseases

- Fibrosis occurs within the lungs.
- Patient presents with a dry cough and dyspnoea.

Telegram - @nextpreladdernotes

A. Usual interstitial Pneumonia / Idiopathic Pulmonary Fibrosis

- Mutations in following genes,
  - TERT
  - TERC
  - MUC5B
  - Surfactant protein C
  - Temporal heterogeneity
    - → Old scar and new Fibrotic foci are seen at the same time.
    - → **Location:** Subpleural and interlobar areas.

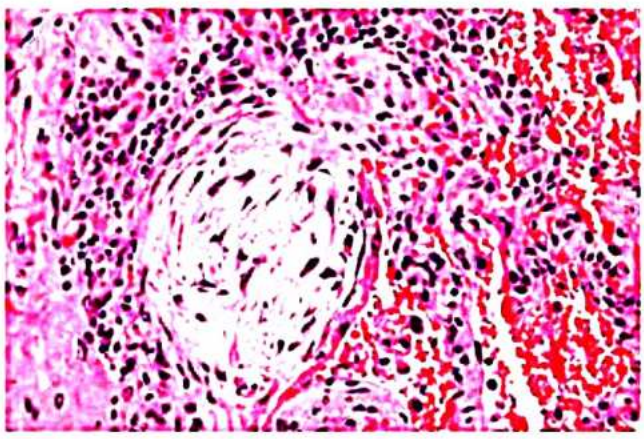
- **Treatment**
  - TGF beta inhibitor - Pirfenidone
    - → Because in Fibrosis TGF beta increases.
  - Tyrosine kinase receptor inhibitor - Nintedanib.
    - → To reduce Fibrosis foci.
  - Lung transplantation.

B. Nonspecific Interstitial Pneumonia

- Similar to IPF.
- Only 1 difference - it has no heterogeneity.
  - Either an old scar is present or a new fibrotic foci is present.
- Treatment includes steroids.

C. Bronchiolitis Obliterans with Organizing Pneumonia (BOOP) / Cryptogenic Organizing Pneumonia (COP)

- Bronchioles are obliterated in this condition.
- In many of the cases the cause is not known so called cryptogenic.
- It is a fibrotic disease - presence of fibrous plugs in:
  - Bronchioles
  - Alveoli
  - Alveolar sacs
- These fibrous plugs are called Masson bodies.



Important Information

- Masson fontana - Stain for melanin.
- Masson Trichrome - Stain for collagen.
- Masson bodies - Seen in BOOP or COP.

2. Granulomatous Diseases

- Granulomas are formed.

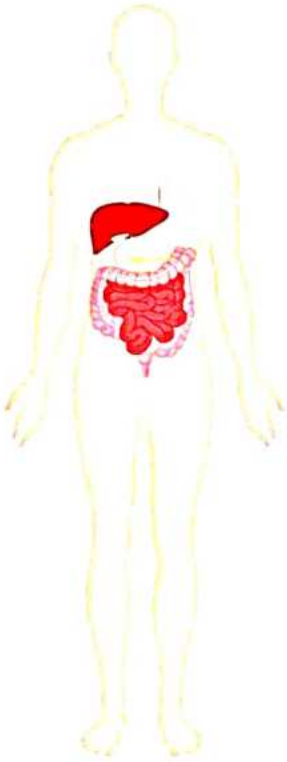
A. Hypersensitivity pneumonitis

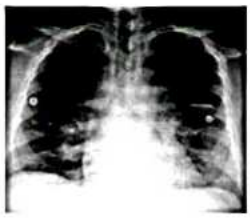
- Combination of type 3 and 4 hypersensitivity reactions.
- Type 4 hypersensitivity so granulomas are formed.
- Occurs due to inhalation of various occupational exposures.
  - **Moldy hay** - Micropoly spora faeni inhalation.
  - **Moldy sugarcane** - Thermoactinomyces sacchari inhalation.
  - **Mouldy Maple Bark** - Cryptostroma corticale inhalation.
  - **Bird Fancier's lung** - Bird feathers (proteins) inhalation.
  - **Hot Tub Lung** - Mycobacterium avium complex.
- Inhalation of all these microbes leads to various infections.

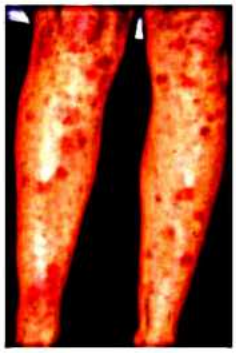
B. Sarcoidosis

- Ag is Unknown
- It is a multisystem disorder.
- It is a diagnosis of exclusion.

Organ Involvement in Sarcoidosis







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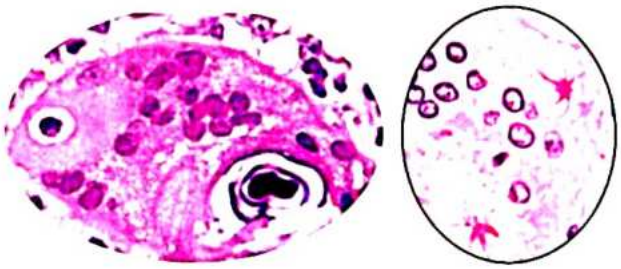


Organs involved

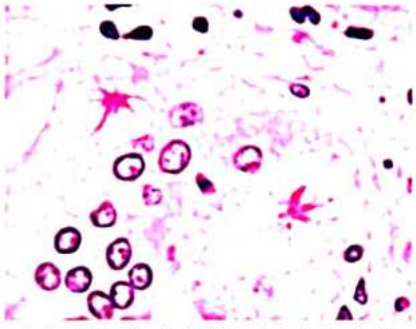
- **Lungs** - Most common
- Lymph nodes (Potato nodes)
- **Eyes** - Uveitis
- **Glands** - Salivary glands and Lacrimal glands affected (mikulicz disease).
- **Hepatitis** - Non infective
- Also has Lofgren syndrome

LOFGREN Syndrome

- **Clinical features**
  - Mnemonic: L O F GR EN
  - **L** - Lymphadenopathy
  - **O** - Ouch (Joint Pains)
  - **F** - Fever
  - **GR** - Granulomas
  - **EN** - Erythema Nodosum
- **Erythema Nodosum**
  - Mnemonic: Nodosum, Nobel.
  - Noble - Good prognosis.
- **Lupus Pernio**
  - Sarcoidosis is involved with skin.
  - Mnemonic: Pernio, Pernicious (mischievous).
  - Mischievous - Bad prognosis.

Diagnosis of Sarcoidosis

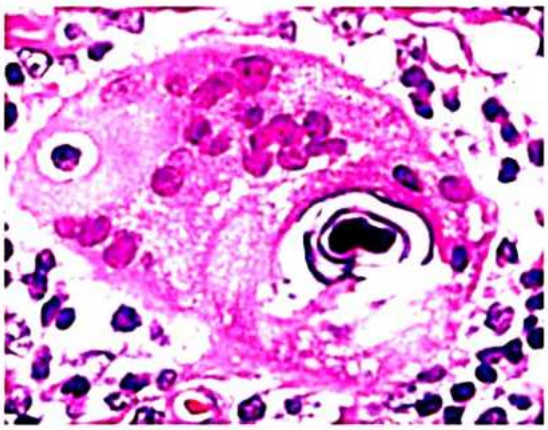
- **Biopsy** (Different Granuloma are seen)
  - Non-caseating granuloma - Characteristic feature
  - (Non-caseating granuloma >>> Caseating granuloma)
  - Naked granuloma (No lymphocyte covering)
  - **Asteroid bodies** (looks like star)
    → Seen inside Multinucleated giant cell



Asteroid bodies in Multinucleated giant cell

Schaumann bodies

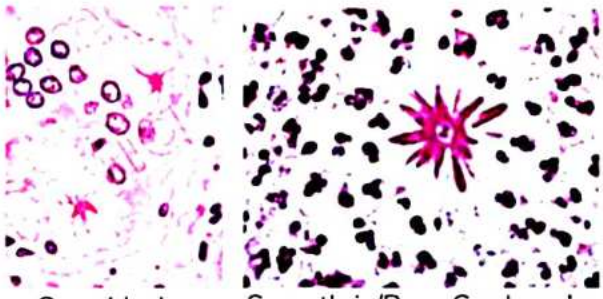
- S for Sarcoidosis
- C for Ca^{2+}



Schaumann bodies in Multinucleated giant cell

  - Lamellar bodies

Important Information



Sarcoidosis | Sporothrix/Rose Gardener's disease

- **Asteroid bodies are seen in**
  - Sarcoidosis
  - Sporothrix/ Rose gardener's disease
- **How to differentiate?**
  - Sarcoidosis - Intracellular Asteroid body (inside multinucleated giant cell)
  - Sporothrix/ Rose gardener's disease - Extracellular Asteroid body
  - Mnemonic: Rose gardener should do work roaming outside, thus extracellular.

- **Skin test**
  - Known as Kveim test
  - Not performed nowadays
- **Blood test**
  - 3 important tests
  - ↑**ACE**
  - ↑Ca^{2+} (Non-caseating granuloma leads to Vit D production leads to Ca

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- ↑CD4:CD8 ratio
  → Normal - 2:1
  → Sarcoidosis - can be up to 15:1
  → Thus CD4 is elevated.

Important Information

- Sarcoidosis leads to Metastatic calcification.

Treatment of Sarcoidosis

- **Spontaneous remission**
- **Steroids**

3. Smoking Related

Pulmonary Langerhans Cell Histiocytosis (LCH)

- Pulmonary LCH is related to smoking.

Related to occupation

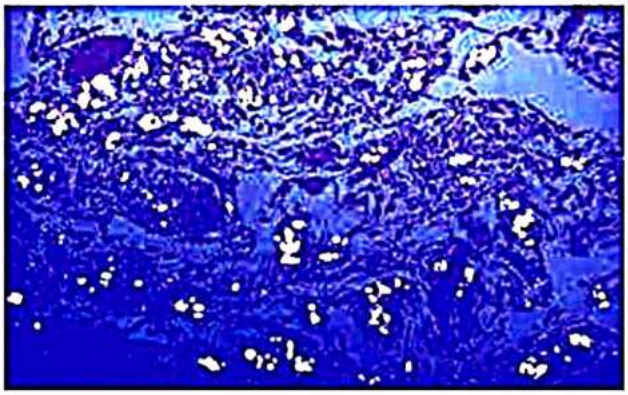
Pneumoconiosis

- Most common - Silicosis.
- Particle size
  - Medium size (0.5 to 5 microns) - Dangerous.
- Most affected lobe is the upper lobe.

Exception: In asbestosis the lower lobe is affected more
Mnemonic: AsBASEtosis, base for lower

A. Silicosis/ Grinder's Disease/ Miner Disease

- Seen in glass industry workers.
- **Common form:** Quartz.
- **Microscopy:** Polarizing Microscopy.



Silicon particles in the lungs

- **Organs affected**
  - Lungs - Nodular fibrosis
  - Lymph nodes - Egg shell calcification

- **Increased Risk of,**
  - Tuberculosis
  - Cancer

B. Coal Workers Pneumoconiosis (CWP) 01:30:19

- Seen in coal industry workers.
- **Initial** (beginning of work)
  - No symptoms
  - Lungs show anthracosis (black lung) due to anthracotic pigment (carbon).
- **Short time** (4 or 5 years)
  - Symptoms of simple CWP
  - Centriacinar emphysema is seen.
- **Long time** (many years)
  - Symptoms of complicated CWP.
  - Progressive massive fibrosis is seen.

Important Information

- **Caplan Syndrome:** CWP/ Silicosis + Rheumatoid Arthritis
- **Erasmus Syndrome:** Pneumoconiosis + Scleroderma

C. Asbestosis

- In asbestosis the lower lobe is affected more
- Mnemonic: AsBASEtosis, base for lower.
- Seen in shipyard industry.
- Forms: 2 main

Serpentine/ Chrysotile	Amphibole/ Crocidolite/ Amosite
Mnemonic • I am crying (Chrysotile) • After looking at a snake (Serpentine)	Mnemonic • I am on a site (Amosite) • Saw a Crocodile (Crocidolite) • Which is an Amphibian
Wavy (snake is wavy)	Rigid (crocodile is straight)
More common (snake can be seen more common)	More pathogenic

- **Rare type:** Erionite - mostly seen in Turkey.

Prognosis of Asbestosis

Pleural plaque (1st lesion)
↓
Pleural nodule
↓
Asbestos body is seen
Asbestos bodies
↓
Asbestos fibers are formed
↓
Body deposits iron around the Asbestos fibers
↓
Ferruginous body formed

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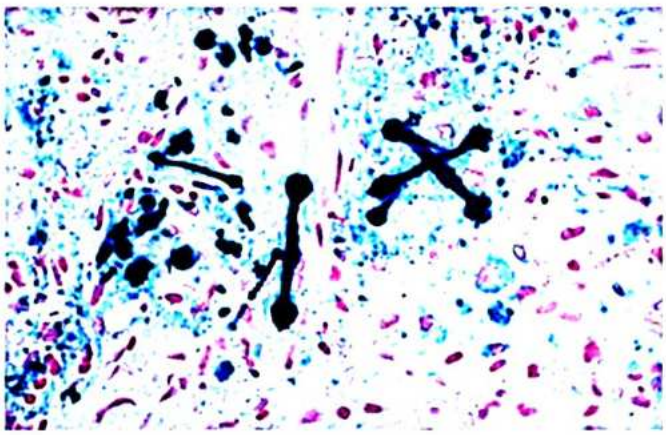
↓

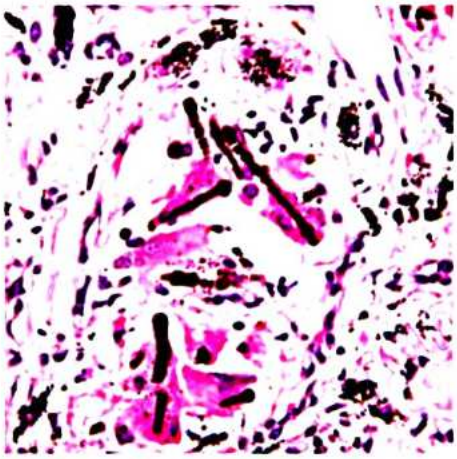
Cancer (after long time)

- As iron is deposited - Prussian blue/ pearl stain can be used.



Prussian blue stained asbestosis body

- Asbestos bodies are dumbbell shaped.



Dumbbell shaped asbestosis body

Important Information

- All Asbestos bodies are Ferruginous bodies, but all Ferruginous bodies are not Asbestos bodies.
- **Iron can cover**
  - Silica
  - Coal
  - Cotton

Let's Exercise Our Brains

Dumbbell shaped bodies

- **Lungs** - Asbestos body
- **Urine crystal** - Calcium oxalate monohydrate (Mnemonic: Mona is Dumb)
- **Microbiology** - Pox virus, dumbell DNA core.
- **Radiology** - Schwannoma of spinal cord.

Tumors Associated with Asbestosis

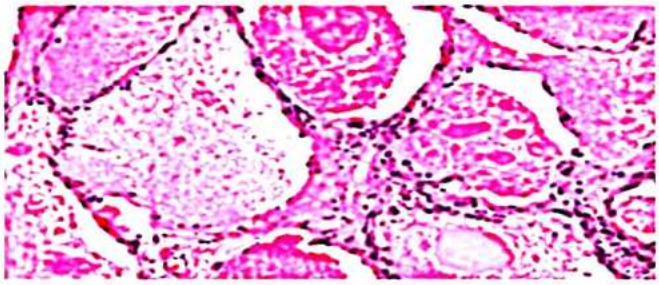
- **Lungs** - 2 types
  - **Lung adenocarcinoma** - Most common
  - **Lung mesothelioma** - More specific
- **Larynx** - Laryngeal carcinoma
- **GIT**
  - Stomach carcinoma
  - Colon cancer
- **Urinary system** - RCC urinary tumors

D. Miscellaneous

- **Baritosis** - Barium exposure.
- **Berylliosis** - Beryllium exposure
  - Can also lead to Non-caseating granuloma.
  - **Differential diagnosis:** Sarcoidosis.
  - Can be differentiated based upon industry exposure
- **Byssinosis** - Cotton dust/ organic dust exposure.
  - Patients have Monday chest syndrome
    - → Maximum symptoms are on Monday morning
    - → Symptoms reduce as the week progresses
    - → This cycle is repeated.
- **Bagassosis** - Sugarcane dust exposure.
- **Siderosis** - Iron exposure.
- **Stannosis** - Tin/ Lead exposure.

5. PAP - Pulmonary Alveolar Proteinosis

- **Neonatal**
  - **Cause:** ABCA3 mutation (ATP Binding Cassette)
  - Mnemonic: Kids learn ABC, so ABCA3 problem.
  - Improper production of surfactant is seen
- **Adult**
  - Loss of GM CSF signaling



Histopathology of PAP

- Protein inside alveolar space
- Comes out as sputum

Electron microscopy of PAP

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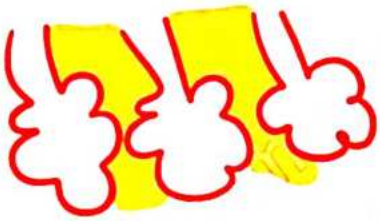
- Round concentric structures called the Lamellar body are seen.
- Usually seen inside type-2 pneumocytes.

Pneumonia

01:52:30

- Infection of the lung parenchyma.

Typical vs Atypical Pneumonia

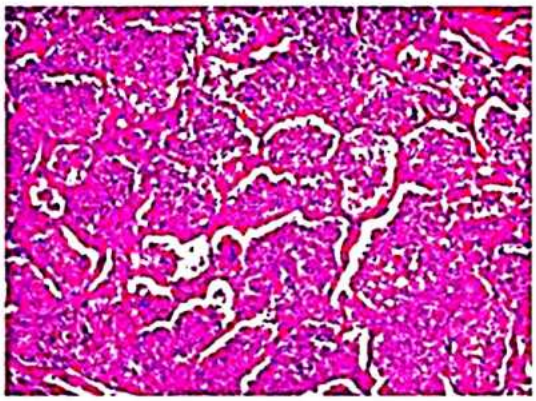
Typical	Atypical
Cause • Bacterial infection: o Streptococcus pneumonia o Klebsiella pneumoniae o Haemophilus influenzae)	**Cause** • **Most common:** Mycoplasma (bacteria) • Others: Virus, like measles (Hecht's Pneumonia) • Mycoplasma pneumonia is also called Walking pneumonia (as patient is not bedridden)
Cell involved: Neutrophils	**Cell involved:** Lymphocytes
Hallmark features	**Hallmark features**
	
• Intra alveolar exudate • Productive cough	• Interstitial exudate • Dry cough is seen

Types of typical Pneumonia

Lobar Pneumonia	Bronchopneumonia
Lobe consolidation	Patchy (Bronchi and Bronchial) involvement. Bilateral Basal Bimodal (young and old)
	

Laennec Stages of Pneumonia

- 4 stages with a 10 day cycle.
  - **Congestion**
    → 1 to 2 days
    → RBC + inflammation
  - **Red hepatization**
    → 2 to 4 days
    → RBC (red) + Fibrin (hepatization)
    → Lung becomes solid like the liver.
  - **Gray hepatization**
    → 5 to 8 days
    → RBC lysis (gray) + Fibrin (hepatization)
  - **Resolution**
    → 8 to 10 days
    → Resolving
- Mnemonic: Multiply with 2 to get the days of the next stage.



Red hepatization histopathology

- Lot of exudate
- Full congestion due to RBC
- Also fibrin is seen

1. Pneumonia associated with Infections

- **Common cause of community acquired pneumonia** - Streptococcus pneumoniae
- **Alcohol associated** (3Ks)
  - Klebsiella pneumoniae
  - Red (K) currant jelly sputum
  - (K) Chronic alcoholism
- **COPD exacerbations** - Haemophilus influenzae
- **Burns patient** - Pseudomonas (Blue green color)
- **ICU patient** - Legionella, Pseudomonas
- **Fungal pneumonia**
  - Dimorphic fungus (histoplasma, blastomycosis, aspergillus, mucormycosis)
  - Opportunistic infections
  - Pneumocystis Jiroveci Pneumonia (PJP)
- **Viral pneumonia**
  - Influenza
  - RSV (Respiratory Syncytial Virus)

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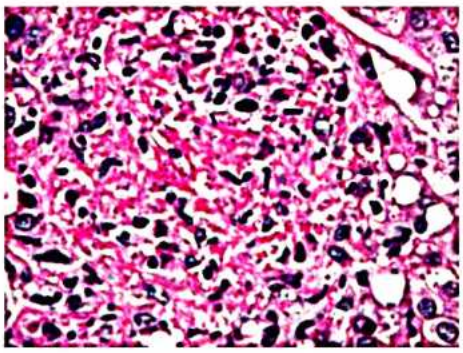
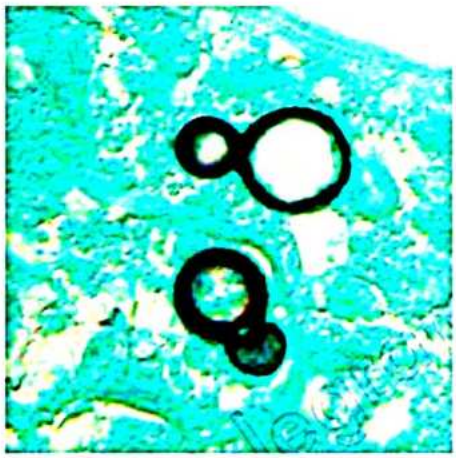
- Measles - Hecht's Pneumonia
- CMV (CytoMegaloVirus)

Images Based on Fungal Pneumonia



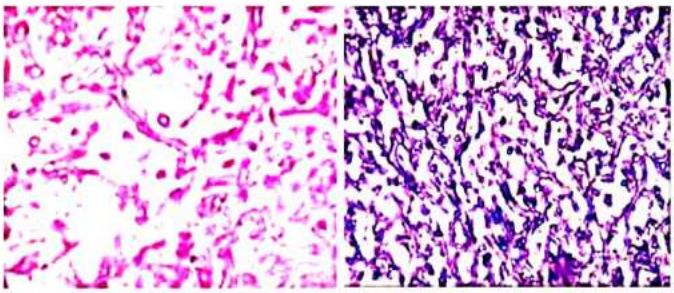
Histoplasma capsulatum yeast

- **Size:** 2-4 microns
- **Location:** Inside macrophages



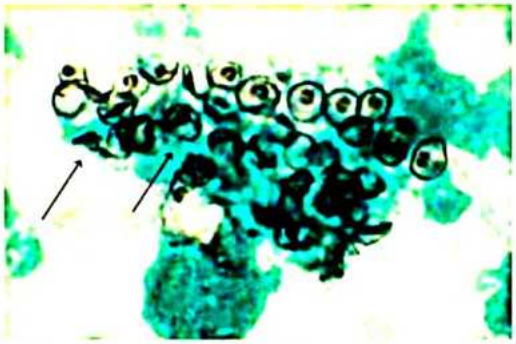
Blastomycosis

- Figure of 8 appearance



Aspergillus and Mucormycosis

- **Aspergillus**
  - Acute angle branching
  - Septate
  - Mnemonic: A for Acute, sp for Septate
  - Thin
- **Mucormycosis** (Opposite to Aspergillus)
  - Right or Obtuse angle branching
  - Aseptate
  - Thick
  - Mnemonic: MucoR, M for Mota, R for Ribbon

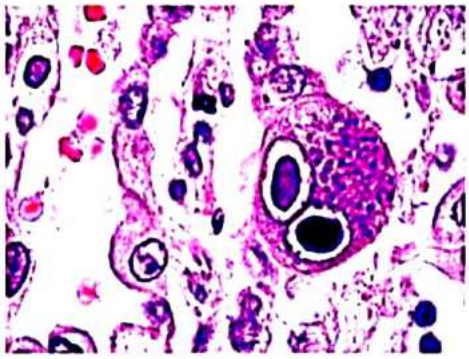


Pneumocystis Carinii Pneumonia

- Mnemonic: PCP
- **Other name:** PCP - Plasma Cell Pneumonia

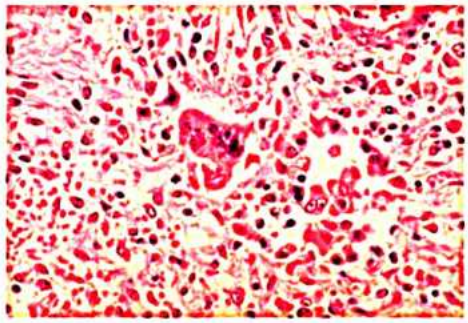
- **Histopathology:** PCP - Crushed Ping Pong Balls.
- **Treatment:** PCP - For Pneumocystis Cotrimoxazole and Pentamidine (severe) are given.

Images Based on Viral Pneumonia



CMV Pneumonia

- Owl eye appearance - CMV Pneumonia
- Intranuclear and intracytoplasmic inclusions



Measles

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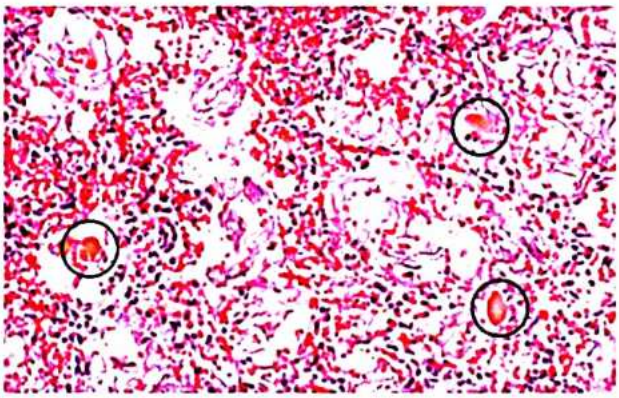
- No Owl eye
- Intranuclear and intracytoplasmic inclusions
- Has Warthin's Finkeldey Giant cells

2. Pneumonia Associated with Other Reasons

A. Meconium Aspiration Pneumonia/ Syndrome

- Meconium Aspiration is usually seen in a child who is passing the birth canal.
- **Other things** can also aspirated like
  - Amniotic fluid
  - Exfoliate skin cells
  - Lanugo hair



MAC

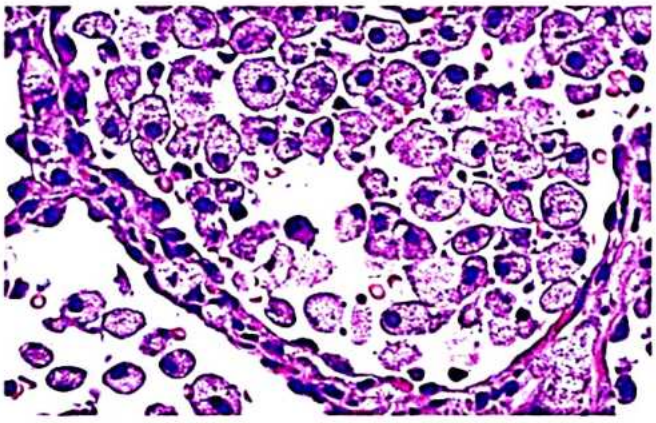
- Golden color rugby balls are seen

B. Mendelson Syndrome

- Aspiration of gastric contents in general anesthesia occurs especially during pregnancy.

C. Lipoid Pneumonia/Golden Pneumonia

- **Association**
  - Oil aspiration - Mineral oil industry
  - Destruction of cells (cell membrane have lipids)
- Macrophages will eat up the lipids



Golden pneumonia

Tuberculosis

Salient features of Tuberculosis

- **Organism** - Mycobacterium Tuberculosis
- **Virulence factor** - CORD factor
- **Acid fastness**
  - ZN stain test
  - Mycolic acid is the reason for acid fastness.

Types of TB (Usual)

- Pulmonary TB
- Extra Pulmonary TB (Lymph node TB is most common)

Multiple Types of TB

Primary TB	• 1st time TB
	• Location
	  ○ Subpleural region
	  ○ Lower part of upper lobe and upper part of the lower lobe

Secondary/ Reactivation TB	• 2nd time reactivated • **Location:** Apex • **Appearance:** Shows cavity (cavitary lesions)
Miliary/ Disseminated TB	• Spreads all over the body • **Hematogenous** - Spreads through blood· • **Hematogenous** - Spreads through blood • **Spine** - Pott's spine • **Kidney** - Putty kidney (PYQ) • **Urinary bladder** - Thimble bladder

Important Information

- Cavities in Lungs can be seen in,
  - Fungal infections
  - Histoplasma
- Squamous cell carcinoma

Foci of TB

Ghon focus	Lung
Ghon complex	Lung + Lymph node
Ranke's complex	• Calcification in Ghon complex • Lung + Lymph node + Ca^{2+}
Simon focus	At apex of lung
Puhl's focus	Supraclavicular region of lung

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Assman focus	Infraclavicular region of lung

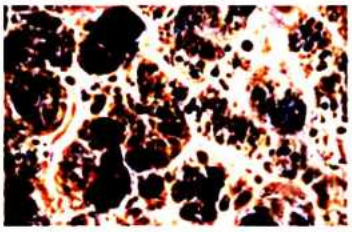
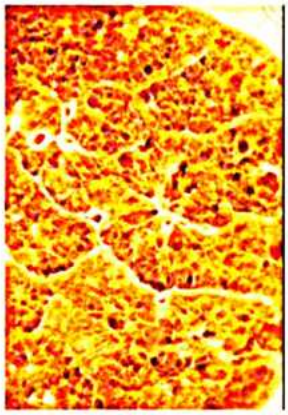
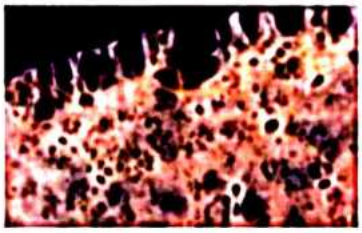
Simmond focus	• Liver
	• Mnemonic
	  ○ Lung has less letters, Simon has less letters
	  ○ Liver has more letters, Simmond has more letters

Weighert focus	• Blood vessels • Mnemonic: Vessels and Weighert same sounding.
Rich's focus	• Brain • Mnemonic: Rich people are brainy

Diagnosis of TB

- **Sample**
  - **Pulmonary TB** - Sputum
  - **Lymph Node TB** - FNAC
  - **Tubercular meningitis** - CSF
- **Concentration**
  - **Name:** Petroff's Method
  - **Chemicals:** NaOH + NaLC (especially for sputum)
- **Stain** - ZN stain (acid fast)
- **Culture**
  - Lowenstein Jensen medium
  - 6 to 8 weeks to grow

Table 27.1

Centriacinar Emphysema	Panacinar Emphysema	Distal Acinar or Paraseptal	Irregular Emphysema
• Respiratory bronchioles are disrupted. • Alveolar ducts and sacs are spared. • M/C clinically • M/C smokers • Upper lobe  Little damage and little normal	Total RDS is disrupted M/C **α 1 Antitrypsin deficeincy** lower lobe  Everything is damaged	• Distal alveolar ducts and sacs are disrupted. • Respiratory bronchioles are spared. • Spontaneous pneumothorax  Only distal part is damaged	Irregular involvement M/C histologically

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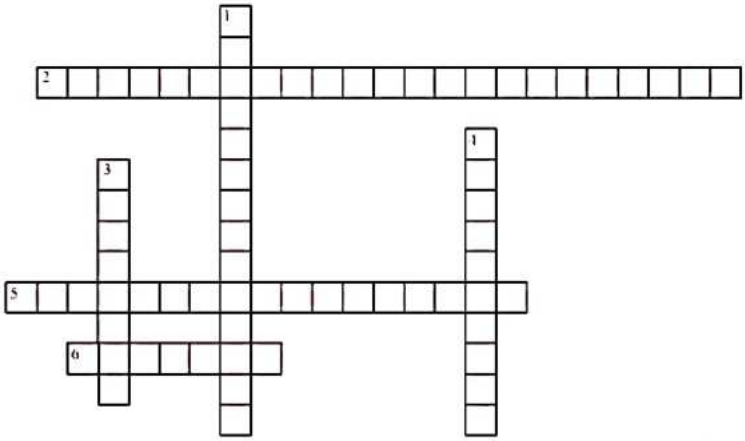
CROSS WORD PUZZLES

Crossword Puzzle



Across

2. Extra lung tissue present within the lungs
5. The ciliated cells get degenerated
6. Breaks elastase tissue

Down

1. The permanent dilation of bronchi or bronchioles
3. Type of Emphysema where everything is damaged
4. Thickness of submucosal gland layer ÷ overall thickness of the wall

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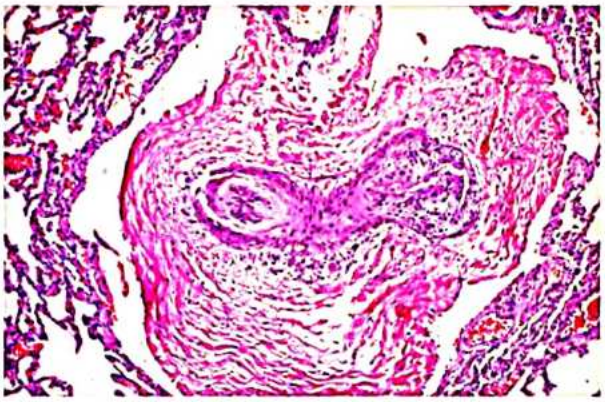
28 LUNG PATHOLOGY PART-2

Pulmonary Hypertension

- Primary - genetic
- Secondary - to some other conditions

Primary Pulmonary hypertension

- BMPR - bone morphogenetic protein receptor
- Normal BMPR2 - apoptosis of smooth muscles
- It makes smooth muscle die
- BMPR- defective → smooth muscle apoptosis not occurring → increase in smooth muscle → plexiform lesions



- Medial hypertrophy
- Tunica media - smooth muscle

Secondary Pulmonary hypertension

- Underlying Hypoxemia
  - High altitude
  - Left ventricular failure
    → Oxygen concentration goes down
  - Hypoxia → vessel dilate → to increase flow
  - **Hypoxia → Pulmonary vasculature - vasoconstriction** → increase in resistance → **pulmonary hypertension**

ARDS

00:04:04

Also called

- DAD - Diffuse Alveolar Damage
- ALI - Acute Lung Injury
- ARDS: Acute respiratory distress syndrome
  - Within 1 week

Causes

- Infections
  - Bacterial
  - Viral
    → COVID - 19
- Near drowning
- Gastric aspiration
- TRALI - transfusion related acute lung injury
  - FFP

Pathogenesis

- Hallmark - Endothelial activation

- Alveoli lined by pneumocytes
- Capillary lined by - endothelial cells

Endothelial cell activation by some causes - bacteria → cytokines
↓
Neutrophils adhere to blood vessels
↓
Endothelial cell damage
↓
Blood/ plasma → interstitial space → alveoli
↓
Non - cardiogenic pulmonary edema
NCPE
↓
(Hungry cell) - macrophage gets activated
↓
Release IL-8,TNF
↓
IL-8 -Recruits - neutrophils
↓
Neutrophils come into alveoli
↓
Release proteases and Free radicals(mainly)
↓
Cause damage to
Pneumocytes type 1 and type 2
↓
Type 1 - gaseous exchange gets disturbed
Type 2 - surfactant production gets down - lung collapse

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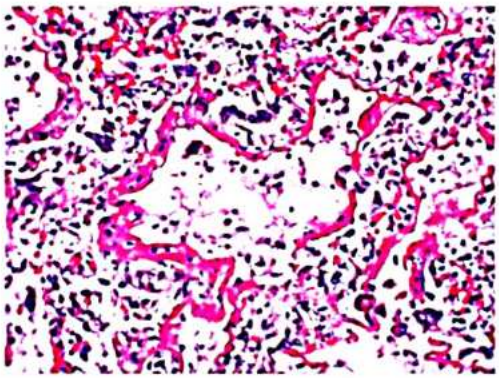


- ARDS - Hyaline membrane disease - formation of hyaline membrane
- Hyaline membrane - Made up of debris, fibrin,necrotic cells

Treatment

- Treat the underlying cause
- Mechanical ventilation
  - With PEEP
  - Positive end expiratory pressure
- Many patients may not survive
- If survive - chronic lung injury

Lung tumors

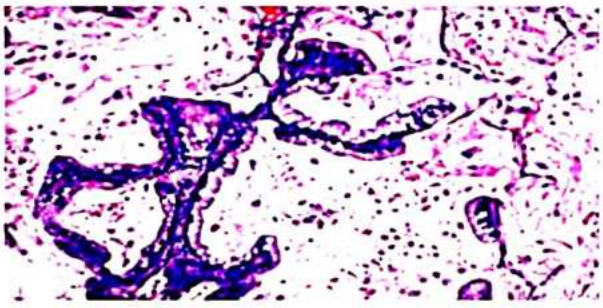
00:16:50

- Benign
- Malignant
- **Most common lung malignancy - metastasis**
  - Multiple nodule - cannonball metastases

Refer Table 28.1

Precancerous stages of adenocarcinoma

1. AAH: Atypical Adenomatous Hyperplasia
- <5 mm
2. AIS: Adenocarcinoma in situ
- <3 cm
- Earlier called as bronchoalveolar carcinoma-BAC



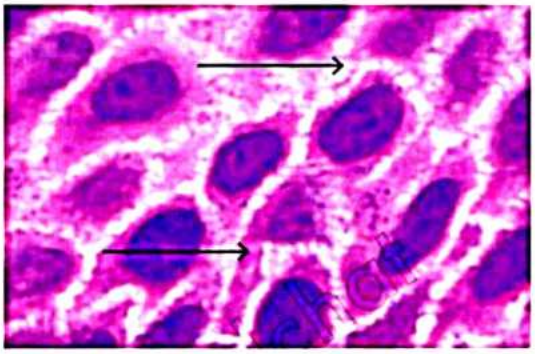
AIS

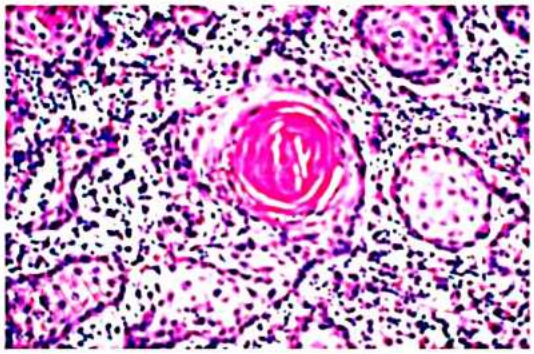
- No invasion
- Butterfly on fence appearance
- Lepidic pattern
- Lepidic cell: cardiac tumors - myxoma of the heart
- Lepidic pattern: AIS

Butterfly on fence appearance

- PAP smear - endocervical cells
- AIS - adenocarcinoma in situ of lung

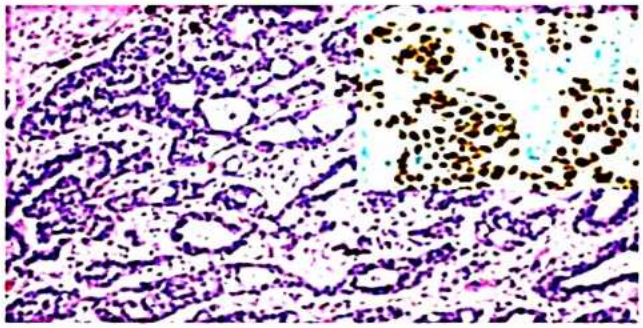
Squamous cell carcinoma





- Keratin pearls
- Intercellular bridges between sq cells - desmosomes

Adenocarcinoma



- Glands +
- Black pigments - carbon
- **IHC - Brown**
  - TTF1
  - MUC1
  - NAPSINA

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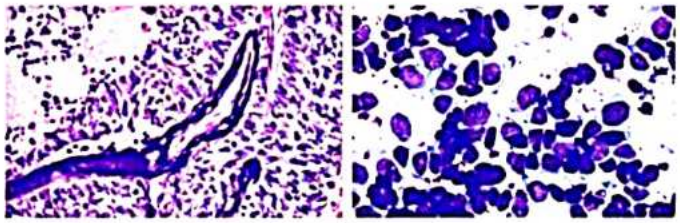
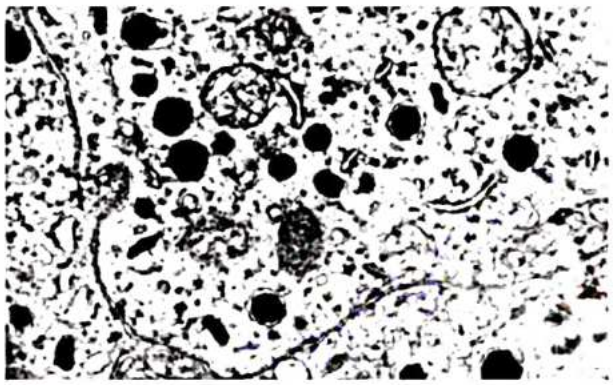
Small cell carcinoma



- Has maximum markers
- Neuroendocrine tumors
- Salt and pepper appearance of chromatin
- Azzopardi Effect: blue blood vessel
  - Molding and smudging of tumor cells
  - The DNA particles come into blood vessels

Molding and smudging of tumor cells
↓
DNA on blood vessel
Special stain for DNA - Feulgen Stain
DNA goes from one Generation to next Generation - FUELGEN (mnemonic)

Electron Microscopy



- Neurosecretory granules

Neuroendocrine tumors

- Lungs
  - Small cell carcinoma
  - Carcinoid
  - GIT - carcinoid tumor
- Thyroid: Medullary carcinoma thyroid
- Adrenal: Pheochromocytoma
- Extradrenal: Paraganglioma
- Skin: Merkel cell carcinoma

Lung cancer in non smokers - Never smokers

- Mostly females
- Mostly adenocarcinoma
- Most likely to have EGFR mutation
- Never likely to have KRAS mutation
  - Kabhi nahi - KRAS

Clinical features of lung cancer

- Cough
- Hemoptysis
  - Blood tinged sputum
- Dyspnea
- Hoarseness of voice
  - Recurrent laryngeal nerve
- Horner's syndrome

Horner' syndrome

- Tumors near apex
- Compression of sympathetic chain
- AMPLE / MAPLE
  - Anhidrosis
  - Miosis
  - Ptosis
  - Loss of ciliospinal reflex
  - Enophthalmos (Sunken eyes)

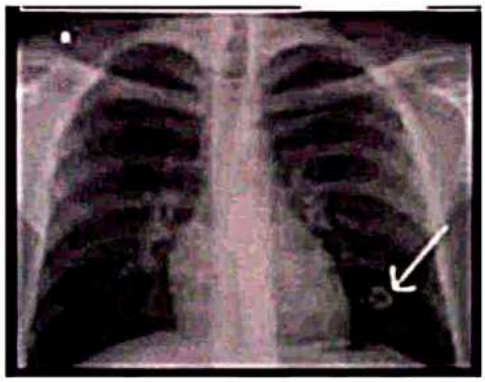
Spread of lung cancer (Robins 10th edition)

- No organ is spared but for obscure reasons, adrenal glands are involved in more than half cases.
- Then liver (30-50%), brain(20%), bone(20%) then anywhere in body
- Most aggressive - small cell carcinoma

Benign tumors

Pulmonary hamartoma/ Pulmonary chondroma

- Most common benign tumor
- Radiologically



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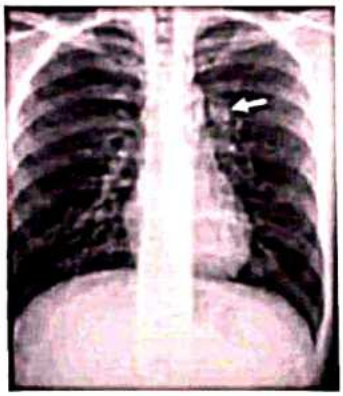
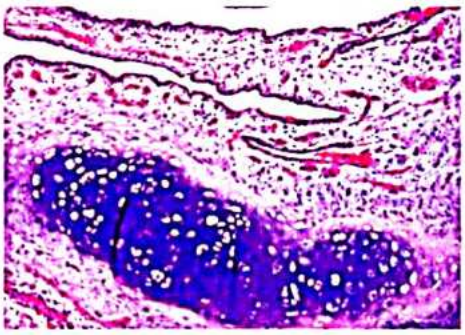
Pulmonary hamartoma/ Pulmonary chondroma

- Most common benign tumor
- Radiologically



- Coin lesion
- Popcorn calcification

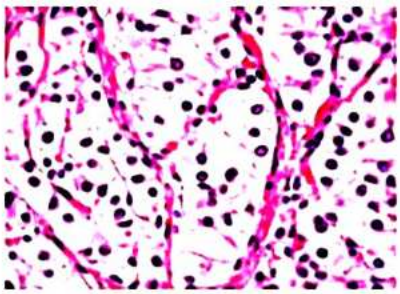
M/E



- Native cells
- Associated with chr 6 & 12

Sugar tumor - PECOMA

- Perivascular Epithelioid Cells Tumors
- M/E: clear cells
- HMB 45



- Pecoma - in lung - sugar tumor
- Pecoma - In kidney - AML - angiomyolipoma
- Both are Positive for HMB45

Carcinoid tumor

- Origin - neuroendocrine cell - KULCHIT SKYCELL

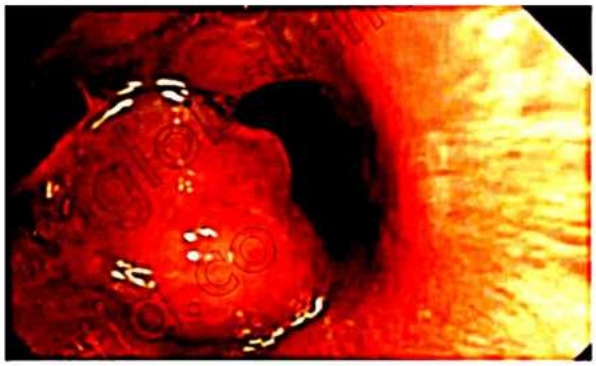
1. Typical
   - Mitosis <2/hpf
2. Atypical
   - Mitosis 2-10/hpf
   - Coat button lesion
   - Nesting
   - Salt and pepper appearance
   - IHC
   - Neuroendocrine tumor

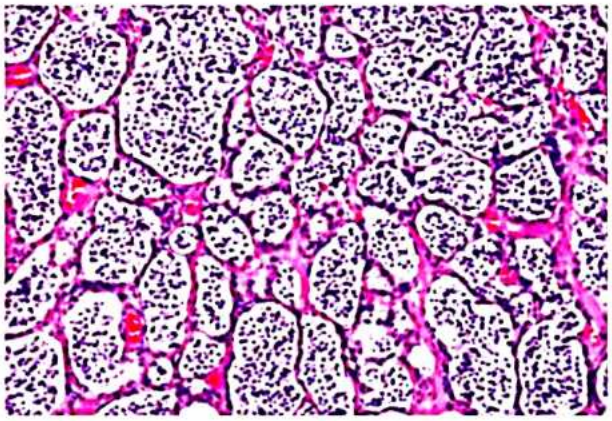
Popcorn calcification

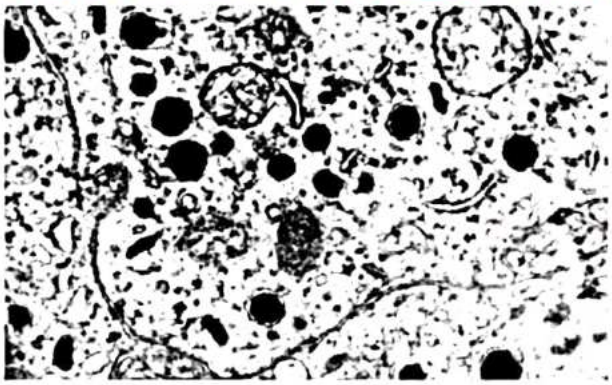
- Pulmonary Hamartoma
- Breast tumor: Fibroadenoma

Carcinoid tumor

- Neuroendocrine tumor







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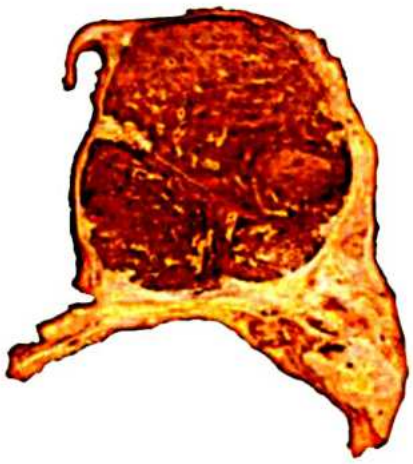


- Electron microscopy - Neurosecretory granules
- Microscopy - Nesting of cells

Pleural tumors 01:06:50

Benign - Solitary Fibrous Tumor

- Cd34 +
- STAT 6 +
- NAB2-STAT6 fusion
- Inv 12

CD34+ in EFGH

- E- Endothelial cells
- F - solitary fibrous tumor (lung - pleural tumor)
- G - GIST
- H - hematopoietic stem cell - HSC

Malignant tumor of pleura - malignant mesothelioma

- Risk factors - asbestosis
- Loss of chr9 (M/C)
- BAP 1 mutation
- SV40 - DNA virus
- Markers - CALRETININ

Mesothelioma

- Outside of lung - tumorous growth more towards the base of pleura
- Asbestos = base of lung
- Like a Cage
- Presents with Pleural effusion
- Psammoma bodies

Adenocarcinoma lung and mesothelioma

- Adenocarcinoma - non branching microvilli

→ Mesothelioma - long branching microvilli

MCQs

Q. Which if these markers can be used in mesothelioma

A. Calretinin

B. TTF-1

C. CK-8/9

D. Glypican

Q. A pleural fibroma is differentiated from mesothelioma by the presence of the following in the former

A. Cd14

B. Cd24

C. Erb positive

D. Cd34

- Pleural fibroma: Cd34+

Q. Marker of small cell cancer of the lung

A. Chromogranin

B. Cytokeratin

C. Desmin

D. Vimentin

- Neuroendocrine tumor positive for chromogranin

Telegram - @nextprepladdernotes

Q. A 63 year old woman with chronic bronchitis presents with shortness of breath, a chest x-ray reveals a 2 cm coin lesion in the upper lobe of left lung. A CT - guided lung biopsy is obtained. Which of the following describes the histologic features of this lesion if the diagnosis is hamartoma?

A. Benign neoplasm of epithelial origin

B. Disorganized normal tissue

C. Ectopic islands of normal tissue

D. Granulation tissue

- It is not epithelium but mesenchyme (mostly cartilage origin)

Telegram - @nextpreladdernotes

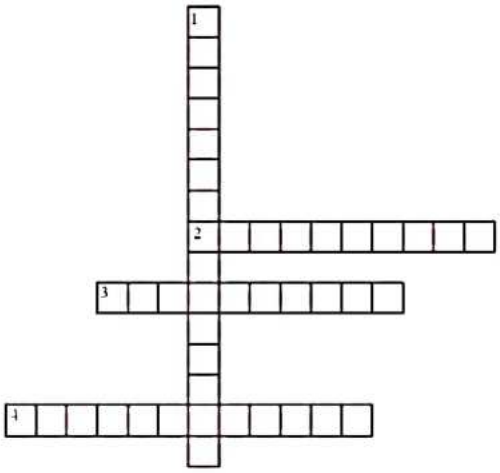
Table 28.1

Features	Squamous cell carcinoma	Adenocarcinoma	Small cell / oat cell	Large cell	Key to remember
Common		Most common			
Smoking	+		+		SSS - Smoking Sq,Small
Gender		Most common in male and female			
Location	Central	Peripheral	Central	Peripheral	Central - squamous , small
Genetics	Loss of • chr3 • chr17p • p53	Gain of function of • EGFR 1 • ALK • KRAS	Loss of chr 3p Almost all L myc amplification P53 inact RB inact		KRAS - KPL tumors: • Colon • Pancreatic • Lung cancer
Precancer	CIS Carcinoma in situ (common with every squamous cell ca)	**AAH** **AIS**	Neuroendocrine tumor DIPNEH Diffuse idiopathic pulmonary neuroendocrine hyperplasia		
Markers IHC	P40 is the best marker p63	menmonic- all A and I NAPSIN A MUC 1 TTF 1	Synaptophysin Chromogranin Bombesin NSE CD56 CD57		
PNS (Para neoplastic syndrome)	hypercalcemia	Migratory thrombophlebitis • itis - inflammation • Thrombus - clot • Phlebo - vein • Awara -migratory (mnemonic) Trousseau phenomenon	• SIADH - syndrome of inappropriate ADH secretion • Cushing's syndrome • Lambert eaton syndrome Most aggressive Lung cancer	Gynaecomastia	Trousseau phenomenon- • Lung adeno • PANCREATIC adenocarcinoma

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. Most common lung malignancy
3. Intercellular bridges between sq cells
4. Special stain for DNA

Down

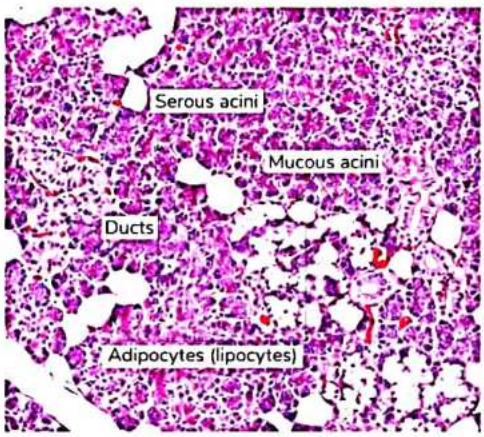
1. Made up of debris, fibrin,necrotic cells

Telegram - @nextpreladdernotes

29 SALIVARY GLAND PATHOLOGY

Histology of Salivary Glands

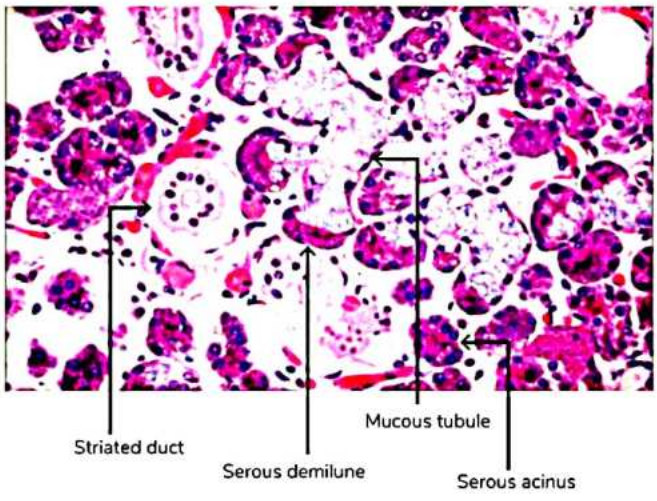
00:00:22



- **3 main Salivary glands:**
  - **Parotid gland** - scrous gland.
  - **Sublingual gland** - mixed gland (serous + mucinous).
  - **Submandibular gland** - mixed gland

- **2 main structures:**
  - **Acini -**
    → Serous acinus - contains zymogen granules, appear blue pink in color.
    → Mucinous acini - looks almost empty because of more mucin.
  - **Ducts -**
    → Has cuboidal epithelium.

- **Demilune structure:** The serous acini is present on the mucinous acini like a mixed structure.
- The white spaces on the image are adipocytes.
  - With increase in age the adipocytes count is increased.



Salivary Gland Tumors

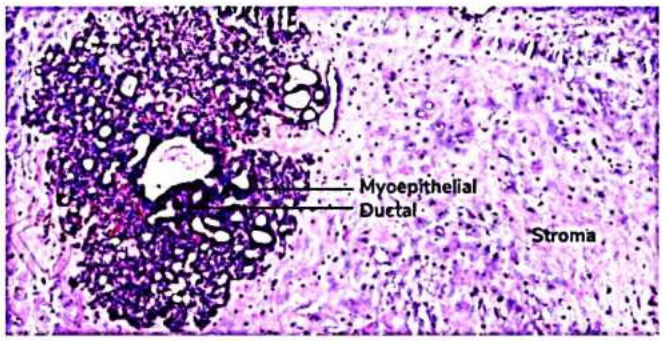
00:06:45

Divided as:

- **Benign**
  - Pleomorphic adenoma
  - Warthin's tumor
  - Oncocytoma - tumor with lots of mitochondria (mentioned in previous chapters).
- **Malignant**
  - Mucoepidermoid carcinoma
  - Adenoid cystic carcinoma
  - Acinic cell carcinoma
  - Carcinoma ex pleomorphic adenoma.
    → The benign pleomorphic adenoma converts to malignant.

Benign Tumors

01. Pleomorphic Adenoma

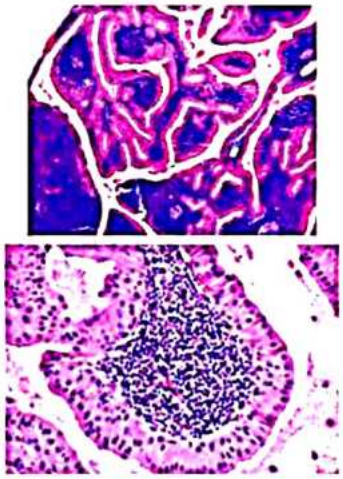


- PLAG1 fusion or HMGA2 fusion is present in 70% of cases.
- Most common benign salivary gland tumor.
- Most common in the parotid gland.
- Most commonly seen in females.
- Also called benign mixed tumor - has 2 components.
  - **Epithelial component**
    → Epithelial cells
    → Myoepithelial cells
  - **Mesenchymal component**
    → Chondromyxoid stroma
- The tumor forms finger-like projections into the gland.
  - So just removal of the tumor is not effective.
  - Complete affected lobe should be removed - superficial lobectomy or parotidectomy.
- If only the tumor is removed and some part of it is left inside then:
  - Patients come with recurrence.
  - May develop carcinoma ex pleomorphic adenoma (must have a past history of pleomorphic adenoma).

Telegram - @nextpreladdernotes

02. Warthin's Tumor 00:14:03

- **Also known as:** Papillary cystadenoma Lymphomatosum.
  Microscopically:
  - Papillary projections are seen.
  - Papillae are lined by 2 rows of oncocytic cells.
  - Lymphocyte count is very high.
  - A tumor exists as a cyst filled with fluid.
- **Most affected gland** - parotid gland.
- **History:**
  - Adult male
  - Chronic Smoker
  - Bilateral parotid gland swelling.
- On aspiration murky fluid is drained out.

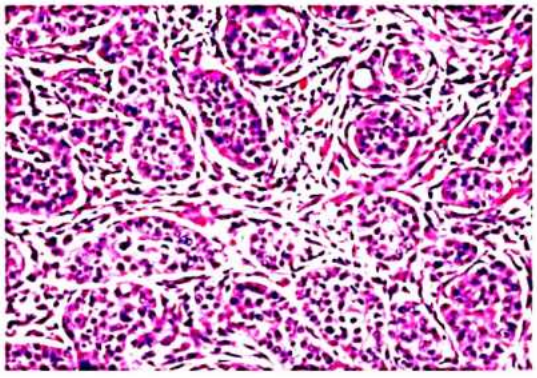


Important Information

- Child comes with bilateral parotid gland swelling - **Mumps disease.**
- Adult smoker with bilateral parotid swelling - **Warthin's tumor.**
  - Can also be unilateral.

Malignant Tumors

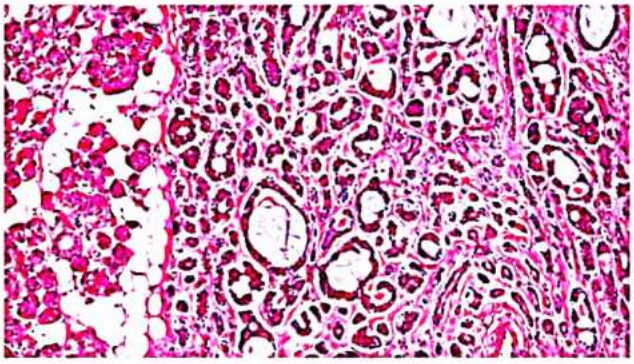
01. Mucoepidermoid Carcinoma



- MAML2 gene defect.
- **Microscopy shows:**
  - Mucin containing cells.
  - Squamoid cells
    → Pink appearance.
    → More epidermoid/Squamoid cells - higher grade of tumor.

02. Adenoid Cystic Carcinoma (ACC)

- **Microscopy shows:**



  - Cribriform pattern - sieve like pattern with basement membrane like material.

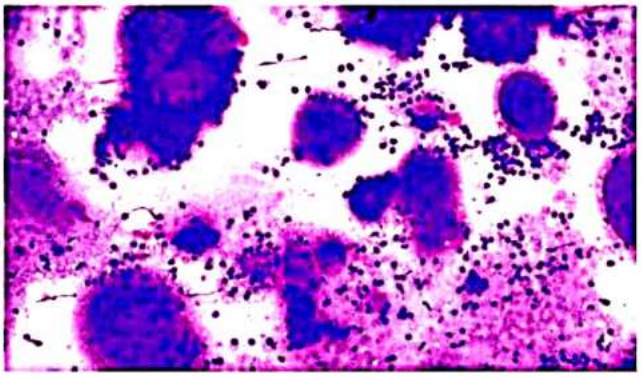
Cribriform Pattern also present in

- Adenoid cystic carcinoma
- Prostate cancer
- Breast cancer- ductal carcinoma in situ.
- **Patient presents with:**
- Lot of pain Because of a perineural invasion.

Perineural invasion is shown by:

- Prostate cancer
- Adenoid cystic carcinoma
- Pancreatic cancer
- Mnemonic - PAP

- **FNAC shows:** Cup shaped fragments with hyaline globules.



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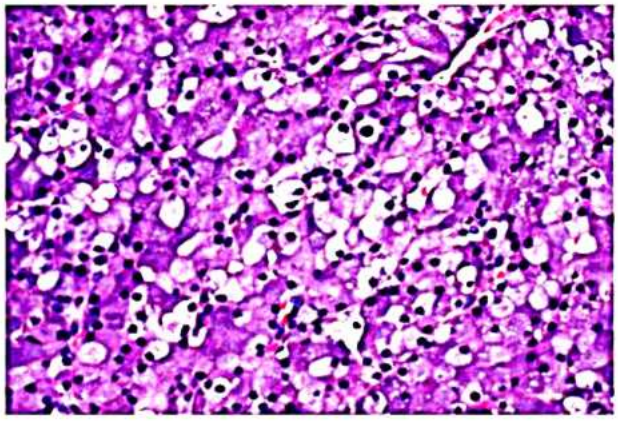
ACC on FNAC

- ACC occurs due to translocation 6:9.

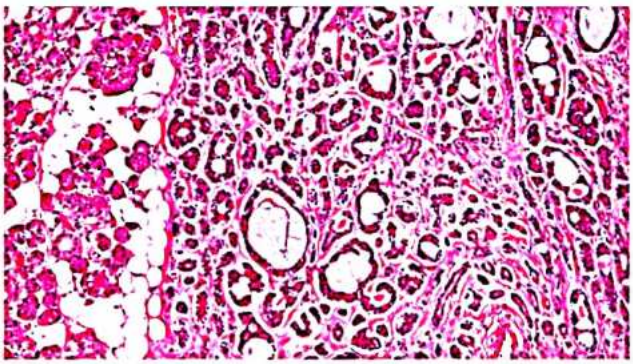
03. Acinic Cell Carcinoma



- Acinar cells give rise to cancer.

MCQs

Q1. A 36-year-old male patient presents with a mass in the parotid region which was 1x1 cm when he first noticed it 3 months ago. The mass is gradually increasing in size to the current size of 5x4cm. The patient also gives history of the selling being painless earlier but now he is experiencing excruciating pain. FNAC shows presence of hyaline globules with cup shaped fragments of cells. Excision biopsy shows the following histopathological image. What is the likely diagnosis?



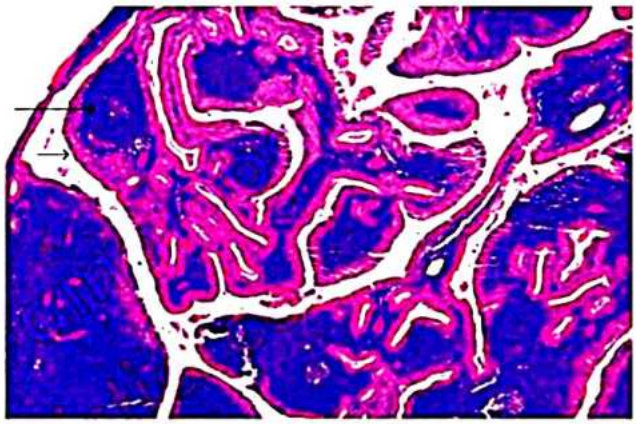
a. Warthin's Tumor
b. Pleomorphic Adenoma
c. Mucoepidermoid Carcinoma
d. Adenoid Cystic Carcinoma

Answer: d. Adenoid Cystic Carcinoma

Explanation

- Adenoid cystic carcinoma first begins without pain, later the pain is more.
- Also, the histopathological image shows cup shaped fragments of cells.

Q2. 40-year-old chronic smoker presents with bilateral swellings in the parotid region. On examination the swellings were soft, cystic in consistency with right jaw swelling measuring 3×2 cm and left jaw swelling measuring 2×2 cm. The swellings were nontender. FNAC revealed the murky fluid in the syringe. Smears prepared showed presence of lymphocytes scattered in a fluid background. Excision biopsy revealed the following histopathological image. What is your diagnosis?



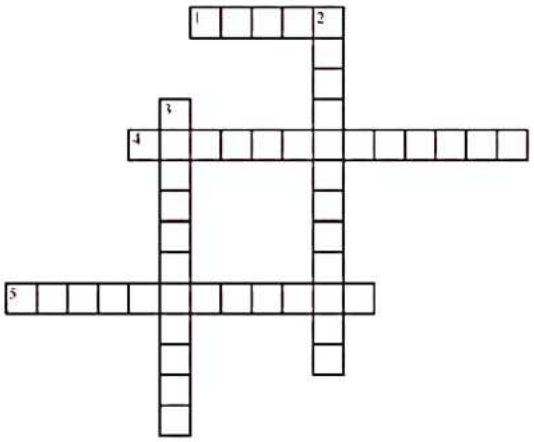
a. Warthin's Tumor
b. Pleomorphic Adenoma
c. Mucoepidermoid Carcinoma
d. Acinic Cell Carcinoma

Answer: a. Warthin's tumor

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

1. Has cuboidal epithelium
4. looks almost empty because of more mucin
5. Serous gland

Down

2. Contains zymogen granules, appear blue pink in color
3. Child comes with bilateral parotid gland swelling

Telegram - @nextpreladdernotes

30 GIT PART-1

Introduction 00:00:44

Parts of GIT

Parts	Epithelia
Oral Cavity	Squamous Epithelia
Esophagus	Squamous Epithelia
Stomach and intestines	Glandular/Columnar Epithelia
Anal Canal	Squamous Epithelia

Layers of GIT

- Four layers:
  - Mucosa - First layer
  - Submucosa - This is not present in Gall bladder
  - Muscularis Propria
  - Serosa - Esophagus does not have this layer
- Nerve Plexus groups
  - Meissner plexus - present in the submucosa (Submucosal)
    → Controls secretory activities
  - Muscular/Auerbach's plexus - present in muscularis propria
    → Controls motor activities
  - Ganglion cells are normally present in the nerve plexi.

Note -

- **Aganglionosis** or missing of ganglion cells is associated with Hirschsprung's disease (occurs due to failure of migration of neural crest cells)
- There is a constricted aganglionic segment in the intestine where there are no ganglion cells which causes this disease.
- Failure to pass meconium or stool is the presenting complaint.

Q. Main defect in Hirschsprung's disease and why?
Ans. Aganglionosis and it is due to failure of migration of neural crest cells.

Pacemaker cells

- Cells of Cajal are pacemakers of GIT
- If there is a mutation in the cell of Cajal, it leads to the gastrointestinal stromal tumor (GIST)

Esophagus 00:11:50

- Muscular tube without Serosa layer.
- Length of esophagus - 25 cm.
- Function - contract and relax to move food.
- Acetylcholine does contraction.
- Vasoactive intestinal peptide (VIP) and nitric oxide (NO) relaxes it.

Achalasia cardia / Mega Esophagus 00:14:00

Definition -

- Loss of inhibitory neurons (neurons that release NO and VIP) i.e. no relaxation will occur.
- There is going to be a constriction - maximally seen at the lower esophageal sphincter (LES).

Causes -

- Primary -
  - Idiopathic
- Secondary -
  - Infections
    → Trypanosoma cruzi causes Chagas disease
    → Herpes
  - Diabetes Mellitus

Clinical features -

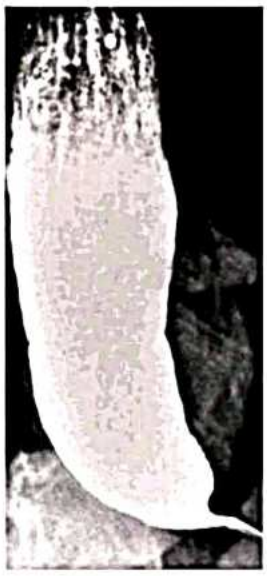
- Dysphagia
  - More to the liquids than to the solids.

Note –

- If dysphagia is more to the solids than to the liquids, it is Esophageal cancer.

Diagnostic modalities

- Barium study
  - Bird beak appearance



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- Esophageal manometry
  - Detects pressure changes in the esophagus
  - High pressure at a narrow area

Treatment
- Medicine
  - Injection of Botulinum Toxin
  - Botulinum decreases acetylcholine, making everything relaxed (flaccid).
- Surgery
  - Heller's myotomy

Extra information-
Allgrove Syndrome
- A - Achalasia
- A - Alacrimia
- A - ACTH resistant adrenal insufficiency
- Grow - All the symptoms will grow with time
- AAAS Gene

Esophagitis 00:24:17
- Inflammation in the esophagus
- Bisphosphonates chemical causes this inflammation.
- Reflux
  - Chronic reflux causes GERD causing Barrett's esophagus.
  - Reflux esophagitis is the most common.
- Infective
- Eosinophilic

Infective Esophagitis
- Causes - Candida, CMV and Herpes
- Mostly occurs in immunosuppressive (HIV positive) patients
- Candidal esophagitis
  - Pseudomembrane formation
  - Candida has long Pseudohyphae.
  - Curdy white lesion is formed wherever candida appears.
- CMV esophagitis
  - Shallow ulcers in esophagus
  - Owl eye appearance
  - CMV shows both intranuclear and intracytoplasmic inclusions
- Herpes esophagitis
  - Punched out ulcers in esophagus
  - Three M's:
    → Multinucleation
    → Moulding
    → Margination - margins of the nucleus are darker
  - Can occur in immunocompetent persons also

Eosinophilic esophagitis
Q. Why? - Allergies cause it.
Q. Which interleukin is responsible? - Interleukin 5
Q. How many eosinophils? - Microscopically, at least 15 eosinophils per high power field
- Eosinophils look orange in color.
- The final appearance of the esophagus has stripes like cats called Feline Esophagus.
- Treatment - Remove the allergen.

Alcohol-induced lacerations 00:35:58
Mallory Weiss Syndrome
- History of alcohol binge drinking.
- M - Mucosal tear/Superficial tear
- AL - Alcohol
- LO - Longitudinal direction of tear
  - Below the gastroesophageal junction
- Bleeding in the vomit (Hematemesis)

Boerhaave syndrome
- Full thickness tear
- Occur 2.5 cm above the gastroesophageal junction
- Hematemesis occurs

Mackler's triad
- Hematemesis
- Chest pain
- Hematemesis and chest pain makes painful Hematemesis
- Subcutaneous emphysema - Hamman's crunch heard on Auscultation.

Note -
- Painless hematemesis - a condition of rupture of esophageal varices (dilated channels - blood vessels) - not causing pain
  - Caused due to portal hypertension.

Barrett's Esophagus 00:42:35
- It is a type of metaplasia.
- Epithelial of the esophagus - Squamous epithelium.
- Patient suffering from GERD (Gastroesophageal reflux disease).
- Acid of the stomach is coming to the esophagus.
- Squamous epithelium changes into the intestinal columnar epithelium/glandular metaplasia.
- There are goblet cells in intestinal epithelium.
- Goblet cells contain acidic mucin
  - Special stain: Alcian blue stain

Q. What is the hallmark of Barrett's Esophagus?
Ans: Goblet cells

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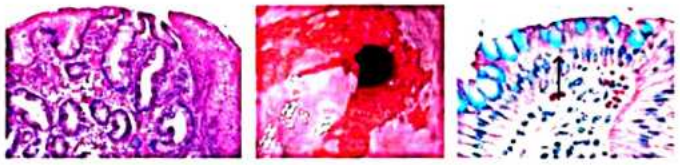
Clinical features
- Heartburn due to acid reflux

Endoscopy

BARRETT'S ESOPHAGUS (METAPLASIA)



- Pale pink area - normal esophagus
- Red velvety or salmon color region - Area of Barrett's Esophagus
- If the area is less than 3 cm, then it is short segment Barrett's Esophagus.
- If the area is more than 3 cm, then the area is long segment Barrett's Esophagus.
- It is a precancerous/pre-malignant condition.
- When Glandular metaplasia becomes dysplasic, it results in adeno-carcinoma.
- Reidel and Vienna classification is for the classification of dysplasia.

Esophageal tumors 00:51:28
- Divided into benign and malignant
- Benign
  - Most common - Leiomyoma (smooth muscle tumor)
- Malignant
  - Most common - Squamous cell carcinoma
    - → Upper ⅓ rd - Squamous cell carcinoma
    - → Middle ⅓ rd - Squamous cell carcinoma
    - → Lower ⅓ rd - Adenocarcinoma

Carcinoma 00:53:20
1. Squamous cell carcinoma
2. Adenocarcinoma

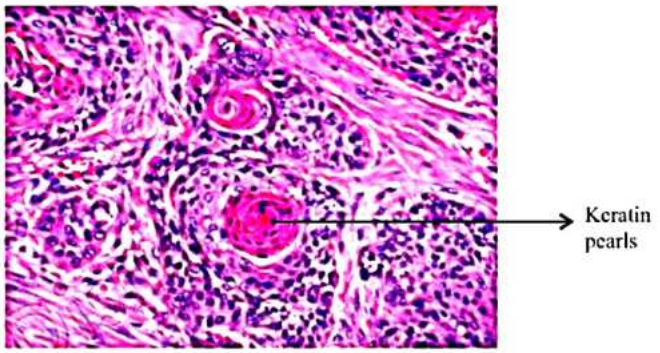
Risk factors of Squamous cell carcinoma
- Alcohol
- Smoking
- Hot beverages
- Celiac disease
- Drugs/radiation
- Epidermolysis bullosa
- Fermented milk - Mursik (consumed in Kenya)
- HPV
- Tylosis palmaris et plantaris - thickening of palm and soles (Hyperkeratosis)
- Plummer Vinson Syndrome
- SOX2 mutation

(Mnemonic - ABCDEF – PPP)

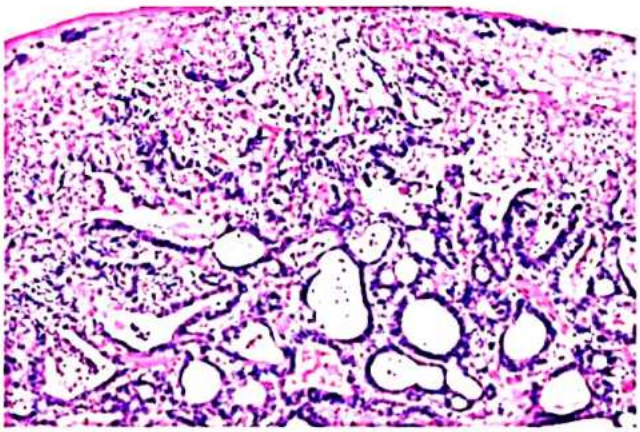
Plummer Vinson syndrome
- Also known as Patterson-Brown Kelly Syndrome or Sideropenic dysphagia
- History is of a middle-aged female.
- Sideropenic means Iron deficiency anemia causing fatigue.
- Dysphagia (difficulty in eating) due to esophageal webs which results in Squamous cell carcinoma.
- Glossitis, and cheilitis can be present.

Risk factors of Adenocarcinoma
- Barrett's esophagus
- p16 and RB gene mutations
- H. pylori has protective action against adenocarcinoma of the esophagus

Gold standard for diagnosis of esophageal carcinoma
- Biopsy
  - Squamous cell carcinoma - Keratin pearls (pink color)



  - Adenocarcinoma - Glands



MCQ
Q. Histopathological difference between Barrett's epithelium and gastric mucosa?

a. Barrett's mucosa is acidic and stains alcian blue positive

b. Barrett's is alkaline and stains prussian blue positive

c. Barrett's alcian blue negative because its neutral

d. Gastric mucosa is alkaline and stains alcian blue positive

Telegram - @nextpreladdernotes

Q. A 48-year-old man from Argentina presents to your office complaining of difficulty swallowing from the past few months. The patient says that he feels uncomfortable no matter what he eats or drinks. He also has lost 5kg in 2 months. The patient is afebrile, and his vital signs are within normal limits. A barium swallow study along with esophageal manometry is performed, and the results are shown in the image below. Manometry shows very high pressure at the lower esophageal sphincter. Which of the following is the most likely etiology of this patient's symptoms?

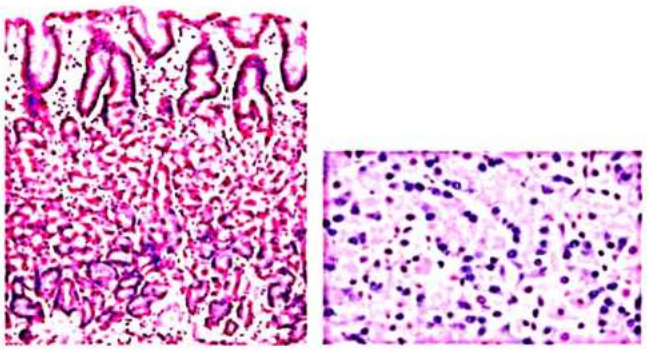
a. Pyloric stenosis
b. Trypanosoma cruzi infection
c. Outpouching of the mucosa and submucosa
d. Malignant proliferation of squamous cells

Note - Chagas infection is caused by Trypanosoma cruzi infection.

Stomach

01:05:55

- Cells of stomach



  - Foveolar cell - containing mucin
  - Parietal cell (pink color) - release
    → HCL acid
    → Intrinsic factor or castle factor - absorbs vitamin B12
  - Blue cell - chief cell

Gastritis

01:08:15

- Types
  - Acute
    → Associated with alcohol and smoking
    → Tea, coffee and spicy food
    → Drug intake - aspirin and NSAIDS
    → Stress
  - Chronic

Acute gastritis

Stress ulcers

- Curling ulcer
  - Burns
    → occurs in Duodenum
    → Causes: Hypovolemia and Ischemia
  - Superficial ulcers

- Cushing ulcer
  - Increased intracranial tension
    → Pressure on the vagus nerve
    → Increase in activity of Parasympathetic system - increased acid secretion in stomach
  - Occurs in the Cardia of the stomach.
  - Deep ulcers

Chronic gastritis

- Type A
  - Autoimmune phenomenon
  - Antibodies are present against parietal cells.
  - HCL and intrinsic factors will go down.
  - Vitamin B12 will not be absorbed, causing Pernicious Anemia/Megaloblastic Anemia
  - Most common in the fundus and body of the stomach

- Type B
  - Bacteria involved
  - H. pylori acts on the antrum of the stomach
  - Increased HCL production

Helicobacter pylori

- Flagellated organism
- Urease positive organism
- Causes
  - Gastritis
  - Peptic ulcer disease (PUD)
  - Adenocarcinoma of the stomach
  - Maltoma of the stomach
- Cag A and Vac A of Helicobacter Pylori cause cancers
- PUD is associated with blood group O
- Adenocarcinoma of the stomach is associated blood group A

Telegram - @nextpreladdernotes

How to identify Helicobacter Pylori?
- Warthin starry silver stain gives black color
- Modified giemsa stain
- Urease positive test (pink)

On M/E, initial observation:
- Epithelium contains Neutrophils
- Subepithelium shows plasma cells

Peptic Ulcer Disease

Protective Factors	Damaging factors
Mucus	HCL
Bicarbonate	Pepsin
COX1 (Gastroprotective)	H.pylori

- NSAID and Aspirin inhibits COX1 causing peptic ulcer diseases

Duodenal ulcer
- More common
- Along the anterior wall of the first part of the duodenum.
- Nocturnal pain - relieved by taking food (increases alkaline secretion in the intestine)
- Most common complication is bleeding (gastro-duodenal artery)
- No cancer risk

Gastric Ulcer
- Less common
- At lesser curvature
- Most common complication is bleeding (left gastric artery)
- Cancer risk
- Complication associated with death is perforation.

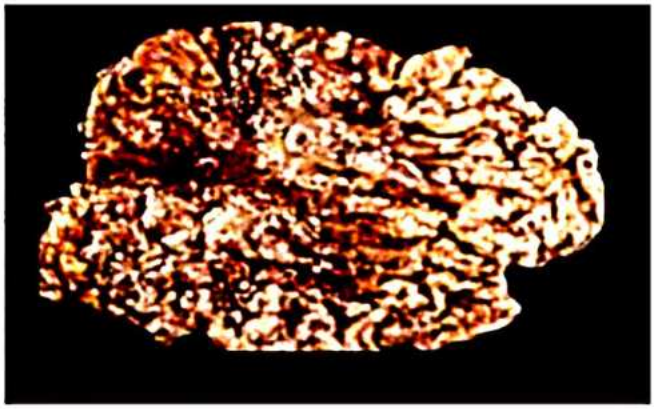
Gastric outlet obstruction
- Associated with duodenal ulcers.
- Most common cause:
  - Children - infantile hypertrophic pyloric stenosis
  - Adults - stomach cancer followed by PUD
- There is an obstruction in the stomach, causing repeated vomiting.
  - Loss of HCL causing metabolic alkalosis.
- Paradoxical aciduria occurs because of renin-angiotensin-aldosterone system (RAAS)

Infantile hypertrophic Pyloric stenosis
- Earlier called congenital hypertrophic pyloric stenosis because it is associated with Trisomy 18 and Trisomy 21.
  - Can occur in 1-2 weeks of life if the child is exposed to Erythromycin or Azithromycin therefore named Infantile.

Clinical features:
- Vomiting from the stomach (Non bilious vomiting)
- Demanding repeated feeding
- Olive lump (1-2 cm) can be seen

Treatment:
- Pyloromyotomy

Menetrier's Disease 01:40:00
- Gender
  - Most common in males
- Cause
  - Increase in TGFα
- Clinical
  - Protein Losing enteropathy
  - Hypoproteinemia causing edema
- Gross



  - Giant Cerebriform Rugae
- Microscopy
  - Foveolar cell hyperplasia

Important Information

TNFα = No appetite
TGFα = Gastric disease Menetrier's disease
TGF β = Most Fibrogenic (big time fibrosis)

Zollinger Ellison Syndrome (ZES) 01:44:08
- Associated with gastrin secreting tumors in the pancreas and small intestine.
- Gastrinoma (malignant) – Pancreas, small intestine
- Gastrin increases acid production & results in duodenal ulcers.
- If Sporadic (75%) - Single tumors
- If Familial (25%)

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- Associated with MEN-1 syndrome
- Multiple tumors 2

- Microscopic findings - Parietal cells are present
- Diagnosis - S. gastrin level is above 1000 pg/ml
- Treatment - Surgery to remove tumor

Gastric Tumors

01:48:15

Q. Most common malignant gastric tumor?
Ans. Gastric adenocarcinoma

Q. Most common malignant mesenchymal gastric tumor?
Ans. Gastrointestinal stromal tumor

Gastric adenocarcinoma

Risk factors

- Alcohol
- Smoking
- Hot beverages
- Smoked and salted food
- Pernicious anemia
- Adenomatous polyps
- Blood group A
- H. pylori (adenocarcinoma)
- EBV - lymphoepithelial carcinoma
  (Most common carcinoma associated is gastric carcinoma)

Q. Most common site of gastric cancer?
Ans. Gastric antrum

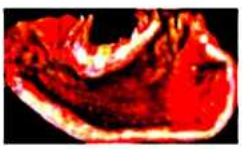
Q. Most common site of gastric cancer in a patient of pernicious anemia?
Ans. Fundus

Gross types

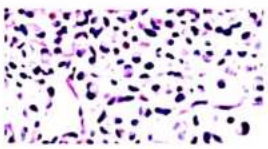
- Proliferative - cauliflower
- Flat
- Ulcerated
- Linitis Plastica
  - Cancer grows within the wall
  - Leather bottle appearance

Microscopic types/Lauren's classification

- Intestinal type
  - Cells arranged in glands
- Diffuse gastric cancer
  - E-cadherin is lost
  - Shows Linitis plastica
  - Microscopic observation - Signet ring cells
    → Nucleus at the periphery
    → Full of mucin at the center



Linitis plastica → LEATHER BOTTLE


Signet ring cells

Note -

- E- cadherin is lost in:
  - Diffuse gastric cancer
  - Invasive lobular carcinoma (Breast)

Clinical feature

- Earliest clinical feature - Early satiety

Spread?

- Pouch of Douglas - Blummer shelf
- Ovary - Krukenberg tumor
- Left supraclavicular lymph node - Virchow Node
- Periumbilical nodule - Sister Mary Joseph Nodule
- Axillary lymph node - Irish node

Gastrointestinal stromal tumor

Cell of origin

- Cell of Cajal

Mutations

- Most common - CKIT/CD117
- Others
  - PDGFRβ
  - Succinate dehydrogenase (SDH)
    → (Pediatric GIST)

Mechanism

- CKIT mutation
  - Increase in tyrosine kinase activity resulting in GIST

Treatment

- Surgery
- Tyrosine kinase inhibitors - Imatinib

Q. Where can we give Imatinib?
Ans. Given in GIST and CML

Q. Where do we see CKIT/CD117 mutation?
Ans. GIST, AML, Mastocytosis, Melanoma, and Seminoma

Most common site

- Stomach
- Intestine

Telegram - @nextpreladdernotes

Markers
- CKIT/CD117 - Most sensitive
- DOG1 - Most specific
- CD34 - miscellaneous

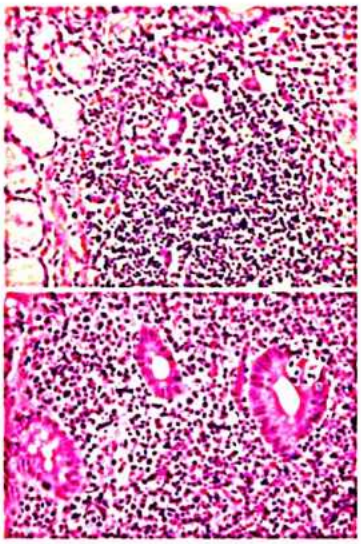
Syndromes
- NF1
- Carney Triad
  - GIST
  - Paraganglioma
  - Pulmonary chondroma

- Carney stratakis syndrome
  - GIST
  - Paraganglioma

Prognosis
- Tumor size
  - Less than 5 cm (less aggressive)
  - More than 10 cm (more aggressive)
- Gastric location which is a more common location is said to be less aggressive.

Gastric lymphomas
- DLBCL - Diffuse large B cell lymphoma
  - Most common lymphoma in the stomach
- Maltoma
  - Most common location - Stomach
  - Mucosa-associated lymphoid tissue
    → Peyer's patches present in ileum

Maltoma



- It is a marginal zone lymphoma.
- Can occur due to H. pylori
  - Early cases can be treated with antibiotics
- Can also occur due to t(11;18)
  - Antibiotic-resistant, so the treatment is with
    → Chemotherapy
    → Surgery

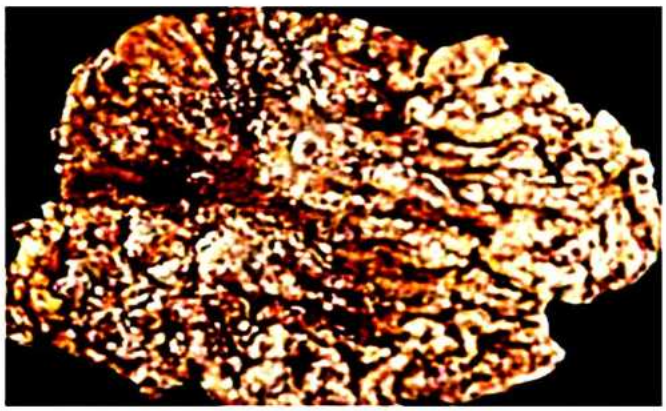
Microscopic analysis
- Lympho-epithelial lesion
  - Lymphoma cells invading epithelial cells

MCQs

Q. Which H. pylori protein is associated with a role in the development of gastric neoplasia?

a. SRC
b. CagA
c. PARI
d. SHP2

Q. 40-year-old male patient presents with upper abdominal discomfort, loss of appetite, significant weight loss, and occasional intermittent episodes of peripheral edema. The most discomforting complaint for the patient is intractable emesis. The gross appearance of the stomach is shown below. What is the most common mediator of this condition?



a. TGF beta
b. TGF alpha
c. Interferon alpha
d. Interferon beta

Q. Which mutation is most commonly associated with hereditary diffuse gastric cancer?

a. CDIII
b. SMARCA4
c. BRCA2
d. NF1

Q. A 30-year-old woman is found to have a germline CDH1 mutation and undergoes a prophylactic gastrectomy. Which other malignancy is she at a higher risk of developing?

Telegram - @nextpreladdernotes

a. Melanoma
b. Osteosarcoma
c. Lobular carcinoma of breast
d. Clear cell renal cell carcinoma

Q. Which of the following statements is false regarding lymphoepithelial lesion-containing tumor?
a. Associated with H. pylori
b. It's a subtype of marginal zone lymphoma
c. Can be associated with t(8;14)
d. Early cases of may show response to antibiotics

Gastrointestinal Pathology – Intestine 02:23:25

- Malabsorption Syndrome
- Ulcers of intestine
- IBD
- Polyps of intestine
- Intestinal carcinoma

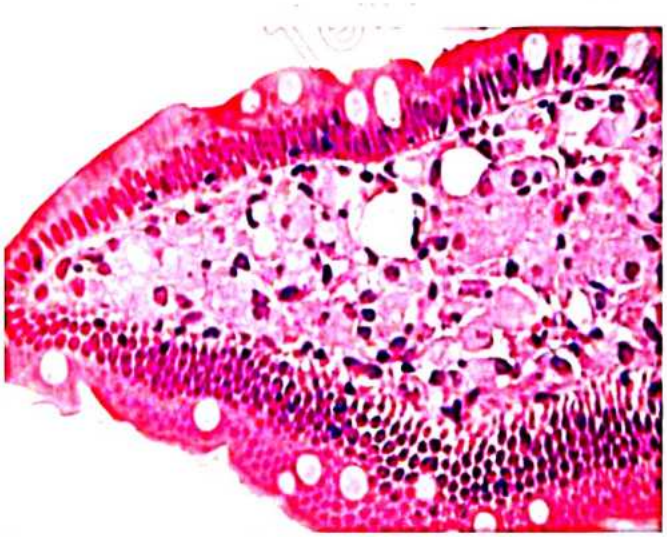
Malabsorption Syndrome

- Hallmark: Steatorrhea
- Steatorrhea: fat in stools (stool is bulky, frothy, foul smelling, & flush-difficulty).

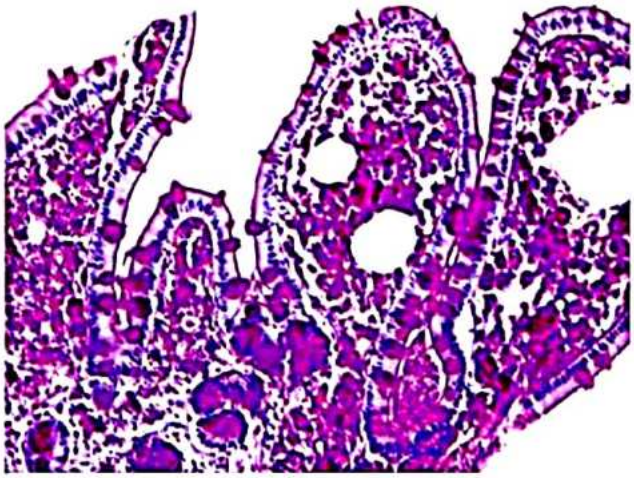
Diseases due to this:

Whipple's disease:

- Organism – Gram + (Tropheryma whipplei)
- Pathogenesis -
  - Macrophages will eat Tropheryma whipplei.
  - It will become foamy cells.
  - So, foam cells are macrophages with engulfed organisms.
  - Lymphatic transport defect.



- Organs - involves GIT, CNS, Joints, CVS
- Diagnosis - PAS+ stain to see the macrophages in the Lamina Propria. Need to do a Biopsy.



- Treatment - Cotrimoxazole (Trimethoprim and Sulfamethoxazole)
- Whipple's disease Vs TB
  - Whipple & TB both show foam cells.
  - But TB foam cells will be positive for stain (ZN stain +)
  - While Whipple will show a negative ZN stain.

Celiac Disease/Gluten Sensitive Enteropathy

- Commonly referred to as Celiac Sprue
- Gluten food - BROW cereals
  (B= Barley, R= Rice, O= Oats, W= Wheat)
- These patients have HLA DQ2 and DQ8 polymorphism.
- Diarrhea on consumption of BROW cereals.

Pathogenesis
Gluten
↓
Alpha-Gliadin
↓
Tissue transglutaminase
↓
Deamidated gliadin
↓
Interact with HLA DQ2,8
↓
Activate CD4 cells
Cytokines
↓
Cellular damage

Alpha-gliadin
↓
Express IL15 on cells
↓
Activate CD8 T cells
↓
Cellular damage
↓
Cellular damage
↓
Makes way for more gliadin inside

$CD_4 + CD_8$

- Alpha-Gliadin - only Alpha Gliadin is a problem, and when it enters the intestine.
- Tissue Transglutaminase (TTG) - Tissue Transglutaminase will convert Alpha-Gliadin into Deamidated Gliadin.
- But for people having HLA DQ2,8 polymorphism, it will activate the CD4T cell.

Telegram - @nextpreladdernotes

- The CD4T cell will then release mediators and damage these cells.
- Alpha Gliadin - it will make the cell express IL15 on the cell.
- IL15 is famous for activating CD8 T cells.
- Then, CD8 T cells will come and damage the cell.
- Makes way for more gliadin inside.

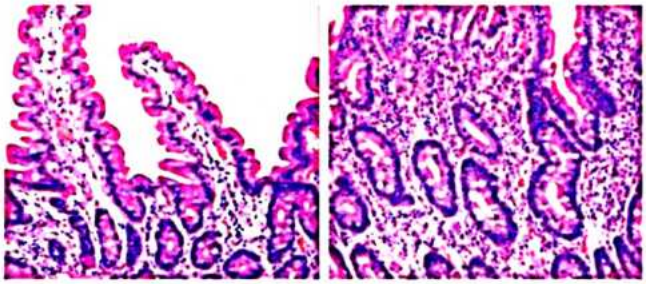
Antibodies formed:
- Anti TTG antibody = most sensitive
- Anti gliadin antibody
- Anti endomysial antibody = most specific

They are most commonly IgA types.

The important Four D's:

D- Diarrhea (problem occurs after gluten)
D- Duodenum - most impacted (Iron deficiency anemia)
D- Dermatitis herpetiformis (skin disorder due to IgA)
D- The treatment for Dermatitis herpetiformis is Dapsone

Duodenum biopsy



- It follows the Marsh criteria:
  - Villous Atrophy
  - Crypt hyperplasia
  - Overall thickness will remain same
  - IEL - Intra epithelial lymphocytes (CD8 T lymphocytes)

Treatment
- Diet modification
  - Do not consume BROW cereals.
  - Rice and Maize can be consumed.
- Otherwise,
  - Steroids is given.

Risk of cancer
- Yes, cancer known as EATL (Enteropathy Associated T Cell Lymphomas).

Environmental Enteropathy/Tropical Sprue
Cause
- associated E-Coli bacteria.

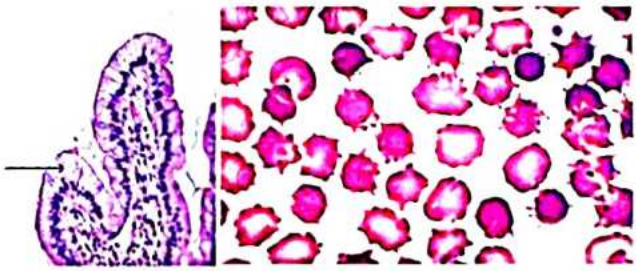
Intestine affected
- Duodenum (Fe), Ileum(B12), Jejunum (FA)

Anemias
- Megaloblastic Anaemia
- Iron Deficiency Anaemia

Treatment
- Antibiotics

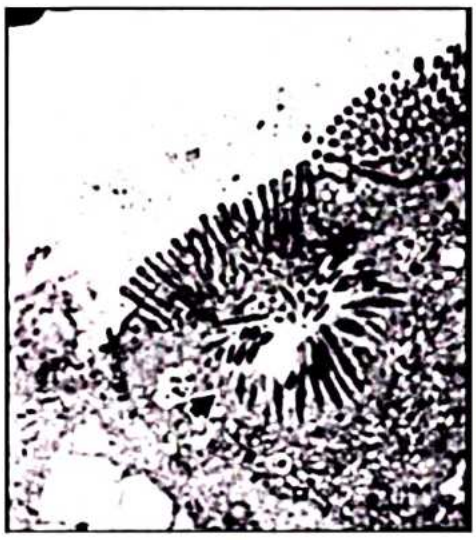
Risk of cancer
- NO

Abetalipoproteinemia
Cause
- It is a Mutation in MTTP
  (MTTP- Microsomal triglyceride transfer protein)
- Due to this, two organs will be affected: intestine & RBC



- Intestine: fat
- RBC: spiky cells - Spur cells/Acanthocytes

Microvillus Inclusion Disease/Davidson Disease
- MYO5B gene - AR
- Patient is going to have intractable diarrhea.
- Microvillus Inclusions are seen under microscope.



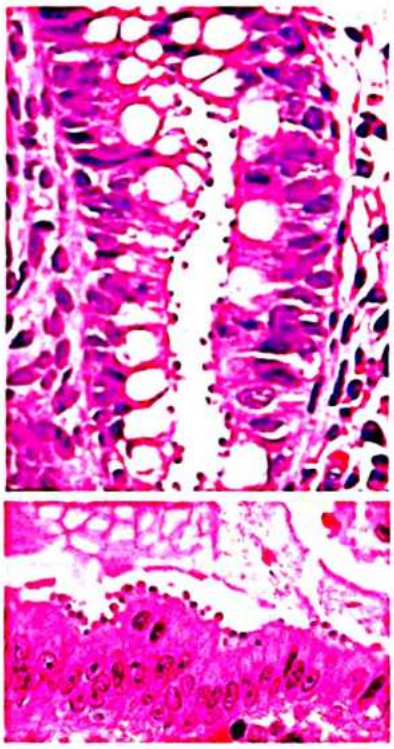
- IHC - CD10+

Telegram - @nextprepladdernotes

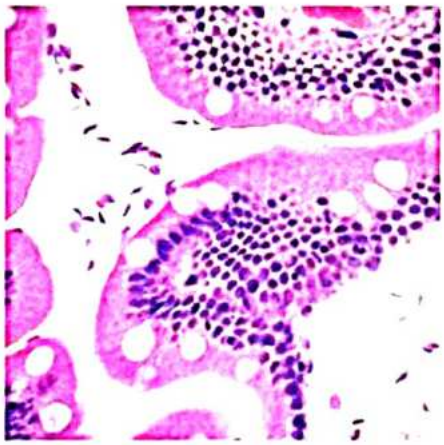
- Treatment:
  - Total parentral nutrition
  - Small bowel transplant

Infections

- Infections can also cause malabsorption.
- Infections such as:
  - **Coccidian parasites** - Cryptosporidium - remains along the brush border.



  - **Giardia lamblia** - two Nuclei & four pairs of flagella. It is a luminal organism. It looks sickle-shaped.



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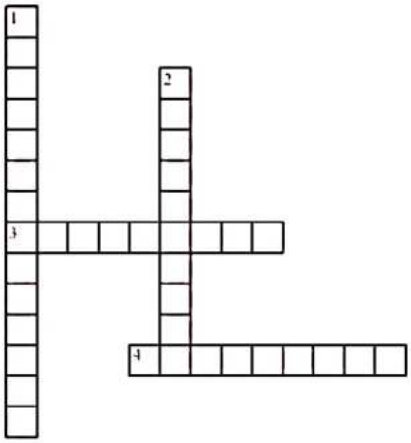
CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Squamous
4. More to the liquids than to the solid.

Down

1. Cells of Cajal are pacemakers of GIT
2. Squamous Epithelia

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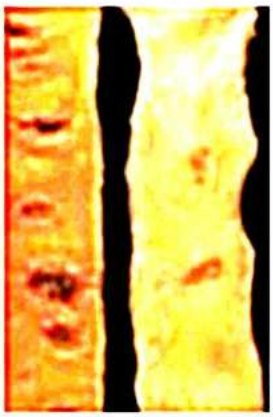
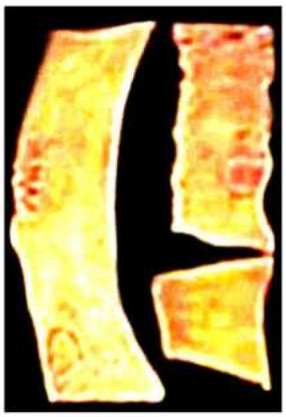
31 GIT PART-2

Ulcerative Disorders

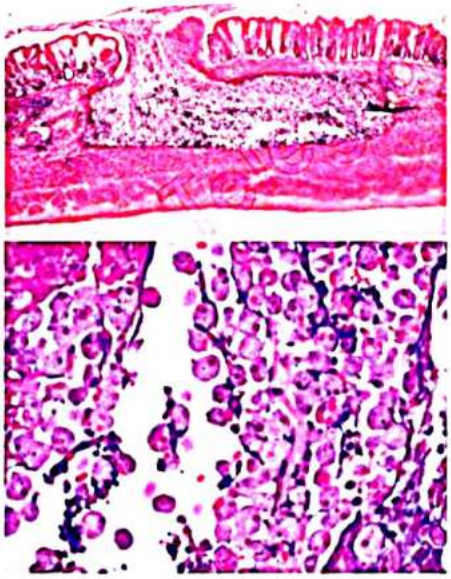




1. TB ulcer - Transverse ulcer
2. Typhoid ulcer - Longitudinal ulcer
3. Amoebic ulcer - Caecum

Both TB and typhoid ulcers occur in the ILEO-caecal junction.

Entamoeba histolytica



- Entamoeba histolytica has a flask-like shape.
- This flask contains dark red spots known as Trophozoites of Entamoeba.
- Trophozoites show the phenomenon of Erythrophagocytosis.
- It has a flask shape because the muscularis layer (at the end after mucosa and submucosa) cannot be broken.

MCQs

Q. Characteristic histopathology finding in Whipple's disease is:

a. Blunting and flattering of the mucosal surface and absent villi
b. Mononuclear infiltration at base of crypts
c. Shortened thickened villi with increased crypt depth
d. PAS-positive macrophages and rod-shaped bacilli in lamina propria
e. Antibiotics

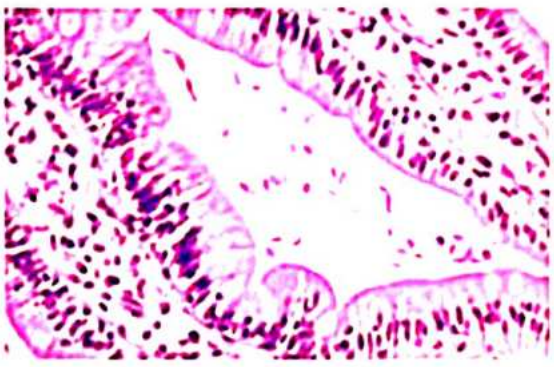
Q. A 13-year-old boy is brought to the pediatrician by his parents, who are concerned about his short stature. He also has had recurrent episodes of diarrhea. Past medical history is significant for iron deficiency anaemia, diagnosed 6 months ago. Serum anti-tissue transglutaminase (anti-tTG) antibodies are positive. An upper endoscopy, along with small bowel luminal biopsy, is performed. Which of the following histopathologic changes would most likely be in the mucosa of the duodenal biopsy in this patient?

a. Cuboidal appearance of basal epithelial cells
b. Crypta oplasia
c. Granulomas extending through the layers of the intestinal wall
d. Blunting of the intestinal villi

Q. HIV-positive patient with intractable diarrhea undergoes a GI biopsy. On high power, a cluster of small dot-like structures is seen along the brush border of the intestine. The most likely pathogen is?

a. Cryptosporidium
b. Cytomegalovirus
c. Giardia lamblia
d. Microsporidia

Q. 43-year-old FACTORY WORKER presented with complaints of abdominal pain, diarrhea, and weight loss. Hemoglobin level was 9OM/DL, MCV 88 fl. Small Intestine biopsy shows features as given in the picture below. The most likely diagnosis is?



Telegram - @nextprepladdernotes

a. Entamoeba
b. Giardiasis
c. Whipple disease
d. Celiac disease

Inflammatory bowel disease 00:09:22

- Inflammatory bowel disease includes Crohn's disease and Ulcerative colitis
- Both showed ulcers.
- Both show intestinal and extraintestinal involvement.

Extra-intestinal:

Crohn's Disease	Ulcerative Colitis
RASH - pyoderma gangrenosum	RASH - pyoderma gangrenosum
Uveitis	Uveitis
Arthritis	Arthritis
Kidney stones - Oxalate	PSC - primary sclerosing cholangitis

Crohn's Disease
mnemonic - [SISTER-MAC]

S- Skip Lesion, skin nodules (Metastatic Crohn's disease)
I- Most common organ affected is Ileum

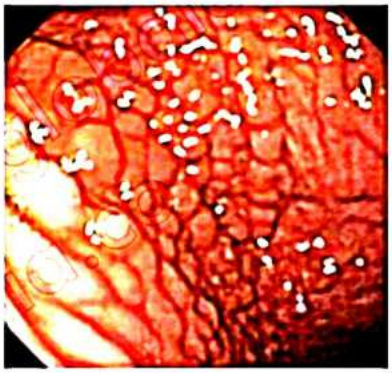
S:

- Radiologically - String sign (Barium study)
- associated with Saccharomyces cerevicae in some cases.
- Immunologically - Anti Saccharomyces cerevisiae positive (ASCA+)

T- Full thickness } Fistures
E- Entire wall } Fistulas
R- Rectum is spared

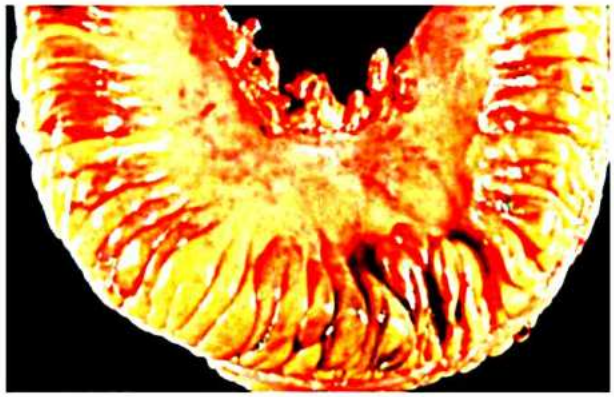
Non caseating granulomas

M- Microscopy: shows Hallmark - Non-Caseating Granuloma
A- Aphthous ulcer (first lesion). It will further progress and form Serpiginous ulcers.
C- Inside of the intestine will show a Cobblestone appearance.



Cobble stone appearance

C- From the outside, it will show creeping fat.



C- Cytokine: TNF is elevated.

Treatment

- Anti-TNF drugs like Infliximab.
- If there is an overall bacterial infection, antibiotics are used.
- Steroids - if there are antibodies.

Telegram - @nextpreladdernotes

Ulcerative Colitis

- These are shallow Ulcers.
- They form Pseudopolyp.
- Pseudopolyp is a regenerating mucosa. (only in Ulcerative colitis)
- Regenerating mucosa will start building and will lead to Bridging.

C- Continuous Lesion

O: Often Rectum

- Rectum is where the disease starts.
- Retrograde involvement process continues
- Backwash ileitis (Disease will affect ileum)

L- Lead pipe appearance (Loss of Haustra)

I- Immunity: P-ANCA

Note: Ulcerative Colitis & PSC both are P-ANCA+

T- Complications: Toxic Megacolon

I- Increased risk of cancer

S- Smoking is going to be protective

Treatment

- Medications
  - 5-aminosalicylic acid (5-ASA)
  - Mercaptopurine (6MP)

Crypt Abscess 00:33:06

- When Crypt Abscess is present, it is both Ulcerative colitis and Crohn's disease.
- It is an indication of active disease.

Marker for intestinal inflammation

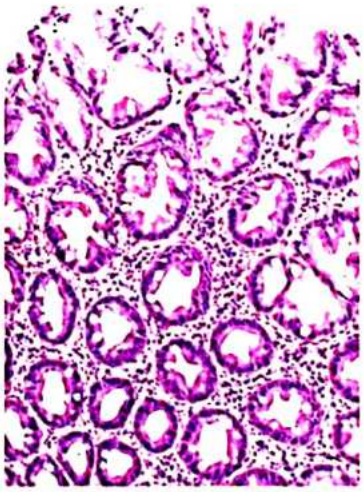
- C-reactive protein(CRP)
- Fecal Lactoferrin
- Fecal calprotectin

Polyps - Classification 00:35:13

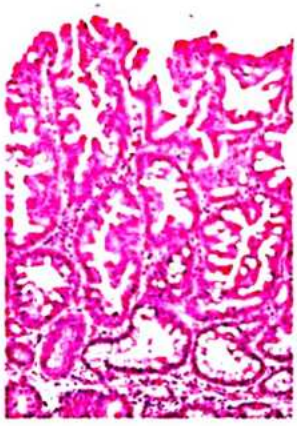
- Non-neoplastic
  - Inflammatory (eg: Ulcerative Colitis)
  - Hyperplastic
  - Hamartomatous
- Neoplastic
  - Includes name "Adenoma"
  - Sessile Serrated Adenoma
  - Tubular Adenoma
  - Tubulovillous Adenoma
  - Villous Adenoma

Hyperplastic Polyps

- Happen between age 50 to 60s
- Increase in the number of cells
- Rectosigmoid
- The distorted zig-zag thing is known as Serrations (only in the upper part)



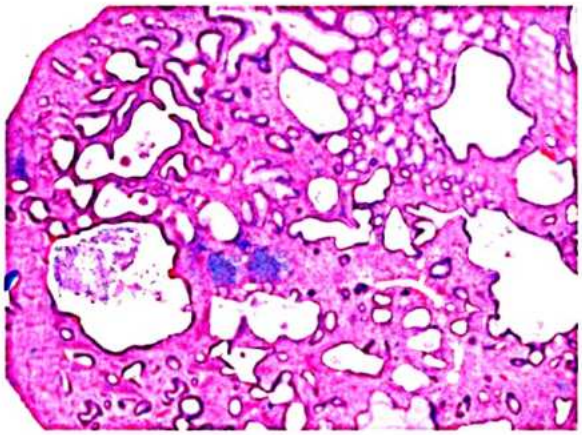
- Serration everywhere with full thickness - Sessile serrated adenoma (boot-shaped crypts). It can lead to cancer.



Telegram - @nextprepladdernotes

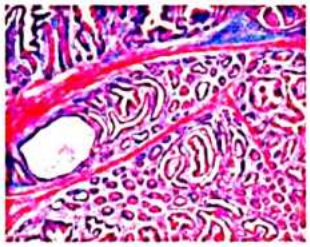
Hamartomatous Polyps

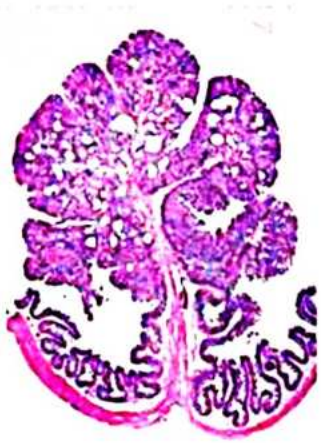
1. Juvenile Rectal Polyp



- Commonly seen in Rectum.
- Age = less than 5 years.
- Clinical feature; Bleeding PR
- Sporadic (Single polyp is seen) or Syndromic (multiple polyps)
- Mutation:
  - SMAD 2
  - SMAD 4
  - BMPR1A
- Extra-intestinal:
  - Digital clubbing
  - Vascular malformations

2. Peutz Jeghers Syndrome





- Age - 11 years
- Mutation?
  - Loss of function - STK11/LKB 1 gene
  - MNEMONIC- P
    → P: Pigmentation, Polyp: Arborishing Polyp- tree-like structure-branches- smooth muscles.
  - MNEMONIC- JE
    → Most common location Jejunum (in the small intestine)

3. Cowden Syndrome

- The PTEN gene is present in Chromosome Ten.
- It causes Cowden Syndrome
- Endometrial cancer
- Thyroid cancer
- Trichilemmoma
- Thickening of skin
- Polyps
- Breast cancer

Trick: TEN TEN DEN and mnemonic PTEN.

4. Bannayan Ruvalcaba Riley Syndrome (BRR)

- Happens because of the PTEN mutation.
- Along with Polyps, mental retardation is going to occur.

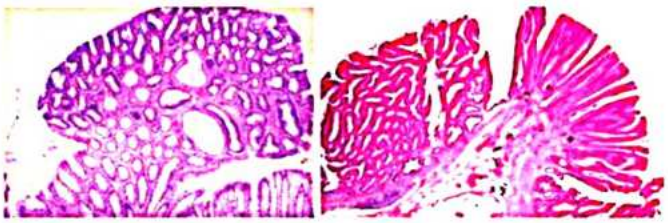
5. Tuberous Sclerosis

- Due to two genes: TSC 1 gene & TSC 2 gene.
- TSC 1 gene = Hamartin protein
- TSC 2 gene = Tuberin protein
- It will have Hamartomatous polyps in the intestine.

6. Cronkhite Canada Syndrome

- Non-hereditary.
- Have polyps + skin + hair + nail.
- Happens at age above 50 years.

Neoplastic: Adenomas



Tubular Adenoma - Round structure
Tubulovillous Adenoma - combo of both
Villous Adenoma - Long structure

- Max Risk of Cancer - Villous Adenoma

FAP - Familial Adenomatous Polyposis 01:00:56

- Minimum no > 100 polyps, thus named Polyposis.
- 100% risk of cancer
- Cancer usually occurs within 30 years of age
- Prophylactic Colectomy - Reduces the risk of cancer (does not completely eliminate)

Telegram - @nextpreladdernotes

- There is the involvement of genes.
  - APC gene - chr 5q21
  - Rarely NER defect can be seen

Types:

1. Classical FAP
   - >100 polyps
   - Congenital hypertrophy of RPE (Retinal Pigmentation Epithelium)

2. Turcot FAP
   - >100 polyps.
   - Additionally, Brain tumors. These include Medulloblastoma and Glioblastoma.
   - Mnemonic TUR- TURBAN= BRAIN TUMOR
   - COT- COlon Tumor

3. Gardner FAP
   - >100 polyps
   - Epidermal cysts
   - Fibroma
   - Osteoma
   - Supernumerary teeth (extra teeth will be present)

Q. What is the inheritance of classical FAP, Turcot FAP, & Gardner FAP?

Ans. Autosomal Dominant (AD)

Mutyh Associated Polyposis (MAP)

- Base-excision repair gene - MUTYH.
- When this gene has a problem, it is known as Autosomal recessive disorder.
- In contrast to FAP, MAP is characterized by fewer than 100 polyps, which appear at later ages.

MCQ

Q. A 16-year-old boy presents after suffering a tonic-clonic seizure. He says it had a duration of 2 minutes and a postictal period of 10 minutes. The patient denies any similar episodes in the past. Past medical unremarkable. Family history is significant for his mother and older brother's history is who died of colorectal cancer at ages 50 and 15 years, respectively. On physical examination, the patient is drowsy. An MRI of the brain reveals an Infratentorial cerebellar mass. Which of the following is the most likely diagnosis in this patient?

a. Cowden syndrome
b. Turcot syndrome
c. Gardner syndrome
d. Lynch syndrome

Q. Osteomas, adenomatous polyps of the intestine, and supernumerary teeth are seen in?

a. Gardner syndrome
b. FAP
c. Cowden syndrome
d. Peutz Jeghers syndrome

Intestinal Carcinoma

01:11:50

- Most common GI cancer = Colonic Adenocarcinoma

Risk Factors:

- High fat
- Low fiber
- Alcohol
- Smoking
- Ulcerative Colitis
- Crohn's disease
- FAP
- HNPCC
  - (Hereditary Non Polyposis Colon Cancer)
  - Also referred to as Lynch Syndrome.
  - It includes Colon cancer, endometrial cancer & ovarian cancer.

Mechanisms

It is a multistep carcinogenesis

1. AK-53: Adenoma carcinoma sequence.

- Genes include Ak53
- A- APC mutation (2 Hits)
- K- KRAS
- 53- p53, SMAD 2 & 4

2. SSA Pathway

- MMR (Mismatch repair gene) - MLH 1, MSH 2,6 & PMS 1,2.
- If these MMRs are working well, all good.
- If any defect, it will cause MSI (Microsatellite instability).
- BRAS mutation

Telegram - @nextprepladdernotes

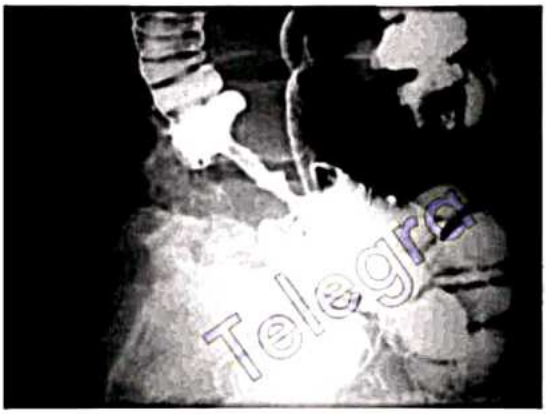
3. **CIMP - BRAF**
   - No MMR mutation
   - MSI without MMR mutation
   - CpG island hypermethylation phenotype
   - CpG - C & G has undergone methylation.
   - BRAF

4. **CIMP-KRAS**
   - No MSI, No MMR mutation
   - CpG island hypermethylation only.
   - KRAS

Types

- **Proximal/Right**
  - Proliferative mass
  - IDA (Iron deficiency anemia)
  - Looks like proximal colon
- **Distal/Left**
  - Napkin ring
  - Occurs bleeding and change in bowel habits.
  - Looks like distal colon

Diagnosis



- Barium enema - Apple core deformation
- Tumor marker = CEA (Carcinoembryonic antigen)
- Occult blood testing in stool.
- The test preferred for this is the Guaiac test.
- Gold STD: Biopsy - Adenocarcinoma

Anal Canal Cancer

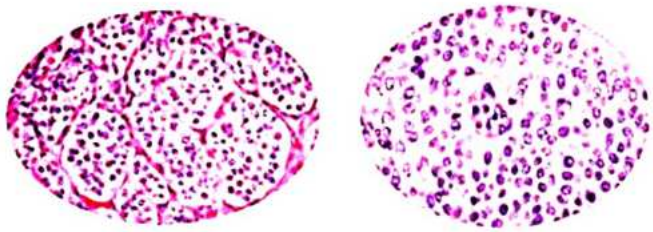
- Lining of the anal canal is squamous epithelium
- Squamous cell carcinoma occurs.
- Virus present is HPV 16,18.
- Famous regime = NIGRO Regime

Miscellaneous Tumor: Carcinoid Tumor

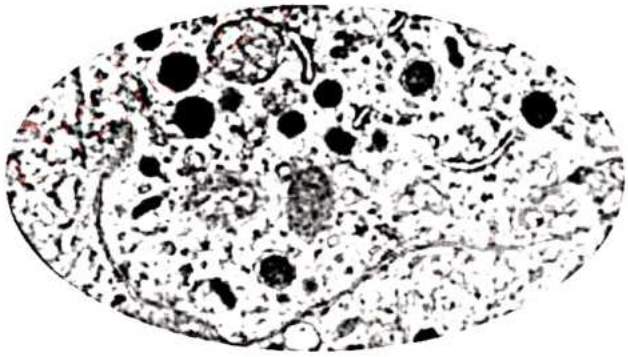
- **Sites**
  - Present in GIT, Lungs (Bronchus)
- **Cell of origin**
  - Neuro-endocrine Tumors
  - APUD cells/ Kulchitsky cells/ Enterochromaffin cells
  - Enterochromaffin cells release 5HT/Serotonin.

Diagnosis:

- **Light Microscopy**
  - Nesting pattern
  - Salt & pepper nucleus



- **IHC (Immunohistochemistry)**
  - SynaptophysIn, chromogranIn, bombesIn
  - NSE, CD56, Cd57
- **Electron Microscopy**



  - Neurosecretory Granules (NSG)
- **Tumor Marker**
  - 5HIAA

Hormones released:

- 5HT
- CCK
- Substance P
- Polypeptide YY

Not related to prognosis

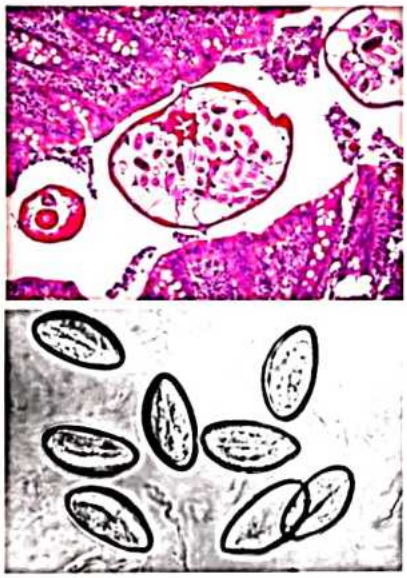
Carcinoid Syndrome

- 10% of hormones(5HT) will show Carcinoid Syndrome.
- When there is an excessive amount of serotonin release leads to Systemic fibrosis.
- **S**= Systemic fibrosis

Telegram - @nextprepladdernotes

- **H**= Hepatomegaly Heart (TR)
- **I**= Intestine motility
- **V**= Vasodilation - Flushing of Face
- **A**= Asthma (bronchoconstriction)

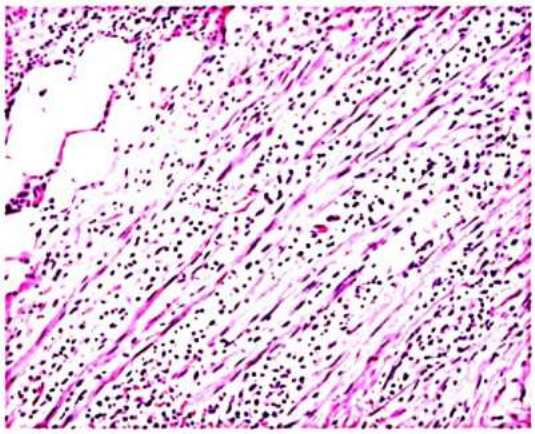
Q. One parasite which goes into the vermiform appendix?
Ans. Enterobius Vermicularis



- Worm - Egg (Dee shaped)
- Egg is plano-convex
- Adult worms have a spiky projection known as Cuticular Alae - 3.

Diagnosis of Appendicitis

- **For microscopic examination:**

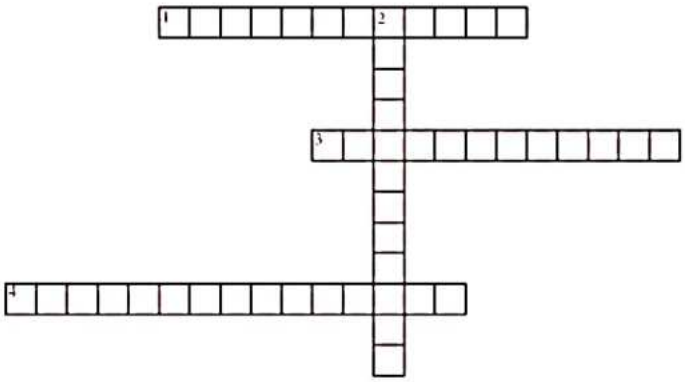


- Acute appendicitis in the presence of Neutrophils in Muscularis Propria.

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

1. Crohn's Disease
3. Caecum
4. TB ulcer

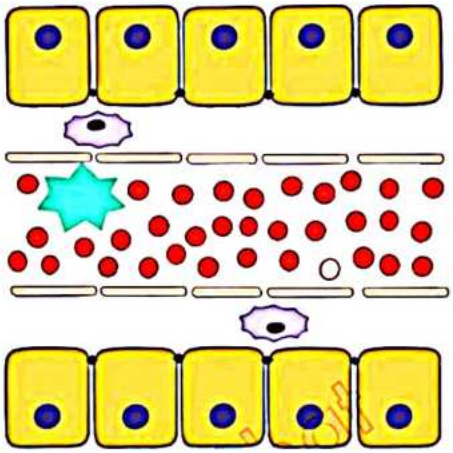
Down

2. Longitudinal ulcer

Telegram - @nextpreladdernotes

32 HEPATOBILIARY PATHOLOGY

- The hexagonal lobule is a functional unit/ functional part of a liver.
- The center has a central vein, and the periphery has these portal triads.
- Hepatocytes lie between the central vein and portal triads in the form of chords.
- These are known as chords of hepatocytes.
- The blood vessels in the liver are sinusoids.
- The space between hepatocytes and sinusoids is known as the space of DISSE.



Space of Disse 00:02:20

It can be divided into two types:

1. Ito cells/stellate cells
2. Amyloid deposition

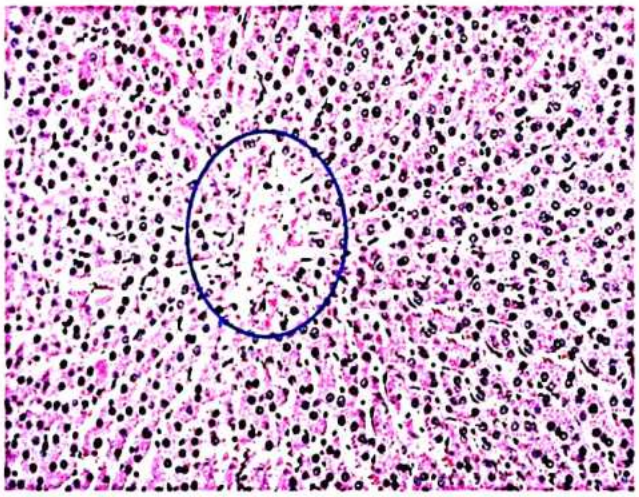
- Location: Between hepatocytes and sinusoids.
- Ito cells act as vitamin A storage.
- Stellate cells will form collagen.
- Cirrhosis of the liver is the state in which collagen is formed.
- The combo of collagen 1 and 3 forms collagen in stellate cells.

Sinusoids

- Kupffer cells are present on the wall of sinusoids.
- These are the macro fudges of the liver.

Canals of herring

- It connects hepatocytes and cholangiocytes.
- These contain oval cells of the liver or the stem cells of the liver.
- The liver can regenerate because of these stem cells.



Bilirubin Metabolism 00:08:40

Heme
↓ Heme oxygenase
Biliverdin
↓ Biliverdin reductase
Bilirubin (unconjugate/indirect/insoluble)
↓ Albumin
Bilirubin enters liver
↓ UGT (UDP glucuronosyl transferase)
conjugated bilirubin

- The bilirubin exits out of the liver through the MRP2 & ATP family.
- A part of it known as Urobilinogen is going to urine.
- Another part will go to stool, referred to as Stercobilinogen.
- The rest goes back to the liver. It is known as Enterohepatic recirculation.
- Entero → Entero Hepatic → Liver

Total Bilirubin

- The reaction for testing total bilirubin is van den berg.
- Total bilirubin = Direct Bilirubin + Indirect Bilirubin
- The direct one is soluble, and the indirect or unconjugated one is insoluble.

Hyperbilirubinemia / Jaundice

- The total bilirubin increase can be due to a rise in unconjugated bilirubin or an increase in conjugated bilirubin.
- Bilirubin has an increased affinity for elastin fiber, so skin, sclera, and the mucous membrane are checked to detect jaundice.

Telegram - @nextprepladdernotes

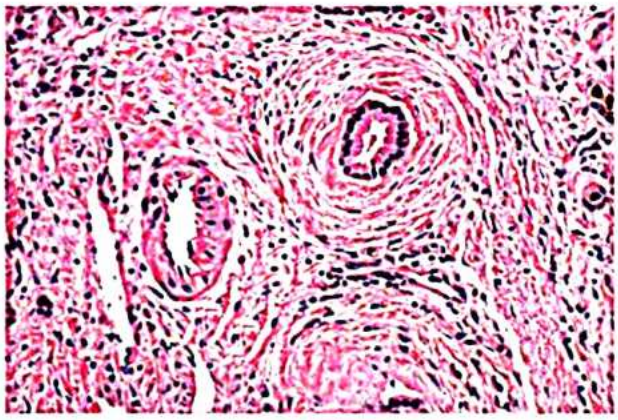
Causes of Unconjugated hyperbilirubinemia

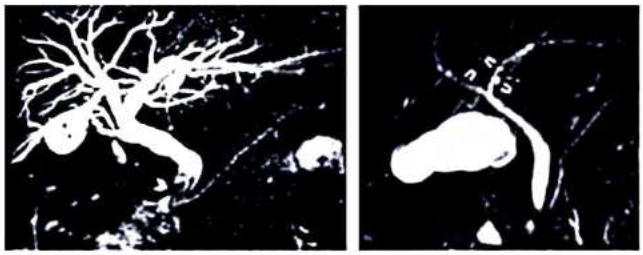
- Hemolytic anemia.
- Syndromes:
  - Criggler Najjar syndrome type 1
    - → Complete deficiency of UGT
    - → 100% fatal
  - Criggler Najjar syndrome type 2
    - → Partial deficiency of UGT
    - → Treatment - The drug phenobarbitone helps increase the activity of UGT.
  - Gilbert syndrome
    - → Very mild dficiency of UGT
    - → Mostly Asymptomatic, but this will only manifest under stress, fever, pregnancy

Causes of Conjugated hyperbilirubinemia

- Obstruction
  - Types of obstruction:
    - → Clonorchis/Opisthorchis
      - It affects the cholangio system. It can even cause cancer. They belong to the Trematoda family.
    - → Cancer
    - → Stricture
    - → Stone

- Biliary Diseases
  - Primary Biliary Cholangitis
    - → Above 50 years old
    - → Common antibodies- AMA (Anti mitochondria), ANA (AntiNuclear antibody)
    - → Associated with Ulcerative colitis
    - → Common in Females
    - → Radiology - Normal
    - → Intrahepatic bile ducts affected
  - Primary Sclerosing Cholangitis
    - → 30 years old
    - → Common antibodies- p-ANCA
    - → Associated with Sjogren
    - → Common in Males





- → Radiology - pruning small bits of bile ducts, stricture, and beading of large bile ducts. Onion skinning can be seen here.
- → Intrahepatic and extrahepatic bile ducts are affected.
- → The effect is onion skinning of the bile duct.
- Syndromes:
  - Dubin Johnson syndrome
    - → MRP defect
    - → Darkly pigmented liver
    - → The pigment is epinephrine which gives the dark-colored
  - Rotor syndrome
    - → Defect in organic anion transporter (OATP)
    - → Non pigmented liver

Note: All the disorders are autosomal recessive except Criggler Najjar syndrome type 2 which is the dominant one.

Onion Skinning in Pathology (Recap)

- Cell injury - myelin figures
- Lysosome - Tay Sachs disease
- SLE spleen
- Hyperplastic arteriolosclerosis
- PSC
- Chronic inflammatory polyradiculoneuropathy (CIDP).

Q. Conjugated hyperbilirubinemia is seen in ?
A. Criggler najjar type 1
B. Criggler najjar type 2
C. Gilbert syndrome
D. Dubin johnson

Q. Which hereditary hyperbilirubinemias are autosomal dominant?
A. Criggler najjar type 1
B. Criggler najjar type 2
C. Gilbert syndrome
D. Dubin johnson

Telegram - @nextprepladdernotes

Q. Which of the hereditary hyperbilirubinemias are always total?

A. Criggler najjar type I

B. Criggler najjar type 2

C. Gilbert syndrome

D. Dubin johnson

Q. First space of amyloid deposition

A. Canals of herring

B. Space of disse

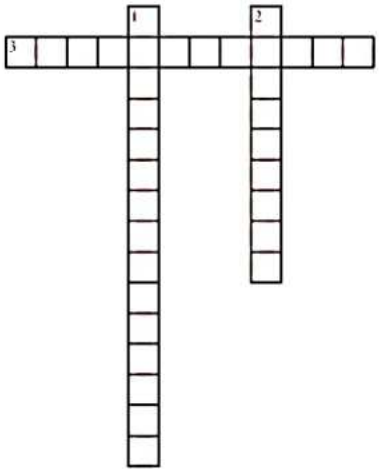
C. Periportal

D. Centrilobular

Telegram - @nextpreladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Between hepatocytes and sinusoids.

Down

1. Criggler Najjar syndrome type I

2. Kupffer cells are present on the wall of sinusoids.

Telegram - @nextpreladdernotes

33 LIVER CIRRHOSIS , TUMORS AND MISCELLANEOUS TOPICS

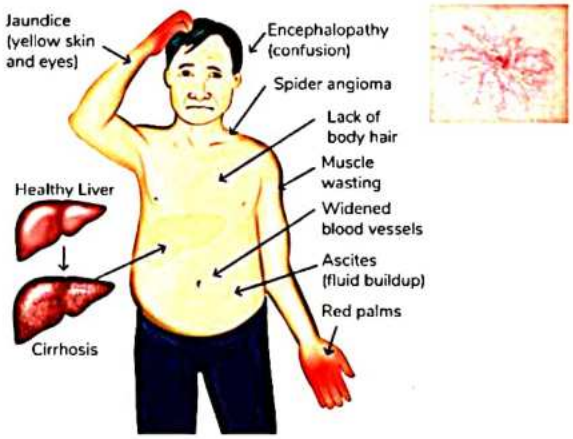
- Damage to the liver parenchyma due to;
  - Alcohol,
  - Hepatitis,
  - Some toxins, and
  - Certain drugs.
- Liver fights back, forming bridging fibrous septa.
- Regeneration of parenchymal nodules.

Q. What type of collagen is present in the fibrous septa?
Ans. Collagen 1+3

Q. Who makes collagen in the fibrous septa?
Ans. Stellate Cells present in the Space of Disse

Cirrhosis – Features 00:02:50

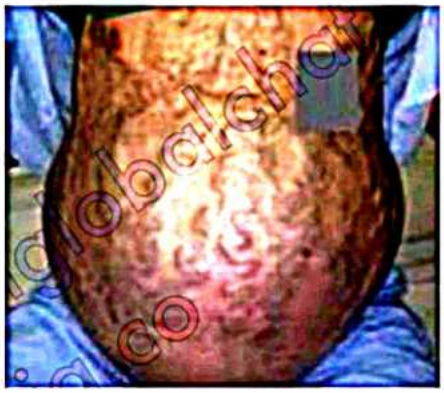
- **Decreased synthesis** of;
  - **Proteins**
    - → Low levels of Albumin
    - → Decreased A: G ratio
    - → Leads to oedema
  - **Clotting factors**
    - → Bleeding
    - → High PT and APTT
- **Decreased Metabolism** increases the levels of
  - **Estrogen**
    - → Palmar Erythema
    - → Gynaecomastia in males
    - → Gonadal atrophy
    - → Spider angioma
  - **Ammonia**
    - → Crosses BBB and causes Hepatic Encephalopathy
    - → Asterixis or flapping tremors are seen



Classical Cirrhotic Liver

All these features can lead to complications - Portal Hypertension.

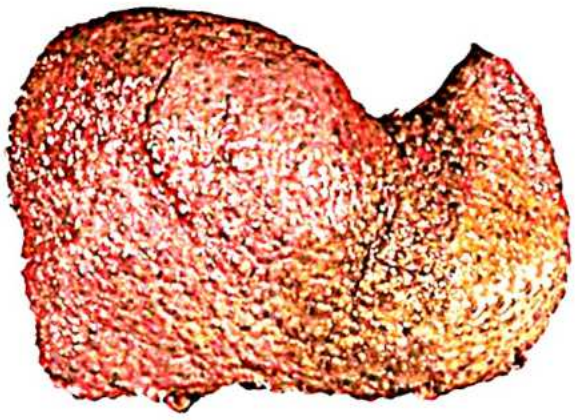
Cirrhosis
↓
Increased Resistance of Portal Hypertension
↓
Portal Hypertension



- Portal Hypertension occurs due to a protuberant abdomen.
- There are two reasons
  - Congestive Splenomegaly.
  - Ascitis.
- Portal systemic stunts will be created, resulting in
  - Caput medusae
  - Hemorrhoids
  - Esophageal Varices, If ruptured, it can result in death.

Micronodular Cirrhosis 00:09:16

- Granular surface on the liver.
- Size of the granule is <3mm.



Telegram - @nextpreladdernotes

Causes

- ALD (Early stage of Alcoholic Liver Disease).
- ICC (Indian Childhood Cirrhosis) - Not so Common.
- PBC (Primary Biliary Cirrhosis).
- Hemochromatosis.

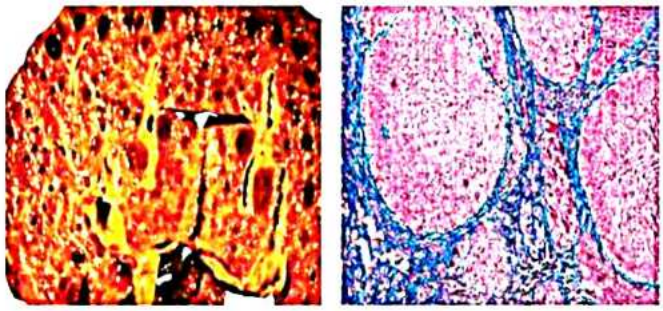
Macronodular Cirrhosis 00:09:35

- The granule size is >3mm.

Causes

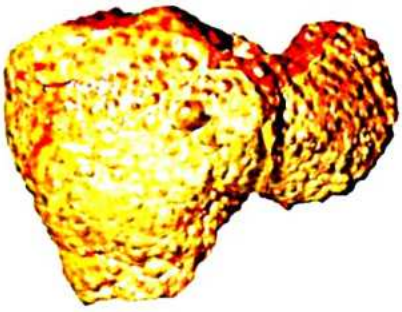
- ALD (Late stage Alcoholic Liver Disease).
- Viral hepatitis.
- Wilson disease.

Masson Trichrome and Image 00:11:51



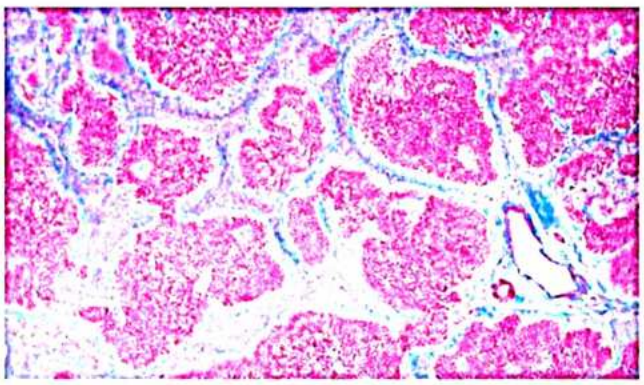
- Masson Trichrome stains
  - **Muscle - Red** color.
  - **Collagen - Blue** color.
- If collagen or fibroid bands are seen in liver histology, it confirms
  - Cirrhotic etiology.

Biliary Cirrhosis 00:12:50



- Bile duct damage.s
- Liver color changes to green.

Nodules - Jigsaw Puzzle 00:13:21

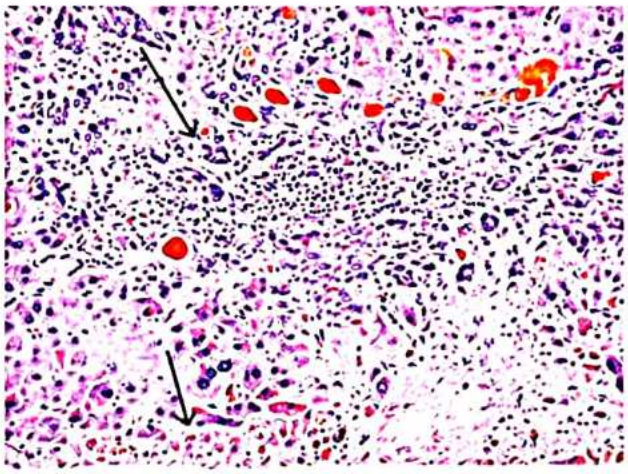


- Under a microscope, the muscle will be red, and collagen will be blue because of Masson Trichrome.
- Nodules show a Jigsaw Puzzle pattern.

Q. Where in the Pathology do you see the Jigsaw Puzzle pattern?
Ans.

- Biliary cirrhosis.
- Cylindroma, a skin tumor.

Bile Plugging 00:15:01



- It confirms the presence of bile in the liver in biliary cirrhosis.
- Hall Stain positivity comes from bile.
- It mark's the bile in Golden color.

Causes of Cirrhosis 00:15:37

1. ALD (Alcoholic liver disease)
2. AIH (Autoimmune Hepatitis)
3. Biliary diseases
4. Cryptogenic cirrhosis (now called NALD)
5. Drug-induced

Telegram - @nextpreladdernotes

6. Enzyme deficiency
   a. Alpha1 antitrypsin deficiency
7. Hepatitis
8. Hemochromatosis
9. Wilson's disease

01. Alcoholic Liver Disease and Image 00:17:03

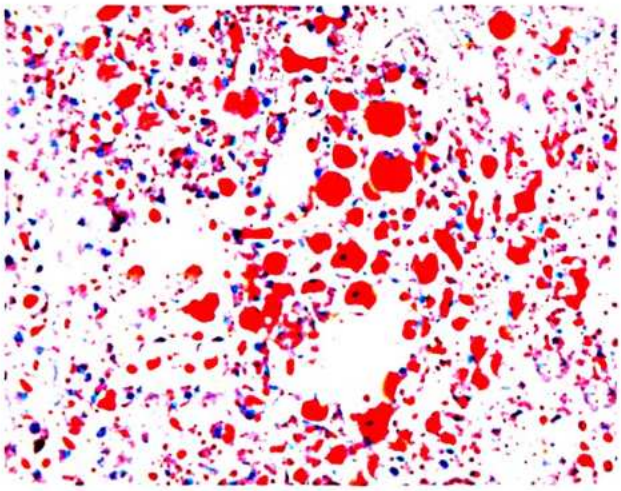
- Progressive disease.
- If alcohol consumption is sustained, the disease will progress in severity.

Stages of ALD

- Steatosis or Fatty Liver → SteatoHepatitis → Cirrhosis (Particularly, Laennec's Cirrhosis)

- **Steatosis or Fatty Liver** 00:18:18
  - Microvesicular steatosis (Tiny white holes), seen in
    → Fatty liver of pregnancy
    → HELLP syndrome
    → Reye's syndrome (Jamshedpur fever)
  - Microvesicular steatosis (Big white holes), seen in nutritional and metabolic changes in:
    → Obesity
    → DM
    → TPN (Total Parenteral Nutrition)
    → Starvation
    → Hepatitis C

Stain - Oil Red O 00:19:32



- Confirms fat is present in the holes.
- It stains the fat into the color Red.

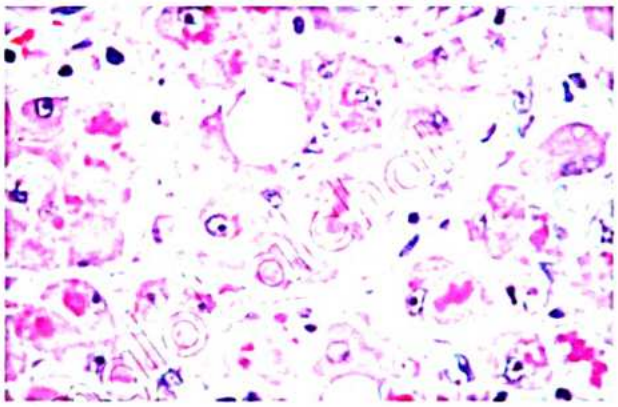
Q. What is the status of hepatitis viruses in the fatty liver?
Ans.

- Hepatitis B doesn't cause fatty liver; the rest can.
- Hepatitis C - causes Macrovesicular fatty liver
- Hepatitis D - causes Microvesicular fatty liver

- **SteatoHepatitis** 00:22:39
  - Mallory Hyaline or Denk bodies are seen.

Alcoholic Hepatitis 00:22:57
Q. What is it made up of?
Ans. Intermediate filaments (Cytokeratin 8/18 or CK 8/18).

Mallory Hyaline / Denk Bodies 00:23:59
They can be seen in



- NAFLD and ALD
- ICC
- Wilson's disease
- Alpha1 antitrypsin deficiency
- Hepatocellular carcinoma
- Cholestasis
- Hepatic or primary biliary diseases.

Not seen in

- Hemochromatosis
- Secondary biliary diseases.

Chicken Wire Fibrosis and Image 00:27:01

- ALD shows Chicken Wire Fibrosis.
- Masson Trichrome is used to see collagen.
- It is seen in SteatoHepatitis.

02. Non-Alcoholic Liver Disease 00:28:36

- No alcohol consumption.

Telegram - @nextpreladdernotes

- **Causes**
  - Obesity
  - Increase in triglycerides
  - DM
  - HTN

Stages of NALD

- Non-alcoholic Steatosis or Fatty Liver → Non-alcoholic SteatoHepatitis → Cirrhosis

- **Blood Test**

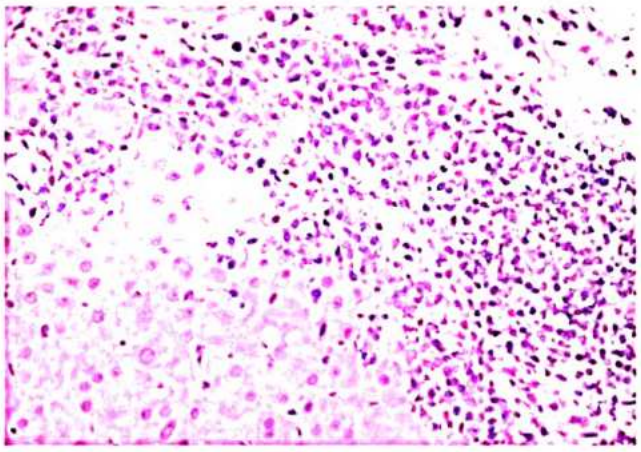
AST/ALT Levels	Condition
<1	NALD
>2	ALD

- **Most common cause of death:** Cardiac complications.

03. Autoimmune Hepatitis 00:31:12

AIH I	AIH II	AIH III
• ANA - Antinuclear antibody • ASMA - Anti smooth muscle antibody • SLA - Soluble liver antigen	• Anti LKM 1 - Hepatitis C • Anti LKM 2 - Drug-induced hepatitis • Anti LKM 3 - Hepatitis D	• Anti SLA

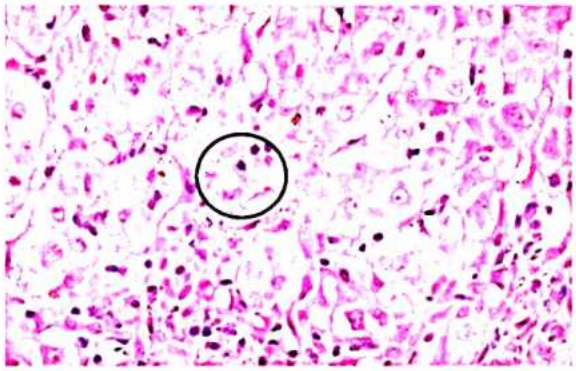
AIH - Interphase Hepatitis 00:34:34



- Mostly inflammation occurs at Interphase (between portal triads and chords of hepatocytes).
- All the cells (Lymphocytes and Plasma cells) die at Interphase, called Piecemeal Necrosis.

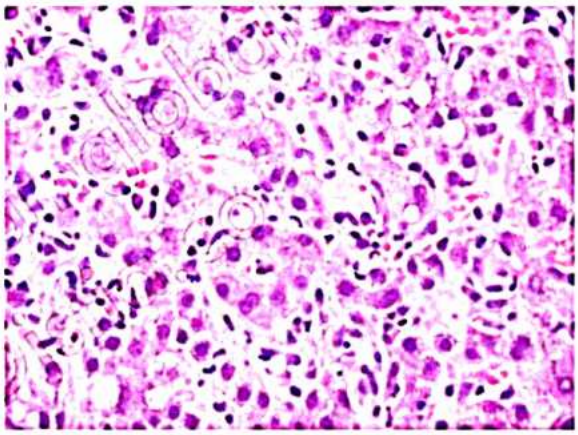
AIH - Emperipolesis (Hepatocyte Eating Lymphocyte) 00:36:16

- Cell within cell appearance.



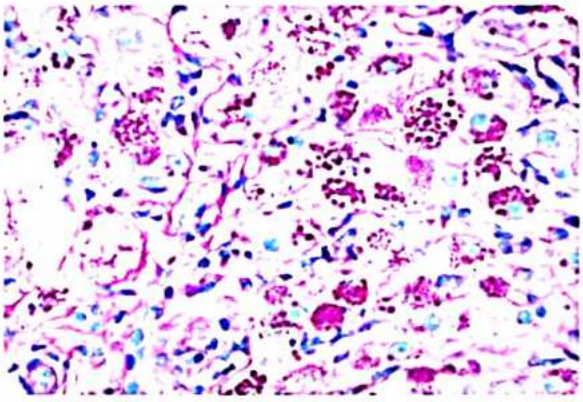
AIH - Hepatic Rosette 00:37:20

- Hepatocytes arranged in round alignment are called Hepatic Rosette.



04. Alpha1 Antitrypsin Deficiency 00:39:10

- Gene on Chromosome 14;
  - **PiMM - Normal**
  - **PiZZ - Diseased**
  - **PiMZ - Carrier.**
- It is inherited in an autosomal recessive manner in children.
- Organs affected
  - **Lungs** - Panacinar emphysema.
  - **Liver** - Cirrhosis.



Telegram - @nextpreladdernotes

Stain 00:42:44

- **PAS + A Diastase** Resistant inclusion.
- Pink color inclusions are seen.

05. Hemochromatosis 00:44:16

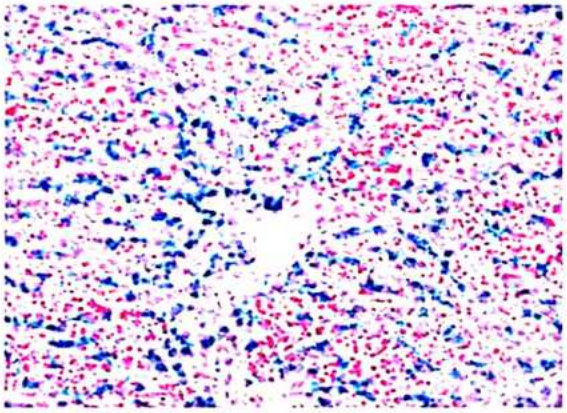
- Iron Overload
- Also known as **Bronze Diabetes**.

Causes

- **Genetic causes:**
  - HFE gene, present on chromosome 6.
  - HJV gene (HemoJuvelin gene) - Causes juvenile hemochromatosis.
- **Acquired causes:**
  - Frequent blood transfusions
  - Bantu Siderosis

Patients present with; 00:46:48

- Cirrhosis.
- Shuts off cell functions causing DM.
- Skin pigmentation (Bronze),(melanin>>>Fe)
- Causes both dilated and restrictive cardiomyopathy.
- Hypogonadism.



Perl's Stain or Prussian Blue 00:49:48

- Liver biopsy and histology are seen under the microscope.

Treatment of Hemochromatosis 00:50:05

- **Drug of Choice** - Iron chelator (Desferrioxamine)
- **Treatment of Choice** - Phlebotomy (Taking out 1 unit of blood)

06. Wilson's Disease 00:51:44

- Copper overload.
- **ATP7B Gene** mutation.
- It is inherited in an **autosomal recessive** manner.
- Copper should be incorporated with Ceruloplasmin. Otherwise, Wilson's Disease is seen.

K - F Ring 00:53:25

Copper deposits in other organs like;

- **Liver** - Causes cirrhosis.
- **Eyes** - KF Ring formed at Descemet's membrane of cornea.
- **Basal ganglia in the brain** - Psychosis.

Stains 00:54:44

Stains

For Copper;

- Rhodanine
- Rubeanic acid.

For Ceruloplasmin;

- ORCEIN Shikata stain, also used to stain
  - HbsAg
  - Cerruloplasmin
  - Elastin

Diagnostic Modality 00:56:21

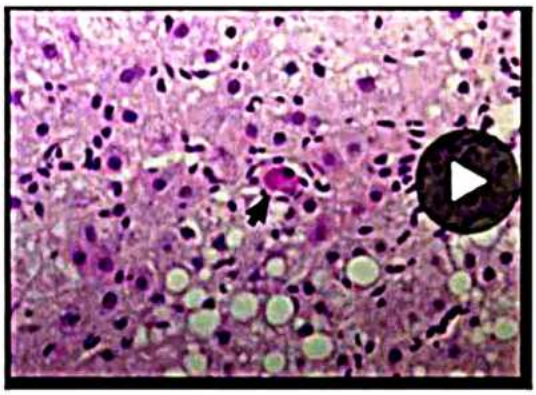
- Serum copper levels - Unreliable test.
- Copper urinary excretion.
- Copper in dry weight of liver tissue.

Treatment 00:57:06

- Copper chelator - **Trientine**.

07. Viral Hepatitis

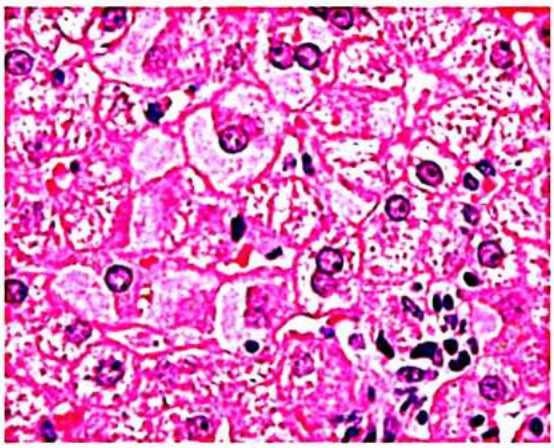
- **Acute Hepatitis** 00:58:01
  - Councilman bodies are seen.
  - Spotty necrosis and dropout necrosis is seen.
  - Kuffer cell hyperplasia.



Telegram - @nextpreladdernotes

- **Chronic Hepatitis** 00:59:27
  - Stellate cells release TGF (Most Fibrogenic) and cause fibrosis, leading to chronic hepatitis.

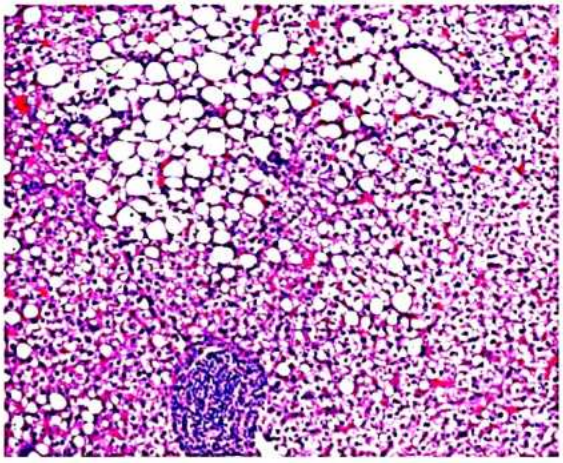
- **Hepatitis B** 01:00:20
  - It shows diffusely pink Ground glass Hepatocytes.
  - ORCEIN shikata stains are responsible for this.



- **Hepatitis C**
  - Causes

→ Macrovesicular Steatosis.

→ Prominent lymphoid follicles are seen.



- **Hepatitis D**
  - It always depends on Hepatitis B.
  - It causes microvesicular steatosis.

Staging and Grading 01:03:05

- Staging depends on
  - Fibrosis
- Grading depends on
  - Inflammation
  - Necrosis

Hepatic Tumors

- Most common malignant tumor - Metastatic Tumor from colorectal carcinoma.

Appearance

- Primary Cancer
  - 1 or 2 nodules are present.
  - No umbilication is seen.
- Metastatic Cancer
  - Multiple nodules are seen.
  - Umblication is present.

Hepatic Tumor - Benign 01:06:23

There are two types

- Hepatic adenoma
  - Most presented with a history of

→ In females with OCP.

→ In males on anabolic steroids.

- Cavernous haemangioma
  - Most common benign tumor in the liver.
  - Associated with VHL syndrome.

Refer table 33.1

Hepatic Adenoma - Bordeaux Classification 01:07:28

- HCA - Stands for HepatoCellular Adenoma.
- Maximum risk of hepatocellular carcinoma is - Catenin HepatoCellular Adenoma.
- Inflammatory HepatoCellular Adenoma is the most common subtype.

Q. Which subtype shows the negativity for LFABP (Liver Fatty Acid Binding Protein)?

Ans. H-HCA.

Q. Which subtype shows all the inflammatory changes?

Ans. IHCA.

Q. What are different inflammatory markers?

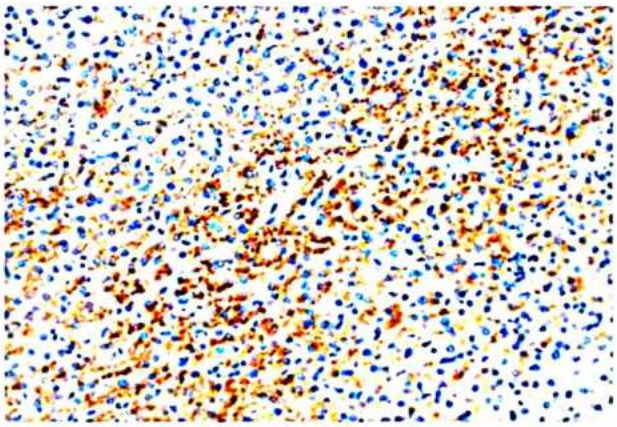
Ans.

- Interleukin-6
- C - Reactive Protein
- SAA IHC - Serum Amyloid Associated IHC.

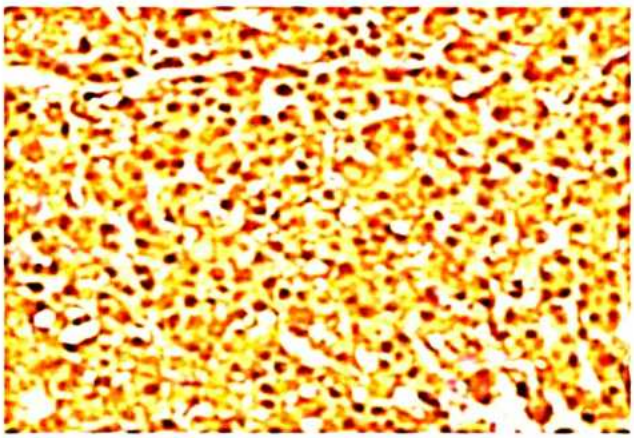
SAA IHC 01:11:05

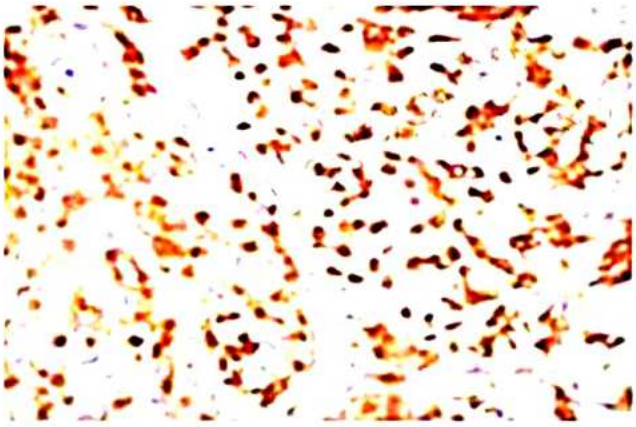
- Serum Amyloid Associated.
- In IHCA, **SAA is positive,** showing brown color if ImmunoHistoChemistry.

Telegram - @nextpreladdernotes



Beta Catenin 01:11:59

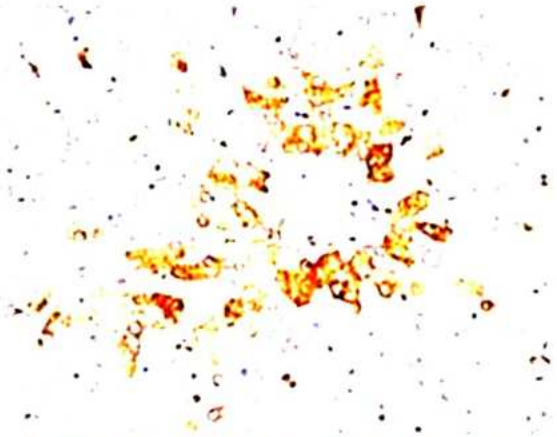


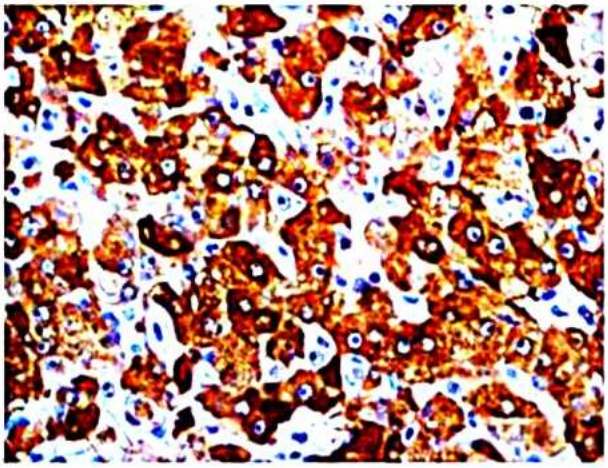


- In normal condition
  - o β Catenin hovers between cytoplasm and nucleus.
  - o Histology shows overall brown color in the hepatocyte.
- In diseased conditions
  - o β Catenin activating mutation occurs, and it is limited to the nucleus.
  - o IHC positive - Only in the nucleus.

Q. What is the home of β Catenin?
Ans. Cytoplasm.

Glutamine Synthetase Stain 01:14:58





- Normally seen in perivenular regions.
- In abnormal conditions
  - o Glutamine synthetase diffuses all over the hepatocytes.

Hepatocellular Carcinoma 01:16:40

- Malignant variety.

Risk Factors:

- ALD
- NAFLD
- Hepatitis B, C
- Hereditary hemochromatosis
- Tyrosinemia
- Aflatoxin (Fungal Infection)
  - o Infection caused by Aspergillus flavus (peanut or groundnut contaminant).
  - o Mutation in the **p53 gene at codon 249**.

Q. Which gene in HbB causes HCC?
Ans. HbX gene.

Q. Which codon on p53 is affected by aflatoxins?
Ans. Codon 249.

Telegram - @nextpreladdernotes

Classical HCC	Features	Fibrolamellar Variant HCC
>40 years	**Age**	19 - 20 years
♀>>♂	**Gender**	♀=♂
Hepatitis B, C positive.	**Associated with Hepatitis**	Hepatitis negative.
-	**Gene Mutation**	DEL 19 DNAJB1-PRKACA (Deletion)
• High levels of AFP (Alpha FetoProtein) are seen.	**Tumor marker**	• AFP (Alpha FetoProtein) levels are normal. • Neurotensin levels are increased.
Hematogenous	**Spread**	Lymphatic spread
Poor prognosis.	**Prognosis**	Good prognosis.

Premalignant Changes in Liver 01:23:21

- **Dysplasia** - Precancer or Premalignant.

Liver Cell Dysplasia	Dysplastic Nodules
Large Cell Change	Low-Grade Dysplastic Nodule
Small Cell Change	High-Grade Dysplastic Nodule

New Tumor Markers HCC?? 01:24:33

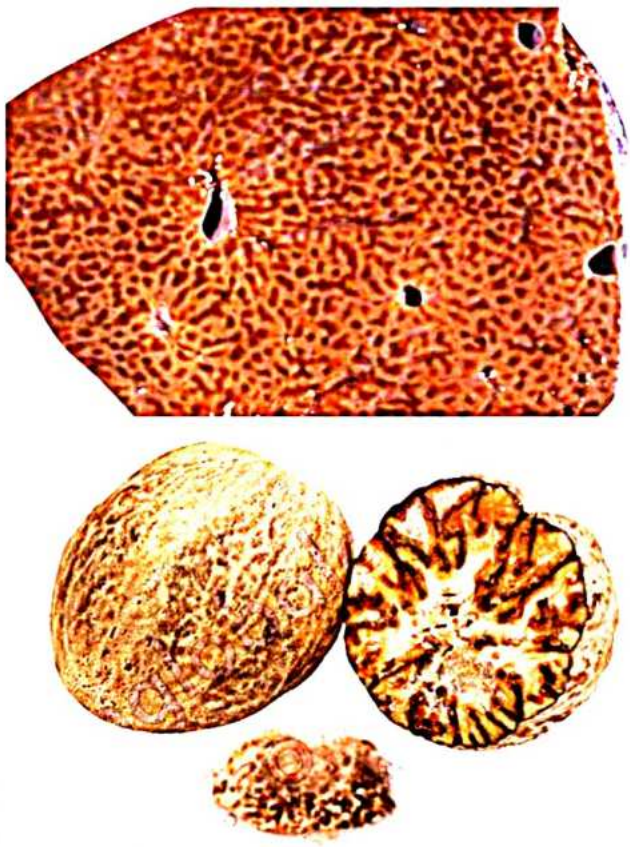
- Arginase 3
- HEP-PARI
- GLYPICAN3

Hepatoblastoma 01:25:33

- Moat common Primary malignant tumor seen in children < 3 years.
- WNT pathway activation.
- Beckwith Wiedemann syndrome (Wilms Tumor).
- Increased levels of AFP (Tumor Marker). Also seen in
  - **YST (Yolk Sac Tumor)**
  - **HCC**
- Treatment includes
  - **Surgery**
  - **Chemotherapy**
- The prognosis is better than HCC.

Miscellaneous Topics

Nutmeg Liver



- Seen due to CVC (Chronic Venous Congestion).
- Dilated and congested hepatic venules with centrilobular hemorrhagic necrosis of hepatocytes at center.
- Periphery shows fatty changes in the liver.

Q. Which part is going to show the hemorrhagic necrosis?
Ans. Centrilobular part.

Central Stellate Scar Seen In? 01:30:13

- **Liver**
  - In conditions like;
    → FNH (Focal Nodular Hyperplasia)
    → Fibrolamellar Carcinoma
- **Kidney**
  - Oncocytoma - Benign
  - Chromophobe RCC (Renal Cell Carcinoma) - Malignant
- **Pancreas**
  - Serous Cystadenocarcinoma.
- **Breast**
  - Radial scar

FNH Liver 01:30:42

- Central Stellate Scar (star shaped) is seen.
- Presented in conditions like;

Telegram - @nextpreladdernotes

- OCP
- Middle-aged female

- No malignant potential, unlike hepatic adenoma.

MCQ 01:32:31

Q. Bordeaux's classification is for?
a. FNH
b. HCC
c. Hepatic adenoma
d. Cholangiocarcinoma

Q. Which of the following variants of hepatic adenomas shows negativity for LFABP?
a. HNF 1 alpha inactivating HA
b. Inflammatory HA
c. Catenin activating HA
d. Unclassifiable HA

Q. Which of the following variants of hepatic adenomas have the maximum risk of conversation to HCC?
a. HNF 1 alpha inactivating HA
b. Inflammatory HA
c. Catenin activating HA
d. Unclassifiable HA

Q. Which of the following variants of hepatic adenomas show diffuse staining of GS?
a. HNF 1 alpha inactivating HA
b. Inflammatory HA
c. **β Catenin activating HA**
d. Unclassifiable HA

Q. What is the mutational hotspot of aflatoxin for causing HCC?
a. Codon 249 of RB gene
b. Codon 249 of the p53 gene
c. Codon 129 of p53 gene
d. Codon 129 of RB gene

Telegram - @nextpreladdernotes

Table 33.1

Subtype	Genotype	Phenotype (usual)	Immunohistochemistry	Frequency
H-HCA	HNF1A-inactivating mutations	Steatosis	LFABP negative	30-40
IHCA	IL6ST, STAT3, GNAS,FRK, Jak1-activating mutations (20% unknown)	Inflammation, sinusoidal dilation, ductular reaction	SAA/CRP Positive	50
-HCA	-catenin-activating mutations	Cellular atypia, rosettes (inconstant)	GS positivity, aberrant nuclear/cytoplasmic -catenin expression	10-15
UHCA	Unknow	Unspecific	None	10

Telegram - @nextprepladdernotes

PREVIOUS YEAR QUESTIONS

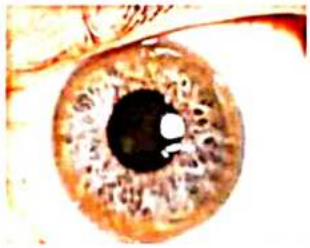
Q. Which of the following is true about nodular regenerative hyperplasia? (JIPMER Nov 2018)

A. Nodule size 0.1 to 1cm
B. Fibrosis septa present
C. Portal hypertension seen in 50% of patients
D. AST and ALT are markedly elevated

Q. α 1-antitrypsin deficiency chromosome is located at? (JIPMER - Nov - 2017)

A. 10
B. 14
C. 17
D. 11

Q. Comment on diagnosis? (FMGE Dec 2020)

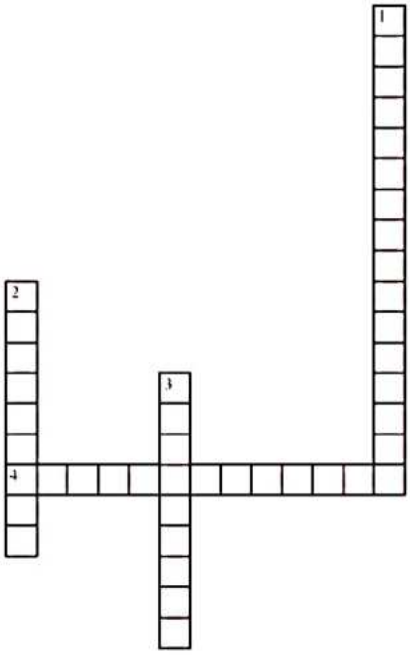


A. NF-1
B. Arcus Senilis
C. Wilson disease
D Myotonic dystrophy

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

4. Who makes collagen in the fibrous septa

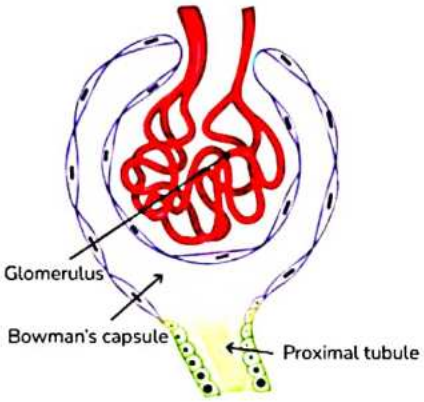
Down

1. Bile duct damage
2. Damage to the liver parenchyma
3. Fatty liver of pregnancy

Telegram - @nextpreladdernotes

34 RENAL PATHOLOGY INTRODUCTION AND SPOTTERS

Kidney- Functional Unit:



Glomerulus

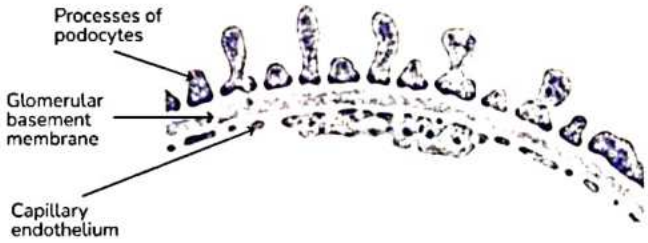
- An arteriole enters the Glomerulus, which is known as the afferent arteriole.
- It comes in and makes a tuft of capillaries.
- Some cells are present between the capillaries, known as mesangial cells, which are the macrophage and amyloid in kidney deposits into it.
- Then it goes out as an efferent arteriole.

Bowman's Capsules

- Bowman's Capsule surrounds Glomerulus
- It has 2 layers:
  - Outer layer: Parietal Epithelial Layer
  - Inner layer: Visceral Epithelial Layer
- The space created by both layers is known as Bowman's Space. It is where urine accumulates and goes to the other parts of the kidney.

Filtration Membrane

The urine formation occurs at the junction of the visceral layer of Bowman's Capsule and capillaries, which is known as the filtration membrane.



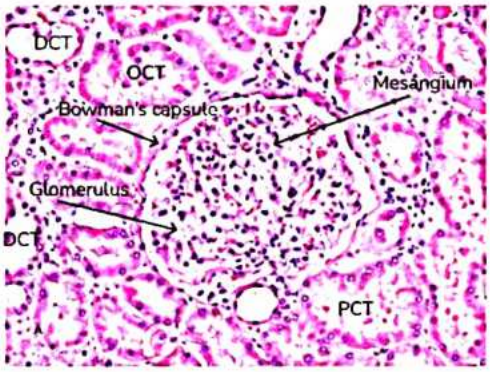
- It is a trilaminar membrane.

Refer table 34.1

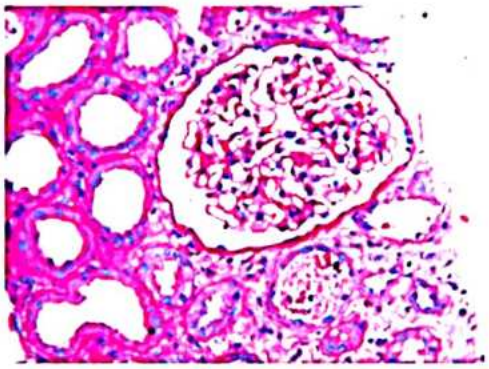
Subendothelial deposits- deposits below the endothelium
Sub epithelial deposits- deposits below the epithelium
Intramembranous deposits- deposits within the basement membrane

Stains used in renal pathology:

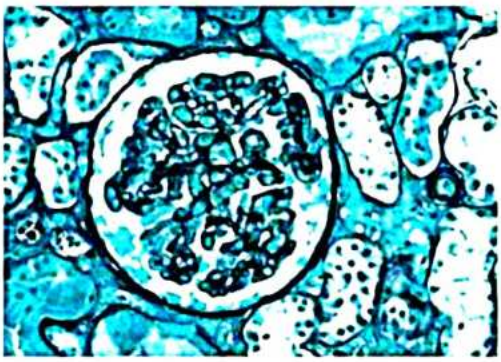
1. H (blue) & E (pink)



2. PAS (pink)



3. GMS (Gomon Methenamine Silver stain) (black)

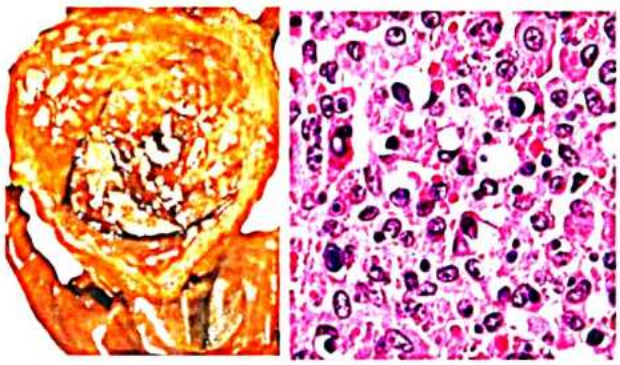


Telegram - @nextprepladdernotes

Gross appearances of the kidney 00:19:59

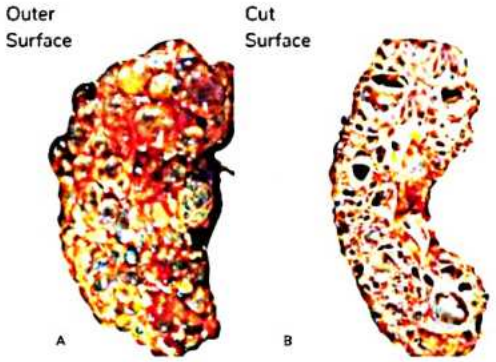
Refer table 34.2

Malakoplakia



- Phagolysosome defect present
- Yellow, white mucosal plaques present that tend to mimic cancer
- Macrophages want to eat e coli but could not because of phagolysosome defect and because of this the calcium deposits around the e coli, this calcium is known as Michaelis-Gutmann bodies.
- The entire cell that eats up the Michaelis-Gutmann bodies is known as the Von Hansemann Cell.

Polycystic kidney disease 00:38:20



In ADPKD disease:

- Cysts are shown at both outer and Inner surfaces.
- Adult Onset
- Chromosomes number: 16 and 4
- The affected Gene is PKD- 1,2. PKD 2 is less severe than PKD 1 and is seen all over the kidney, whereas PKD 1 is seen in the distal.
- The affected protein is polycystin.
- Causes: aortic dissection, > in boys, increased BP, Increased blood in urine, berry aneurysm, colonic diverticula, cysts

In ARPKD disease

- The outer surface looks normal and cysts are shown on the inner surface.
- Childhood Onset
- Chromosomes number: 6
- The affected Gene is PKHD
- The affected protein is fibrocystin.
- Congenital Hepatic Fibrosis is present on these cytes.

Medullary cystic kidney

- It shows cortico medullary cysts.
- Shrunken kidney
- 2 forms:
  - AD (in adults)
  - AR (in children) / Familial Juvenile nephronophthisis

Medullary sponge kidney 00:48:16

- It shows only medullary cysts.
- Increased risk of renal stones

Urine Examination (M/E)

Crystals & Casts

Crystals:

Refer table 34.3

Casts

Refer table 34.4

MCQ's

Q. Which of the following layers does not contribute to the filtration membrane?

a. Visceral epithelial layer
b. Fenestrated endothelial layer
c. Lamina rara interna
d. Parietal epithelial layer of bowman's capsule

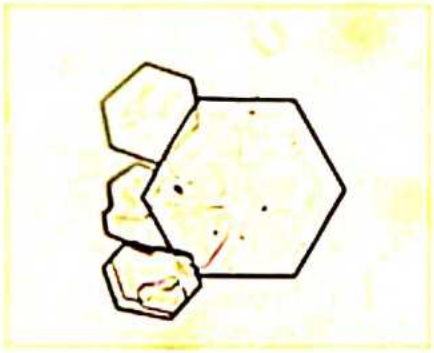
Q. Match the following appearances with their respective diseases.

a.	a. Putty kidney	1 Amyloidosis
b.	b. Flea bitten kidney	2 TB
c.	c. Leather grain kidney	3 Malignant HTN
d.	d. Asymmetrically scarred kidney	4 Benign HTM
e.	e. Waxy kidney	5 CPN

A a-2, b-3, c-4, d-1, e-5
B. a-2, b-3, c-4, d-5, e-1
C. a-3, b-2, c-1, d-5, e-4
D. a-5, b-2, c-1, d-3, e-4

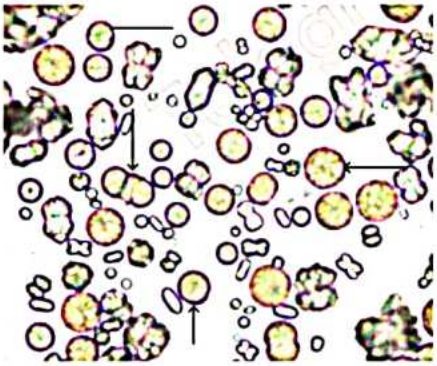
Q. Identify the crystal

Telegram - @nextpreladdernotes



a. a.Cholesterol
b. Cysteine
c. Calcium
d. Triple phosphate

Q. Identify the crystal.

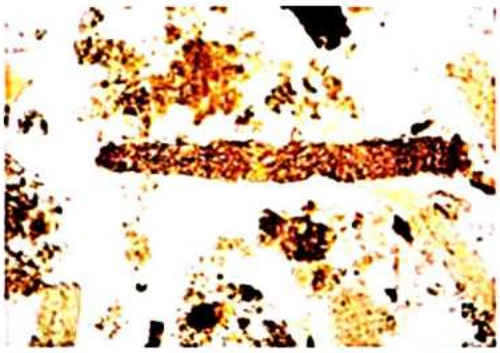


a. Calcium oxalate monohydrate, calcium oxalate dihydrate, calcium carbonate.
b. Calcium oxalate dihydrate, calcium oxalate monohydrate, calcium carbonate.
c. Calcium carbonate, calcium oxalate hydrate, calcium oxalate monohydrate.
d. Calcium carbonate, calcium oxalate monohydrate, calcium oxalate dihydrate

Q. Identify the crystal

a. Leucine
b. Tyrosine
c. Bilirubin
d. Triple phosphate

Q. Identify the cast.

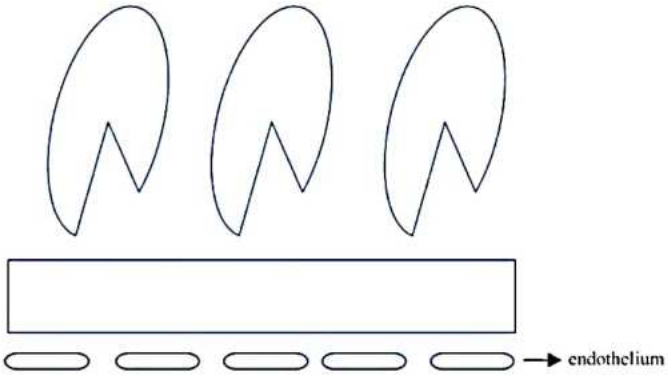


a. Hyaline cast
b. Rbc cast
c. Muddy brown cast
d. Granular cast

Telegram - @nextprepladdernotes

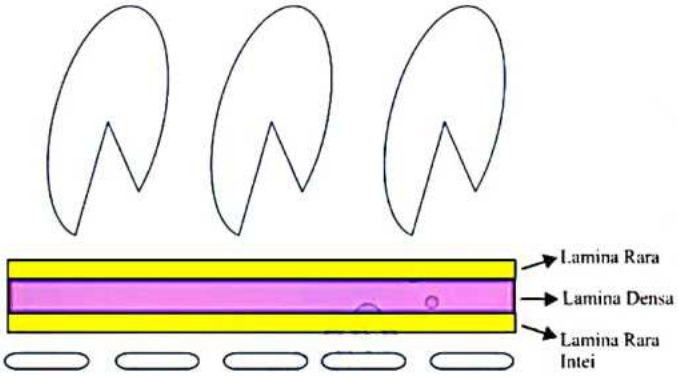
Table 34.1

Capillary endothelium



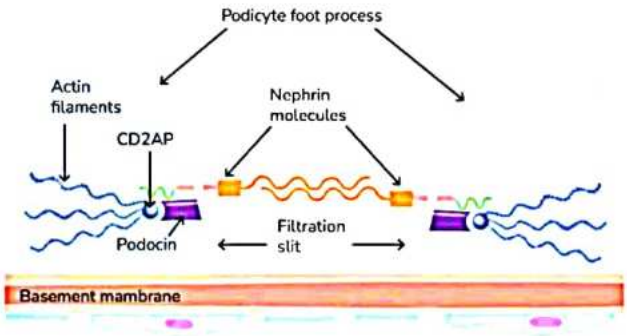
- It is not a straight line; there are gaps of 70 to 100 nm in between, known as Fenestrated capillary endothelial cells.
- Through these holes, various waste particles go out from the blood to the urine.
- These holes are tiny, so proteins and fats can not go out from these.
- 2 low molecular weight proteins, Albumin and Transferrin, can leave out these holes, but that does not happen because they are negative in charge, and so does the basement membrane. The Basement membrane repels these proteins and sends them back to the Capillary endothelium.

Glomerular basement membrane



- It is made up of collagen four.
- Various proteins, including collagen, laminin, heparan sulfate, and other glycoproteins, are present to provide a negative charge to it and bounce back Albumin and Transferrin to Capillary endothelium.
- The most common collagen in this is collagen four.
- The most common protein is laminin.
- It has 3 layers: Lamina rara externa, Lamina densa, Lamina rara interna

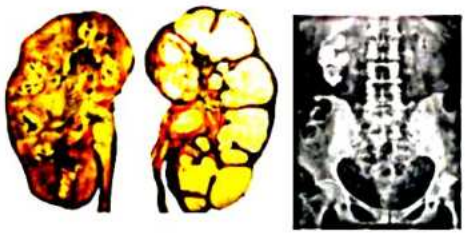
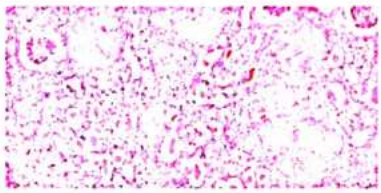
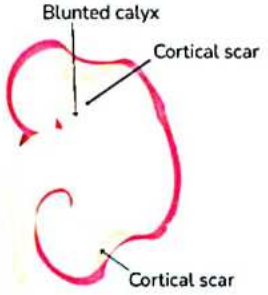
Visceral foot process/ processes of podocytes



- There are 2 podocytes, and a filtration slit separates these.
- The Nepharin molecule stays outside the podocytes, and for other proteins, podocin stays inside.
- Podocin is connected to CD2AP and further connected to actin filaments.

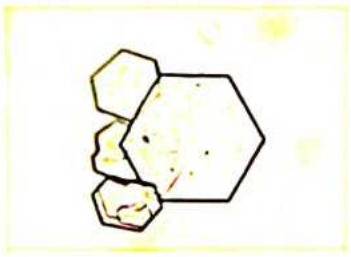
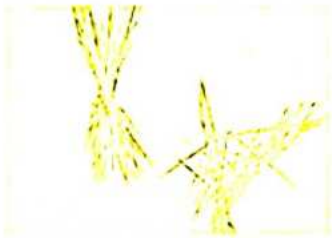
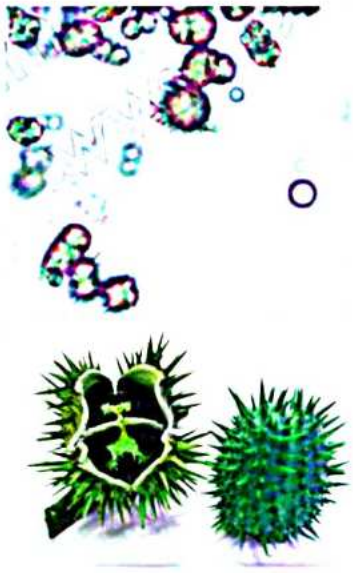
Telegram - @nextprepladdernotes

Table 34.2

Putty kidney		• Presents with Sterile pyuria
Leather grain kidney		• It is seen in Benign Hypertension (benign nephrosclerosis) • Symmetrically contracted kidney
Flea bitten kidney		• It is seen in Malignant Hypertension (Malignant Nephrosclerosis) • Symmetric contracted Kidney • Red dark dots are present on the surface of the kidney, which are known as pinpoint hemorrhages. • Caused behind this : Wegener's Granulomatosis, HSP, HUS/TTP, PSGN, PAN, SLE, SABE, Malignant (HTN) **(We Hate PSM)**
Waxy kidney		• It is seen in Amyloid • Kidney is part of GI dracula so that all GI organs on amyloidosis show organomegaly (organs become big). • One exception is there, in the late stage of renal amyloidosis the kidney shrinks.
Chronic pyelonephritis (CPN)	 	• Granular contracted (asymmetric) • Asymmetrically scarred kidney • It is a tubular disorder. • Tubules undergo atrophy and are filled with pink material known as Thyroidisation of Tubules. • These scars cause peri glomerular fibrosis around the Glomerulus.

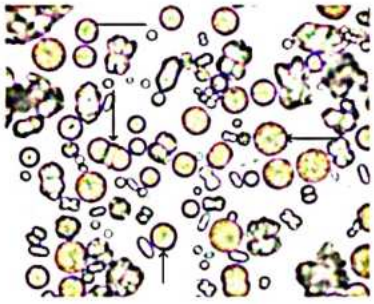
Telegram - @nextpreladdernotes

Table 34.3

Cysteine crystal		Hexagonal
Tyrosine crystal		Thin needles
Bilirubin crystal		Pigmented thin needles
Ammonium biurate crystal (Seen in laxative abuse)		Apple thorn appearance
Leucine crystal		Lamellated appearance

Telegram - @nextpreladdernotes

Calcium carbonate crystal

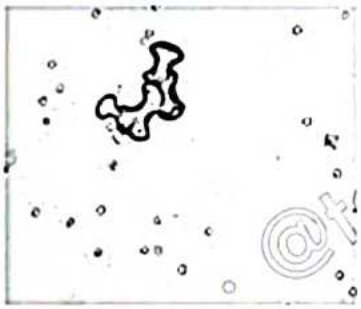


Round wheel

Calcium oxalate monohydrate crystal

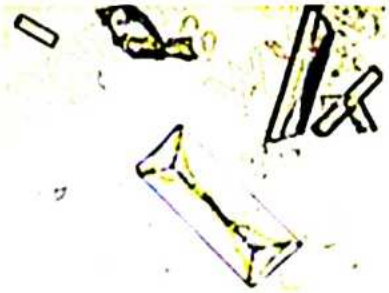
Dumb-bell

Calcium oxalate dihydrate crystal

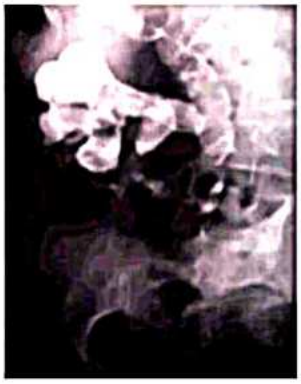


Envelope Shaped

Triple phosphate crystal/ Struvite Crystal



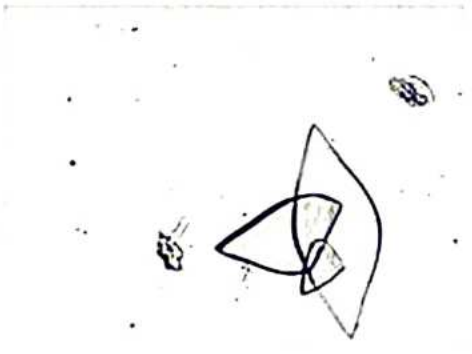
- Coffin lid appearance
- Triple phosphate crystal terminates into staghorn calculus(deer/ antler appearance)



- Associated with proteus infection
- Proteus functions in alkaline urine

Telegram - @nextpreladdernotes

Uric acid crystal



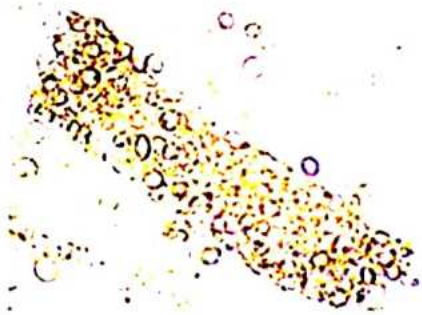
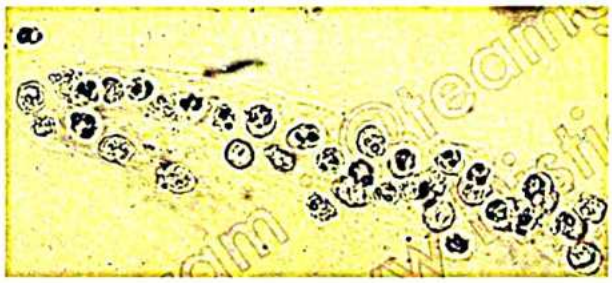
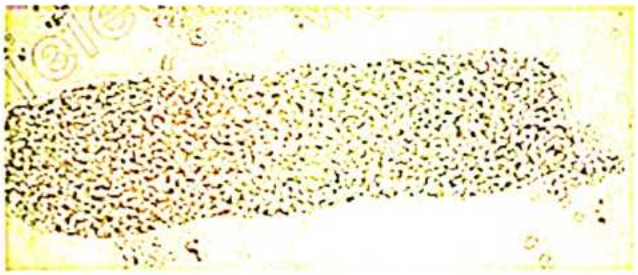
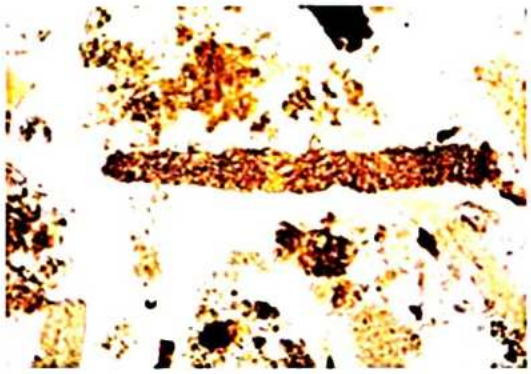
Rhombus, triangle, parallelogram, etc.

Cholesterol crystal

Broken chips

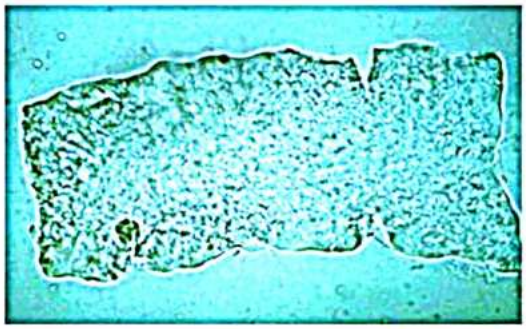
Telegram - @nextpreladdernotes

Table 34.4

Hyaline cast		• Cylindrical shape • Made up of Tamm Horsfall protein. • Tamm Horsfall protein comes from a loop of Henle. • It is seen in normal routine examination, fever, dehydration, and pregnancy.
Red cast		• Tamm Horsfall protein gets along with red blood cells. • It is seen in glomerulonephritis.
WBC cast		• Tamm Horsfall protein gets along with white blood cells. • It is seen in chronic pyelonephritis (CPN).
Granular cast		• Degenerated cast
Muddy brown cast		• Seen in Acute tubular necrosis • Eg. Toxin abuse (ethylene glycol used as antifreeze)

Telegram - @nextprepladdernotes

Broad waxy cast

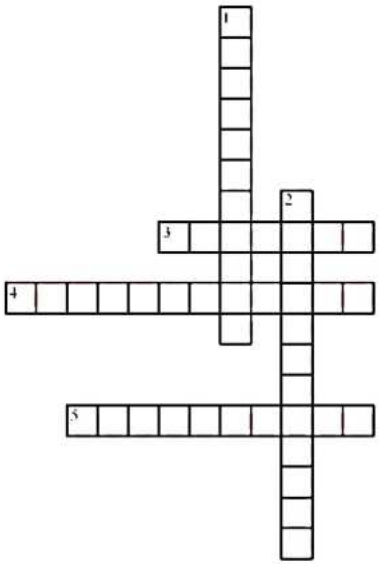


- Seen in CRF

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Tamm Horsfall protein gets along with white blood cells.
4. The outer surface looks normal and cysts are shown on the inner surface.
5. An arteriole enters the Glomerulus, which is known as the afferent arteriole.

Down

1. It is seen in normal fever, dehydration, and pregnancy.
2. Yellow, white mucosal plaques present that tend to mimic cancer

Telegram - @nextpreladdernotes

35 NEPHRITIC AND NEPHROTIC SYNDROME

Nephrotic Syndrome	Nephritic Syndrome
Massive proteinuria (>3.5gm/24-hour urine sample)	Mild proteinuria (<1gm/ 24-hour sample)
Lipiduria	H- HTN (hypertensive)
Hypoalbuminemia	O- Oliguria (Less urine output)
Edema (causes are sodium water retention and hypoalbuminemia)	H- Hematuria (blood in urine)
Thrombotic events (removal of ATIII through urine)	A- Azotemia (Increase in blood urine nitrogen levels)

Cola-color urine- hematuria (Nephritic Syndrome)
Frothy urine- protein and fat presence (Nephrotic Syndrome)

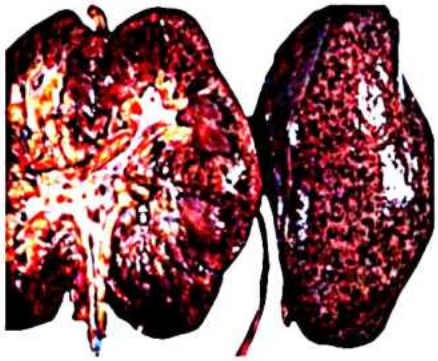
Nephritic Syndrome

00:05:25

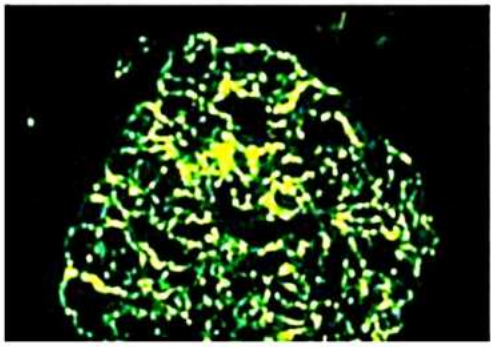
- Most common Pediatric- PSGN (Post Streptococcal Glomerulonephritis)
- Most common Adults-
  - IgA Nephropathy/ Berger's Disease
  - Most common nephritic all over the world

Post-Streptococcal Glomerulonephritis (Hypersensitivity III)

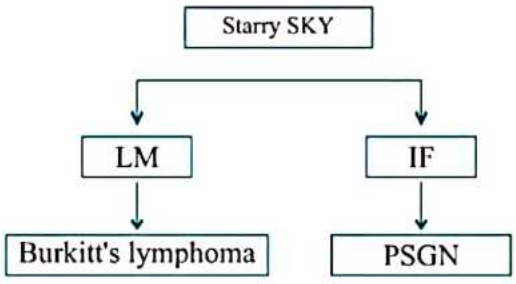
- AKA- Post Infectious Glomerulonephritis/Acute Proliferative Glomerulonephritis
- **Caused by Streptococcus Pyogenes** (Group A β Hemolytic streptococcus): Strain 12, 4, 1
- **Incubation period-** 1-4 weeks
- **Clinical Features**
  - Sore throat —10 to 14 days→ cola colour urine (hematuria)
- **Blood Test-**
  - **Antibody; Anti DNAse B (preferred)**, Antistreptolysin O (ASO)
  - Transient Hypocomplementemia blood test (Transient decrease in c3)
- **Gross Finding-** Flea Bitten appearance



- **Light Microscopy-** Increase in endothelial cells,
  - Increase in mesangial cells
  - Increase in neutrophils
  - Increase in macrophages
  - hypercellular glomeruli noted
- **Immunofluorescence:**

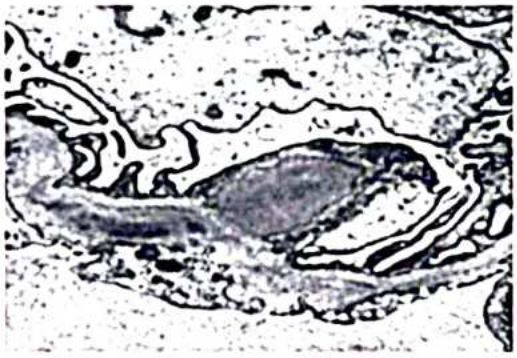


- Immunofluorescence shows starry sky appearance



Telegram - @nextpreladdernotes

- **Electron Microscopy**-Subepithelial Humps



- **Treatments**
  - Self-limiting disorder
  - Supportive treatment is required as in most cases, in 6 weeks, patients start to heal
  - In just 1% of cases, the condition can turn chronic

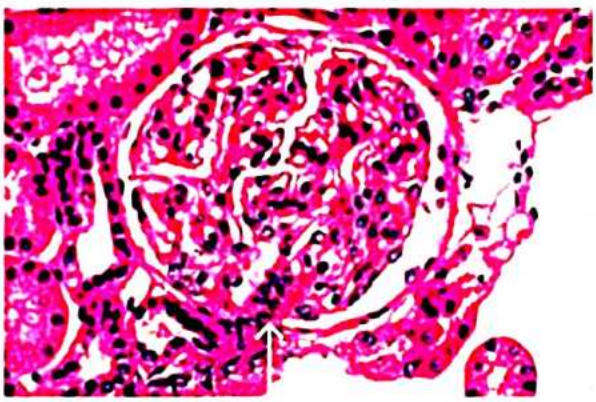
IgA Nephropathy/ Berger's Disease 00:21:41

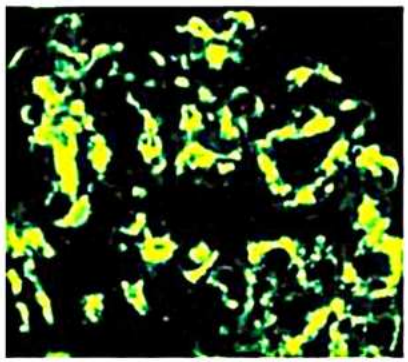
- M/C in Adults
- M/C worldwide
- Associated with infections, Ig A1 antibodies are made which are galactose deficient (an underlying genetic condition)
- Further antibodies are produced against Ig A1, and the antibody-antigen complex gets deposited in the kidney; Mesangial deposition takes place

Symptoms - Sore Throat $\xrightarrow{3 \text{ to } 4 \text{ days (adults)}}$ Recurrent Hematuria in 3-4 days

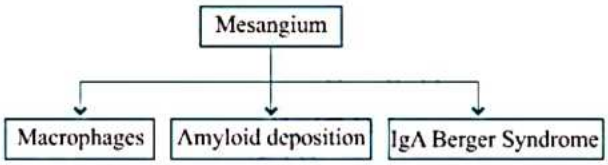
IgA Vasculitis	IgA Nephropathy
Henoch Schonlein purpura	Berger's disease
Purpuric rash in lower limbs, abdominal pains, arthralgia, renal(hematuria)	Affects Kidney

Under Microscope-





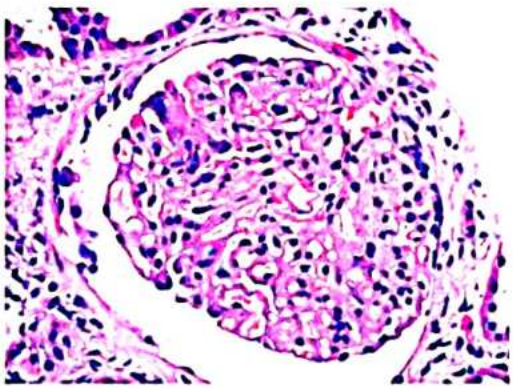
IgA1 immunocomplex getting deposited in mesangial cells



IgA nephropathy can progress into Rapidly Progressive Glomerulonephritis

Rapidly Progressive Glomerulonephritis 00:32:50

- Aka Crescentic Glomerulonephritis



Composition- Fibrin, Leukocytes, and Parietal epithelial(most common) and visceral epithelial cells(least common) (FLaP)
Prognosis: increased number of crescent- poor prognosis

Types

Refer Table 35.1

Goodpasture Syndrome/Anti-Glomerular Basement Membrane Ab Disease 00:41:49

1. Type I RGPN
2. Type II Hypersensitivity reaction

Telegram - @nextpreladdernotes

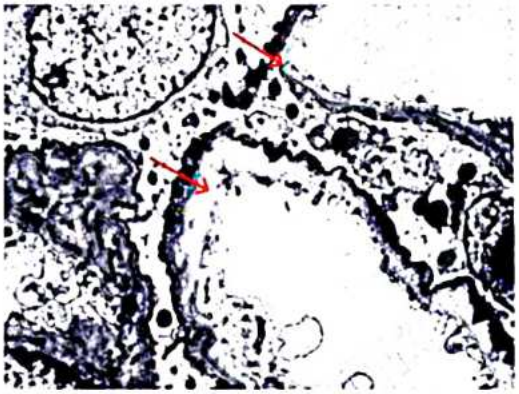
3. Ab against Alpha α3 chain of
↓
4. Type IV collagen
   - GPS can affect the lungs leading to hemoptysis (blood in sputum) or hematuria (blood in urine)
   - Most common cause of death is uremia
   - Linear Immunofluorescence

Thin Basement Membrane Disease (<200 nm thin)

- AKA- Benign Familial Hematuria 00:46:36
- Defect- α4 chain of Type IV collagen

Alport Syndrome

- Defect- α5 chain of Type V collagen; all inheritances are possible (AD,AR,XLR,XLD)
- Triad- Kidney, eyes (anterior lenticonus), and ears (Sensorineural hearing loss) get affected as well
- Electron Microscopy is gold standard for diagnosis and shows basket weave appearance of lamina densa of basement membrane



Type 4 collagen

- α3→ GPS
- α4 → BFH/thin BM
- α5→ M (alport) weave

MCQs

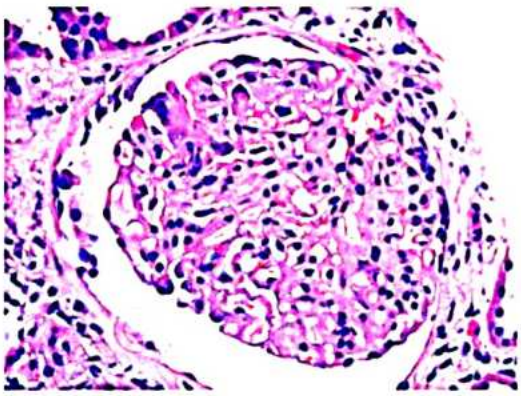
Q. Regarding the pathogenesis of crescentic glomerulonephritis, which of the following is the first and most crucial event that leads to crescent formation?

a. Podocyte injury
b. Epithelial-mesenchymal transition
c. Podocyte replacement
d. Endothelial Injury

Q. An 8-year-old boy presents with a sore throat. No medication was taken for the same, and the child seemed apparently well until two weeks later when he developed cola-colored urine. Mother rushed to the doctor for a urine examination, which revealed full of RBC and mild proteinuria. Based on the clinical history above, which hypersensitivity reactions seem to be likely?

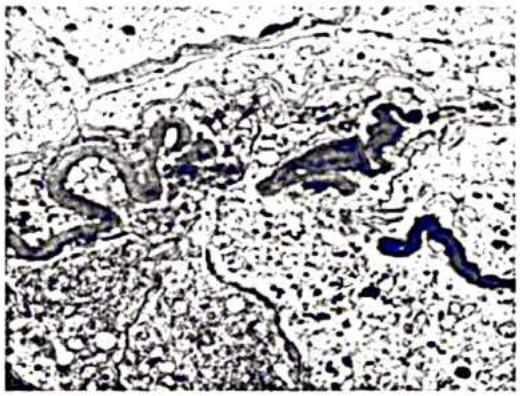
a. Type I
b. Type II
c. Type III
d. Type IV

Q. A 29-year-old male presents with haematuria and mild proteinuria. Serum creatinine levels are raised. Histopathology shows the following finding. Which of the following causes is least likely?



a. Microscopic polyangiitis
b. Goodpasture Syndrome
c. Wegener's granulomatosis
d. PAN

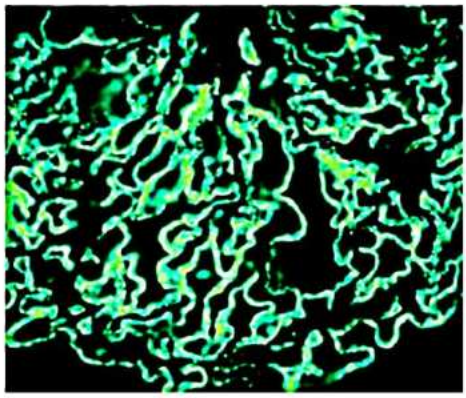
Q. 55-year-old male presents with hematuria, mild proteinuria, and hemoptysis. Serum creatinine levels are markedly raised. Electron microscopic findings are shown below. Which of the following best defines the next line of action?



a. Wait and watch
b. ASO levels
c. ANA levels
d. Anti-GBM antibodies

Telegram - @nextpreladdernotes

Q. A 55-year-old male presents with hematuria, mild proteinuria, and a single episode of hemoptysis. Serum creatinine levels are markedly raised. The patient is hypertensive. Immunofluorescence findings are shown. Which of the following is incorrect?

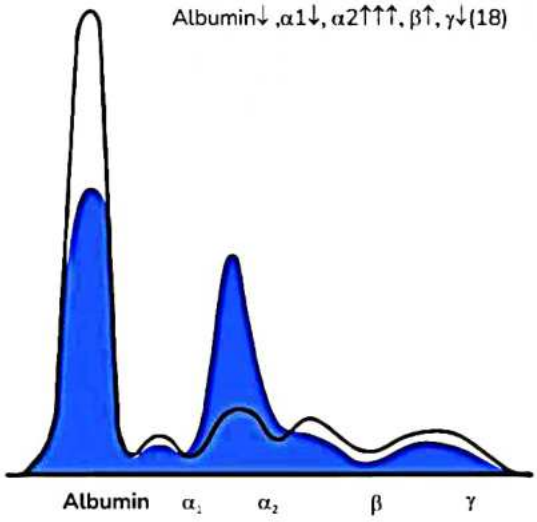


a. Type II hypersensitivity reaction
b. IF shows lumpy, bumpy deposits
c. Antibodies are seen against the alpha 3 chain of Type IV collagen
d. Crescent formation can be noted

Q. Mark the correct combination for thin basement disease.
a. Alpha 1 chain of type 4 collagen
b. Alpha 2 chain of type 4 collagen
c. Alpha 3 chain of type 4 collagen
d. Alpha 4 chain of type 4 collagen

Nephrotic Syndrome

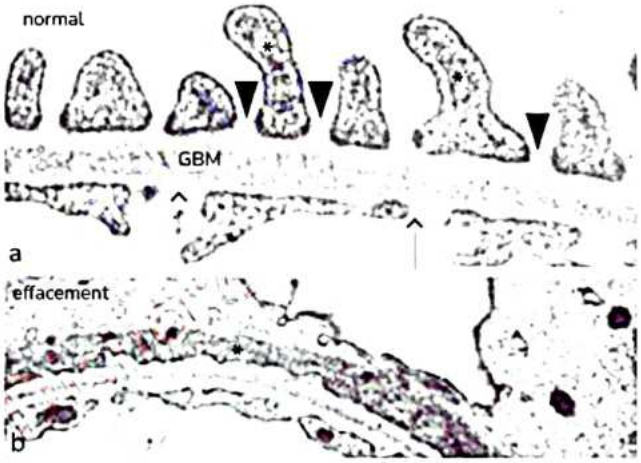
01:01:05



- Diagnosis: SPEP: Serum Protein Electrophoresis
  - In case of nephrotic syndrome, albumin levels decrease, and alpha 2 protein increases (compensatory increases)
- M/C Children- Minimal Change Disease (MCD)
- M/C Adults- Focal Segmental Glomerulosclerosis
- M/C Elderly Population- Multiple Glomerular Nephropathy

Minimal Change Disease / Lipoid Nephrosis / Nil Deposit Disease

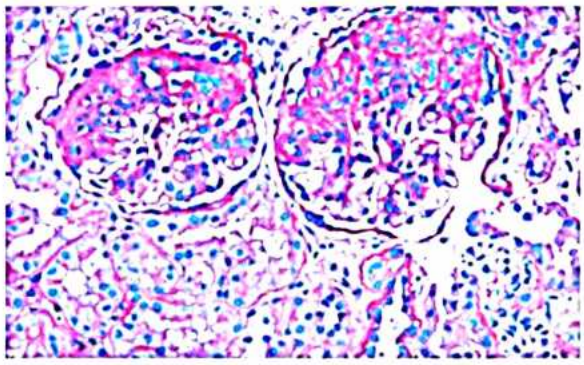
- **Age:** In children between 2-6 years
- **Cause:** Poorly understood: associated with atopy, Hodgkin's Lymphoma: Increase in mediators, increases level of lymphokines
- **Clinical Feature:** Selective proteinuria (Albumin secreted out), episodes of edema,
- **Gross:** Nothing; normal kidney observed
- **Light Microscope:** Normal glomerulus, presence of lipid in PCT (Lipid Nephrosis)
- **IF:** Nil Deposit
- **E/M:** gold standard, Effacement of foot processes



- **Treatment**
  - Excellent 100% response is shown when treated with steroids

Focal Segmental Glomerulosclerosis (HIVAN: HIV Associated Nephropathy

Definition: The definition can be divided as Focal (When Few Glomeruli are affected) and Segmental (When only a part of the glomeruli segment being affected), it is known as Focal Segmental Glomerulosclerosis



Telegram - @nextpreladdernotes

Types

- Primary type: Idiopathic
- Mnemonic- H3R2S1
  - HIV positive
  - Hypertension
  - Heroin drug abuse
  - Reflux nephropathy
  - Renal tissue loss
  - Sickle Cell Anemia

Classification

Columbian Classification

1. FSGS NOS (Not Otherwise Specified) (M/C)
2. FSGs, Glomerular Tip (Best Prognosis)
3. FSGS, Glomerular Collapsing Type (Worst Prognosis) HiVAN (HIV-associated nephropathy)
4. Perihilar Type
5. Cellular Type

Congenital Nephrotic Syndrome 01:20:38

- NPHS-1 Gene→ Nephrin protein→ congenital NS of FINNISH type
- NPHS-2 Gene→ Podocin protein → AR FSGS
- Alpha Actinin 4 Gene → Actin protein→ AD FSGS
- CD2AP → No disease caused
- TRPC6 Gene→ Adult Onset FSGS
- APOL1 Polymorphism→ associated with HIVAN

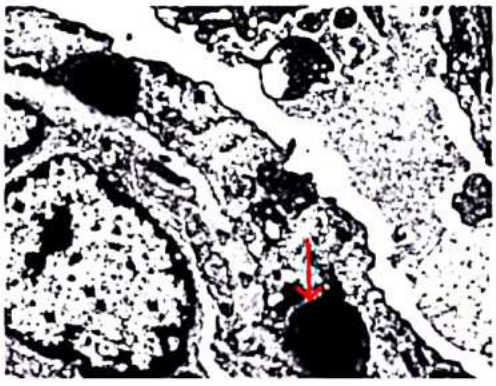
Membranous Glomerularnephropathy (Thickened Basement Membrane)

Causes

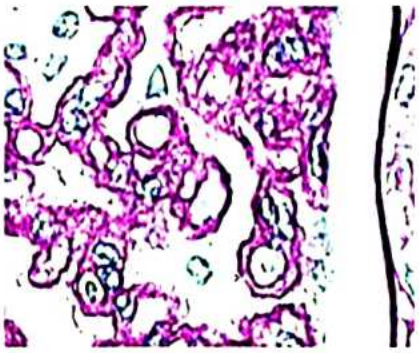
- Primary/ Idiopathic
  - Antibodies against phospholipase A2 receptor(PLA2Rc)
  - Thrombospondin THSD7A- associated with MGN
  - CD10 (neural endopeptidase)
- Secondary
  - Drugs (Gold, NSAIDS, Penicillamine)
  - Infections (HBV, HCV, Syphilis, Schistosoma, Malaria, Leprosy)
  - Cancers (Colon, lung, melanoma)
  - Systemic Diseases (Diabetes Mellitus, SCE)

In humans, MGN - Ab against PLA2Rc

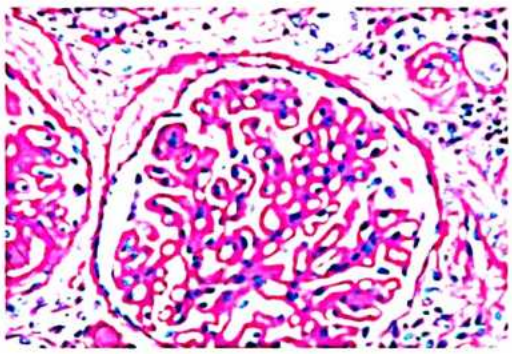
In rats & mice, Heymann Nephritis- Ab against megalin Ag



- Subepithelial Deposits
- Formation of Spikes; Spike and Dome Pattern

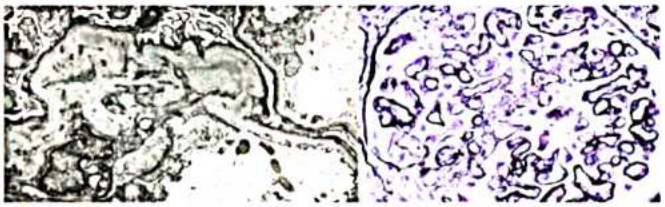


- Upon using Glomerimethiminine silver stain, the basement membrane stains black

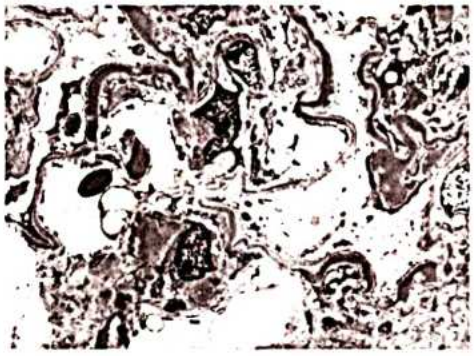


MPGN (MembranoProliferative Glomerulonephritis)/ (Mesangiocapillary Glomerulonephritis)

- MPGN I



  - Primary- Idiopathic
  - Secondary- AATD(alpha1 antitrypsin deficiency), Hep B, Hep C, HIV, Schistosoma, Cryoglobulinemia, CLL
- MPGN II

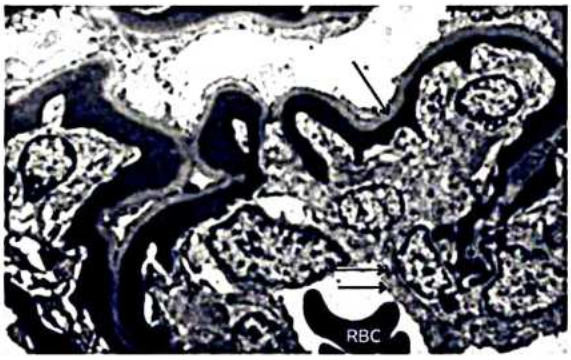


Telegram - @nextpreladdernotes

- C3 nephritic factor (C3NeF) that binds the alternative pathway C3 convertase
  This favors persistent C3 activation and hypocomplementemia

Image

- Tram Tracking (splitting of basement membrane)seen in MPGN I
- E/— DDD Dense Deposit Disease: Intramembranous disease seen in MPGN 2

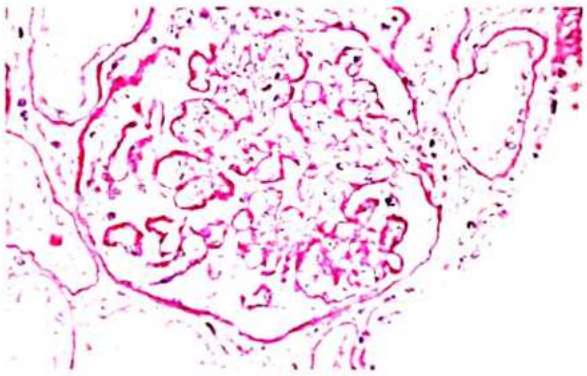


SLE- Kidney Lupus Nephritis

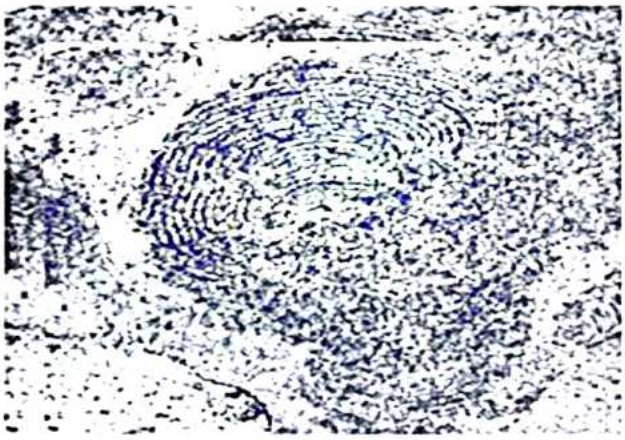
01:48:25

Classification

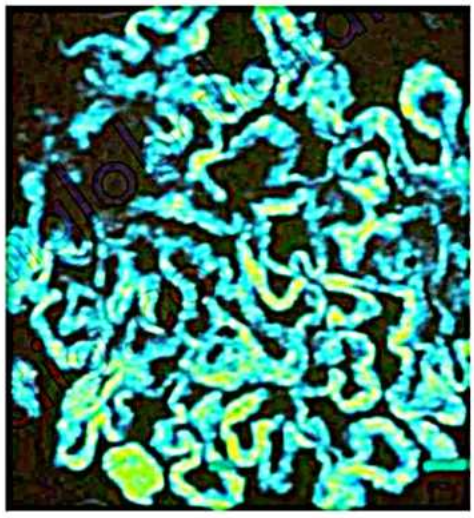
Class	Abbreviated ISN/ RPS classification of LN
Class I	Minimal Mesangial LN
Class II	Mesangial proliferative LN
Class III	Focal LN (<50% glomeruli)
Class IV	Diffuse LN (>50% glomeruli)
Class V	Membranous LN
Class VI	Advanced Sclerosing LN



- SLE- Wire Loop Lesions observed in Class III, IV, V (Subendothelial deposition Maximum wire loop lesions are seen in class 4)
- E/—Thumb Print



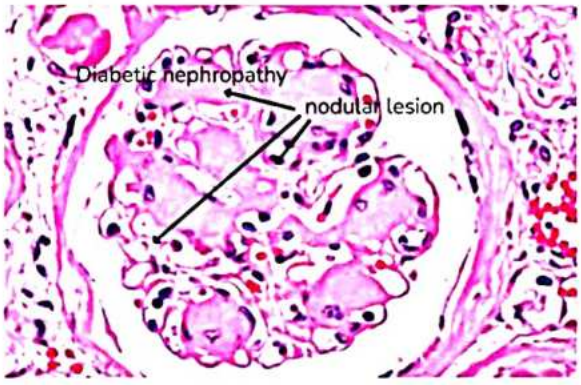
- SLE- Full House Effect (IF)



Diabetic Nephropathy Mnemonic "THE DN"

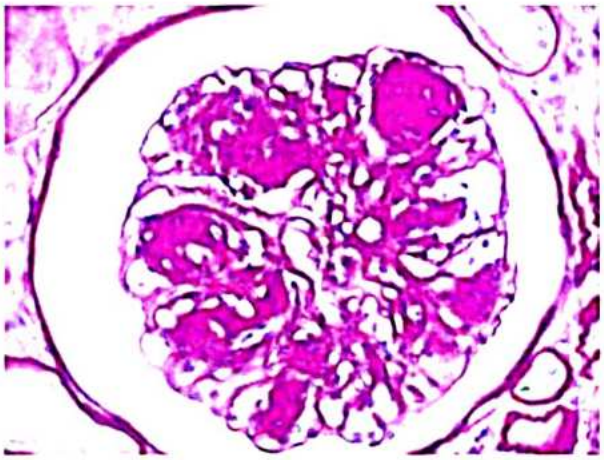
- Thickening of Capillary Basement Membrane
- Hyaline arterioSclerosis (bening hypertension, diabetes mellitus)
- Armani abstain lesion (glycogen deposition in PCT)
- Diffuse glomerulosclerosis, Fibrin Cap, Capsular Drop
- Nodular glomerulosclerosis (KW lesion), Papillary Necrosis

Kimmelstein Wilson Lesion



Telegram - @nextpreladdernotes

- Non-enzymatic glycosylated end products
- Most characteristic lesion
- PAS staining

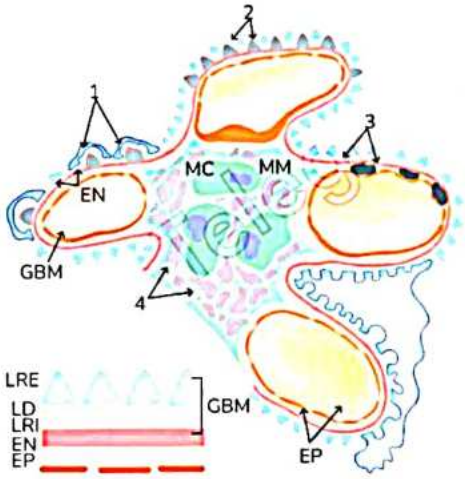


Renal Papillary Necrosis 02:01:05

Mnemonic PAPER DOSA

- Diabetes Mellitus
- Obstructive pyelonephritis
- Sickle Cell Anemia
- Analgesic Overdose

Summary



- Subepithelial Deposition- PSGN, MGN
- Mesangium- Berger's Disease
- Intramembranous Deposit- DDD (MPGN II)
- Subendothelial Deposits- MPGN I, Wire Loop/ SLE
- Hep C virus associated with cryoglobulinemia >> MGN

MCQs

Q. HIVAN is associated with?

a. RPGN
b. Goodpasture syndrome
c. Minimal Change Disease
d. FSGS

Q. HIVAN is associated with which type of FSGS

a. Glomerular Tip
b. Collapsing FSGS
c. Perihilar FSGS
d. Cellular FSGS

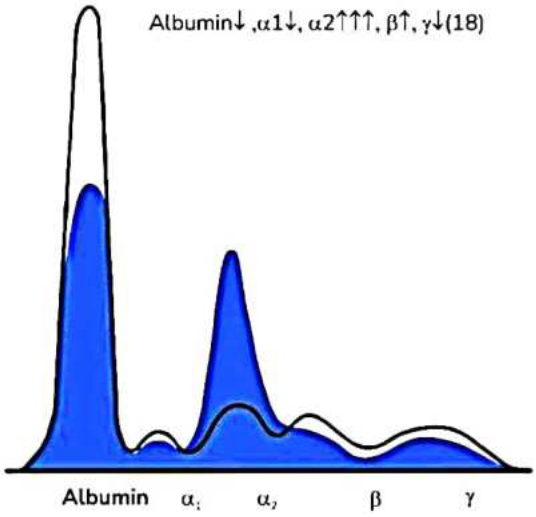
Q. Which of the following signs and symptoms is common in minimal change disease?

a. Azotemia
b. Hypertension
c. Microproteinuria
d. Selective Proteinuria

Q. If present on kidney biopsy, which variant of focal segmental glomerulosclerosis has a significantly poorer prognosis and supersedes the presence of others (of other variants present in the biopsy) in the FSGS classification?

a. Cellular variant
b. Collapsing variant
c. Hilar variant
d. Tip variant

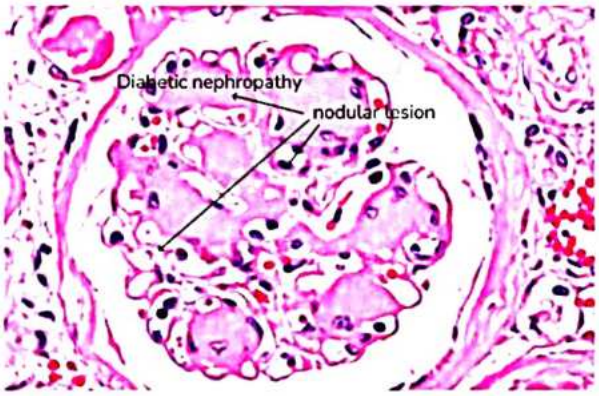
Q. A 6-year male child presents with frothy urine and edema. The urine examination is attached herewith. Appearance- frothy, RBC nil, WBC nil, Epithelial Cells nil, Casts 0-1 hyaline cast, crystals nil, Protein 3+, bacterial nil, Serum protein electrophoresis pattern is shown below. Histopathological examination reveals no obvious abnormality. Which of the following causes is most likely?



a. RPGN
b. Goodpasture syndrome
c. Minimal Change Disease
d. FSGS

Telegram - @nextpreladdernotes

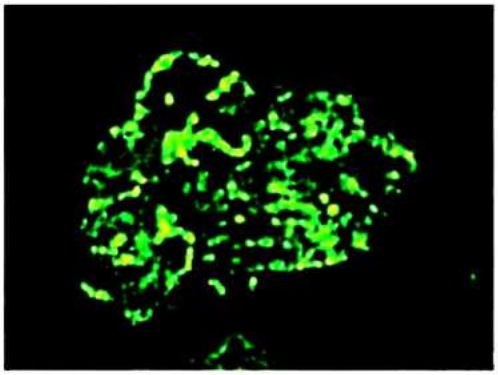
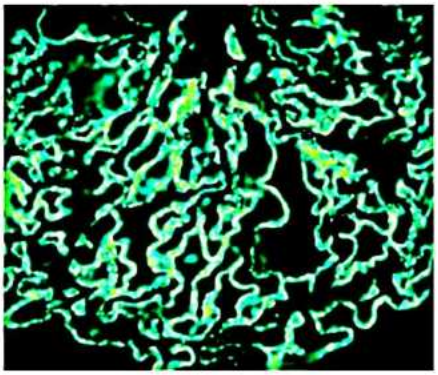
Q. A 55-year-old comes for a health checkup at a pathology laboratory. Her CBC findings are Hb 12gm/ dl, TLC 10000/mm3, DLC N60/L30/M6/E4, and Platelet count 170000. Her HbA1c is 11%. Urine examination shows protein 2+, glucose 3+, case nil, and crustal nil. Renal biopsy shows the following finding. Which is the special stain for the same?



a. VVG
b. blue
c. PAS
d. ZN stain

Telegram - @nextpreladdernotes

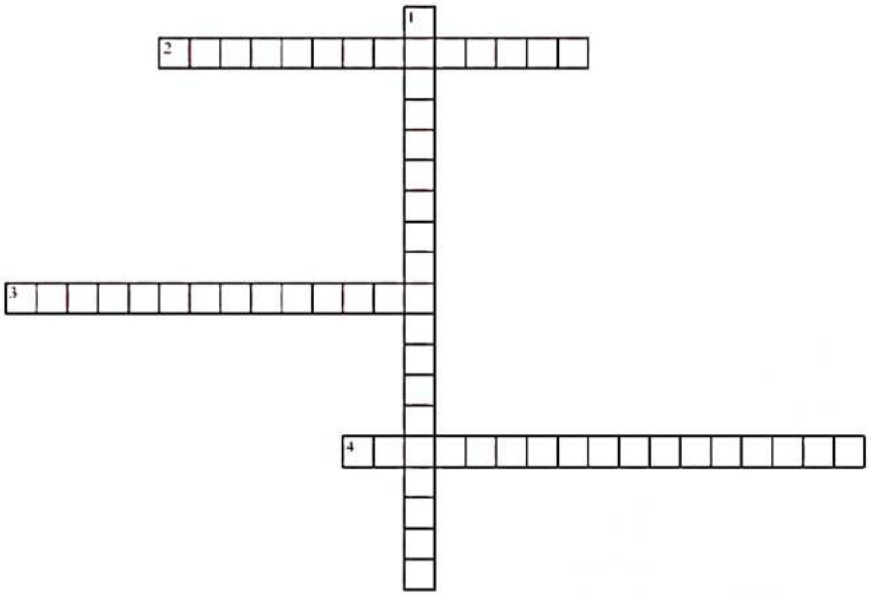
Table 35.1

Feature	RGPN I	RGPN II	RGPN III (Most common)
Includes	Goodpasture Syndrome (Anti Glomerular Basement Membrane Disease)	PSGN, IgA, SLE, HSP (antigen-antibody complex)	Microscopic polyangiitis, Wegener's Granulomatosis (Pauci Immune Vasculitis)
IF	Linear (image) Ribbon Pattern	Lumpy/ Bumpy/ Granular/ Starry Sky (Image) Garland Pattern	Nil
			
EM	Common: R R R (**R**GPN causing **R**upture and w**R**inkling of basement membrane)		

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. Clinical Feature: Selective proteinuria (Albumin secreted out), episodes of edema,
3. Defect- α5 chain of Type V collagen; all inheritances are possible
4. Diagnosis: SPEP: Serum Protein Electrophoresis

Down

1. Nodular glomerulosclerosis (KW lesion), Papillary Necrosis

Telegram - @nextprepladdernotes

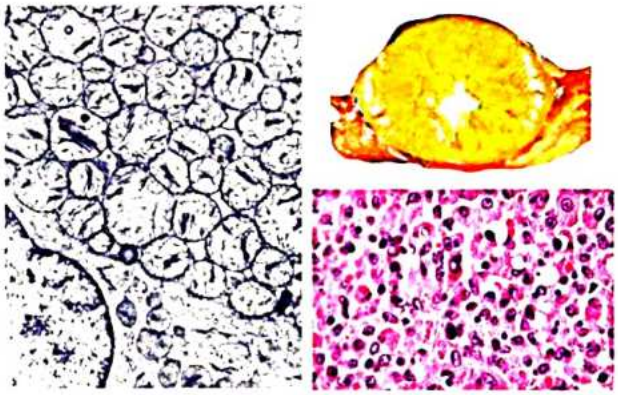
36 RENAL TUMORS

Classification

00:00:25

- **Adults**
  - Benign
    - → Renal papillary adenoma: Carries risk of RCC
    - → Oncocytoma
    - → Angiomyolipoma
  - Malignant
    - → Renal Cell carcinoma (RCC)
- **Children**
  - Wilms tumor

Renal Oncocytoma



- Increase in mitochondria
- Origin: collecting duct of the kidney
- Mahogany brown colour
- Centre part- Central stellate scar
- In H&E stain: Diffusely pink cells are observed
- The electron microscope shows a lot of Mitochondria structure.

Central Stellate scar	
Parts	Tumours
Kidney	Oncocytoma, Chromophobe RCC
Liver	FNH, Fibrolamellar, Carcinoma Liver
Pancreas	Serous Crytsdenocarcinoma Pancrease
Breast	Radial Scar

Mahogany Brown:

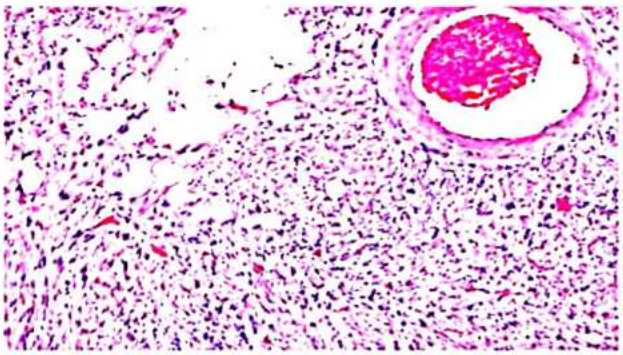
- Lugol's I2 on Amyloidosis specimen
- Oncocytoma

An abundance of mitochondria:

- Hepatocytes-liver cells
- Hurthle cell (Thyroid)
- Oncocytoma

Angiomyolipoma (AML)

00:06:48



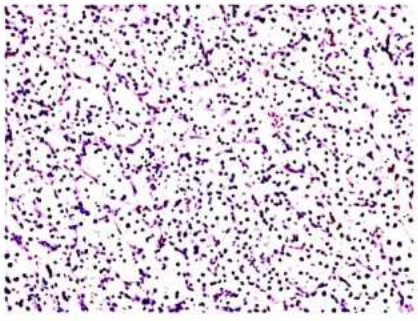
- It has circular blood vessels, muscles, and fat.
- PEComa (Perivascular Epithelloid Cell) tumor
  - Lung tumor- Sugar tumor
  - Kidney- AML
- It is positive for the HMB45 marker

Renal Cell Carcinoma

- Also known as Grawitz Tumor/ Hypernephroma
- Adult patient
- **Risk factor:**
  - Sporadic: Tobacco (most common)
  - Genetic
- **Clinical features:**
  - Abdominal mass
  - Flank pain
  - Hematuria (blood)

Types of RCC

1. **Clear Cell RCC**
   - Most common



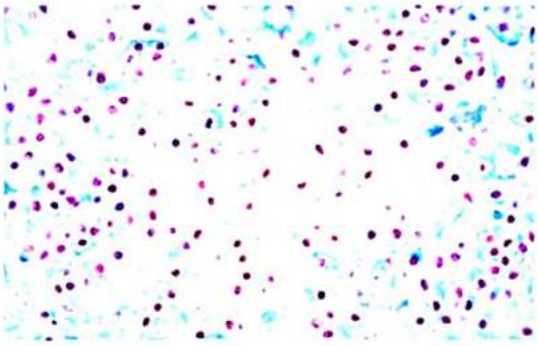
Telegram - @nextprepladdernotes

- Deletion of chromosome 3p - leads to VHL (Von Hippel Lindau) syndrome.
- VHL can have Bilateral clear RCC- both kidneys are affected.
- M/E: Clear cells - fat & glycogen

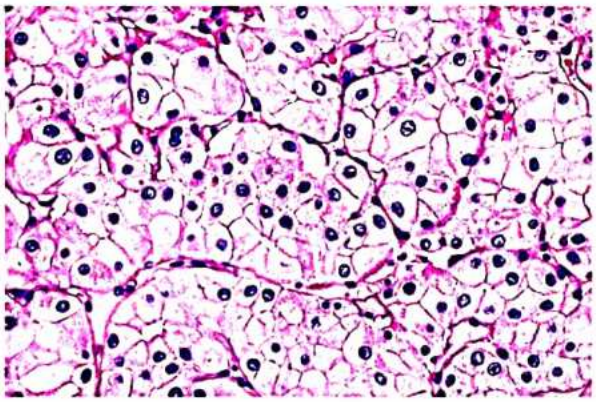
Stain:

- Fat OILRED O positive
- Glycogen- PAS positive
- A grossly golden yellow (fat) tumor is visualized.

2. Chromophobe RCC



- Genetics:
  - → Multiple chromosomal losses (loss of 1,5,7)
  - → Birt-Hogg-Dube syndrome (RCC+ Fibrofoliiculoma +)
- Microscopically, its shows plant cells

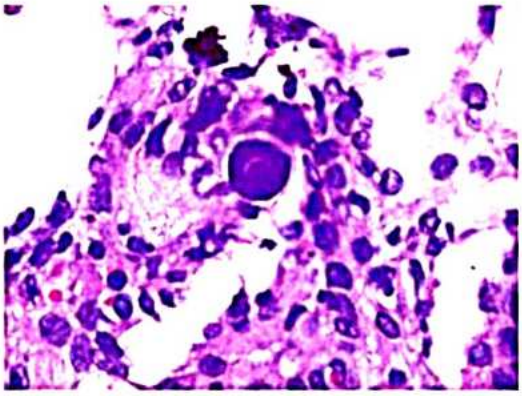


- → Well-defined outlines
- → Dark irregular nucleus- Raisanoid nucleus
- → Pheriperies have cytoplasm
- → Around the nucleus- Perinuclear Halo

Special Stain:

- Hale's Colloidal Iron positivity
- Best Prognosis

3. Papillary RCC



- Shows Psammoma bodies
- Associated with
  - → Dialysis/hemodialysis patient
  - → Trisomy 7- Extra C-MET gene (hereditary)
  - → Trisomy 17
  - → Loss of Y
  - → HPRCC (Hereditary Papillary RCC) -Trisomomy 7
  - → HLRCC (Hereditary Leiomyomatosis Papillary RCC)
    - Lots of Leiomyomatosis
    - Fumarate Hydratase Mutation
- Renal Papillary adenoma causes papillary RCC.
- One of the most important predictors is size:
  - → Size<1.5cm ⇒ Adenoma
  - → Size >=1.5cm ⇒ PRCC

4. Bellini/collecting duct RCC

- Worst prognosis
- Microscopically, it shows Hobnail cell

5. Medullary RCC

- Associated with Sickle Cell trait patients (HbAS) & not with sickle cell anemia (HbSS)

6. Pediatric RCC:

Associated with:

- Translocation of Xp
- TFE3 gene
- Microscopically, shows a combination of clear and papillary cells.

Spread of RCC

- Spreads via Hematogenous (blood)
- Examining the Renal vein is important.
- All carcinoma spread via lymphatic spread except; HCC & RCC

Telegram - @nextpreladdernotes

Paraneoplastic Syndrome 00:32:00

- RCC shows:
  - Hypercalcemia
  - Endocrine problem- Cushing's disease
  - Polycythemia
  - Hepatic Dysfunction- Stauffer syndrome (in the liver due to RCC in the kidney)

Microscopic Grading of Renal Cell Carcinoma

- Fuhrman Nuclear Grade

Wilm's Tumor 00:34:18

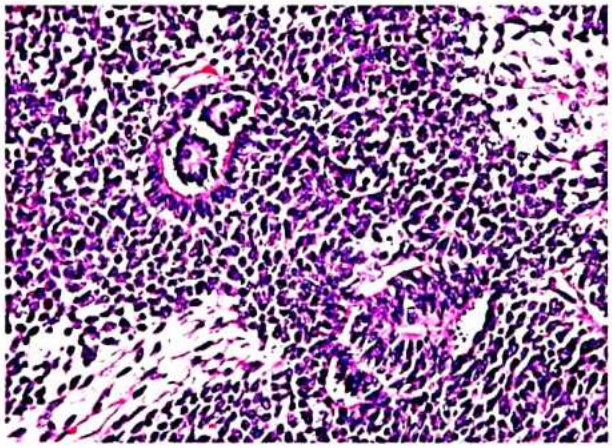
- Also known as Nephro blastoma/ Tripahsic tumor
  - Epithelial component (Abortive glomeruli/tubules)
  - Mesenchymal (spindle cell)
  - Blastema (blue color)

- **Clinical Feature:**
  - Abdominal mass
  - Hematuria
  - Fever

- WT1 gene in chr. 11p13 and WT2 gene in chr. 11p15, respectively.
- WT1 defect:
  - WAGR syndrome:
    → Wilms tumor
    → Aniridia (no iris)
    → Genital abnormalities
    → Mental Retardation
  - Denys Drash Syndrome:
    → Diffuse mesangial sclerosis
    → Dysgenesis of the gonads

- WT2 defects- Beckwith Weidemann syndrome:
  - Both maternal & paternal Insulin growth factors are active, leading to way too much proliferation of cells
  - Loss of maternal imprinting
  - This leads to wilms tumor, adrenal cortical tumor, and hepatoblastoma
  - Hemihypertrophy
  - Macroglossia (tongue)

- Grossly it has a fleshy appearance.
- m/e E- Epithelial component, abortive glomeruli if tubules present
  —Mesenchymal
  B- Blastoma



- Synovial Sarcoma is a biphasic tumor
- Wilms tumor + Anaplasia
  - p53 mutation
  - Resistance to chemotherapy

- Wilms tumor + nephrogenic rests
  - Embryological remnants
  - Risk of Contralateral Wilms tumor

MCQ

Q. A 40-year-old man presented with painless hematuria. Bimanual examination revealed a ballotable mass over the right flank. Subsequently, a right nephrectomy was done, and the mass was seen to be composed of cells with clear cytoplasm. Areas of hemorrhage and necrosis were frequent. Cytogenetic abnormalities of this mass are likely to reveal the normality of?

a. Chr 1
b. Chr 3
c. Chr 17
d. Chr x

Q. Which chromosomal abnormality is most often associated with clear cell renal cell carcinoma?

a. Deletion of Y and trisomy 7 and 17
b. Germline mutation of C-MET
c. Loss of one copy of chromosomes 1,2,6, 10, 13, and 17
d. Loss of the short arm of chromosome 3

Q. Which of the following RCCs is associated with Birt Hogg Dube syndrome?

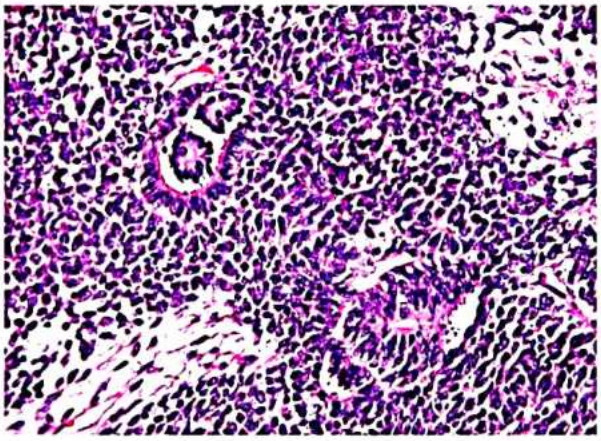
a. Papillary RCC
b. Clear cell RCC
c. Chromophobe RCC
d. Medullary RCC

Telegram - @nextpreladdernotes

Q. Which of the following Histologic features is most important for risk stratification in nephroblastoma (Wilms tumor)?

a. Anaplasia
b. Botryoid growth
c. Fetal rhabdomyomatous pattern
d. Teratoid elements

Q. 4-year-old boy with fever, 3 episodes of hematuria and abdominal mass. The histopathology reveals the following findings as shown. On further examination, the child also has Macroglossia. The gene for this disorder is on the chromosome?



a. 11p13
b. 11p15
c. 11q13
d. 11q15

Q. Which of the following organelle is seen in abundance in the following renal tumor?

a. Lysosome
b. Ribosomes
c. Mitochondria
d. Endoplasmic Reticulum

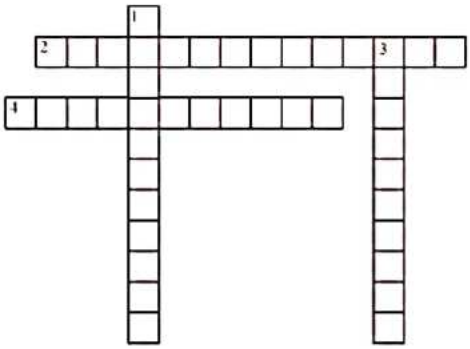
Q. Find the incorrect match with regard to the renal tumors and their chromosomal translocations.

a. Clear Cell RCC	VHL syndrome
b. Papillary RCC	Loss of Y
c. Chromophobe RCC	**HLRCC**
d. Pediatric RCC	TFE3 Gene

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. It has circular blood vessels, muscles, and fat.
4. Birt-Hogg-Dube syndrome (RCC+ Fibrofoliiculoma)

Down

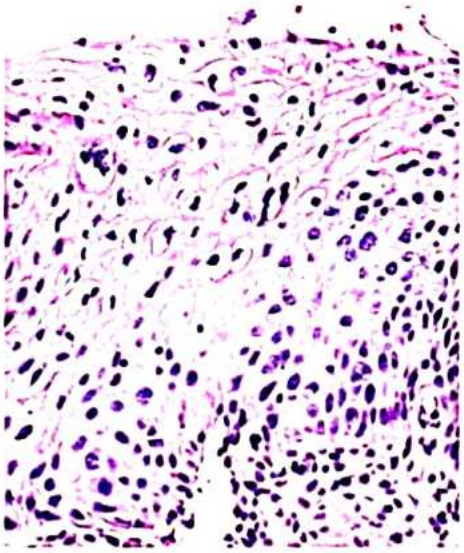
1. Also known as Nephro blastoma/ Tripahsic tumor
3. In H&E strain: Diffuserly pink cells are observed

Telegram - @nextpreladdernotes

37 PATHOLOGY OF FEMALE GENITAL TRACT

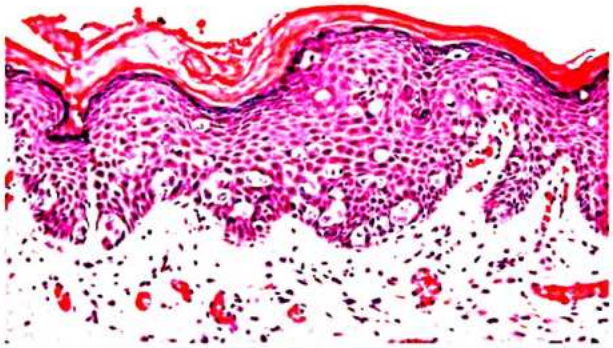
1. Vulva
2. Vagina
3. Cervix

Vulval Tumor



- Condyloma acuminatum/warts → Low risk HPV 6,11
- VIN (Vulval Intraepithelial neoplasia)/ Bowen's disease (pre-cancer condition) High-risk HPV 16, 18
- Vulval carcinoma (cancer) High-risk HPV 16, 18
- All of them are associated with human papillomavirus (HPV) because the lining of vulva is squamous epithelium and HVM go and target this squamous epithelium
- HPV shows koilocytes, a cell that has a dark blue nucleus called Raisinoid nuclei around this nucleus, white area is present which is called perinuclear halo.
- Koilocytes are seen in both high risk and low risk HPV.

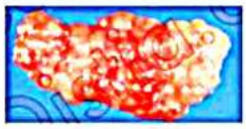
Extramammary Paget's Disease

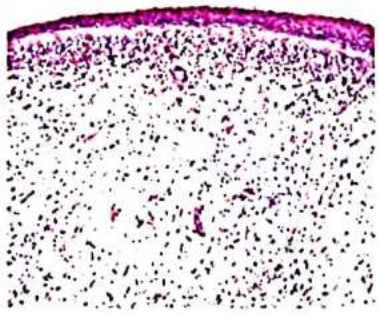


- Two layers are there, one is dermis and the other one is epidermis.
- Someclear cells are present on this epidermis layer, and these cells are referred to as Paget's cells.
- Underlying Tumor (vulval tumor) from where tumor cells reaches skin.
- Skin of the patient presents with red itchy or pruritic lesions.
- Paget's cells are positive for PAS, MUCICARMINE, and Ck 7.

Vaginal Tumor

- **Squamous cell carcinoma**: It is associated with HPV.
- **Clear cell Adenocarcinoma**: It is associated with DES ((Diethylstilbestrol). Example: DES drug was given to the mother but the cancer occurred in her daughter.
- **Sarcoma botryoides/Embryonal Rhabdomyosarcoma**: It is malignant and it is a skeletal muscle tumor that occurs in children. This tumor looks like a bunch of grapes that is why it is known as botryoides.





A Cambium layer is present in it that contains a lot of cells & below this layer very few cells are present and this area is called hypo cellular.

Rhabdomyosarcoma: Deals with skeletal muscles

- Special stain: PTAH (PhosphoTungstic Acid Hematoxylin)
- IHC: Desmin, Mydo1, Myogenin.

Cervical Cancer- Risk Factors

- HPV Infection
- Smoking
- Immunodeficiency
- OCPs (oral contraceptive pills)

Before cervical cancer occurs, CIN (cervical intraepithelial neoplasia) is there and it is in the pre-cancer stage. CIN is of 3 types: I, II and III.

Refer Table 37.1

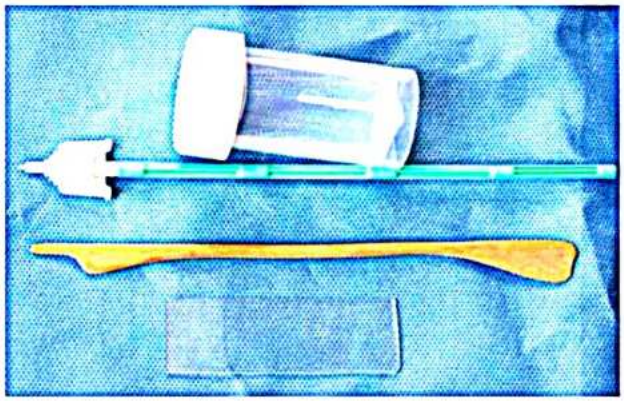
Telegram - @nextpreladdernotes

- CIN I comes in the category of LSIL i.e., Low Grade Squamous Intraepithelial lesion.
- CIN II, CIN III, and CIS comes in the category of HSIL i.e., High Grade Squamous Intraepithelial lesion.

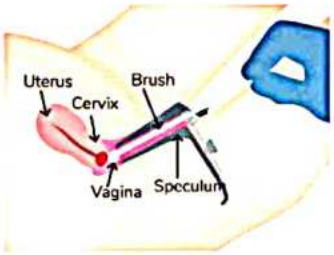
Screening For Cervical Cancer-pap Smear 00:17:17

- Pap is named after the scientist, George Papinacacou.
- Pap Smear includes 2 techniques:

1. Conventional techniques:



- In this Ayre's spatula is used. With the help of a speculum, the area is being exposed and a sample is taken from that.



Cervix is composed of ectocervix and endocervix:

Ectocervix:

- Made up of squamous epithelium
- There are some cells which are round at bottom known as parabasal cells
- Some cells present above these cells are known as intermediate cells
- Above intermediate cells, some cells are present that are called superficial cells.

Endocervix

- Made up of columnar or glandular epithelium and has tall columnar cells.

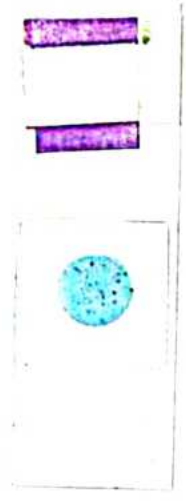
After taking a sample, it is being painted on a slide

2. Liquid Based Cytology (LBC)

- It cut out all the debris i.e., all the blood, mucus, waste and dirty materials.
- It was saving the time of pathologists because in this the slide is just a small circle that is not painted randomly.
- A brush is used to take the sample→ it is being put in a liquid container→ contains alcohol(ethanol)

2 types of LBC:

1. Sure path LBC
   - Drop brush in container
   - The size of circle on the slide is going to be very small (13mm)



Telegram - @nextprepladdernotes

2. Thin prep LBC
   - No brush in container
   - The size of circle on slide will be big (20mm)

Pap Stain Components:
Fixation: fixative is 95% ethanol.
Staining:(HOPE)

- Hematoxylin: blue color
- OG 6: Orange green color
- EA 50 (Eosin Azure): Pink color

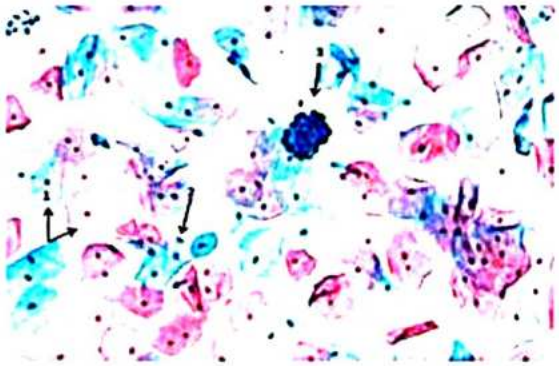
Adequacy For Reporting Of Pap Smear (By Bethesda System)
Conventional PAP smear

- 8000-12000 epithelial cells/ 10 hpf (ectocervix- squamous cells)
- Endocervical cells >= 10 well preserved(endocervix-tall columnar cells)

Liquid based cytology

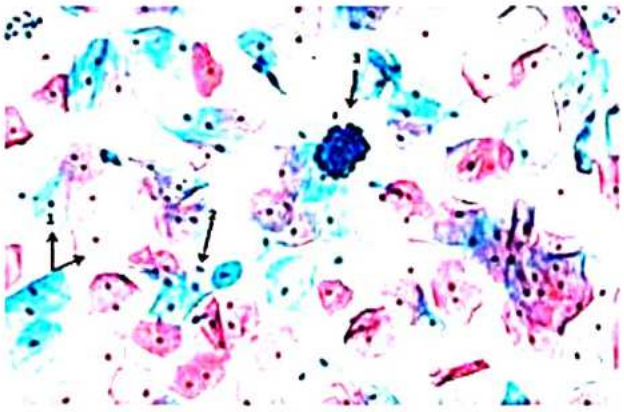
- 5000 cells/ 10 hpf
- Endocervical cells >= 10 well preserved

Superficial Cells



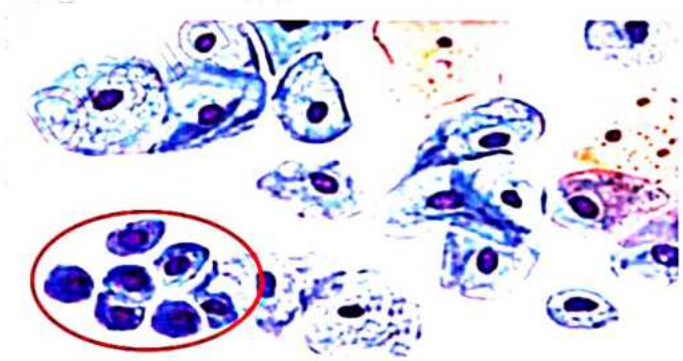
- Polygonal cells
- Pink cytoplasm
- Pyknotic nuclei

Intermediate Cells



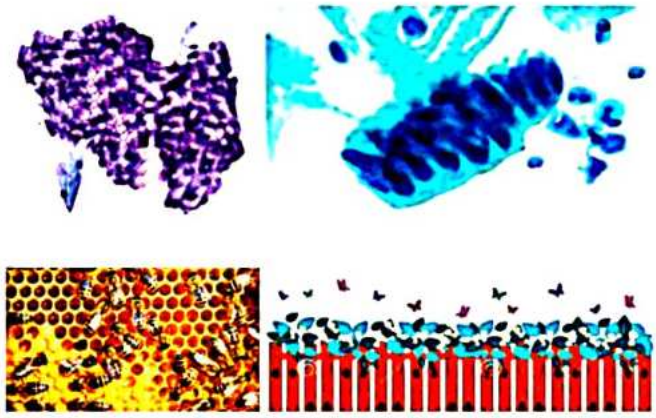
- Polygonal cells
- Blue cytoplasm
- Bigger nucleus

Parabasal Cells



- Cluster of Round cells

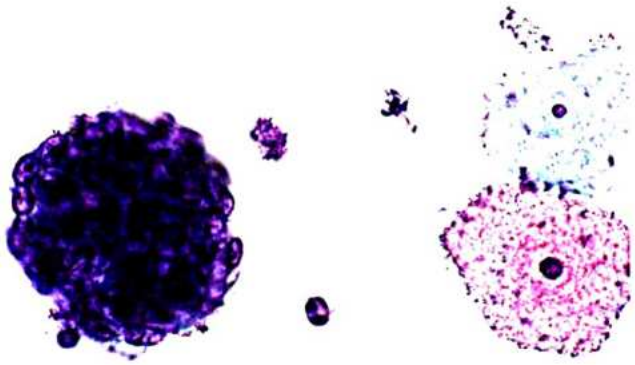
Endocervical Cells



Telegram - @nextpreladdernotes

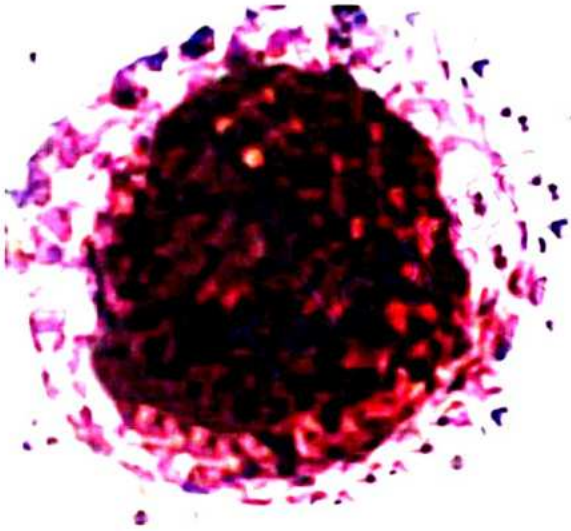
- Butterfly on fence or picket fence appearance
- Honeycomb appearance from top

Endometrial Cells



- 3d Ball Like Cluster
- It is seen from the 1st day of the menstrual cycle to the 5th day()when bleeding is visible)

Exodus Ballaqa



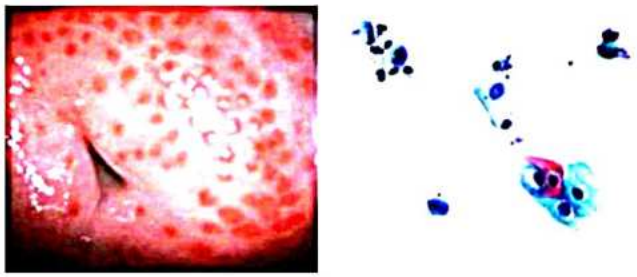
- Remnant of Endometrial shedding
- It is dark pink at the center and light pink at the periphery.
- It is seen from the 6th day of the menstrual cycle to the 10th day.

Maturation Index

- P/I/S
- Shift to right-0/10/90 estrogen predominance
- Shift to left-100/10/0 atrophic smear/Post menopausal
- Shift to mid zone- 0/90/10 progesterone predominance

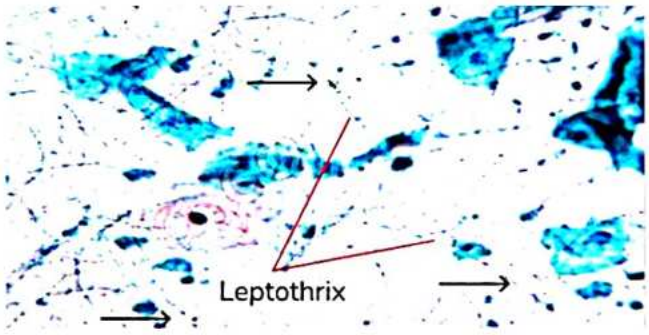
Infections

Trichomonas Infection



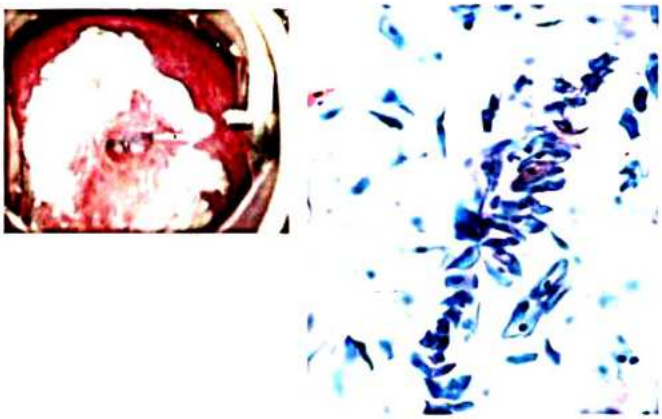
- It is a strawberry cervix or an angry looking cervix.
- Clinical feature: greenish discharge.
- Microscopically:
  - Appears like a pear kite shaped
  - Flagella can also be seen

Trichomonas And Leptothrix



- Round trichomonas come in association with thread like Leptothrix and give a spaghetti(leptothrix) and meatball(trichomonas) appearance.
- Fungus and malassezia furfur also show spaghetti and meatball appearance.

Candida Infection

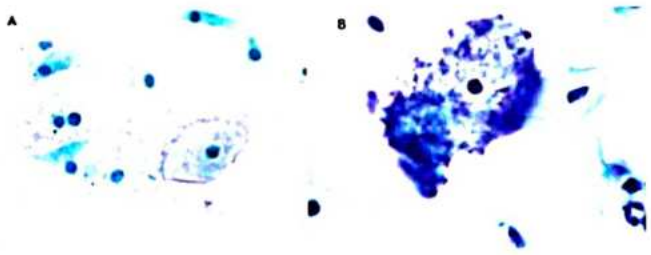


- It seems curdy white and gives a shish kebab appearance.

Telegram - @nextpreladdernotes

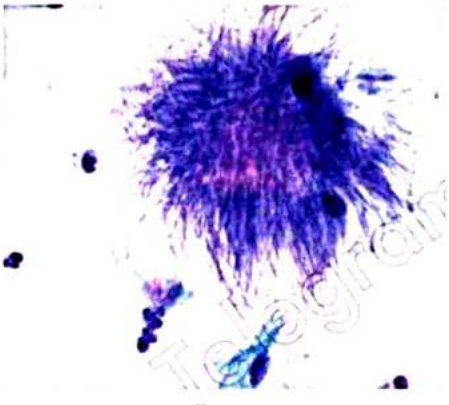
- There is a stick in the middle called pseudo hyphae, on which cervical cells are present.

Bacterial Vaginosis 00:49:38



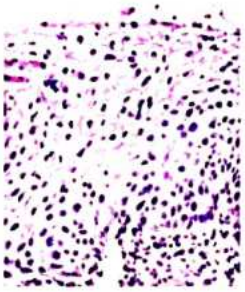
- It is caused by gardnerella vaginalis.
- Two criterias are there for diagnosis of bacterial vaginosis:
  - Amsel's criteria
  - Nugent's criteria.
- Cocoa bacilli stuck on the cells -clue cells
- Cocoa bacilli is present on the background also, this background is known as a filmy background.

Actinomyces



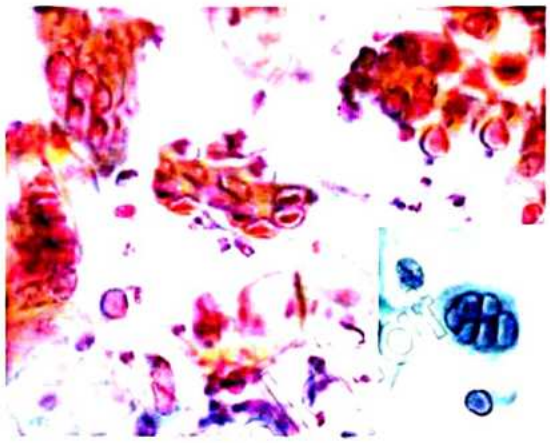
- History of IUCD (Intrauterine contraceptive device)
- It appears like got to be known as:
  - cotton balls
  - wooly balls
  - Sunray
  - dust bunniesgupta bodies.

HPV



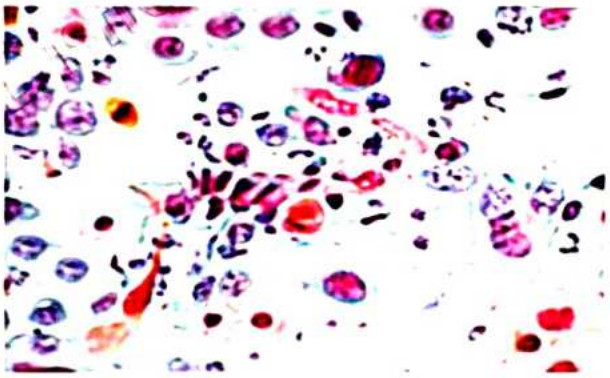
- It shows koilocytes
- It has a dirty dark nucleus called raisinoid nuclei and around this a white area is present that is called perinuclear halo.
- It looks same on PAP and Biopsy

Herpes simplex virus



- Multinucleation
- Moulding
- Margination

Squamous Cell Carcinoma



- Has tadpole cells (Big head and narrow tail)

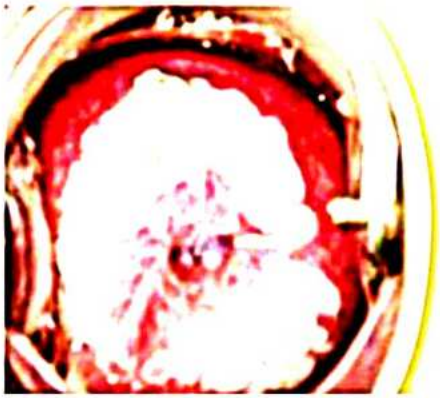
Questions

Q. Adequacy of PAP smear on conventional PAP?
a. 1000 Epithelial cells
b. 3000 Epithelial cells
c. 5000 Epithelial cells
d. 8000 Epithelial cells

Q. Adequacy of PAP smear on smear liquid-based cytology?
a. 1000 Epithelial cells
b. 3000 Epithelial cells
c. 5000 Epithelial cells
b. d 8000 Epithelial cells

Telegram - @nextprepladdernotes

Q. A 28 Year old female comes to your clinical with complaints of Vaginal discharge which on colposcopic examination shows the following appearance. Which of the following findings are you most likely to see on PAP Smear?



a. Spaghetti and meatball
b. Shish kebab
c. Blue blobs
d. String of pearls

Q. A 34 Year old female on PAP smear evaluation shows presence of multinuclearity, moulding and margination of nuclear chromatin?
a. a Bacterial vaginosis
b. CMV
c. HSV
d. d.Trichomonas

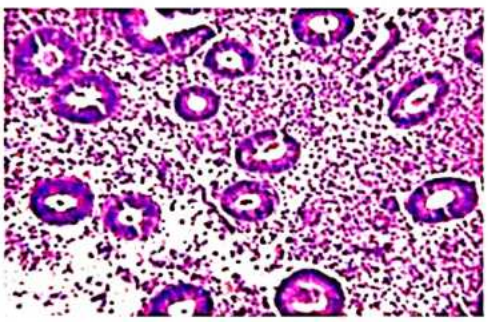
Female Genital System

1. Endometrium
2. Gestational
3. Trophoblastic Tumors

1. Endometrium

- Endometrium comprises two things:
  → Glands
  → Stromas (CD 10+).
- It has 2 phases:
  → proliferative endometrium
  → secretory endometrium.

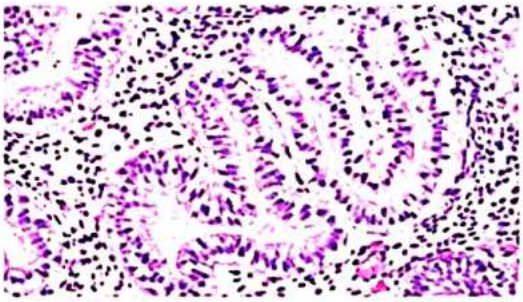
Proliferative Endometrium:



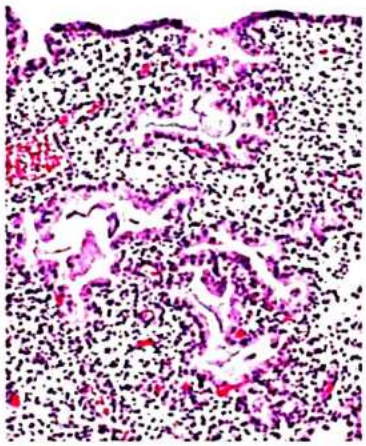
- Glands: Uniform and round
- Stroma: Closely packed/compact

Secretory Endometrium:

- Subnuclear vacuolation (below the nucleus) part of early secretory endometrium
- Piano key appearance



- Secretion is occurring into the lumen then it is called late secretory endometrium.
- Glands: Tortuous
- Stroma: loose edematous



Endometrial Hyperplasia: increase in number of cells

Old classification:

Simple Hyperplasia		Complex Hyperplasia	
Typical	Atypical	Typical	Atypical
			Maximum risk of cancer

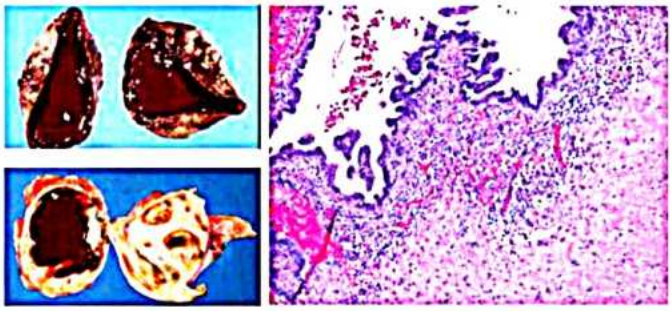
Endometrial Hyperplasia is premalignant and results in endometrial cancer.

Telegram - @nextprepladdernotes

New classification:

- Typical hyperplasia and Atypical hyperplasia

Endometriosis



- It has endometrial glands, stroma, and hemosiderin present outside of the uterus.
- Sites:
  - Ovary (chocolate cyst, old Blood)
  - broad ligament
  - POD, intestine
  - lung, and scars (scar endometriosis)
- Clinical features: Pelvic pain and infertility

Hypothesis theories:

Retrograde Menstruation (Sampson theory):

- Menstruation occurs in the opposite or retrograde direction and blood goes in the ovary.
- But it can't reach lungs and intestines that way. So other theories were proposed.

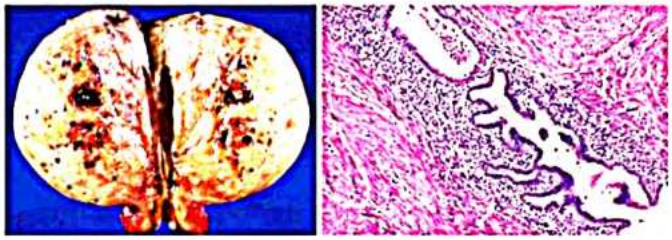
Metaplasia: It says it is happening because the epithelium is changing.

ARID1a: ARID1a: Involved in all cancers related to naari,
PTEN: (chr. 10, COWDEN) it can be seen in all endometrium diseases such as:

- Endometriosis
- endometrial cancer
- endometrioid ovarian tumor.

Stem cell theory: It says it is happening because stem cells are changing.

Adenomyosis:



- It is associated with endometrial glands, stroma and they get shifted to myometrium.
- Glands and stromas should at least be 2.5 mm away from the endometrium myometrium junction.
- It looks like a bag of blood.

Uterine Tumors: 01:22:47

1. Endometrial tumors: Glands (Adenocarcinoma), stroma (Stromal Tumor)
2. Myometrial tumors

I. Endometrial Tumors
   - Epithelial tumors (Adenocarcinoma)
   - Stromal tumors
   - Endometrial Carcinoma- Types And Genetics

Feature	Type I endometrial carcinoma	Type II endometrial carcinoma
Age	50-65 years	65-75 years
Hormone	Estrogen excess	Atrophy
Morphology	Endometrioid	Serious cell, clear cell, MMMT (Malignant Mixed Mullerian Tumor)
Precursor	Hyperplasia	SIEC (Serous intraepithelial carcinoma)
Behavior	Indolent	Aggressive

Type-1 Endometrial cancer: Genetics

Estrogen
↓
PE
↓ PTEN
Non atypical hyperplasia
↓ KRAS, MSI
Atypical hyperplais
↓ ARID1a, Beta catenin
Endometrial carcinoma type 1

Estrogen controls proliferative phase of endometrium

Type-II Endometrial Carcinoma- Genetics

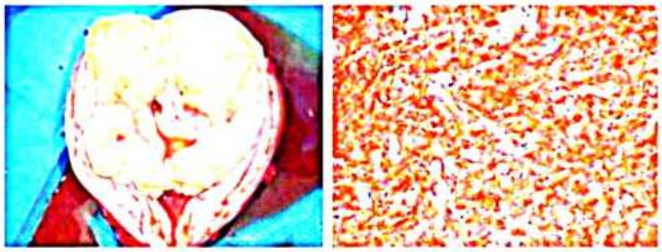
Atrophy
↓ Aneuploidy
SIEC
↓ Cyclin E
Endometrial carcinoma type I

Telegram - @nextprepladdernotes

Endometrial Carcinoma-update:
Pole mutation: associated with endometrial carcinoma types I genetics

Endometrial Stromal Tumors:
- Benign: Endometrial Shomal module (ESN)
- Malignant: Endometrial Shomal Sarcoma (ESS) marker: t(7:17) JAZ-SUZ fusion.

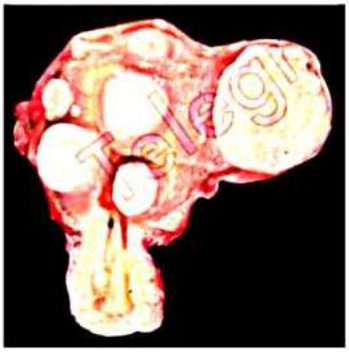
ESM and ESS:



- Gross: Yellow
- IHC: Cd10+

II. Myometrial tumors
  - o Leiomyoma (BENIGN)
  - o STUMP
  - o Leiomyosarcoma (MALIGNANT)

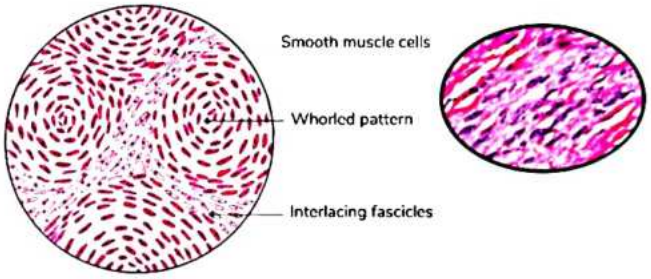
Leiomyoma/ Fibroids



- Genetics:
  - o MED 12 genemutation,
  - o Rearrangement of chromosomes 12 and 6.
  - o Med 12 gene is shown by Fibroids, fibroadenoma in breast, and phyllodes tumor in breast.

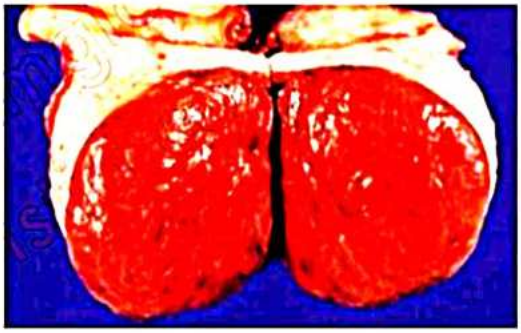
Site:
1. Submucosal leiomyoma (maximum chances of bleeding)
2. Intramural leiomyoma
3. Subserosal leiomyoma

Gross: White, and whorling pattern
Microscopically: fascicles and cigar shaped nuclei



Cigar Shaped Nucleus : Found in smooth muscle, leiomyoma and leiomyosarcoma.
IHC: SMA (smooth muscle actin)

Degenerations In Fibroids:
1. Hyaline Degeneration (pink)
2. Calcific Degeneration
3. Red Degeneration:



- It is beefy red in color and is associated with pregnant females.
- Causes fever, and abdominal pain
- Blood Test shows increase in WBC and ESR.

Leiomyoma:
1. Cellular leiomyoma: Cells are present
2. Symplastic leiomyoma: Atypia present
3. Metastasizing leiomyoma: Benign Tumor (controversial)

Leiomyosarcoma:
1. Atypia
2. Mitosis- most important criteria >10/10 hpf
3. Necrosis
4. Cellularity

Stump (Smooth Muscle Tumor Undetermined Malignant Potential):
1. Atypia
2. Mitosis-low

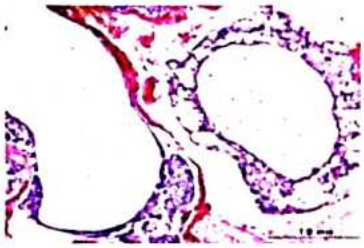
Telegram - @nextpreladdernotes

3. Necrosis
4. Cellularity

2. Gestational trophoblastic tumor

1. Partial mole
2. Complete mole
3. Invasive mole
4. Choriocarcinoma

Hydatidi form mole (H mole):

- Bunch of grapes appearance
- It shows hydropic villi (Edina fluid is there)

Placenta has two cells:

1. Cytotrophoblast (mononuclear),
2. Syncytiotrophoblast (multinucleated)

- Around hydropic villi, trophoblastic proliferation is there that has Cyto and Syncytiotrophoblast.

Differences

Partial mole	Complete mole
Triploid/tetraploid etc (One ova is going to fuse by multiple sperms)	Diploid (ova decides to be empty and 2n is contributed by sperms, only paternal chromosomes are present)
Fetal parts seen	Fetal parts not seen
p57 positive	p57 negative (paternally imprinted)
	Higher risk of choriocarcinoma

Choriocarcinoma:

- Gross- Hemorrhages, Necrosis
- Microscopic- Cytotrophoblast, Syncytiotrophoblast
- Marker- beta HCG
- Metastasis- Lung
- Treatment - Chemosensitive
- NO VILLI formation

Invasive mole:

- From the endometrium it invaded into myometrium

Questions

Q. POLE mutation has been associated with which of the following carcinomas?

a. Cervical carcinoma
b. Endometrial carcinoma type 1
c. Endometrial carcinoma type 2
d. Ovarian surface epithelial tumors

Q. Which of the following is/are features of endometriosis?

1. Endometrial glands
2. Endometrial stroma
3. Hemosiderin

a. 1,2
b. 1,3
c. 2,3
d. 1,2,3

Ovarian Tumors

Classification:

1. Surface Epithelial Tumor
2. Germ Cell Tumor
3. Sex Cord Stromal Tumor
4. Metastasis/ Krukenberg Tumor

1. Surface Epithelial Tumors

Refer Table 37.2

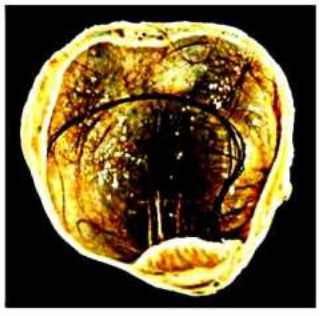
2. Germ Cell Tumors

New markers:

1. CD 117/CKIT
2. D240
3. OCT¾
4. SALL4
5. NANOG

Refer Table 37.3

Teratoma



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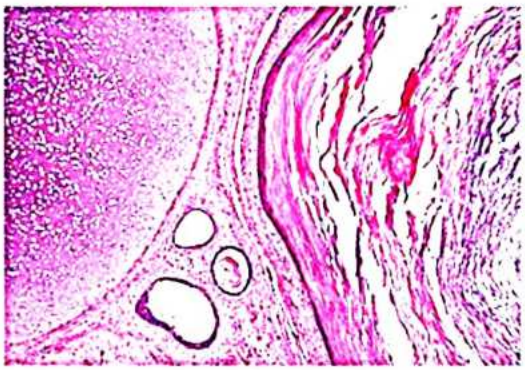
Definition: Tumors arising from 2 or more than 2 germ layers that are endoderm, mesoderm, and ectoderm.

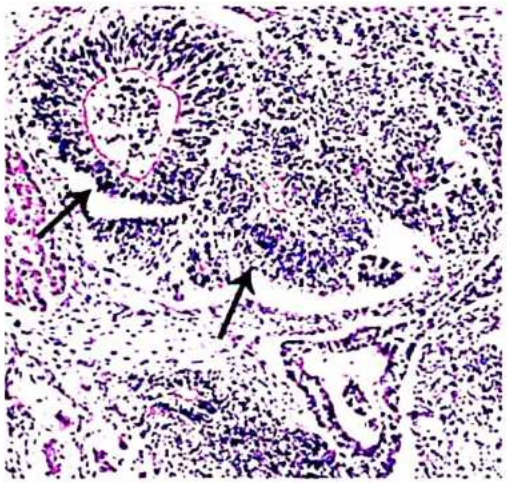
It is of two types:
Mature Teratoma:

- It is benign
- It has cartilage, skin, and gland.
- It is a mature cystic teratoma that is called dermoid cyst.
- Rokitansky protuberance may or may not have foci of cancer.

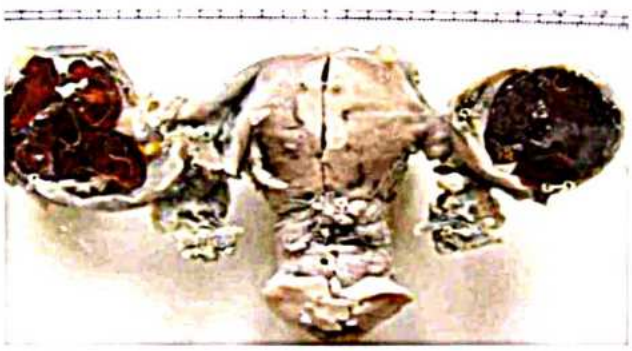


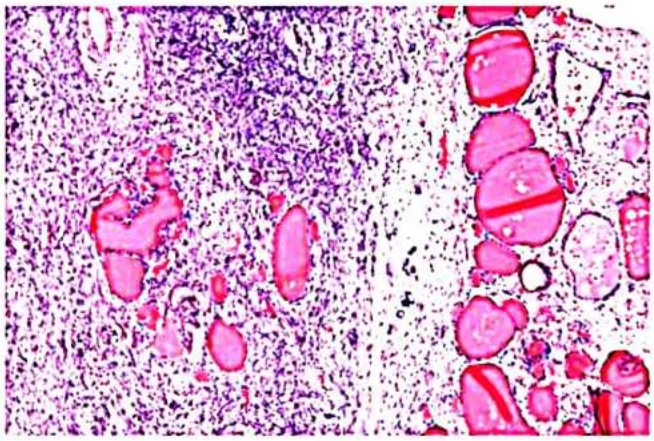
Immature Teratoma:

- It can be malignant
- It has small round blue cells (Immature neural components)



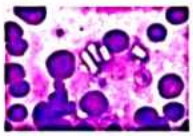
Monodermal teratoma:





- Only one germ layer.
- Its example is struma ovary (thyroid in ovary, that is functional and can produce T3 T4)
- Thyroid follicle present filled with colloids

3. Sex Cord Stromal Tumors 02:51:09

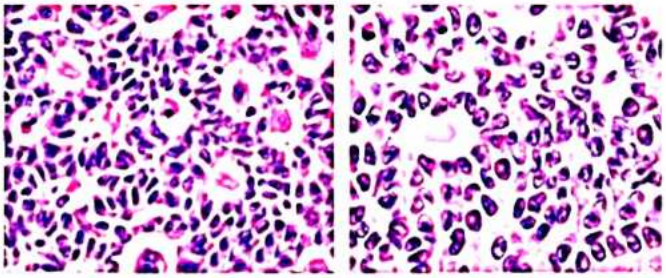
Tumor	Features	Microscopic Appearance	Marker
Sertoli-Leydig Tumor Pure Leydig Tumor	Masculinization	Reinke's crystalloids 	
Fibroma Thecoma	White Yellow	Spindle cell Vacuolated cells	Vimentin + Oil red 0+
Granulosa Cell Tumor			

Fibromas of ovary:

- MEIGs Syndrome:
  - Ascitis
  - Right sided hydrothorax
- Pseudo Meigs Syndrome:
  - Pelvic mass other than fibroma
  - Ascitis
  - Right sided hydrothorax
- Granulosa Cell Tumor:
  - Gene: FOX L2 mutation
  - Age: Post Menopausal, Juvenile
  - Hormone: Increased estrogen

Telegram - @nextprepladdernotes

- Clinical features: Endometrial HP/ Bulky uterus and bleeding
- Microscopically: Call exner Bodies, coffee bean nuclei

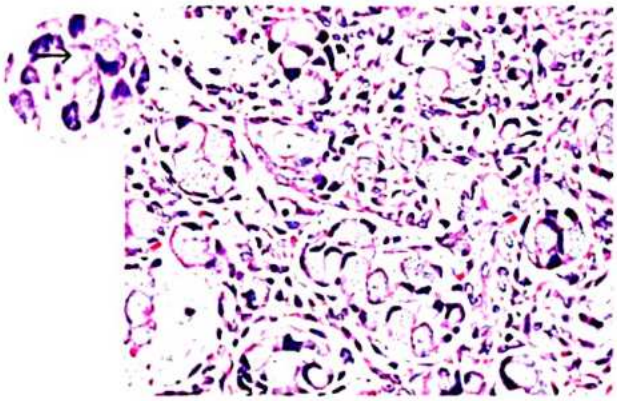


Markers: increase in inhibin, CD 99 +

Update:
Sex Cord Stromal Tumors have DICER 1 mutation

4. Metastatic - Krukenberg Tumor
   - Grossly:
     → Bi-lateral enlargement
     → Symmetrical enlargement
     → Capsule intact

Microscopically: Signet Ring Cells (Nucleus at the periphery because of presence of mucin inside the cell)



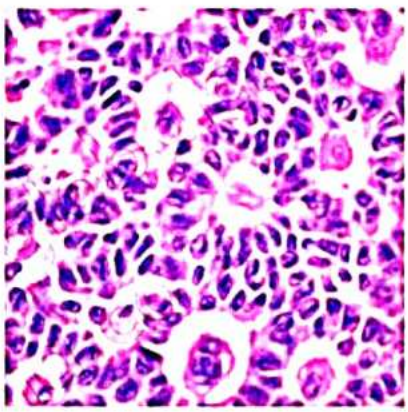
Combination tumors:
- Sertoli-leydig tumor
- Fibro-thecoma tumor
- Brenner's tumor can coexist with mucinous tumor

Questions:

Q. Reinke's crystalloids are seen in?
a. Serous ovarian tumors
b. Mucinous ovarian tumors
c. Teratoma
d. Leydig cell tumors

Q. A 30 year old female presents with abdominal pain. USG reveals an abdominal mass which is resected. Gross appearance shows gray white solid tumor with focal yellow areas. Microscopic examination reveals spindle cells admixed with vacuolated cells Diagnosis?
a. Fibroma
b. Thecoma
c. Fibro-thecoma
d. Granulosa cell tumors

Q. A 54 year old female presents bleeding PV. USG reveals bulky uterus along with presence of an adnexal mass. Hysterectomy with bilateral salpingo-oophorectomy is performed. Microscopic examination is shown below. Which of the following statements is incorrect?



a. Most commonly occurs in postmenopausal women
b. Associated with FOXL2 gene mutation
c. Can show presence of nuclear grooves
d. Shows the features of hypoestrogenism

Q. An adult female recently got unilateral salpingo-Oophorectomy performed which reveals the presence of nests of cells similar to urinary bladder. This ovarian tumor further can show which other component?
a. Serous cystadenoma
b. Mucinous cystadenoma
c. Fibrothecoma
d. Brenner tumor

Q. A 29 year old female is detected with an ovarian cyst on usg examination and is surgically removed. The cyst on the cut is uniloculated and shows presence of clear watery fluid. Histopathological assessment is shown below?
a. Serous cystadenoma
b. Mucinous cystadenoma
c. Fibrothecoma
d. Brenner tumor

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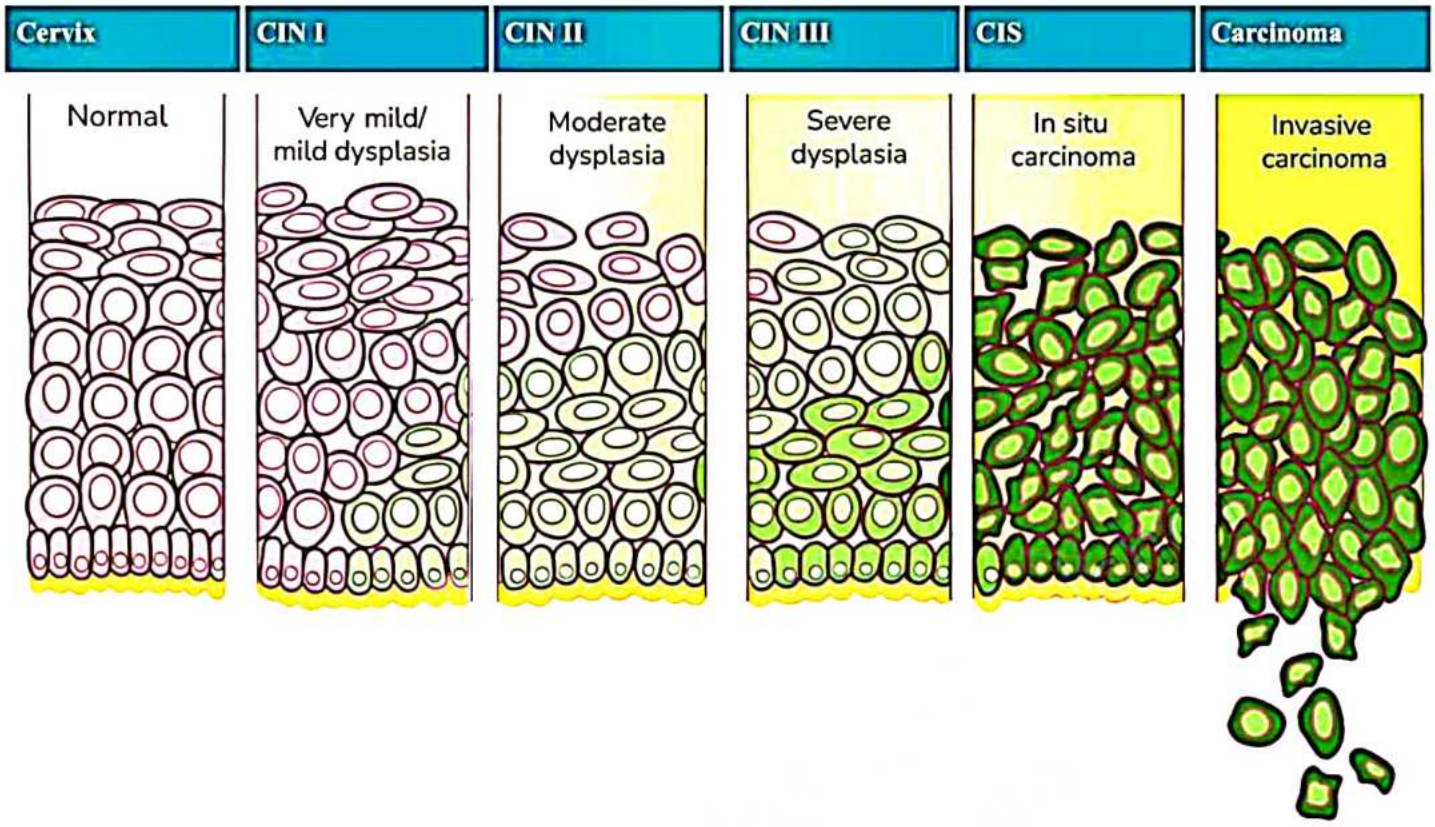
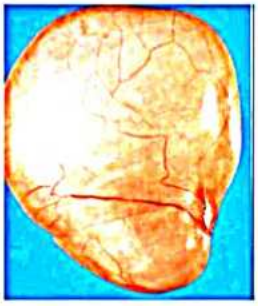
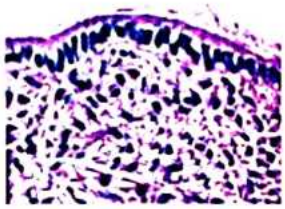
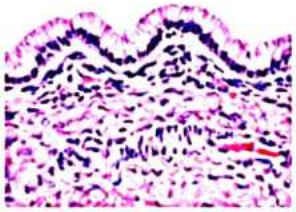
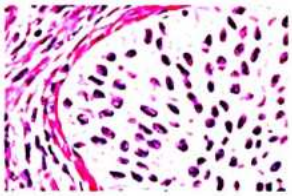
Table 37.1



Normal	Very mild/ mild dysplasia	Moderate dysplasia	Severe dysplasia	In situ carcinoma	Invasive carcinoma
	Lower one-third shows dysplasia	Lower two-third shows dysplasia	Almost full thickness shows dysplasia	Full thickness involvement	Basement membrane is broken
BM intact	BM intact	BM intact	BM intact	BM intact	BM broken

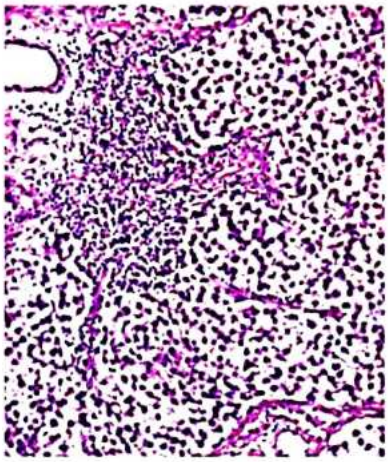
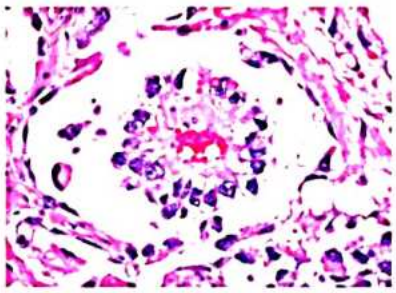
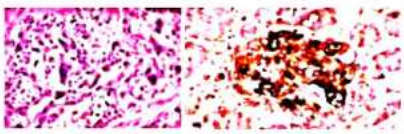
Telegram - @nextpreladdernotes

Table 37.2

Tumors	Gross	Micro	Tumor marker	Extra
Serous Ovarian Tumor Benign/borderline/malignant	Single Watery Cyst, Unioculated 	Ciliated epithelia, Psammoma bodies 	CA 125	B/L R/F Nulliparity BRCA1,2
Mucinous Ovarian Tumor Benign/borderline/malignant	Multi loculated, mucin present 	Mucinous Epithelium tissue 	CA 125	U/L R/F Smoking K-RAS It can be endocervical or intestinal-metastasis from intestine
Brenner Tumor Benign/borderline/malignant	Yellow white	Bladder epithelium/ transitional epithelium, coffee bean appearance/ nuclear groove 	CA 125	Brenner tumor Granulosa cell tumor Both shows coffee bean appearance
Clear Cell Tumor			CA 125	Involvement of DES (Diethylstilbestrol)
Endometrioid Tumors			CA 125	PTEN Endometriosis

Telegram - @nextprepladdernotes

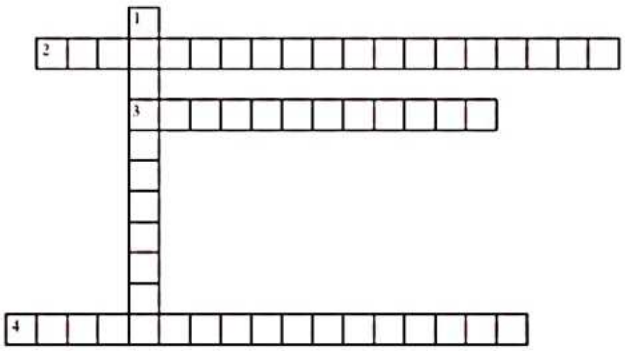
Table 37.3

Tumors	Gross	Microscopy	Tumor Markers
Dysgerminoma/ seminoma (testes)	Cut potato	Fried Egg appearance (clear cells- Glycogen i.e., PAS+ present), Fibrous Septa that contains lymphro- plasmacytic infiltrate 	LDH, PLAP
Yolk Sac Tumor/ endodermal sinus tumor		Schiller Duval/ Glomeruloid Bodies (seen in glioblastoma) Blood vessels are surrounded by 2 rows of germ cells separated by space. 	Less than 3 years, AFP(responsible for hyaline globules) alpha1 AT
Choriocarcinoma	Hemorrhage and Necrosis 	Cytotrophoblast and syncytiotrophoblast (No villi seen) 	Beta HCG
Teratoma	Hair, skin, teeth rokitansky protuberance	Mature, Immature	AFP

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. It cut out all the devry
3. It is associated with HPV.
4. Polygonal cells

Down

1. Condyloma acuminatum/warts

Telegram - @nextpreladdernotes

38 PATHOLOGY OF MALE GENITAL TRACT

Penile Tumours

00:00:30

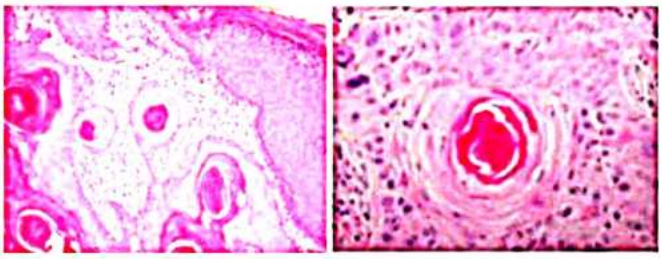
- Condyloma acuminatum
- Warts of penile region
- Low risk HPV 6 & 11
- Condylomata Lata is seen in secondary syphilis.

Pre Cancer or Carcinoma In Situ

- Bowen's disease
- Bowenoid papulosis (Rarest)
- Erythroplasia of Queyrat.

Cancer

- Squamous epithelial carcinoma



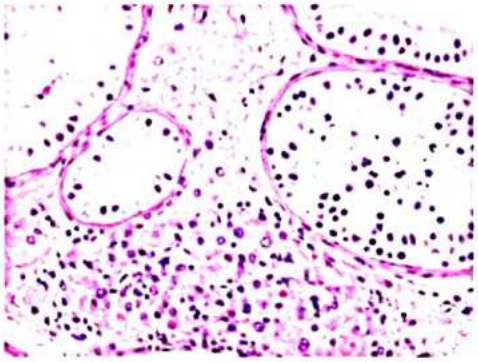
Verrucous Carcinoma

- A variant of squamous cell carcinoma.
- Good prognosis

Characteristic feature:

- The keratin pearls of kp's are pink colour round lesions.
- **In higher power;**
  - Can definitely see these pearls made of keratin.
- Squamous cell carcinoma could be in the;
  - Oral Cavity
  - Skin
  - Esophageal
  - Anal Canal
  - Penile or Anywhere

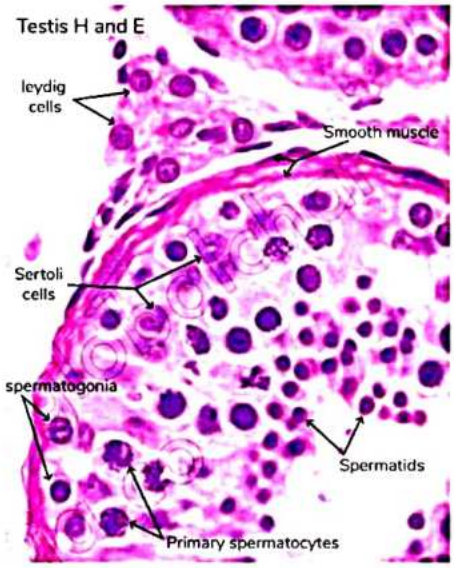
Testes



- Cryptorchism
- Testicular tumours.

Normal Testes

- **Round structures** - Seminiferous tubules, all the primary spermatocytes and secondary spermatocytes are present.
- Inside the seminiferous tubules;



  - The longest and tallest cells are present - Supporting cells called Sertoli cells.
  - Sperm formation stages;
    → Primary spermatocytes
    → Secondary spermatocytes
    → Spermatids
    → Spermatozoa
- A set of cells in between the seminiferous tubules.
  - A cluster of leydig cells
  - They form;
    → Testosterone hormone.
    → Crystalloid which is known as Renke's crystalloid.
  - Leydig cells are most important as they release Renke's crystalloid.
  - Spireme chromatin, seen in spermatocytes
  - Sertoli Only Syndrome
    → Only sertoli cells are present.
    → Leydig cells are normal.
    → Only spermatocytes are missing.

Testes Biopsy and Fixative

- FNAC
- Biopsy
  - Trucut

Telegram - @nextpreladdernotes

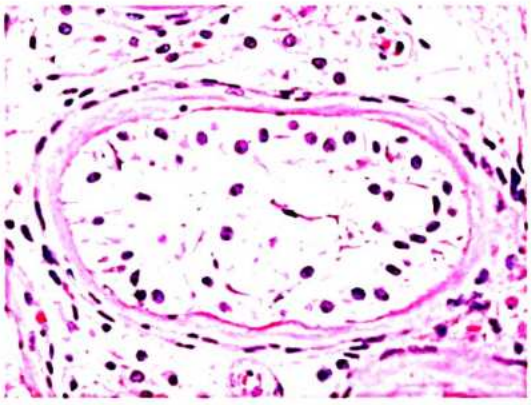
- Excisional
- In infertility cases:
  - None of these are done
  - Risk of spread of tumor
  - If the case is patient with cancer or suspecting cancer;

Fixative

Tissues With Different Diagnosis	Fixative	Uses
Testicular tissues for infertility	Bouin's Fixative (Picric acid)	• Maintains morphology of the germ cell. • Can locate the arrest of the cells. • NBF is not used as it destroys all the cells.
Testicular tissues for tumours	10% NBF (Neutral Buffered Formalin)	• For the tissues under surgery.

Cryptorchidism 00:14:54

- It is a failure of the testis to descend into the scrotal sac.
- Scrotal sac becomes empty.
- The most common site of arrest - Inguinal.
- **Risk of;**
  - No spermatogenesis - Infertility, cancers
- **Treatment**
  - Orchiopexy (Descending testes to scrotum) - 6 Months to 2 Years of life.
    → Nowadays, the upper limit is 18 months.
    → If delayed, the chances of becoming infertile is more.
  - Following treatment the risk of Cancer only decreases, it is not going to become zero.
    → Keep the case under follow ups.
- On microscopic examination



  - Spermatogenesis is affected.
  - Supporting cells are in abundance.
  - Leydig cells hyperplasia.
  - Thickening of basement membrane.

Microbiological Correlation?

- Orchitis infection
  - Syphilis
  - Mumps

Testicular Tumours - Classification 00:18:29

- Germ cell tumours
  - Seminomatous tumours
    → Age - 30s (2nd and 3rd decade)
    → Radiosensitive
    → Spread via lymphatic - Paraaortic group of lymph nodes.
    → Better prognosis.
  - Nonseminomatous tumours
    → Elderly
    → Radioresistant
    → Hematogenous spread via;
      – Lungs
      – CNS
    → Poor prognosis.
- Non germ cell tumours
  - Sex cord stromal tumours
  - Others

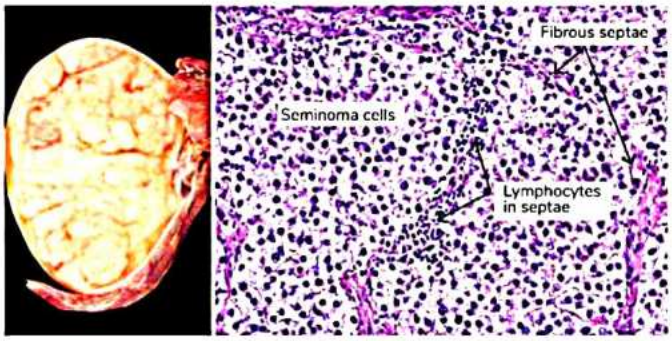
Precursor or Risk factors

- i12p - Isochromosome P (2 same arms).
- Klinefelter's syndrome - Chromosomal abnormality, it causes;
  - Gonadal Germ Cell Tumour
  - Extra Gonadal Germ Cell Tumour
- Cryptorchidism
- Precursor lesion for testicular tumours;
  - ITGCN (earlier) / GCNIS (Latest)
    → GCNIS - Germ Cell Neoplasia In situ.
    → GCNIS result in cancers except;
      – Teratoma
      – Spermatocytic Seminoma
    → Teratoma, spermatocytic seminoma are derived from ITGCN.
- Classical presentation for testicular tumour - Painless Lump.

Telegram - @nextpreladdernotes

1. Seminomatous Germ Cell Tumours

a. Seminoma 00:24:16



- **Gross appearance** - cut potato appearance
- Cells has fried egg appearance
- Lymphocytes and plasma cells in fibrous septic
- **Tumour markers**
  - LDH
  - FLAP
- **New tumour marker**
  - Cd117
  - D240
  - OCT ¾
  - SALL4
  - NANOG

b. Spermatocytic Seminoma

- Above 65 years
- Gain of chromosome 9 - Genetically
- No metastasis
- Slow growing
- Excellent prognosis
- Microscopically - 3 types of cells notes
- Name is because; it shows three types cells with Spireme chromatin (spermatocytes)
  - Small size cells
  - Medium size cells
  - Large multi nucleated cells.

Q. What is the tumour marker in seminoma except?
Ans: AFP - Alpha FetoProtein.

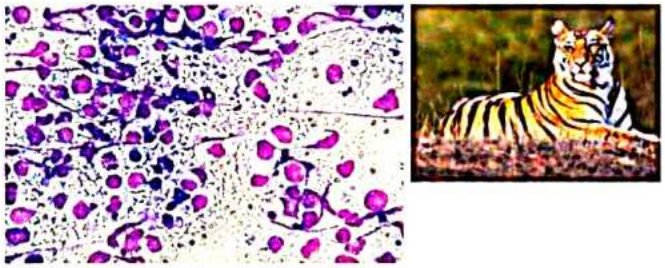
Role of FNAC

Case: 30 year old male comes with a testicular lump along with a right inguinal lymphadenopathy.

Explanation

- FNAC can be done from the right inguinal lymph nodes as it is contraindicated to do from testis.

Q. How to Know it's a Seminoma (Germ Cell Tumour)?

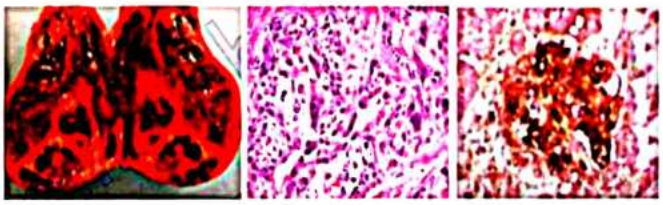


Ans:

- If FNAC from lymphadenopathy is showing a tigroid background, it will confirm that germ cell tumour in testis.
- The background, dark and light areas are everywhere alternatively - Tigroid Background.

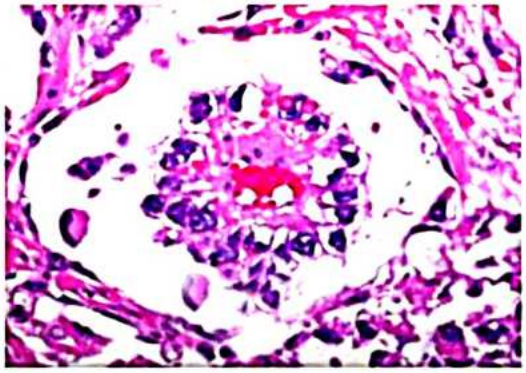
2. Nonseminomatous Tumours

a. Choriocarcinoma



- Gross haemorrhage and necrosis.
- The cell types are;
  - Single nucleated cells are called cytotrophoblasts.
  - Multi nucleated cells are called syncytiotrophoblasts.
  - IHC (Beta HCG) - Tumour marker for choriocarcinoma.

b. Yolk Sac Tumour 00:31:37



- Yolk sac tumour shows;
  - Schiller duval or Glomerular Bodies.
  - Tumour Marker - AFP, Alpha 1 AT.
- Occur in young children.

Telegram - @nextprepladdernotes

c. Teratoma

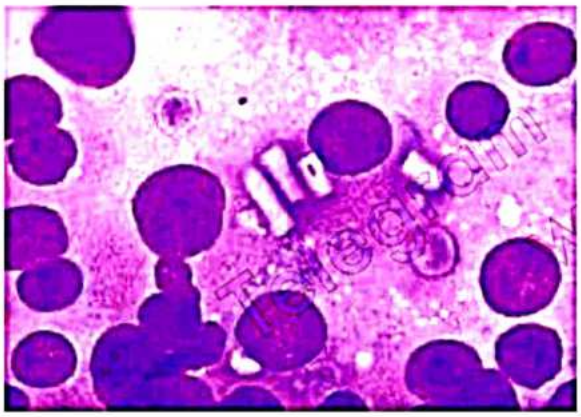
- In ovarian teratoma has;
  - Mature teratoma - Benign tumour
  - Immature teratoma - Malignant tumour
- In testes;
  - Prepubertal teratoma - Benign tumour
  - Postpubertal teratoma - Malignant tumour
  - GCINS is not a precursor.
  - Teratoma can become malignant at postpubertal age.
    → At 1% chances squamous cell carcinoma (most common) can occur.

d. Embryonal Carcinoma

- CD30+ is seen in;
  - Carcinoma
    → Only embryonic carcinoma
  - Lymphoma
    → Hodgkin's lymphoma (Reed-Sternberg cells are CD30+)
    → Non Hodgkin's lymphoma
      – PEL - Primary effusion lymphoma.
      – ALCL - Anaplastic Large Cell Lymphoma.

Non Germ Cell Tumours

Sex Cord Stromal Tumour

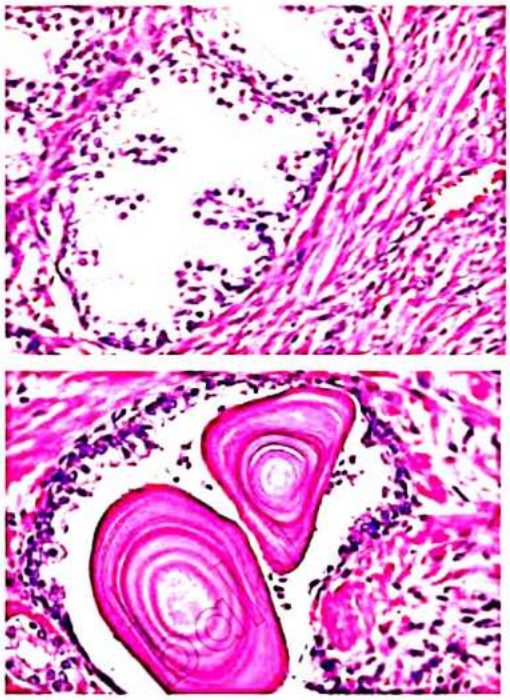


- **Sertoli Cell Tumour**
  - Clinically silent.
  - Tumour Marker - Inhibin.
- **Leydig Cell Tumour**
  - Identified by reinke's crystalloids.
  - Grossly, they have a golden pigment called lipofuscin.
- **Both Sertoli Cell Tumour + Leydig Cell Tumour**

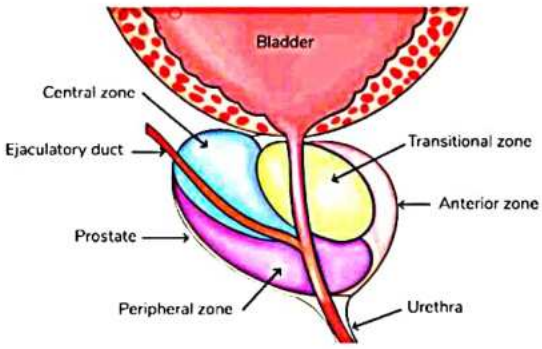
Other Testicular Lymphoma

- Above 60 years
- Bilateral
- DLBCL - B cell lymphoma.
- Bad prognosis.

Prostate Gland



- 20 grams of gland present in the male around the urethra and urinary bladder.



Basic Zones of Prostate Gland

- At extreme periphery - Peripheral zone
- At Center - Central Zone
- In between extreme periphery and Center - Transitional Zone.
- Clinically;
  - In the Transitional zone - Hyperplasia (BPH).
  - Peripheral zone - Prostate cancer.
  - The cancer reaches the urethra from the periphery, this process will take a lot of time.
    → Urinary complaints are extremely late, thus late presentation.
  - Hyperplasia - Very close to urethra.
    → Early presentation of urinary symptoms, like,
      – Urgency
      – Frequency

Telegram - @nextpreladdernotes

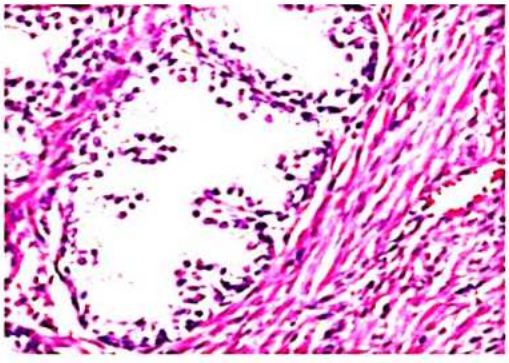
Glands + Stroma

- **Stroma**
  - Prostate has glands and stroma (Fibro-muscular stroma - To secrete and eject the secretions).
- **Glands**
  - The glands have two layers.
    - → First - Epithelial cells on the inside.
    - → Second - Outside the base, Basal cells.
    - → Significance

Cells and Tumour Markers	Normal Prostate	BPH	Cancer
Epithelial cells	+	+	+
Basal cells	+	+	-
Tumour Marker p63	+	+	-
AMACR - Alpha methyl acyl coenzyme A Racimase	-	-	+

- **Fibromuscular Stroma;**
  - Layer on the inside - Epithelium cells.
  - Layer on the outside - Basal cell.
  - If the Basal cells are Present - BPH.
  - If the Basal cells are absent - Cancer.
- Secretions in the prostate - corpora, aymalesia (Important secretions).
- The breast and the endometrium also have the same glands and stroma.
- But, the stroma Differentiating point;
  - In prostate - fibromuscular stroma
  - In breast - fibroadipose stroma (As breast is fat organ).
  - In endometrium - Positive for a CD marker, and that is CD-10 given by pathologist (Discussed in endometrium chapter).

Nodular Hyperplasia Prostate



- Earlier- Benign prostate, or benign prostatic hyperplasia
- Both the layers of the cells are going to be present, including the Basal layer.
- P63 marker is positive.
- BPH occurs not because of testosterone.
- Testosterone → dihydrotestosterone, DHT in the presence of an enzyme called 5 alpha reductase type 2
  - DHT (Hormone) causes benign prostatic hyperplasia.
- Treatment: 5 alpha reductase inhibitor - Finasteride.
- It occurs in the transitional zone - Urinary complaints are going to be in time.

Prostate Cancer 00:50:24

- Peripheral zone
- Late urinary symptoms.
- **Spread via**
  - Batson's Venous Plexus and go to Bones (Vertebra).
    - → Thus, a common complaint is low back pain in the lumbosacral region.
  - Perineural spread (around the nerves)
- **Markers**
  - PSA (Prostate Specific Antigen)
  - PAP (Prostate Acid phosphatase)
  - New marker - NKX 3.1 (JIPMER Exam Question).
- **Risk factors**
  - Advancing age
  - High fatty diet
  - Black population
  - BRCA2 mutation;
    - → Also seen in Male Breast Cancer.
  - Hypermethylation of GST (Glutathione S Transferase)
  - TMPRSS2-ERG fusion

Note:

- TMPRSS2-ERG fusion - Prostate cancer.
- TMPRSS2 - Receptor for COVID.
  - ACE2 and TMPRSS2 helps in binding of COVID.
- TMPRSS6 Gene Mutations- IRIDA (Iron Refractory Iron Deficiency Anaemia).

  - Protective Factors;
    - → Antioxidants like selenium.
    - → Vitamin D
    - → Lycopene

Q. Which cancers show perineural invasion?

- PAP tumours
  - P - Prostate Cancer
  - A - Adenoid Cystic Cancer
  - P - Pancreatic Cancer

Telegram - @nextpreladdernotes

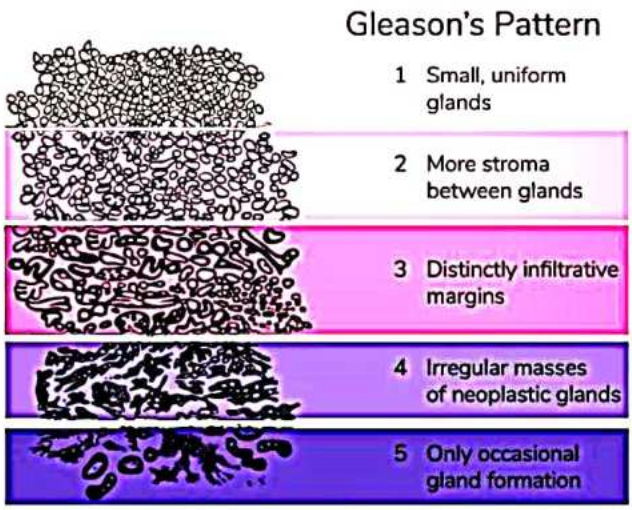
Gleason

Calculate in the below sequence;

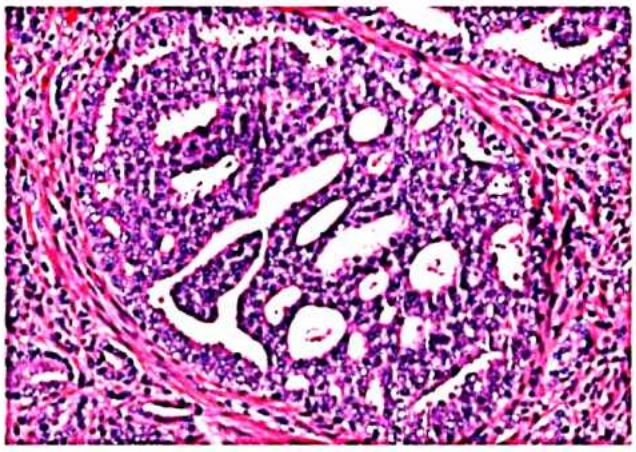
- PSG - Preeti Sharma Group (mnemonic).
  - P - Pattern
  - S - Score
  - G - Grade

Gleason Pattern 00:57:45

Patterns:

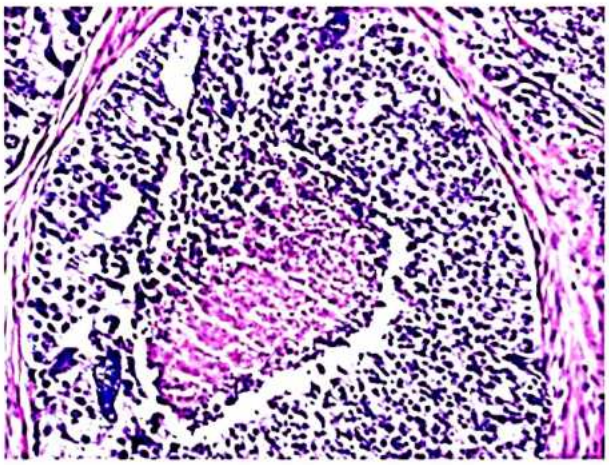


1. Small uniform glands
2. More stroma between the glands
3. Distinctly infiltrative margins
4. Irregular masses of neoplastic glands
   a. Cribriform
   b. Hypernephroid
5. Only occasional gland formation
   a. Single cells
   b. Comedonecrosis



Cribriform Pattern

- Cribriform - Means sieve-like pattern.



Comedonecrosis Pattern

- Comedone - Its centre has lot of necrotic material (Like acne)

Score

If the examiner gives two patterns;

- Predominant pattern (1°) - 80% of the slide.
- Miscellaneous pattern (2°) - 20% of the slide.

Then the score will be the addition of patterns = 1° + 2°.

If the examiner gives only single patterns; the double it up = 1° + 1°.

If the examiner gives three patterns;

- Predominant pattern (1°) - 80%
- Miscellaneous pattern (2°) - 15%
- Others - 15% (1°+2° or 3°).

Grading

Gleason Score	Grade
< = 6	Grade Group I
3 + 4 = 7 • Main pattern is 3.	Grade Group II
4 + 3 = 7 • Main pattern is 4.	Grade Group III
8	Grade Group IV
9 to 10	Grade Group V

Other Test

- Age specific PSA
  - 40-49 = 2.5 ng/ml
  - 50-59 = 3.5 ng/ml
  - 60-69 = 4.5 ng/ml
  - 70-79 = 6.5 ng/ml
  - Free PSA to Bound PSA.

Telegram - @nextpreladdernotes

- PSA velocity should not increase <0.75 ng/ml (Ideal).
- PSA Density = Serum PSA / Volume of Gland (In surgery and radiology).

MCQs

Q. A 67 year old male is found on rectal examination to have a hard, irregular nodule within his prostate. A biopsy of this lesion reveals the presence of crowded small glands lined by a single layer of cells with enlarged, prominent nucleoli. Immunohistochemistry shows absence of outer basal cell layer. From what portion of the prostate did this lesion most likely originate?

A. Anterior zone
B. Central zone
C. Peripheral zone
D. Transition zone

Explanation:

- Given their single layer, there the answer becomes cancer.
- The region cancer originates is the peripheral zone.

Q. Cribriform pattern on prostate cancer biopsy is indicative of?

A. Pattern 2
B. Pattern 3
C. Pattern 4
D. Pattern 5

Q. A 69 year old male is found on rectal examination to have a hard, irregular nodule within his prostate. PSA LEVELS are 100 ng/ml. TRUS guided biopsy shows presence of tumour cells without any glandular formation. 10 percent of the tumour also shows cribriform pattern. What is the Gleason grade?

A. Grade 2
B. Grade 3
C. Grade 4
D. Grade 5

Explanation

- Pattern 5 - Single cell formation - Score 5.
- 2° Tumour - Score 4.
- Total score 9.
- The Grade is Five.

Q. Prostate biopsy shows the presence of adenocarcinoma predominantly in cribriform pattern followed by crowded pattern but separate glands and a minor Component 5 percent of single cell infiltration. What is the Gleason grade?

A. Grade 2
B. Grade 3
C. Grade 4
D. Grade 5

Explanation

- 1° = 4
- 2° = 2
- 3° = 5
- Three patterns: 3°>2°
- Score = 4+5.
- Score is 9 then it is Grade 5.

Q. A surgeon suspecting testicular carcinoma in a patient asks the intern to send A sample for histopathology. What is the fluid in which the intern should send the sample to the pathologist?

A. Bouin solution
B. 10% formalin
C. 95% ethanol
D. Alcohol

Q. Alkaline phosphatase is a tumour marker of which tumour?

A. Seminoma
B. Embryonal carcinoma
C. Lymphoma
D. YST

Explanation

- Tumour markers;
  - YST - AFP, A1AT.
  - Lymphoma has no tumour markers.
  - Embryonal carcinoma - Cd30+.
  - Seminoma - LDH, PAP (Placental Alkaline Phosphatase).

Q. A glomerulus-like structure composed of a central blood vessel enveloped by germ cells within a space lined by germ cells is seen in?

A. Seminoma
B. Embryonal carcinoma
C. Lymphoma
D. YST

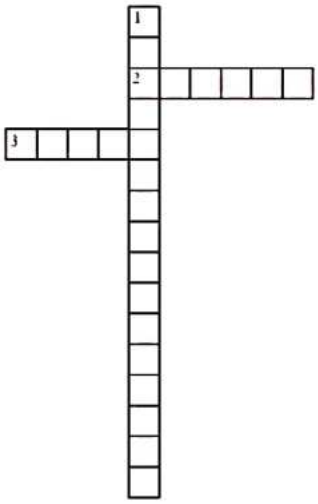
Explanation

- Blood vessel in the Centre.
- Two layers of germ cells and the space between two layers is called the glomeruloid body.

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. Squamous epithelial carcinoma
3. Penile Tumours

Down

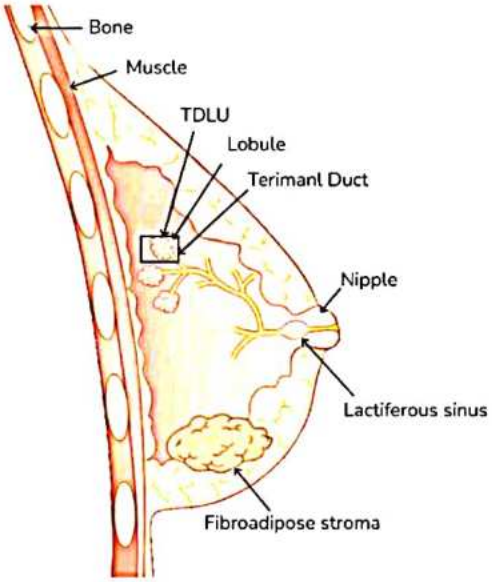
1. Surgery can be done.

Telegram - @nextpreladdernotes

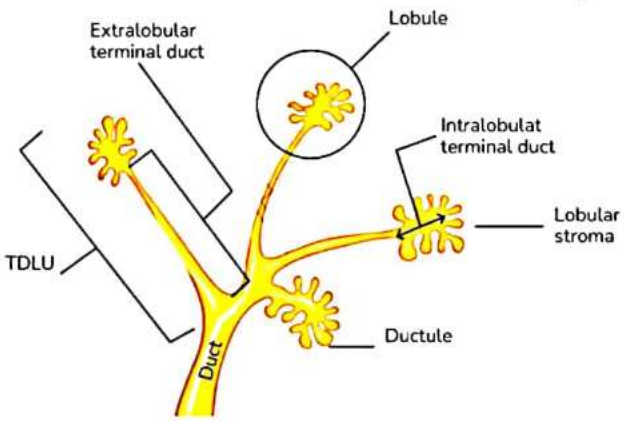
39 BREAST PATHOLOGY

TDLU

00:00:30



- Stands for: Terminal duct lobular unit
- In this nipple-areolar complex is present.
- Lactiferous glands or lactiferous ducts are raised from this complex.



- Division of lactiferous duct
- This duct further divided into
  - Extralobular terminal duct (present outside the lobule)
  - Intralobular terminal duct (present inside the lobule)
- TDLU: L for lobule
  - TD (Terminal duct) divides into extralobular, intralobular
  - Bunch of the acini will make up the lobule
- The no.of acini per lobule increases: fibrosis
- Every lobule contains the stroma
  - If the stroma is present inside: Intralobular stroma
  - If the stroma is in between the lobule: Intercellular stroma
- Breast has fat, stroma is nothing but the adipose tissue

Other Organs with Similar Features

Organs	Glands	Stroma
Breast	Present	Fat: Adipose tissue
Prostate	Present	Muscular stroma: Fibromuscular stroma (Due to amount of secretions it has to do)
Endometrium	Present	CD 10+

Glands of Breast Tissue Image

00:07:25

- All this glands are called TDLU
- Inner layer cells: Epithelial cells
- Set of cells present beneath the epithelial cells (Outer): Myoepithelial cells

Markers

- **Epithelial Cells**
  - CK 7 (Cytokeratin cell positive 7: Present in the inner layer)
  - In the neoplasia chapter, female related cervical cancer, breast cancer, and endometrial cancers have lady luck 7 (CK 7+).

- **Myoepithelial cells**
  - Present only in benign conditions (Fibroadenoma, fibrocystic disease)
  - Absent in malignant conditions
    → Happy when the lizard is present, worried if it vanishes suddenly (if don't know where it gene)
  - CK 5/6
  - P63 (to delineate the outer layer in prostate)
  - SMA
  - S100
  - The CK 5/6, P63, SMA, and S100 markers are present at the outer layer of the cells (Presence or absence is the deciding factor)

Inflammatory Disorders or Lesions

- Acute mastitis
- Duct ectasia
- Lymphocytic mastitis
- Granulomatous mastitis
- Zuska disease Fat necrosis

Telegram - @nextpreladdernotes

Disorders	Description
Acute Mastitis	• Associated with breastfeeding, cracked nipples • When child is feeding, bacteria can enters and then travels into the lactiferous duct) • This infection will results in abscess • Bacteria: Staphylococcus aureus • As it is related to skin and soft tissue (Cellulitis can occur) • Bacteria: Streptococcus
Duct ectasia	• Ectasia: Dilation (by default) • Duct ectasia: Dilation of lactiferous duct • Telangiectasia: Dilation of blood vessel • Discharge of thick creamy paste like material • Microscopically: a lot of foamy macrophages
Lymphocytic mastitis	• The autoimmune disorders association • Examples o SLE o Sjogren's syndrome o Hashimoto thyroiditis o In all this disorders there will be the presence of lymphocytes
Granulomatous mastitis	• Examples. o Most commonly TB (Caseating granuloma) o Sarcoidosis (M/C Non caseating granuloma) o Silicon implant (Foreign body) → Reaction can be granulomatous mastitis
Zuska disease Fat necrosis	• Other Names: Subareolar abscess • SMOLD: Squamous metaplasia of the lactiferous duct (metaplasia disorder) • Lactiferous duct made of cuboidal or columnar epithelium • But it has changed to the squamous metaplasia due to the smoking • Hence, smoking results in SMO-LD • Then this squamous cells will form the keratin (keratin plugs) • Which causes the abscess (typical features: Redness, fever)
Fat necrosis	• Breakdown of the fat (stromal fat) • Due to trauma on the breast • Fats breakdown into fatty acids • This fatty acids attracts the calcium (chalky white color) • **Hint No.1:** Trauma (patient history) • **Hint No.2:** Calcification (Chalky white)

Lesions of the Breast (Sequence Before the Cancer Occurs)

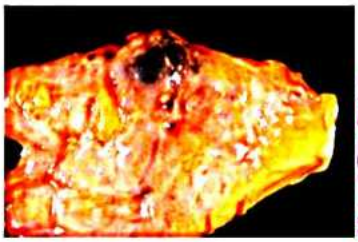
Category	Features	Relative risk and absolute lifetime risk
Non-proliferative breast changes	• Mild HP • Ectasia (dilation) • Cysts (fibrocyst-little proliferation) • FA without complex features	• Relative risk: 1% • Absolute risk: 3%
Proliferative diseases without atypia	No bad looking cells Some diseases include • Moderate hyperplasia • Sclerosing adenosis • Intraductal papilloma (Benign tumor) • FA with complex features	• Relative risk: 1.5-2% (2%) • Absolute risk: 5-7% (6%)
Proliferative breast diseases without atypia	• Atypical ductal hyperplasia • Atypical lobular hyperplasia	• Relative risk: 4-5% (4%) • Absolute risk: 13-17% (>12%)
Carcinoma in situ	• Precancerous condition • Tremendous amount of risk is present	• Relative risk: 8-10 (8%) • Absolute risk: 25-30% (>24%)

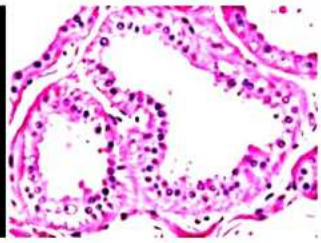
Telegram - @nextprepladdernotes

Fibrocystic Diseases

00:26:18

- Lumpy bumpy appearance (Palpatory appearance)
- Pain is present, mainly cyclic pain (Pain during menstrual cycle)





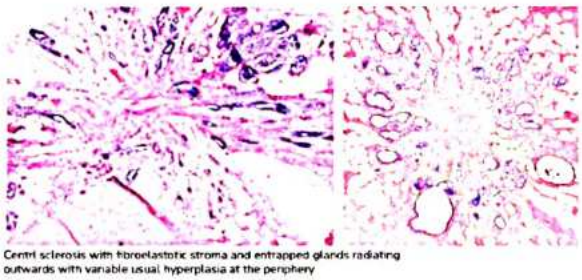
Fibro: Fibrosis

- Cystic: Cyst
  - The cyst is filled with a fluid, due to which it appears like a blue dome cyst
- For any type of breast etiology
  - FNAC - Decreases or disappears on needling (it is temporary)
  - BIOPSY- Change with Apocrine metaplasia
    - → Apocrine cells are pink coloured cells (due to increased eosinophilic cytoplasm)
    - → Projections is called apocrine shouting
    - → All this secretions are migrated to middle forming a cyst like appearance
  - Adenosis-Increased acini per lobule
- Fibrocystic disorder is a combination of
  - Fibrosis
  - Cystic
  - Adenosis
- It comes under the category of Non-proliferative breast changes

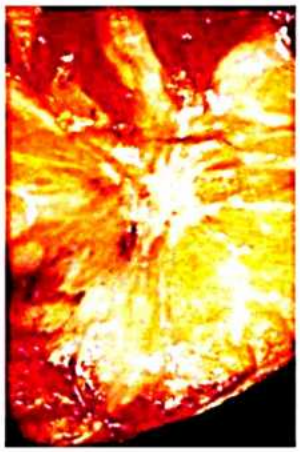
Proliferative Breast Diseases without Atypia

It includes

- Hyperplasia (Moderate)
- **Papilloma (Benign tumor)**
  - In surgery, it is Intraductal papilloma
  - It presents as a subareolar lump
  - Discharge from the nipple is present
- **Sclerosing adenosis**
  - There is sclerosis
  - Increase in the acini cells per lobule (Adenosis)
- **Radial Scar**



Centri sclerosis with fibroelastotic stroma and entrapped glands radiating outwards with variable usual hyperplasia at the periphery



  - Whitish scar is present at the center (central stellate scar)
  - Central stellate scar

Organs in which central stellar scar is present	Region of the Scar
Breast	Radial Scar
Kidney	It is there are two things • **Benign tumor:** Oncocytoma • **Malignant tumor:** Chromophobe RCC
Liver	It also has two • **Benign tumor:** Focal Nodular hyperplasia • **Malignant tumor:** Fibro lamellar HCC
Pancreas	• Serous cystadenocarcinoma of pancreas shows central stellar scar

- Scar is like a stellate has projections in which ducts and glands are trapped
- A central nidus of entrapped glands in a hyalinized stroma is surrounded by long radiating projections into the stroma.
- There are no bad Cells, but the cells are proliferating.

Proliferative Breast Diseases with Atypia

It includes

- ADH (Atypical ductal HP)
- ALH (Atypical lobular HP)
- They occur due to the chromosome changes
  - Loss of 16q
  - Gain of 17p
- ADH goes into ductal carcinoma in situ (CIS), this further leads to ductal carcinoma

Telegram - @nextprepladdernotes

- ALH goes into lobular carcinoma in situ (CIS), this further leads to lobular carcinoma

Reminder

- The myoepithelial cells are absent in carcinoma

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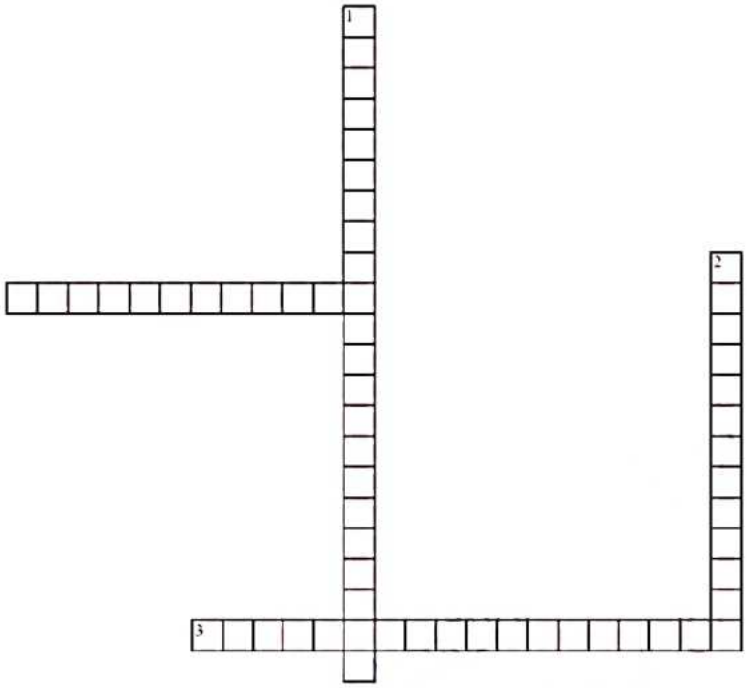
CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Present only in benign conditions

Down

1. May be foreign particles are entered into the body

2. Inflammatory Disorders

Telegram - @nextpreladdernotes

40 BREAST TUMORS

Classification of Breast Tumors

- Classified as two main classes:
  - Epithelial
  - Stromal

Epithelial Breast Tumor	Stromal Breast Tumor
• 2 subtypes: o Ductal o Lobular	• 2 subtypes: o Intralobular tumors o Interlobular tumors
• Ductal is classified as o Ductal cancer in situ (DCIS) - precancerous form. o Infiltrating ductal cancer (IDC).	• **Intralobular Stromal Tumors** Rises from the lobule. o Fibroadenoma o Phyllodes Tumor
• Lobular is classified as o Lobular cancer in situ (LCIS) - precancerous form. o Invasive lobular cancer (ILC).	• Interlobular Stromal Tumors o Lipoma o Angiosarcoma o Myofibroblastoma

Myofibroblastoma: Has equal prevalence in both males and females unlike other breast cancers.

- More cases present with Stromal tumors.

Stromal Tumors

01. Fibroadenoma

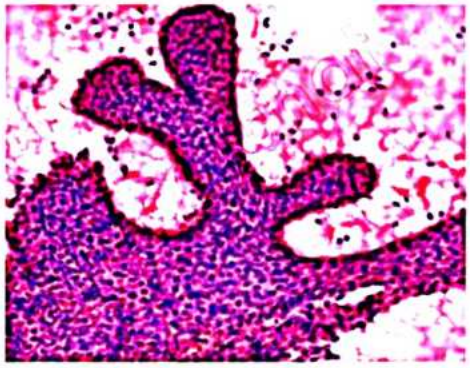
- **Clinical presentation:** Soft to firm lump, mobile.
- **Radiology**
  - Shows breast mouse - The lump is mobile and slippery and hence called breast mouse.
  - Also shows popcorn calcification

Popcorn calcification

- 2 types:
  - **Radiologically Calcification** - seen in fibroadenoma and pulmonary hamartoma.
  - **Pathological calcification**- seen in RS cells of nodular lymphocyte predominant hodgkin's Lymphoma (NLPHL).

- **Molecular alteration:** Alterations in MED12 gene - associated thrice in pathology.
  - Fibroadenoma
  - Phyllodes Tumor
  - Fibroids (leiomyoma)
  - Mnemonic: Family MED
- Drug associated with bilateral fibroadenomas: Cyclosporine

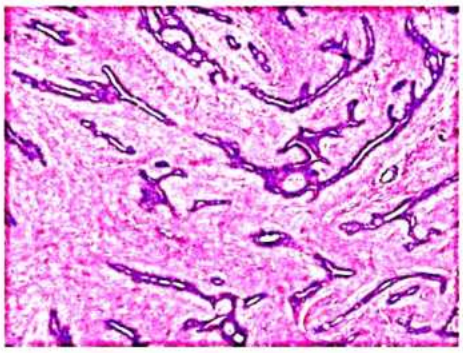
FNAC of fibroadenoma



- When a patient arrives with Breast mouse FNAC is performed. Findings are:
  - Staghorn clusters of cells
  - Bare nuclei
  - Other Staghorns
    - → **Staghorn calculus** - Present on kidneys called struvite calculus, made of triple phosphatase.
    - → **FNAC** - Fibroadenoma
    - → **Blood vessels** - Hemangiopericytoma
    - → **Megakaryocyte** - Essential Thrombocytopenia.

Histopathological Findings

- Intracanalicular **fibroadenoma** - Ducts are compressed.



- Pericanalicular **fibroadenoma** - Ducts are open.

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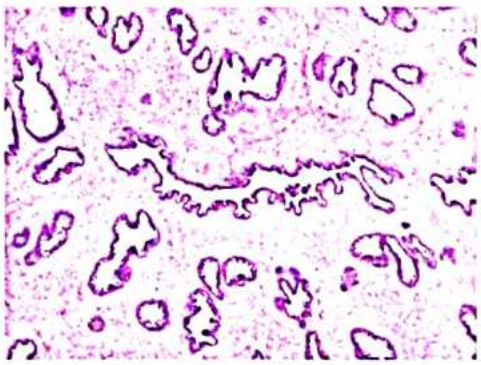
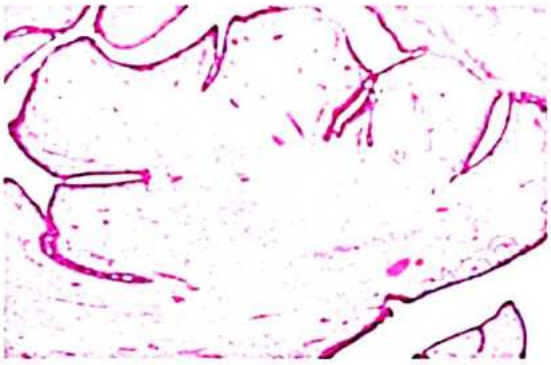


- In both the findings the prognosis remains the same.
- **Complex fibroadenoma:** if it helps fibroadenoma has following features:
  - Cyst > 0.3 cm.
  - Papillary apocrine change (metaplasia change).
  - Sclerosing adenosis.
  - Mnemonic: ComPlexeS.

02. Phyllodes Tumor 00:14:32



- Earlier known as - cystosarcoma phyllodes.
- Phyllodes means phylum which is a leaf-like structure.
- Phyllodes tumors can be benign or malignant.

Criteria (on the basis stromal)	Benign	Borderline	Malignant
Stromal cellularity and atypia	Mild/ minimal	Moderate	Severe/ marked
Stromal overgrowth	Minimal	Moderate	Marked
Mitoses/10 high power fields - definitive criteria	0 to 4 mitoses	5 to 9 mitoses	> or = 10 hpf

Same Mitosis Cut Off

- Both phyllode tumors and leiomyosarcoma of the uterus.
- Malignancy is mitosis > = 10.

Risk Factors of Breast Cancer

- **Sporadic factors:** Non familial
  - Age
  - **Early menarche** - increases estrogen levels.
  - **Late menopause** - increases estrogen levels.
  - Nulliparity
  - Sedentary lifestyle, obesity, high-fat diet.
  - Smoking
- **Genetic factors:** Non Familial
  - Related to BRCA1 and BRCA2 - comprises 90% of Familial breast cancer.
  - Recalling Neoplasm chapter,
    → BRCA1 - present on chromosome 17.
    → BRCA2 - present on chromosome 13.

Gene	Frequency	Tumors
BRCA1	55%	• Breast cancer • Ovarian cancer • Prostate cancer
BRCA2	35%	• Breast cancer • Ovarian cancer • Prostate cancer • Associated with male breast cancer
P53	<1%	Li Fraumeni syndrome (autosomal dominant inheritance)
PTEN (Present on the 10th chromosome)	<1%	Cowden syndrome: Includes: • Polyps, breast cancer • Thyroid cancer, trichilemmoma • Endometrial cancer • **Mnemonic:** PTEN
STK11	<1%	Peutz Jeghers syndrome: Associated with • Breast cancer • Colon cancer
CDH1 (E-Cadherin)	<1%	Invasive lobular carcinoma Diffuse gastric cancer
ATM gene	5%	Ataxia Telangiectasia (blood vessel dilation).
CHEK2	5%	Radiation induced breast cancer

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PALPB2 (monoallelic loss)	<1%	• Pancreatic cancer • Prostate cancer • Breast cancer • **Mnemonic: PALPB2**

Li Fraumeni Syndrome

- The syndrome includes:
  - Breast cancer
  - Brain cancer
  - Bone cancer
  - Blood cancer
  - Mnemonic: BBBB tumors

P53: Most common genetics of sporadic breast cancer.

- Can be included in sporadic risk factors.

E-cadherin

- Hold cells together like glue.
- It's mutation causes:
  - Diffuse gastric cancer
  - Invasive lobular carcinoma
  - Can be remembered as GoLu tumors.

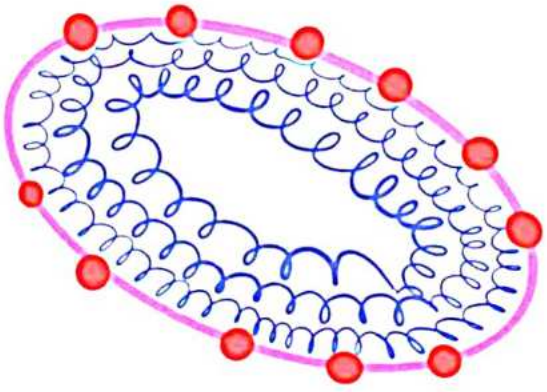
PALPB2

- Has 2 alleles
  - **Monoallelic loss** - Prostate, Pancreatic and breast cancers.
  - **BI Allelic** - Fanconi anemia.

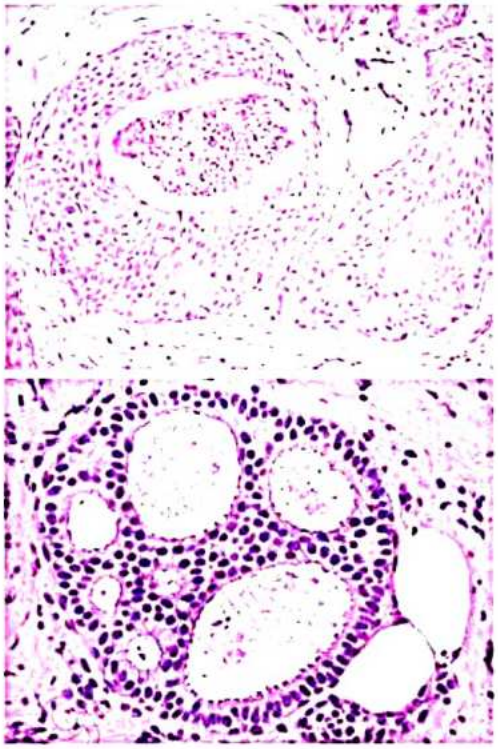
Epidermal Tumors

01. Ductal Carcinoma In Situ

- Precancerous situation.
- The atypical cells fill up the ducts.
- Both the myoepithelial cells and basement membrane are present.
- When the basement membrane is absent, it can be termed as cancer.



Types of Ductal Carcinoma In Situ



Comedo-necrosis, cribriform DCIS.

- **Solid DCIS**
  - Duct is full of cells.
  - Becomes solid.
- **Papillary:** The cells have made small proper papillary.
- **Micropapillary:** The cells make very tiny papillary.
- **Comedo-necrosis:** necrosis in the middle of the duct.

- **Cribriform:** Meaning sieves

Cribriform Patterns in Pathology

- Found in
  - Breast - cribriform DCIS
  - Prostate - carcinoma pattern 4
  - Salivary glands - adenoid cystic carcinoma.

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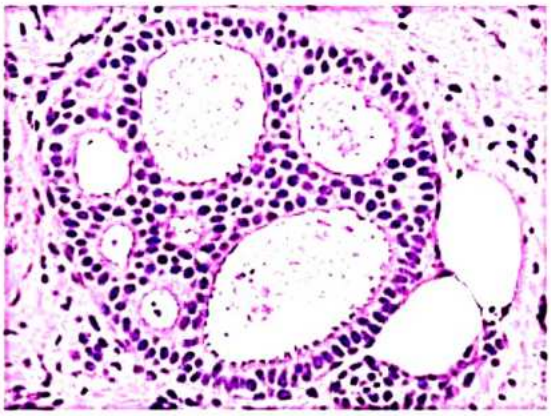
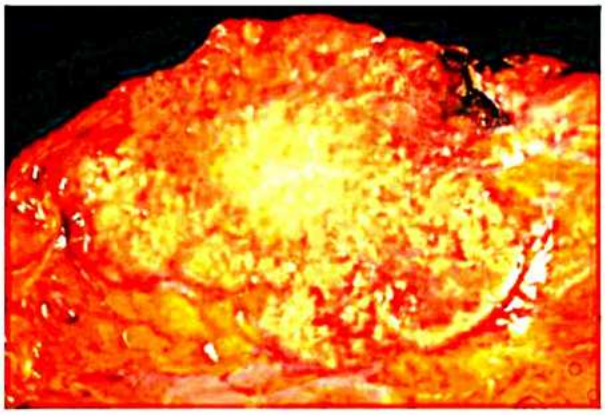


Cribriform Patterns

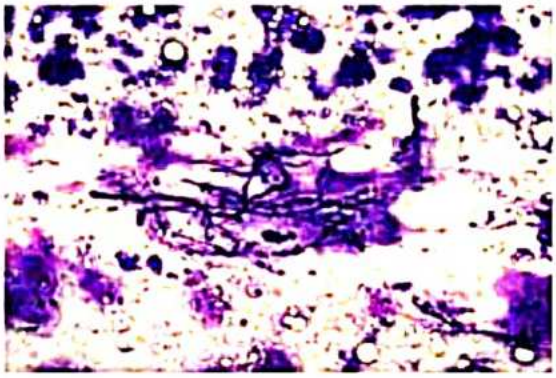
02. Infiltrating Ductal Carcinoma, NOS 00:40:10



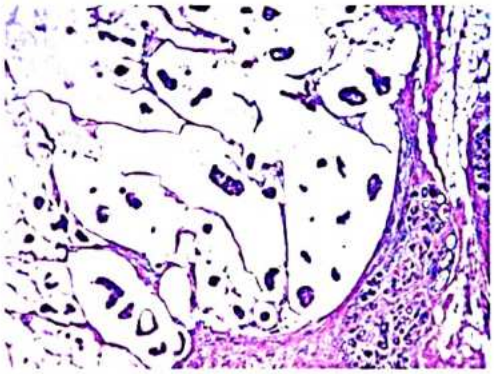
- Infiltrating Ductal Carcinoma, not otherwise specified.
- It is not a special type of cancer.
- **Gross appearance:** Gritty due to calcification, fibrosis.
- Microscopically: Adenocarcinoma

Special Types of IDC

- **Tubular carcinoma:** Shows the presence of regular tubules.
  - Have a good prognosis.
- **Mucinous or Colloid carcinoma:**



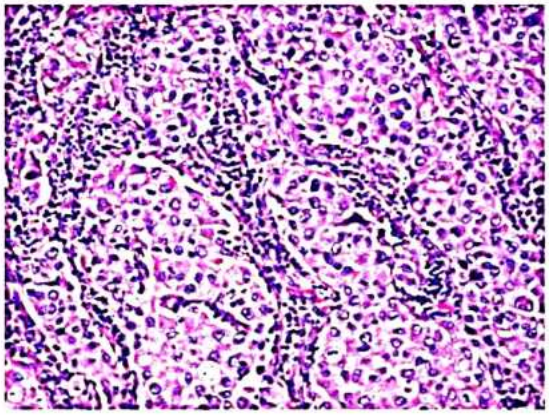
1. FNAC finding of mucinous carcinoma.



2. Biopsy findings

  - Has pools of mucin extracellularly and may be intracellularly (called signet ring cell).
  - FNAC: shows chicken wire blood vessels.
  - Biopsy: Shows more mucin both extracellular and intracellular with signet rings.
    Floating cells.
- **Clinical findings**
  - Soft to firm lump due to mucin
  - So this can be mistaken for a benign tumor.

Medullary carcinoma 00:48:15



- Medullary carcinoma presents with:
  - Pushing membrane
  - Prominent nucleoli
  - Lymphoplasmacytic infiltrate - the only visible presentation.
  - Mnemonic: PPP.

Lymphoplasmacytic infiltrate

- Found in,
  - Breast - medullary carcinoma
  - Testes or ovaries - seminoma or dysgerminoma.
  - Liver - Autoimmune hepatitis.
  - Blood - Lymphoplasmacytic lymphoma
    → Also called waldenstrom's macroglobulinemia.

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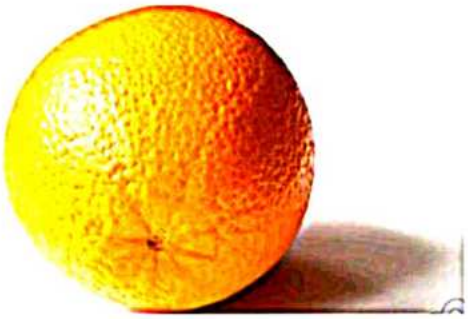
- **Other special types**
  - Apocrine carcinoma
  - Metaplastic carcinoma
  - **Secretary carcinoma:** Occurs in younger people.
  - **Inflammatory carcinoma:** Tumor cells spread to the dermal lymphatics.
    → Skin has Peau d' orange appearance.
    → Poor prognosis.

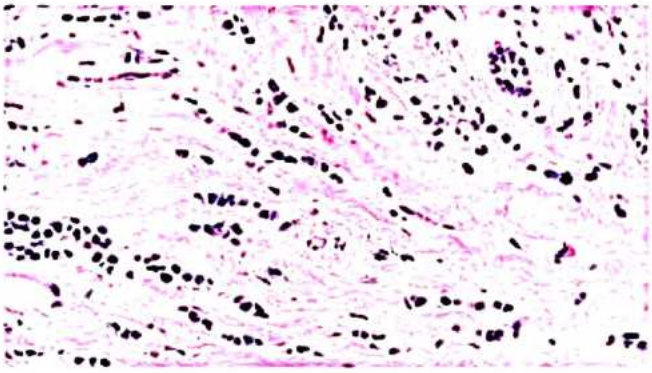


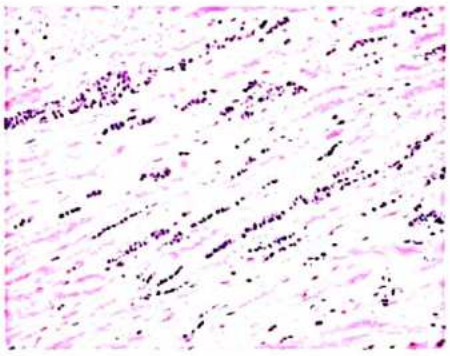
03. Invasive Lobular Carcinoma 00:53:47

- **Genetic cause:** CDH1 gene mutation.
  - E-cadherin levels decrease.
- Patient presents with:
  - Bilateral
  - Multicentric

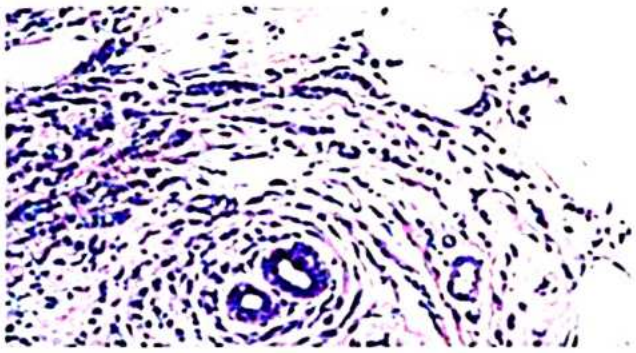
Note: If one breast has ILC the other breast should also be examined and followed up.

- **Microscopy of ILC**

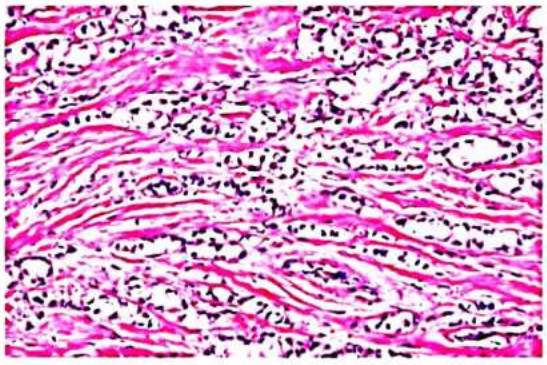




  - Tumor cells are present back to back, just like the army men.
  - Hence, called Indian File Pattern.
  - Also shows minimal desmoplasia (less fibrosis) - tumor is not hard.
  - The ducts are unaffected and normal but surrounded by the tumor cells Called targetoid spread.



  - The tumor cells try to enter the normal ducts called the pagetoid spread.



  - Can also contain signet ring cells - contains little mucin at some places.

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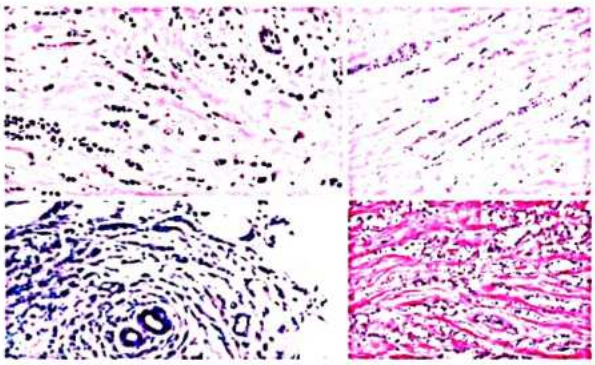
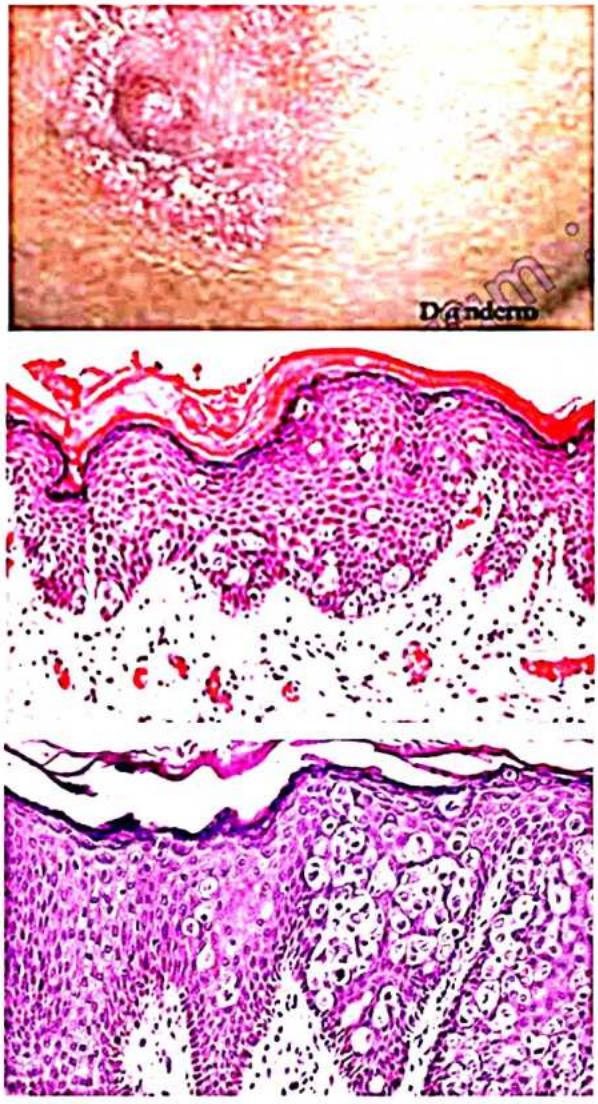


Microscopy of ILC

Sum Up

- Microscopy of ILC shows:
  - Indian File Pattern.
  - minimal desmoplasia (less fibrosis)
  - targetoid spread & pagetoid spread
  - signet ring cells

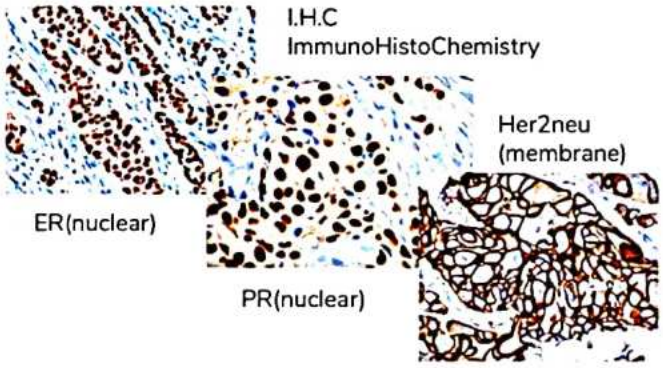
04. Paget's Disease 01:00:18



- The two similar paget's diseases are: Breast and vulva.
- Bone paget's disease is different from the above two.
- Dermatology lesions are seen
  - Crusting
  - Excoriation
  - Pruritus
- **Biopsy** findings shows
  - Empty cells in the epithelium - called paget's cells.
- **Stains:** The cells have clear cytoplasm as it contains:
  - **Glycogen** - PSA positive.
  - **Mucilage** - mucicarmine positive.
  - **Fats** - oil Red positive.
- For paget's disease mainly associated with breast few other things should be considered like: Hormone profile
  - ER negative
  - PR negative
  - Her2neu positive

Molecular Classification of Breast Cancer 01:03:50

- Initially Based on the gene expression profiling.
- Later to make it simple the basis changed to ER, PR and Her2neu.



Measuring of ER, PR and Her2neu

- ImmunoHistochemistry used as staining technique - gives brown color.
- In ER and PR the nucleus becomes brown.
- In Her2neu also known as EGFR2 or ERBB2 - membrane becomes brown.
- Scorings
  - For ER and PR - ALLRED scoring is done.
    → Even 1% brown cells - positive test.
  - For Her2neu
    → 0 to 1 - negative
    → 2 - equivocal (not negative, not positive) - FISH is performed.
    → 3 - positive - treated with trastuzumab.

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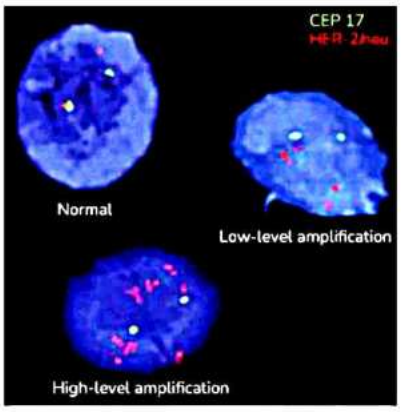
FISH Test 01:09:18



- Chromosome 17 contains Her2neu gene.
- If the Her2neu gene is amplified the test is positive.
- To find the Her2neu application, a formula is given:
  - Her2/CEP17 >2.2 - amplification occurred - positive.

Luminal A	Luminal B	Her2 rich	Triple negative or basal
ER - positive	ER - positive	ER - negative	ER - negative
PR - positive	PR - positive	PR - negative	PR - negative
Her2neu - negative	Her2neu - negative • Sometimes Her2neu is positive. • Also called triple positive.	Her2neu - positive	Her2neu - negative

- To differentiate luminal A from liminal B: Ki67 is used.
  - Ki67 <14% - luminal A.
  - Ki67 >14% - luminal B.

Ki67: MIB1 labeling index.
- Proliferation marker.

Luminal A	Luminal B	HER2 rich	Triple-negative
• Tubular carcinoma • Mucinous carcinoma • Papillary carcinoma • Low grade ILC	High grade ILC	Apocrine carcinoma **Mnemonic:** happy and rich	• Medullary carcinoma • Metaplastic carcinoma

- **Luminal A** - most common tumors.
- **Worst prognosis** - triple negative.
- **BRCA1** - associated with triple negative category.
- **BRCA2** - associated with two luminals.

Update in Molecular Classification

Claudin low
- **Type of triple negative:** ER, PR, HER2NEU negative.
- Low claudin expressions
- Has poor prognosis.

Modified Bloom Richardson Scoring
- Done by pathologist microscopically.
- **Staging**
  - Tubular formation
  - Nuclear pleomorphism
  - Mitosis
- Mnemonic: TNM.

MCQs

Q. A 30-year-old woman suffers a traumatic injury to her breast while playing soccer. Physical examination reveals a 3-cm area of ecchymosis on the left breast. Two weeks later, the patient palpates a firm lump beneath the area where the bruise had been located. Which of the following is the most likely pathologic diagnosis?

a. Duct ectasia
b. Fat necrosis
c. Fibrocystic change
d. Granulomatous mastitis

Explanation
- **Trauma** - Usually Fat necrosis
- Fat necrosis shows calcium deposition and makes the lump more firm.

Q. Cancer detected in one breast which can be screened in contralateral breast

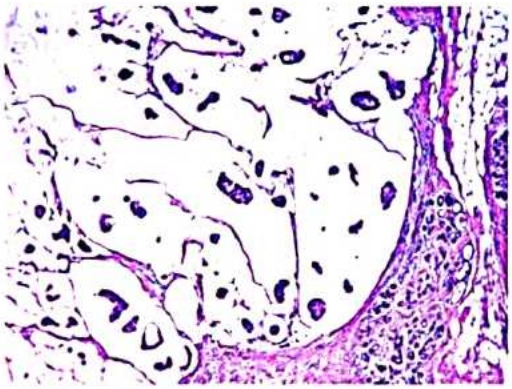
a. Lobular
b. Ductal
c. Medullary
d. Colloid

Q. Only breast tumor having same incidence in males as in females

a. Fibroadenoma
b. Lipoma
c. Angiosarcoma
d. Myofibroblastoma

Telegram - @nextpreladdernotes

Q. A 30-year-old female presented with 4 cm mass in the right breast. Biopsy showed densely packed cells with bland nuclei and mucin infiltrating the stroma. What is your diagnosis?

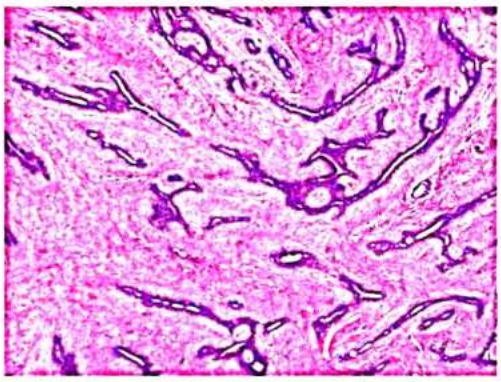


a. Invasive papillary carcinoma
b. Medullary carcinoma
c. Apocrine carcinoma
d. Colloid carcinoma

Explanation

- Image shows pools of mucin - Mucinous carcinoma.
- Mucinous another name - Colloid carcinoma.

Q. A 24/f presented with a painless left sided breast lump for the last 2 months. Local examination revealed soft mobile, non tender mass. Excision biopsy was done and histopathological image is as shown below. What is your diagnosis?



a. Phyllodes tumor
b. Intraductal carcinoma breast
c. Fibroadenoma
d. Ductal carcinoma in situ

Explanation

- Mobile mass - Breast mouse (Fibroadenoma).
- Histopathology shows - compressed ducts.
- Both clinical and histo supports Fibroadenoma.

Q. Radiation induced breast cancer is associated with?

a. PALPB2
b. BRCA1
c. BRCA2
d. CHEK2

Telegram - @nextprepladdernotes

PREVIOUS YEAR QUESTIONS

Q. Which of the following shows breast necrosis and calcification? (FMGE 2017)

A. Comedo subtype of DCIS

B. Cribriform subtype of DCIS

C. Colloid carcinoma

D. Lobular carcinoma in-situ

Q. In a known case of breast cancer, Fluorescent In-Situ Hybridization (FISH) for gene amplification will be done based on which of the following immuno-chemistry (IHC) staining for HER 2/Neu? (AIIMS 2017)

A. 1

B. 2

C. 3

D. Any of the above

Q. A middle aged female presented with a 4cm mass in upper outer quadrant of the breast. Biopsy showed densely packed cells with large lake of mucin. Which of the following is the most likely diagnosis in this patient? (NEET 2020)

A. Medullary carcinoma of breast

B. Colloid carcinoma of breast

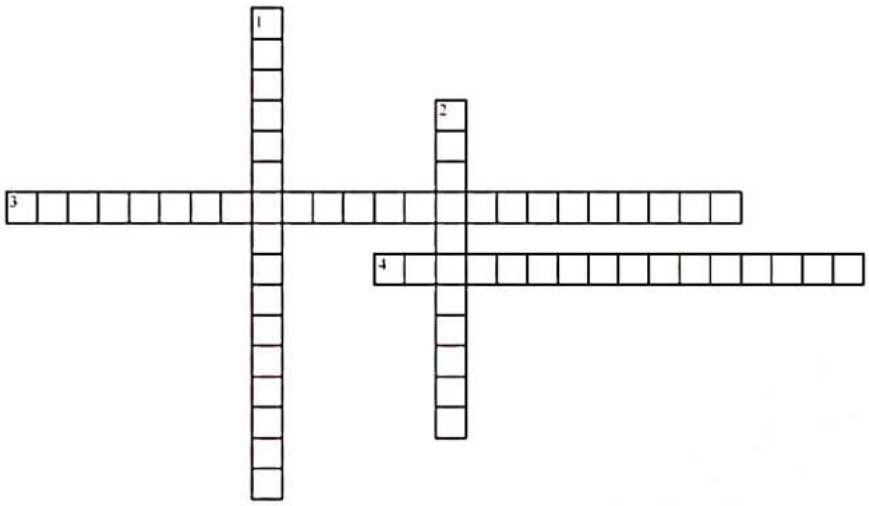
C. Tubular carcinoma of breast

D. Papillary carcinoma of breast

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Its Genetic cause is CDH1 gene mutation.

4. Present on kidneys called struvite calculus, made of triple phosphatase

Down

1. In Inflammatory carcinoma, tumor cells spread to the _____

2. The lump is mobile and slippery and hence called breast mouse.

Telegram - @nextpreladdernotes

41 BASICS OF CENTRAL NERVOUS SYSTEM

Cells of the Central Nervous System 00:00:14

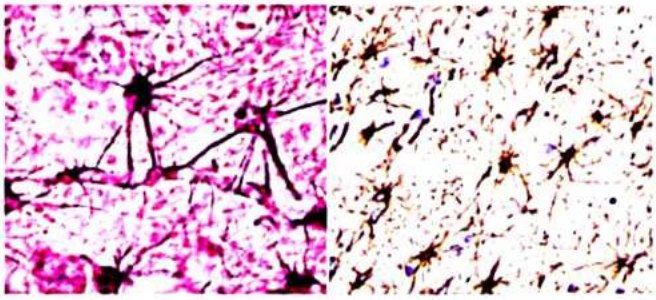
They are classified into two different types

- **Glial cells:** These are further divided into
  - Astrocytes
  - Oligodendrocytes
  - Ependymal cells
- Macrophages

1. Glial Cells 00:00:24

a. Astrocytes 00:00:51

- These are star-shaped cells.
- **Immunohistochemistry stain:** Glial Fibrillary Acidic Protein (GFAP) marker



Functions

- Formation of Blood Brain Barrier (BBB).
- Involved in repair mechanism

b. Oligodendrocytes 00:01:31

- Formation of myelin sheath in the CNS.

Special stain for myeline **Luxol Fast Blue**

Question to be known

Questions	Answers
How myelin sheath is formed in PNS?	Schwann cells

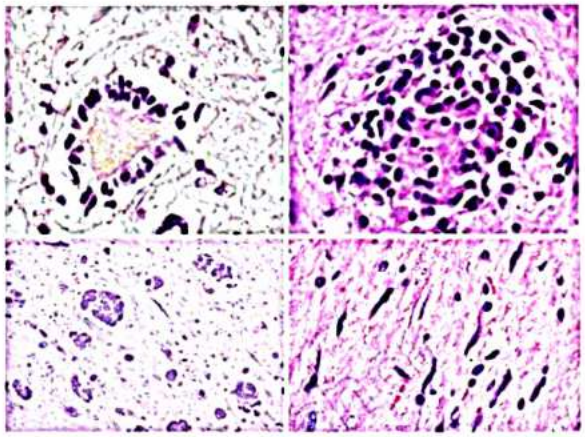
c. Ependymal cells (2:39)

- Present in the brain ventricles
- Formation of Cerebrospinal fluid (CSF).

2. Macrophages 00:03:08

- **Other name:** MicroGlia (in brain)

Modified Macrophages



- Rod cells: Neurosyphilis
- MicroGlial Nodules: HIV positive
- Neuronophagia: Polio, Rabies
- Small clusters (Globoid bodies): Krabbe's disease Lysosomal storage disorder

Questions	Answers
How do brains change in HIV positive individuals?	MicroGlial Nodules are formed
In which condition rod shaped cells are found?	Neurosyphilis (tertiary syphilis I
In which condition neuronophagia occurs? Image: 6:20	Polio and Rabies
What are Globoid bodies? And in which condition they are found?	These are small clusters found in lysosomal storage disorders (KRABBE'S disease)

Response to Injury 00:07:45

- Astrocytes become giant and pink (Gemistocytes)
- Alzheimer (no memory loss) type 1 astrocytes seen in progressive multifocal leukoencephalopathy.
- Alzheimer (no memory loss) type 2 are found in ammonia damage (Hepatic encephalopathy).

Other conditions when injury to the brain is present

- **Rosenthal fibers:**
  - Not specific
  - Seen in tumor called Juvenile Pilocytic Astrocytoma

Telegram - @nextprepladdernotes

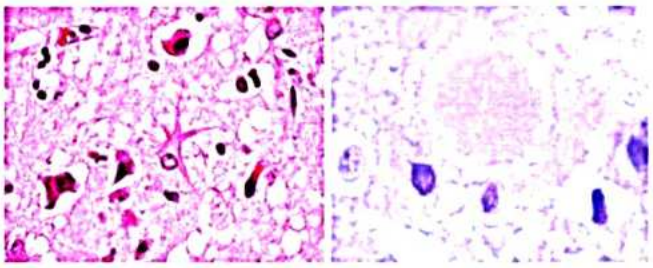
- In Alexander disease
- Gliosis (response to injury)

How are Rosenthal fibers made? — They are made of ubiquitin, alpha beta crystallin, HSP, GFAP (In In P P)

- **Corpora amylacea:** Known as Polyglucan body.

Prion Diseases 00:12:02

- **Other Name:** TSE (Transmissible Spongiform Encephalopathy)
- **Route of transmission:** Iatrogenic route
- **Spongiform:** Vacuolization (inside the cell)



Pathogenesis 00:13:59

- Normal Prion protein are found in nerve cells (alpha helical)
  ↓misfolding
- formation of PrPsc (beta pleated configuration)
  ↓
- Mainly occurs due to the polymorphism at codon 129

Diseases 00:15:33

- Creutzfeldt-Jakob disease
- Fatal familial insomnia (FFI)
- Kuru disease - PAS & Congo red positive
- Scrapie disease (sheep)
- Bovine spongiform encephalopathy (animal association)
- Mink transmissible encephalopathy (animal association)

To Remember: All these show spongiform changes except FFI.

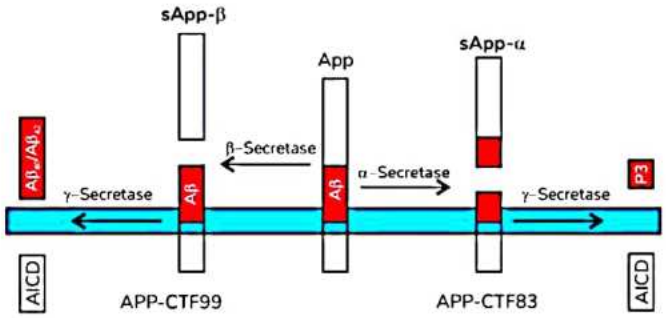
Codons 00:18:10

- Prion disease is caused due to the mutation of codon 129
- Aflatoxin (p53 gene) due to the mutation of codon 249
- BRAF V600 E
- JAK2 V617F is seen in Polycythemia

Neurodegenerative Disorders 00:20:09

1. Alzheimer's Disease 00:20:50

- More prone to old age people (>70 years).



- Amyloid Precursor Protein (APP) gene is present in chromosome no.21
- APP gene has two options: Alzheimer's disease
  → Amyloidogenic pathway
  - Have beta secretase activity (Amyloid beta 40, 42)
  - gamma secretase enzyme

  → Non amyloidogenic pathway
  - Have alpha secretase activity
  - gamma secretase enzyme

A beta 42- key initiator of alzhiemers disease
↓
Beta plaque
↓
Meyernet Nucleus
↓
Reduced Ach
↓
Dementia

Genetics of Alzheimer's Disease 00:26:43

- **APP gene:**
  - If chromosome 21 amount is increased (trisomy)
  - More number of APP
  - Causes Presenile Alzheimer's disease
- **Presenilin 1 gene**
  - Present in chromosome 14
  - Increases the levels of gamma secretase activity
- **Presenilin 2 gene**
  - Present in chromosome 1
  - Activity of gamma secretase enzyme is increased
- **ApoE4 gene**
  - Present in chromosome 19
  - Early onset of Alzheimer's disease kif defect is present)

Pathology of Alzheimer's Disease 00:30:02

- **Neuritic plaques:**
  - Core of Amyloid (Abeta 42) in the center.
  - Damaged nerves are accumulated (dystrophic nerve fibers)

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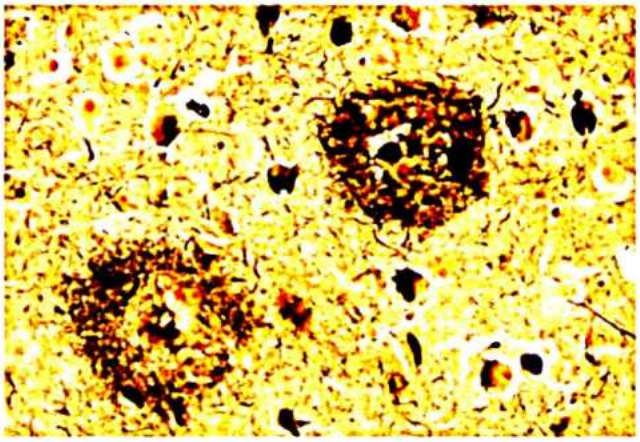
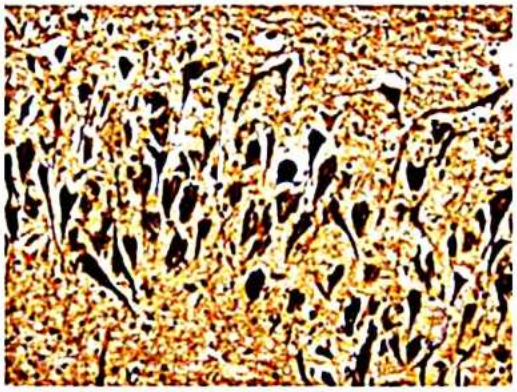
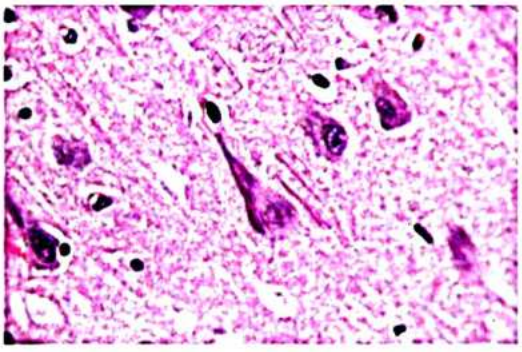
- Bielschowsky Stain is used in the brain to see neuritic process

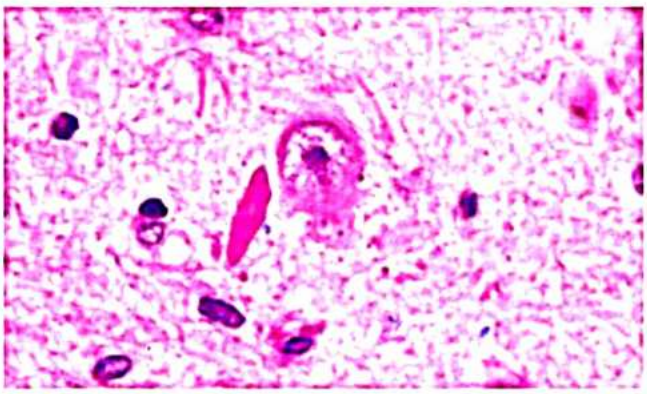


- **NeuroFibrillary Tangles**
  - Flame cells
  - Made of hyperphosphorylated Tau protein





- **Cerebral amyloid angiopathy:**
  - Blood vessels present in the brain are affected due to Abeta 40.
- **Granula-vascular degeneration:**
  - Degeneration of cells
- **Hirano bodies**
  - Needle shaped body
  - Made of actin



ii. Parkinson's Disease 00:35:54

- Decrease in dopaminergic neurons
- Decrease in melanin (pale substantia nigra)
- Symptoms: Tremors, Rigidity, Slow movement (Bradykinesia)

Genetics of Parkinson's Disease

Mutations in the genes.

- Alpha synuclein
  - Increase its function
  - Increased deposition of alpha syn-nucleon
  - Forms lewy bodies
- LRRK2
- PINK
- Parkin

Questions	Answers
In which disease Lewy bodies are found?	Parkinson's disease
What is a lewy body made up of?	Alpha-synuclein

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iii. Huntington's Disease 00:41:46

- **Other Name:** Huntington's Chorea (purposeless jerky movements).
- CAG repeats on the exon of chromosomes 4
- Proteins: Polyglutamate, Huntintin, and Ubiquitin proteins

↓

Accumulate in caudate nucleus

↓

Chorea

↓

Purposeless jerky movements

iv. Amyotrophic Lateral Sclerosis 00:44:20

- Involves both upper motor neuron and lower motor neuron
- Presence of bunina bodies
- SOD 1 gene mutations

Overview - Most Expected Questions 00:45:33

Disease	Summary
Alzheimer's Disease	• APP (Chromosome: 21) • PS 1 (Chromosome: 4) • PS 2 (Chromosome: 1) • APDE4-Hirano bodies: Made of Actin
Parkinson's Disease	• Parkin and pink gene mutation • Lewis bodies: Made of Alpha-synuclein
ALS	• Mutations of SOD1 • BUNINA bodies
Huntington disease	• CAG chromosome 4 • Increase of Huntintin, Ubiquitin proteins

Protein with Disease 00:48:08

Protein	Disease
A beta	Alzheimer's disease
Alpha synuclein	Parkinson's disease
Polyglutamine aggregates	Huntington's disease
FUS	Fronto temporal lobar degeneration
TDP43	Fronto temporal lobar degeneration
Tau (Tau pathies- CCAPP)	• Fronto temporal lobar degeneration • Cortico-basal encephalopathy • Chronic traumatic encephalopathy • Alzheimer's disease • Parkinson's disease • Progressive supranuclear palsy

FTLD: They have C9orf72 expansions.

Multiple Choice Questions

Q. Ubiquitin deposition is seen in which of the following conditions?

a. Alzheimer's disease
b. Huntington's disease
c. Meningioma
d. FTLD

Q. Lewy bodies are composed of?

a. Tau protein
b. Alpha synuclein
c. Beta plaques
d. Actin

Q. Hirano bodies are composed of?

a. TauTau protein
b. Alpha synuclein
c. Beta plaques
d. Actin

Q. Which of the following is/are seen in Alzheimer's disease?

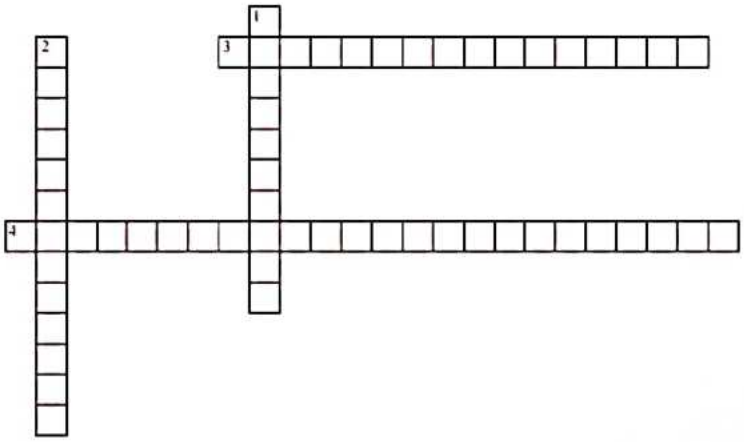
1. CAA
2. Granulovacuolar degeneration
3. Flame cells
4. Hyper phosphorylated tau proteins
5. Hirano bodies
6. Neuritic plaques

a. 1,2,4,5
b. 1,2,4,5,6
c. 1,2,3,4,5,6
d. 2,3,4,5,6

Telegram - @nextpreladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Formation of myelin sheath
4. Small clusters (Globoid bodies)

Down

1. Astrocytes
2. Rod cells

Telegram - @nextpreladdernotes

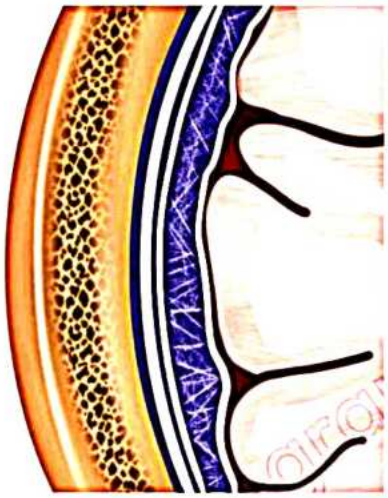
42 BRAIN TUMORS

Q. Which is the most common malignancy in the brain/lungs/heart/liver/bones?
Ans. METASTASIS - not primary tumor

Q. Most common cancer to send brain metastasis?
Ans. Small cell lung cancer

Q. Cancer that never metastasize to the brain?
Ans. Prostate cancer

Q. Which is the cancer that goes to the meningeal part?
Ans. Leptomeningeal carcinomatosis commonly from breast cancer



Primary Brain Tumors - Grading

A - Atypia — Bad looking cell
M - Mitosis
E - Endothelial Cell Proliferation
N - Necrosis

- Grade 1 -AMEN - None of These present - Good tumor
- Grade 2 - Atypia
- Grade 3 - Atypia & Mitosis
- Grade 4 - AMEN [3 Out Of 4] Vey very very bad tumor

Classification Of Brain Tumors

Brain Tumors	Types
Gliomas - Glial cells	• Astrocytoma • Oligodendroglioma • Ependymoma
Neuronal tumors	• Ganglioglioma - most common • Gangliocytoma • Neurocytoma • DNET (dysembryoplastic neuroepithelial tumor) o Floating neurons in pools of mucopolysaccharide
Undifferentiated Tumors	• Medulloblastoma • Atypical teratoid/ atypical rhabdoid o SNF/INI mutation - rhabdoid tumor shows snf mutant
Meningioma	Risk factors • Females • Pregnancy • Radiation • NF2- MISME Progeny receptors POSITIVE

Gliomas - Glial cells

Astrocytoma

- Grade 1
  - o Juvenile Pilocytic Astrocytoma(JPA)- Small tumor

Subependymal - Giant Cell Astrocytoma - (associated with tuberous sclerosis)

Question related SEGA

Q. Which syndrome is it associated with ?

- Tuberculosis

- Grade 2
  - o PXA
  - o Diffuse astrocytoma
  - o Fibrillary astrocytoma

- Grade 3
  - o Anaplastic astrocytoma

- Grade 4
  - o Glioblastoma - very bad

Juvenile Pilocytic Astrocytoma - CCC factor

Age	Child
Site	Cerebellum

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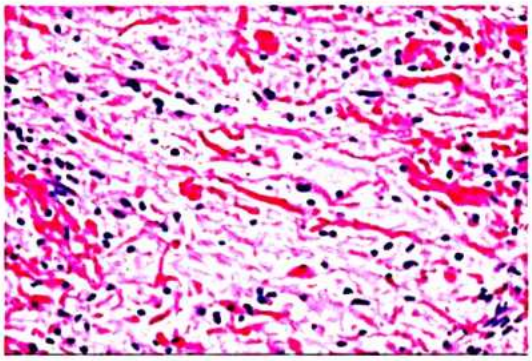
Gross Cystic Lesion with mural nodule

Mutation BRAF- KIAA FUSION
BRAF $V_{600}E$ mutation
- Pick up one - opt for BRAF KIAA FUSION

Microscopic



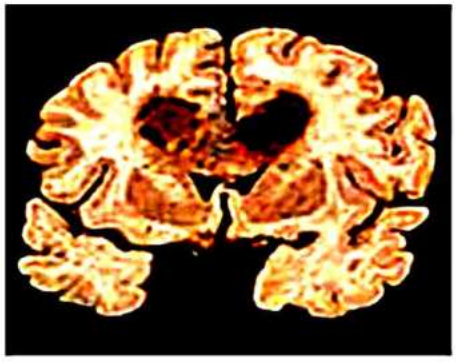
- ROSENTHAL FIBRE - fiber like structure - non-specific -Injury/Alexander disease
- MULBERRY BODY - intagled body of fibers (Robbins 10th edition)
  - Alpha beta crystallin, ubiquitin, HSP & GFAP (Components)

All good - no tumor

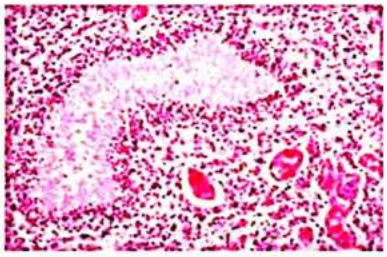
Glioblastoma 00:13:45

Age Most common malignant brain tumor in adult

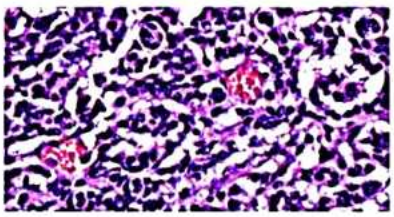
Gross Looks like **butterfly glioma** - cross the midline



Microscopy Serpentine necrosis - surrounded by pseudopalisading of tumor cells



Glomeruloid Body - **Endothelial Cell proliferation**



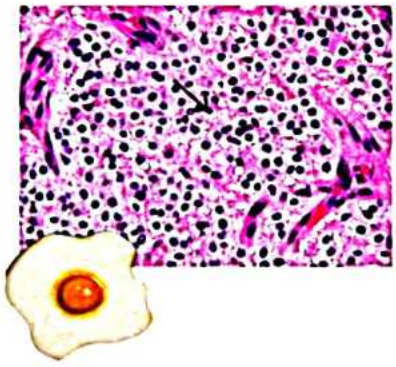
Mutation IDH - wild type predominant (90% normal)
- P53
- PTEN
- EGFR
- TERT

IDH - mutation type (problem)
- P53
- A - ATRX
- T- TERT

Prognosis Grade-IV, Very very bad - Cancerous

Oligodendroglioma
Site - Frontal Lobe
Gross - Calcification +
- **C** - Craniopharyngioma
- - **Oligodendroglioma**
- **M** - Meningioma

Microscopy -
- Fried egg appearance - nucleus & Perinuclear halo



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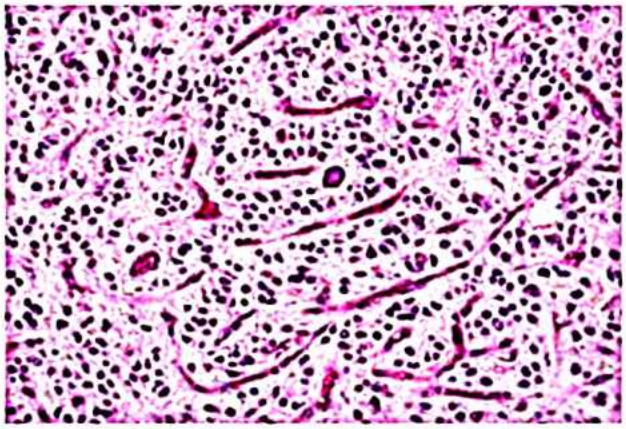
Chicken wire blood vessel



- Cancer spreads ---- perineuronal satellitosis

Mutation -
- IDH mutation - most common
- Codeletion 1p/19q - good response to chemotherapy

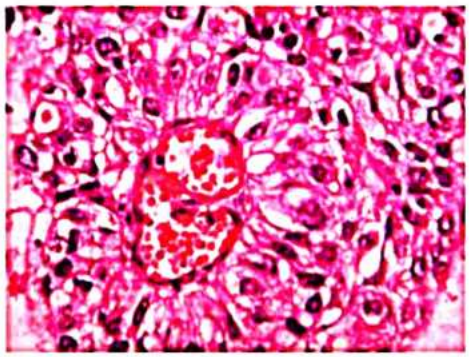
Note:
Fried Eggs Experience
- Brain - oligodendroglioma
- Bone marrow biopsy - hairy cell leukemia
- Testes/Ovary - seminoma/dysgerminoma
- Microbiology colonies
  - Malassezia furfur
  - Mycoplasma

Note:
Chicken Wire
- Blood vessels
  - Oligodendroglioma
  - Breast FNAC - (MUCINOUS/ colloid carcinoma)
- Fibrosis: ALD
- Calcification: chondroblastoma

Ependymoma
Grade II tumor

Site	Children - 4th Ventricle Adults - Spinal Cord
Genetics	MIS M E • Multiple Inherited Schwannoma • Meningiomas • Ependymoma NeuroFibromatosis-2

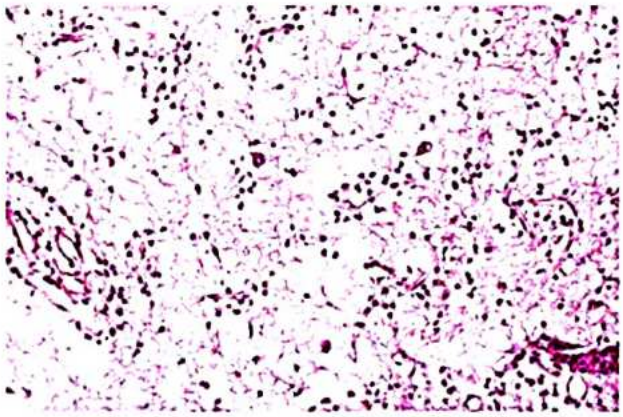
Microscopy Perivascular Pseudorosettes



Updates
Myxopapillary Ependymoma
- Myxoid + papillae
- Located in Filum Terminale

Rela Fusion Ependymoma
R - Rela gene
EL - chr 11
A - above **tentorium in the brian/supratentorial tumor**
- Occurs in children
- Poor prognosis

Neuronal Tumors
- Ganglioglioma - most common
- Gangliocytoma
- Neurocytoma
- DNET (dysembryoplastic neuroepithelial tumor)
  - Floating neurons in pools of mucopolysaccharide



Undifferentiated Tumors
Medulloblastoma - Grade 4

Age	Child
site	Cerebellum not cystic
Drop mets	Cancer drops from cerebellum into spinal cord- CSF

Telegram - @nextprepladdernotes

Microscopy

Homer wright pseudorosette (non-specific)

M- MB

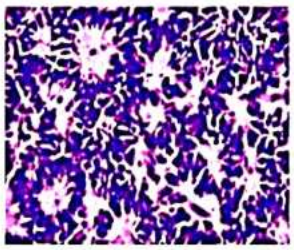
E- Ewing's sarcoma

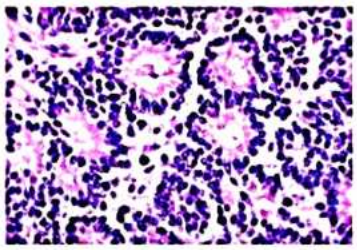
N- neuroblastoma

- Tumor cells are shaped in rose petals

Pink fibrillar material

- When there is something in between the rosettes it is called pseudorosette.
  - Homer Wright pseudorosette (M/E/N) - pink material in centre
  - Perivascular pseudorosette- blood vessel in centre
- When is there is clear and empty centre of the rosette it is called true rosette
  - Flexner wintersteiner rosette





Updates

1) WNT pathway	Best prognosis
2) SHH pathway ↕ **N MYC**	intermediate
3) Non WNT non SHH pathways ↕ **i17q**	Worst Prognosis
4) Non WNT non SHH pathways	intermediate

SHH pathways shows:

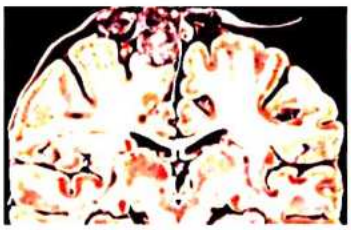
- Desmoplastic Medulloblastoma
- Gorlin Syndrome
  - Ptch gene
  - SHH pathways
  - Basal cell carcinoma
  - Gynae bilateral fibroma ovary
- TUR/COT syndrome
- Cot—colon tumor and tur — turban- hence brain tumor

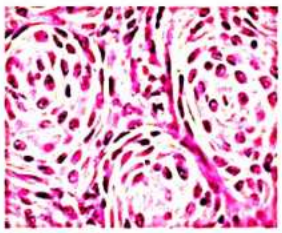
Atypical Teratoid/ Atypical Rhabdoid – AT/AR

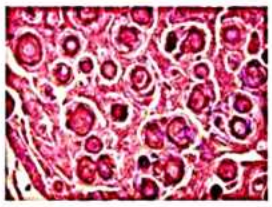
- SNF/INI Mutation
- Sounds like sniffing
- Who shows SNF mutation – RHABDOID tumor shows SNF mutation/ INI mutation

Meningioma

- Dura based tumour -whorling of the tumor cells
- Calcium -psammoma bodies - most common in females
- Grade 1/2/3 of brain tumor – depending on the progression
- Progesterone receptor positive
- Radiation and NF 2 -NeuroFibromatosis

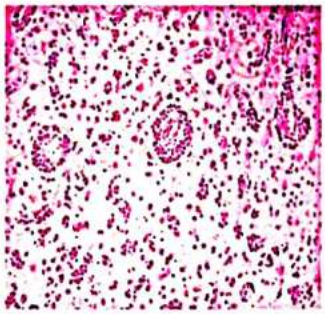


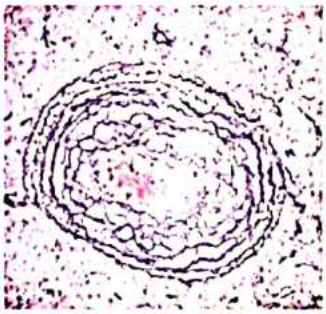




Miscellaneous

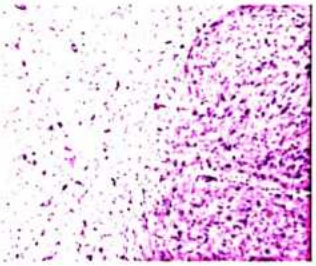
- Lymphoma

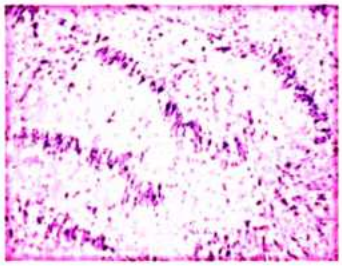




  - EBV infection—HIV+
  - Most common in DLBCL
  - Angiocentric pattern
  - Hooping pattern
- Schwannoma
  - NF2 MISME
  - Can appear in any site
  - Spinal cord — dumbbell shaped\

Microscopically:

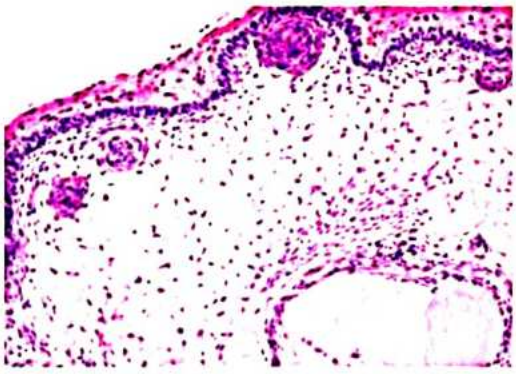




Telegram - @nextprepladdernotes

- Antony A - hypercellular areas
- Antony B - hypoblank
- Verocay bodies
- Malignant peripheral nerve sheath tumor + skeletal muscle rhabdomyoblasts knowns as TRITON tumor

- Craniopharyngioma
  - Origin - Rathke's pouch
  - Site- Suprasellar location + calcification
  - Age - children
  - Clinical feature – vision disturbance & headaches
  - Two types — Adamantinomatous and Papillary

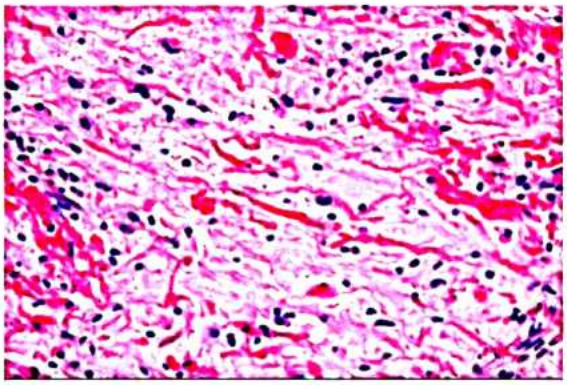


  - Palisading nuclei stellate reticulum
  - Wet keratin – grossly gives brown color like machine-oil appearance

Update - Diffuse Midline Glioma
- Occurs in the midline of brain
- Seen in children
- Spreads out also called glial tumor
- Bad prognosis
- $H_3K_{27}M$- mutation (H3 is histone)

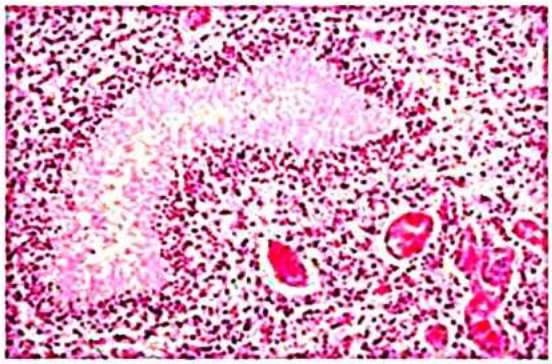
MCQs

Q. 10-year-old male child shows a well-circumscribed cystic lesion in the cerebellum. A mural nodule is identified within the cystic lesion. Biopsy and microscopic evaluation shows the following feature. What is your diagnosis?



a. Oligodendroglioma
b. Glioblastoma
c. Meningioma
d. JPA

Q. A 55-year-old man presents with seizures and muscle weakness but no other neurological signs. CT scan reveals a mass in the left cerebral hemisphere. A left frontotemporal craniotomy is performed. A histological examination of the brain biopsy is shown.



a. Oligodendroglioma
b. Glioblastoma
c. Meningioma
d. JPA

Q. A 35 yrs old female patient presents with epileptic attacks. Radiological investigations reveal the presence of a frontal lobe mass with foci of calcification. Biopsy shows the presence of sheets of cells with a perinuclear halo. The patient is diagnosed with a Grade 2 brain tumor and advised genetic analysis. Treatment of the patient shows a good response to chemotherapy. Which of the following tumors best describes this?

a) Oligodendroglioma
b) Glioblastoma
c) Meningioma
d) JPA

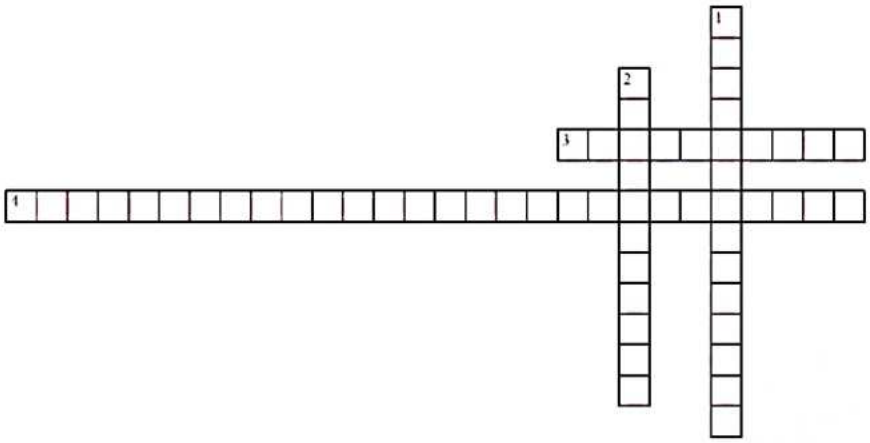
Q.A 33 yr old female with acoustic schwannoma. She also shows the presence of pigmented lesions over the trunk and lower back. On radiological investigations, she has a non-infiltrating brain tumor showing areas of calcification. On biopsy, the tumor shows positivity for PR. Diagnosis.

a. Oligodendroglioma
b. Glioblastoma
c. Meningioma
d. JPA

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Not primary tumor
4. The cancer that goes to the leptomeningeal part

Down

1. Cancer that never metastases to the brain
2. Gliomas

Telegram - @nextpreladdernotes

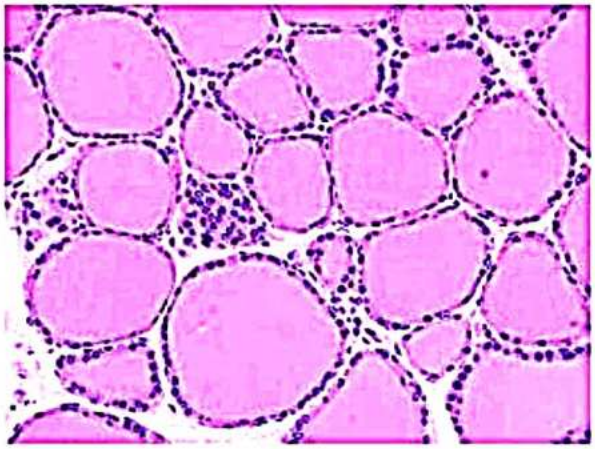
43 THYROID GLAND & TUMOR PATHOLOGY

Endocrine Pathology - Thyroid Gland

- Two techniques used for thyroid gland are
  - FNAC
  - Biopsy
    - → Incisional biopsy
    - → Excision biopsy

Normal thyroid

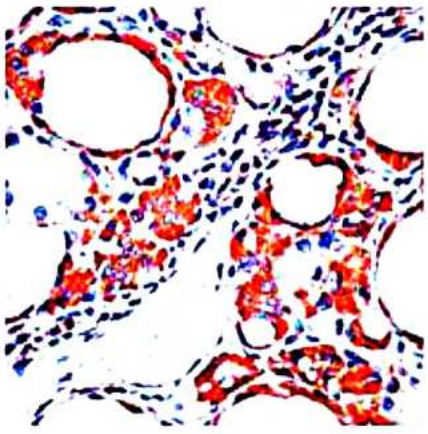
Biopsy specimen



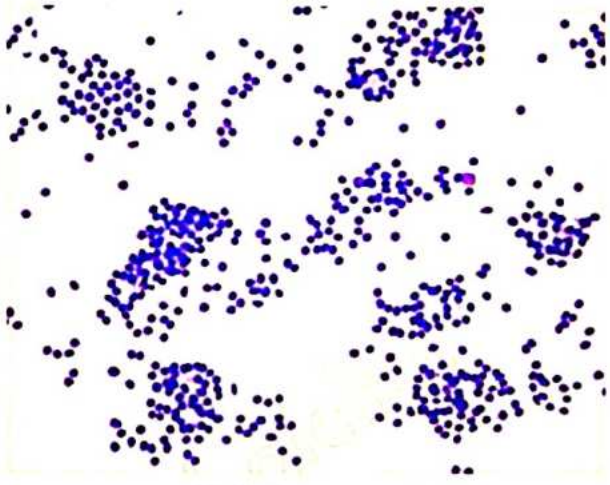
Microscopy

- Thyroid follicle lined by cuboidal lining
- The cuboidal cells are called follicular cells
- Follicles are filled with colloid
- The cells outside the follicle are called parafollicular cells
  - Parafollicular – C cells
    - → Releases calcitonin

- Image showing IHC – brown color



Thyroid - FNAC specimen



- FNAC of thyroid follows BETHESDA system of reporting
- **Adequacy of FNAC of thyroid**
  - At least 6 groups of cells
  - Every group should have at least 10 cells
- **Exception**
  - Presence of Atypical Cell ? (cancer like cells)
  - Inflammation
    - → Hashimoto's thyroiditis - has lot of lymphocytes
  - Colloid cyst or colloid goiter
    - → Cells may be few

BETHESDA system of reporting 00:03:24

- Followed for FNAC of
  - Breast
  - Pap smear
  - Thyroid

Thyroiditis 00:07:42

- Hashimoto's thyroiditis
- De Quervain thyroiditis
- Subacute Lymphocytic thyroiditis
- Riedel's thyroiditis

Hashimoto's thyroiditis / struma lymphomatosum

- Most common cause of hypothyroidism in iodine sufficient areas
- Age group - 4th to 6th decade 45-65 years
  - All autoimmune disorders are common in female
- **Genetics**
  - Polymorphisms
    - → HLA DR3, Dr5
    - → PTPN 22 , CTLA 4

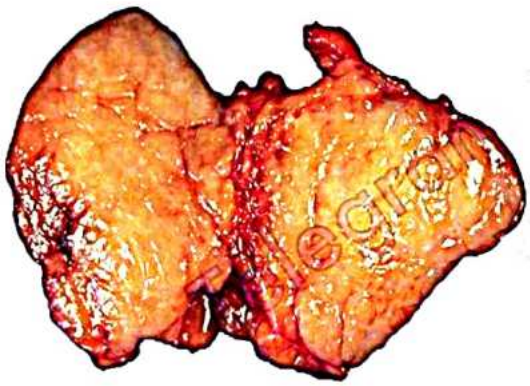
Telegram - @nextprepladdernotes

- **Antibodies**
  - Anti TG antibody - antithyroglobulin antibody
  - Anti TSH antibody

- **Investigations**
  - T3: reduced
  - T4: reduced
  - TSH: increased
    → Hypothyroidism - T3 and T4 low
    → Due to feedback - TSH is high

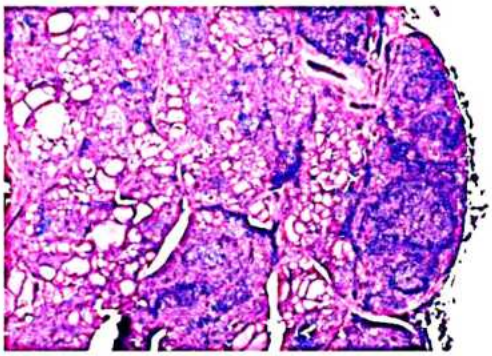
Normal Thyroiditis

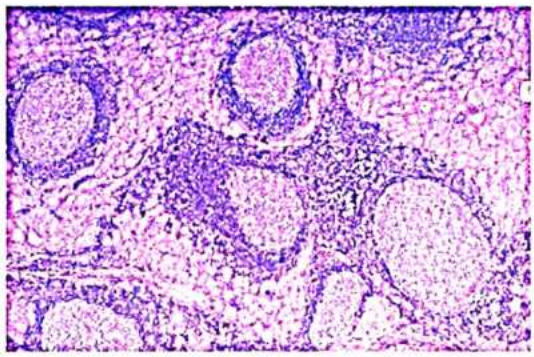
  - RAIU - radioactive iodine uptake
  - Cold nodule - nodule having RAIU < 5 %



  - Diffuse homogeneous enlargement
  - Painless enlargement of thyroid

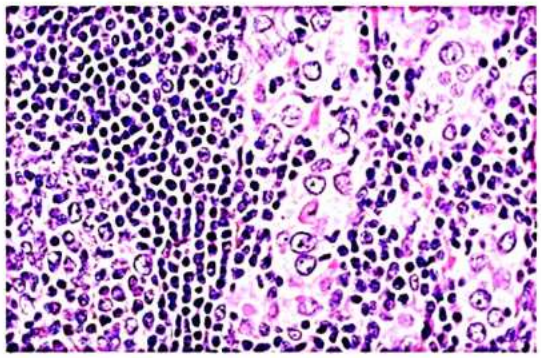
- **Microscopy**
  - Thyroid tissue with lymphoid infiltration





  - Collection of lymphoid follicles
  - With germinal center

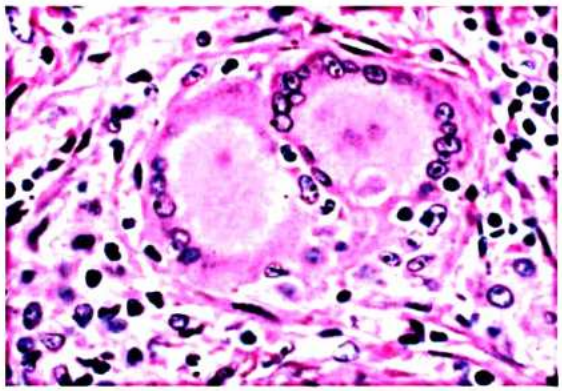
- **Hallmark of hashimoto's thyroiditis**
  - Hurthle cell/Askanazy cell/Oncocytic cell/Oxyphilic cell



- Oncocytic - lots of mitochondria that gives pink color
- Hurthle cells are not specific
- They are seen in other disorders of thyroid as well

Complications of hashimoto's thyroiditis
- Papillary carcinoma thyroid
- Maltoma - marginal zone lymphoma

De quervain thyroiditis
- Painful enlargement of thyroid
- Most common cause of thyroid pain
- Also known as granulomatous thyroiditis



Telegram - @nextpreladdernotes

- **Microscopy**
  - Giant cells have engulfed colloid
- Also called subacute thyroiditis
- Commonly seen Post viral infection like mumps or adenovirus infections
- Association with HLAB5
- Treatment - NSAIDs

Subacute lymphocytic thyroiditis 00:21:09

- Painless thyroid swelling
- Commonly seen Postpartum
- Self limiting condition, do not usually require treatment

Riedel's thyroiditis 00:22:25

- Belongs to IgG4 related disease
- Due to fibrosis - thyroid becomes stony hard
- Due to the hard consistency it may mimic cancer

- **Treatment**
  - Rituximab
  - Tamoxifen
  - Steroids

Other IgG4 related diseases

- IgG4 Causes fibrosis
  - Mikulicz syndrome
    → Affects Salivary gland and lacrimal gland
  - Kuttner syndrome
    → Submandibular gland
  - Ormond Disease
    → Retroperitoneal fibrosis
  - Reidel's thyroiditis
  - Angiocentric fibrosis

Hyperthyroidism

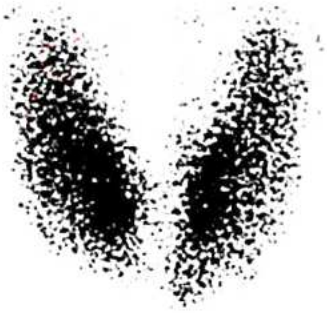
Grave's disease / basedow disease

- Triad in grave's
  - Hyperthyroidism
  - Pretibial myxoedema
  - Proptosis

- **Clinical features**
  - Patient becomes very thin
  - Inability to gain weight
  - Sweating
  - Heat intolerance
  - Menstrual irregularity - occurs with both hypo and hyperthyroidism
  - Palpitation
  - Tachycardia
- **Pathogenesis**
- **Antibodies in grave's**
  - Antibodies - LATS / TSI
  - LATS - Long acting thyroid stimulator
  - TSI - Thyroid stimulating immunoglobulin
  - These antibodies attach to TSH receptor
  - They increase the function of thyroid
  - Thyroid releases more T3 and T4
  - This results in hyperthyroidism
- Type V hypersensitivity
- Earlier was considered type II HS

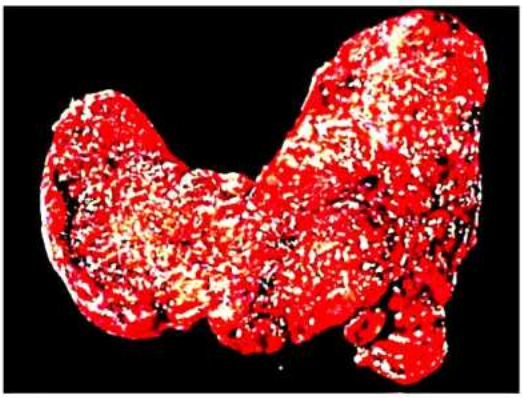
- **Lab tests**

A. Normal



B. Graves' disease

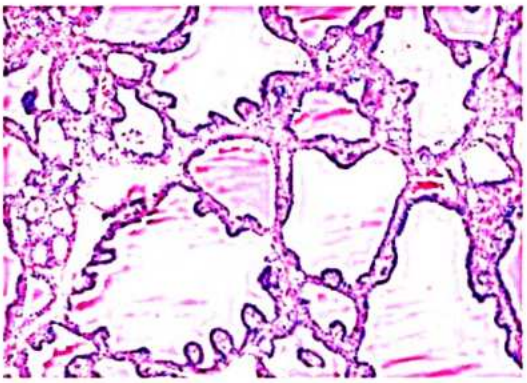
  - T3 - increased
  - T4 - increased
  - TSH - decreased
  - RAIU -
    → In grave's disease - enhanced uptake compared to the normal



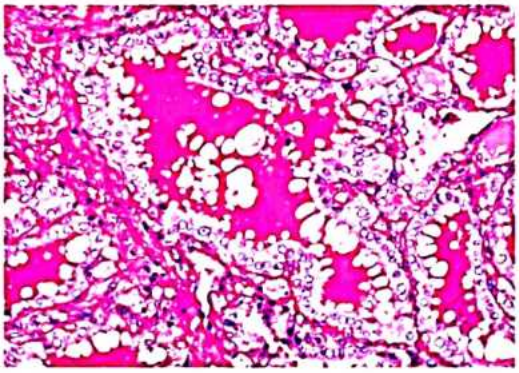
Telegram - @nextpreladdernotes

- Diffuse enlargement of thyroid
- Beefy red color

- **Microscopy**

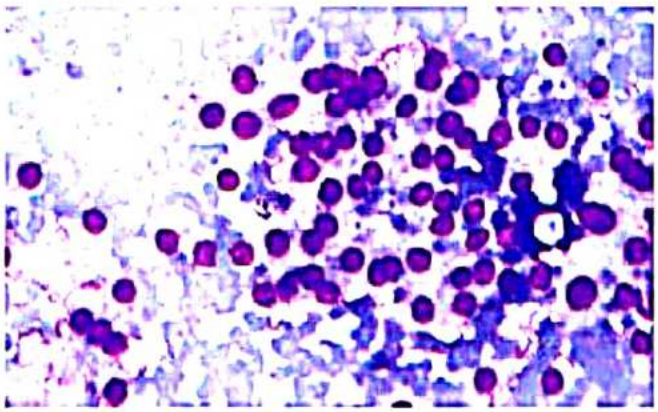


- Pseudopapillae
  → True Papilla is seen in papillary carcinoma
  → True papilla has fibrovascular core



- Colloid being eaten
- This phenomenon is called scalloping of colloid

- **FNAC**



- Around the cells - fire flares can be seen
- These indicate hyperactivity
- These are Dilated endoplasmic reticulum

Thyroid tumors

- Adenoma - benign
- Carcinoma - malignant

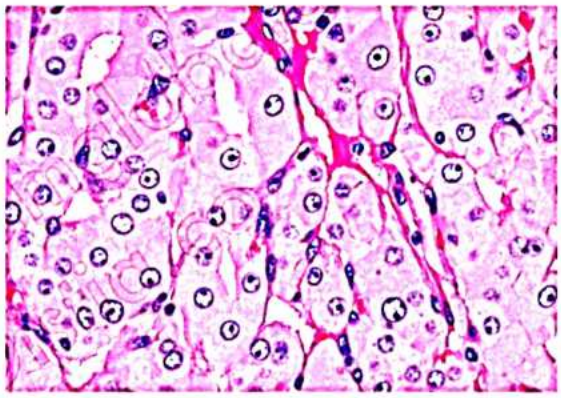
Follicular adenoma

- RAS mutation
- No capsular or vascular invasion

Follicular adenoma vs follicular carcinoma

- Both of them shows RAS mutation
- Capsular and vascular invasion seen in follicular carcinoma
- Cannot be distinguished on FNAC
- Differentiation is done only by a biopsy

Hurthle cell adenoma



- Variant of follicular adenoma
- Shows Hurthle cell / Oncocytic cell / Oxyphilic cell / Askanazy cell
- If a follicular adenoma has > 50% hurthle cells then it is called hurthle cell adenoma
- Recent update says > 75 %

Toxic adenoma

- Follicular adenoma is usually a Cold nodule
- If it has started T3 and T4 production → it is called toxic nodule
- **Genetics**
  - ToXiC
  - TSH receptor mutation
  - GNAS mutation (C→G)
  - EZH1 mutation

Thyroid carcinoma

00:43:19

- **Papillary carcinoma**
  - Most common thyroid cancer
  - Best prognosis
  - Excellent prognosis with 100 %survival

Telegram - @nextpreladdernotes

- **Follicular carcinoma**
- **Medullary carcinoma**
- **Anaplastic carcinoma**
  - Least common
  - Worst prognosis
  - May succumb to illness within 6 months

Papillary carcinoma

- BRAF V600E mutation - most common mutation in PCT
- RET - PTC fusion
  - t(10:17)
- NTRK

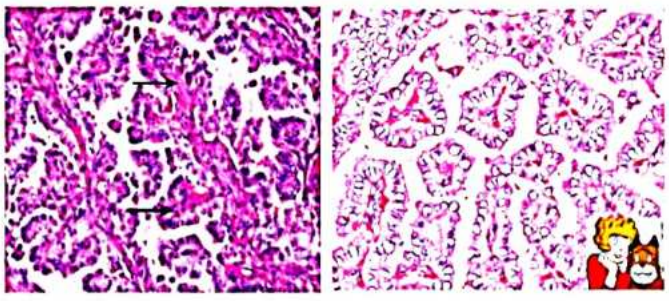
BRAF V600E mutation seen in

- LCH - Langerhans cell histiocytosis
- HCL - hairy cell leukemia
- PCT - papillary carcinoma thyroid
- JPA - Juvenile Pilocytic Astrocytoma - tumor of brain
- Colon cancer
- Melanoma

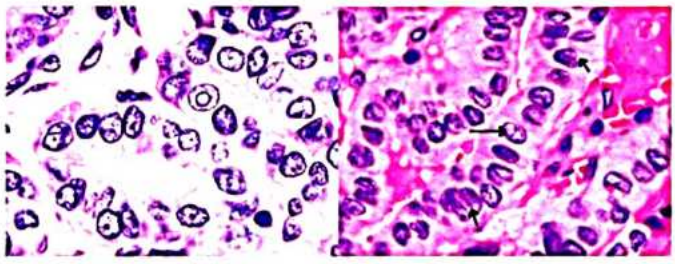
- **Other risk factors**
  - History of Hashimoto's thyroiditis
  - Long standing Thyroglossal cyst
  - History of radiation
    → May be 10 years following radiation to some other organs

- **Clinical features**
  - Nodule in anterior neck
  - Lymphadenopathy
  - Spread - lymphatic
    → All carcinoma show lymphatic spread except Follicular carcinoma
    → Follicular carcinoma - shows hematogenous spread

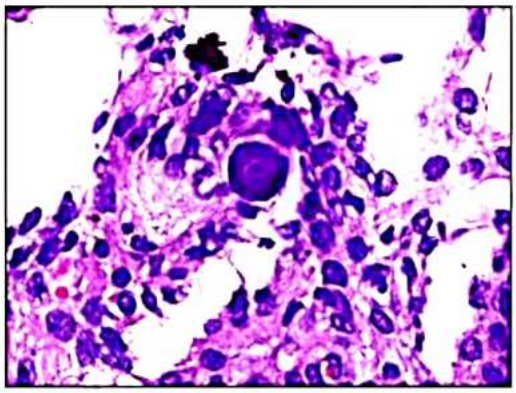
- **Microscopy**



  - Fingerlike projections - true papilla
  - True papilla has blood vessels - Fibrovascular core
    → Optically clear nucleus
  - It is a formalin artifact



  - INCI - Intranuclear cytoplasmic inclusion
  - Nuclear groove or coffee bean nucleus

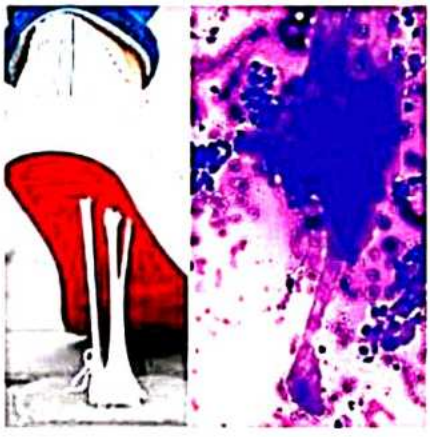


  - Psammoma body

Q. Which of the following is not seen on FNAC of Papillary carcinoma thyroid

a. Orphan annie eye nuclei
b. Psammomma body
c. Coffee bean nucleus
d. Intranuclear cytoplasmic inclusion

- **Feature seen only seen in FNAC**



  - Chewing gum colloid
    → We use very thin needle
    → The colloid gets stuck to the needle

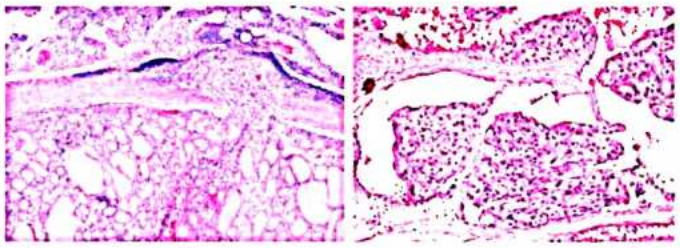
Telegram - @nextpreladdernotes

Variants

- **Microcarcinoma**
  - size < 1 cm
- **Encapsulated variant**
  - Cancers having a well defined capsule
- **Follicular variant of papillary carcinoma**
- **Lindsay tumor**
  - Solid encapsulated follicular variant of papillary carcinoma thyroid
- **Diffuse sclerosing variant of PTC** - bad prognosis
- **Tall cell variant of PTC**
  - Length is 3 times more than width
  - > 30 % of cells should be tall
  - BRAF V600E mutation +

Follicular thyroid carcinoma

- **Genetics**
  - RAS mutation
  - PI3KCA
  - PTEN
  - PAX8-PPAR gamma



- **Criteria for FTC**
  - Capsular invasion
  - Vascular invasion

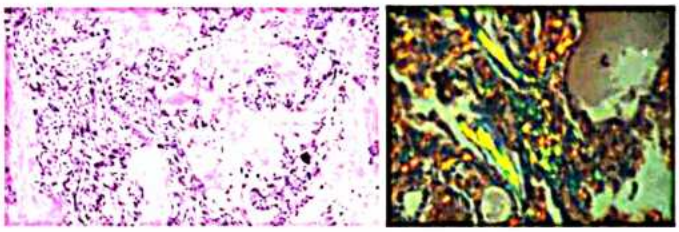
Medullary thyroid carcinoma 01:02:29

- Only tumor to arise from para follicular c cells
- They normally release calcitonin
- **Tumor marker**
  - Calcitonin
  - Carcinoembryonic antigen also used

- **Types and mutation**
  - Familial or sporadic syndrome
- Most common mutation = RET
  - RET - gene
  - TEN - on chromosome 10
  - MED - medullary thyroid carcinoma
  - MEN - MEN 2a and 2b Syndromes associated

- RET mutation- medullary thyroid carcinoma
- RET - PTC fusion - papillary carcinoma

- Type of Amyloid that gets deposited - Acal



- Congo red positive in Polarising microscopy shows apple green birefringence

- **It is a neuroendocrine tumor**
  - Markers -
    - → Synaptophysin
    - → Chromogranin
    - → NSE

Refer Table 43.1

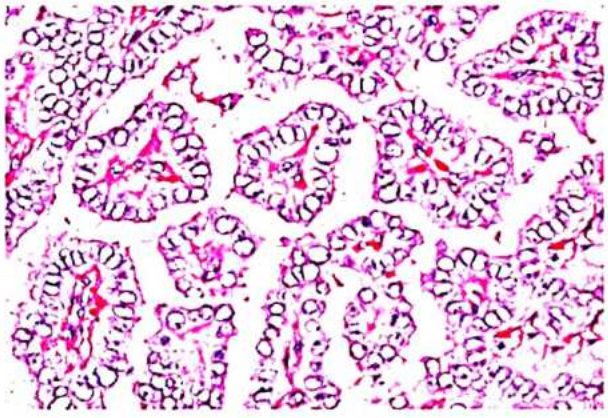
MCQs

Q. The following thyroid cancer cannot be diagnosed on FNAC ?

a. PTC
b. FTC
c. MTC
d. Anaplastic carcinoma

  - Capsular and vascular Invasion cannot be identified on FNAC
  - Biopsy needed to differentiate between follicular adenoma and follicular carcinoma

Q. What is the most common molecular alteration of this thyroid tumor?



Telegram - @nextpreladdernotes

a. BRAF V600E mutation
b. EGFR mutation
c. HRAS mutation
d. TP53 mutation

- Image shows orphan annie eyed nucleus
- This is a feature of PTC
- The common mutation in BRAF V600E

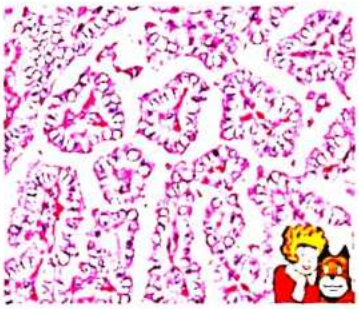
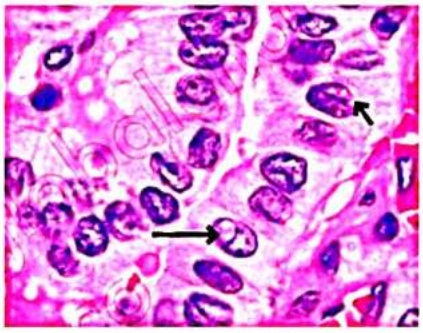
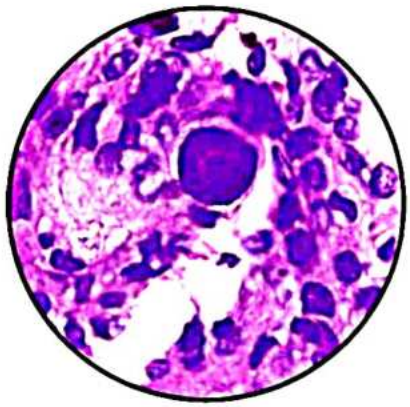
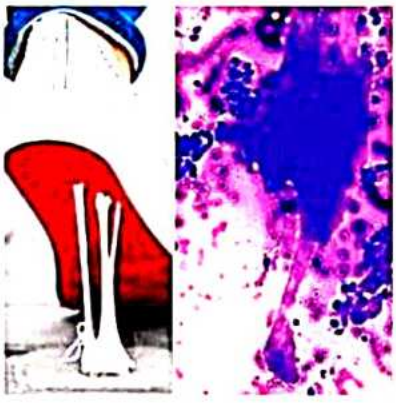
Q. A 54 year old female presents with a thyroid nodule which shows the presence of some tumor cells.The cells are three times as long as their width and eosinophilic cytoplasm. Majority of the tumor cells have this morphology. Mitotic figures are noted. Vascular invasion is noted. Pathologist makes a diagnosis of a variant of papillary carcinoma thyroid and suspects a genetic alteration which is sine qua non for this tumor. What is the most likely genetic alteration?

a. PAXB - PPAR gamma
b. RAS
c. TSH receptor
d. BRAF V600E

- Cells are Three times as long as their width - tall cells
- Majority - > 30 % are tall cell
- So this is Tall cell variant of papillary carcinoma
- For all papillary carcinoma - BRAF V600E
- For tall cell variant it is mandatory to have this mutation for the diagnosis

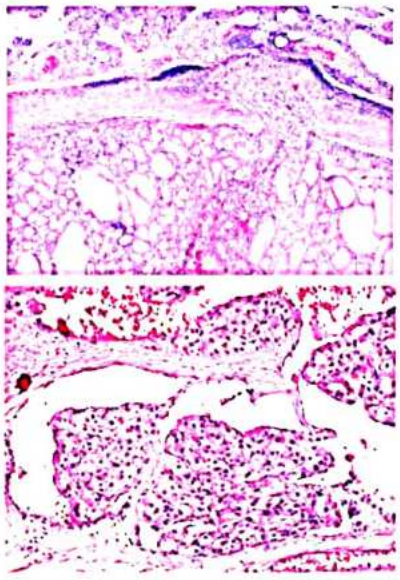
Telegram - @nextprepladdernotes

Table 43.1

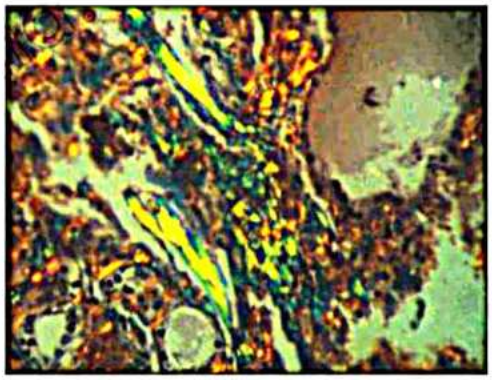
Tumor	Features	Mutation	
Papillary carcinoma	Best prognosis Association with • Hashimoto's thyroiditis • Radiation • Thyroglossal cyst	**BRAF**	• Orphan annie eye nucleus  • INCI - Intranuclear cytoplasmic inclusion  • Coffee bean nucleus • Psammomma bodies  • FNAC - chewing gum colloid 

Telegram - @nextpreladdernotes

Follicular carcinoma	Hematogenous spread	RAS	Capsular and vascular invasion



Medullary carcinoma	RET TEN MED MEN	• Tumor marker - calcitonin • Amyloid - ACal • Stain - congo red • Apple green birefringence

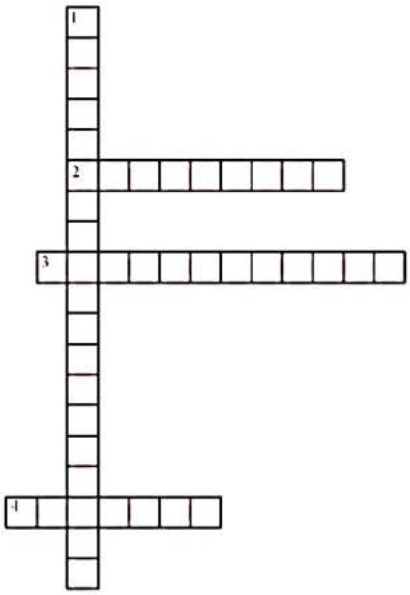


Anaplastic	Worst prognosis	• Rapid enlargement • Severe deterioration

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. Lots of mitochondria that gives pink color
3. Solid encapsulated follicular variant of papillary carcinoma thyroid
4. Marginal zone lymphoma

Down

1. The cells outside the follicle are called?

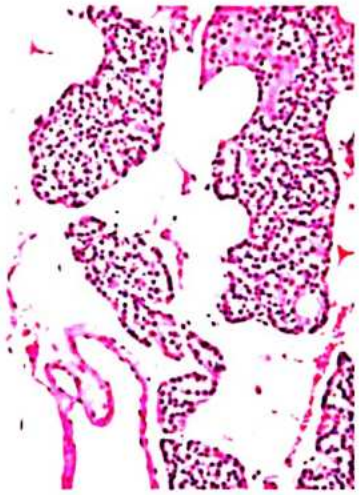
Telegram - @nextprepladdernotes

44 PARATHYROID, ADRENAL & PITUITARY GLAND

Parathyroid Gland

00:00:02

Histology of Parathyroid Gland



- Chief cells/principle cells
  - Light pink cells
  - Secrete PTH (Parathyroid Hormone)
- Oxyphil cells
  - Dark pink cells
- Adipocytes

Physiology of Parathyroid gland

- Release PTH
- **Overall action:** Increases calcium and decreases phosphate levels in the blood.
- **Mechanism:**
  - Initiates bone resorption.
  - Increases calcium and phosphate levels in blood.
  - Calcium reabsorption takes place in kidney.
  - Phosphate is eliminated in urine, reducing phosphate levels in blood.
  - Increased absorption of calcium in GIT.

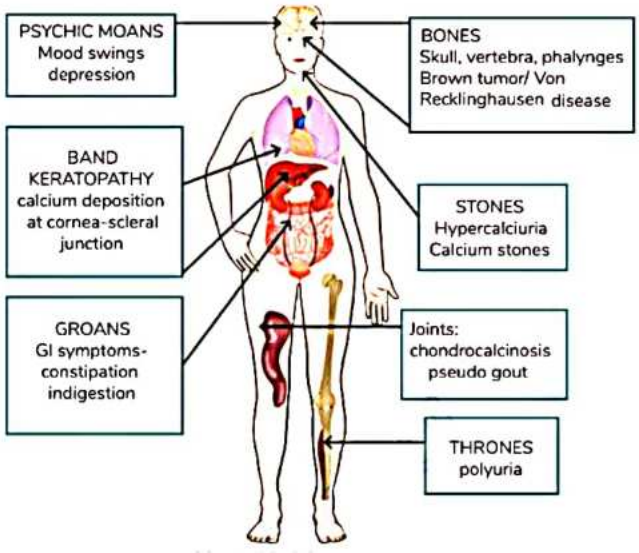
Hyperparathyroidism

00:03:15

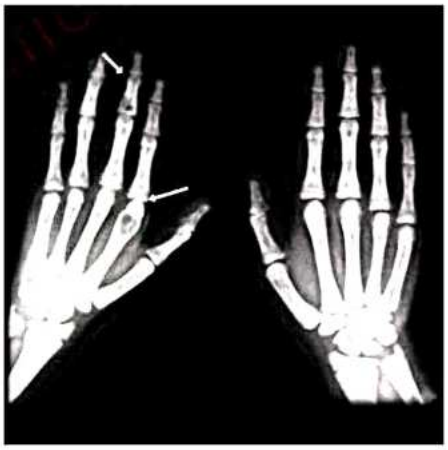
Primary Hyperparathyroidism

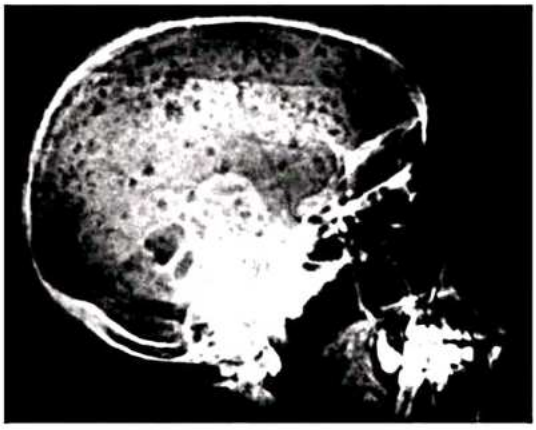
- Most common cause is Parathyroid adenoma.
- Parathyroid adenoma (solitary gland)>>Parathyroid hyperplasia (all glands)

1. **Parathyroid adenoma**
   - Most common cause of Primary Hyperparathyroidism
   - Solitary gland involvement
   - Predilection area: Right inferior parathyroid gland



- **Clinical manifestations**
  - Bones resorption
    - → Increased resorption leads to Osteopenia
    - → Skull, vertebra, phalanges
    - → Density of bones decreased





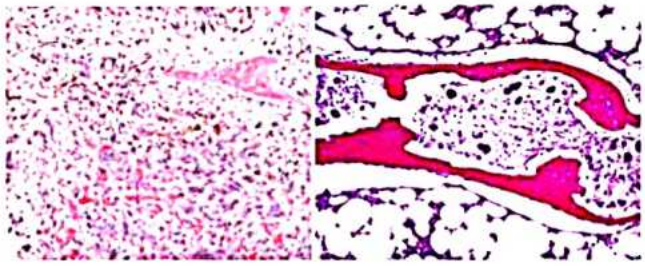
Telegram - @nextprepladdernotes

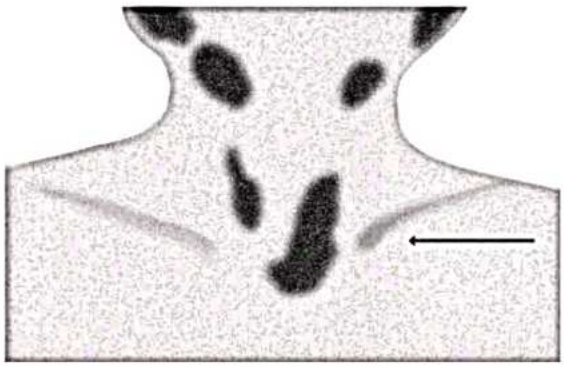
Important Information

Salt and pepper skull is seen in Primary Hyperparathyroidism

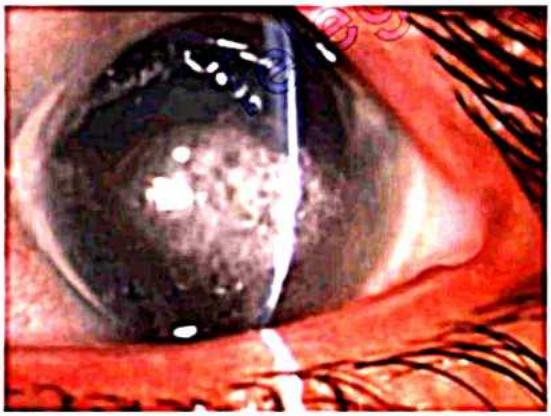
→ **Von Recklinghausen disease**

- Bleeding occurs when the bone fracture which releases Hemosiderin (brown pigment).
- This forms Brown colored tumor or Osteitis fibrosa cystica.
- Osteoclasts invade the medulla of bone, Dissecting osteitis.





- Stones-Increased calcium in kidney leads to Hypercalciuria and Calcium stones.
- Joints - Chondrocalcinosis and pseudogout
- Groans - GI symptoms like constipation, indigestion
- Psychic Moans - Mood swings and depression
- Thrones - Polyuria
- **Band Keratopathy**-Calcium deposition at Cornea-sclera junction



- **Lab investigations**
  - S.PTH: elevated
  - S.Ca: elevated
  - S.PO4: reduced
  - 99 Tc sestamibi scan
    → Increased uptake of radio nucleotide material into affected area

2. Parathyroid Hyperplasia

- All the 4 glands are affected
- MEN syndrome
- Familial hypocalciuric hypercalcemia
  → Autoimmune dominant
  → CASR (Calcium Sensing Receptor) abnormality
  → Decreased calcium in urine
  → Increased calcium in blood

3. Parathyroid Carcinoma

- Very rare
- Presence of invasion and metastasis
- CDC73 gene: Parafibromin protein

Secondary Hyperparathyroidism

PTH increases due to feedback mechanisms:

- CKD
  - Reduced calcium levels
  - Increased phosphate levels
  - Stimulates PTH
- GI malabsorption
  - Reduced calcium absorption
  - Stimulates PTH
- Vitamin D deficiency
  - Bone deformities
  - Stimulates PTH
- Clinical symptoms appear due to another primary disease

Tertiary Hyperparathyroidism

- Autonomous activity of PTH due to long standing secondary Hyperparathyroidism.
- No signals

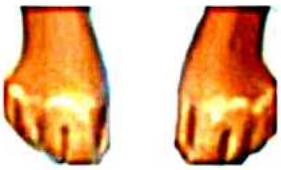
Primary Hyper-parathyroidism	Secondary Hyper-parathyroidism	Tertiary Hyper-parathyroidism
PTH, high Calcium, high PO4, low	Calcium, low PO4, high PTH, high	PTH, very high Calcium, high

Telegram - @nextpreladdernotes

Hypoparathyroidism

00:19:35

Hypopara-thyroidism	Pseudo-Hypopara-thyroidism	Pseudo-Pseudo Hypopara-thyroidism
PTH, low	PTH, high	PTH, normal
Calcium, low	Calcium, low	Calcium, normal
Hypocalcemia: • Chvostek sign • Trousseau sign	Albright Hereditary Osteodystrophy • Archibald sign	Genetic or skeletal abnormalities
Cause: Surgical removal of Parathyroid gland (by mistake during thyroid surgery)	Cause: PTH receptor becomes nonresponsive	



Important Information

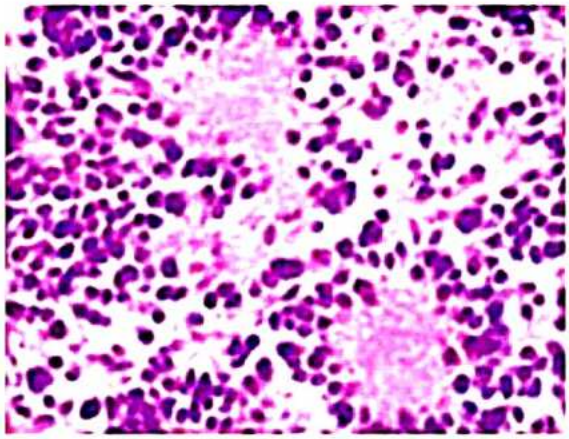
- Chvostek sign, tapping the facial nerve results in muscle twitching.
- Trousseau sign, BP cuff is tied, which results in spasm of limbs.
- Archibald sign. 4th, 5th knuckles are not seen when patient is asked to make a fist.

Adrenal Gland Tumors

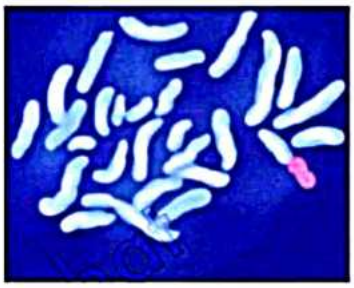
00:25:17

Neuroblastoma

- Most common Extracranial solid tumor of childhood
- Mostly sporadic
- N MYC amplification is seen in Neuroblastoma



- Homer Wright pseudorosettes, Blue tumor cells surround central pink cells. Seen in:
  - Medulloblastoma
  - Ewing's sarcoma
  - Neuroblastoma
- N MYC amplified into
  - Double minutes (outside)
  - HSR (Homogenous Staining Region, inside)



- Amplification can be seen on
  - Karyotyping
  - FISH

Prognostic factors	Good	Bad
Age	<18 months	>18 months
Differentiation	Gangliocytic or shwannian	
MKI	<200/5000 cells	>200/5000 cells
DNA ploidy	Hyperdiploid	Hypodiploid
TRK A	Good	
TRK B		Bad
Chromosome 1p loss, 11q loss		Bad
NMYC		Bad

Pheochromocytoma

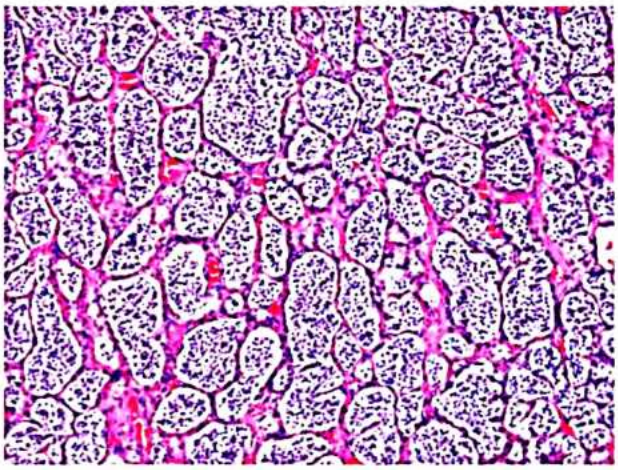
- Neuro endocrine tumors
- **Rule of 10**
  - 10% bilateral
  - 10% children
  - 10% extra-adrenal (Paraganglioma)
  - 10% malignant
  - 10% do not cause hypertension
- Triad (release of catecholamines)
  - Headache
  - Sweating
  - Tachycardia

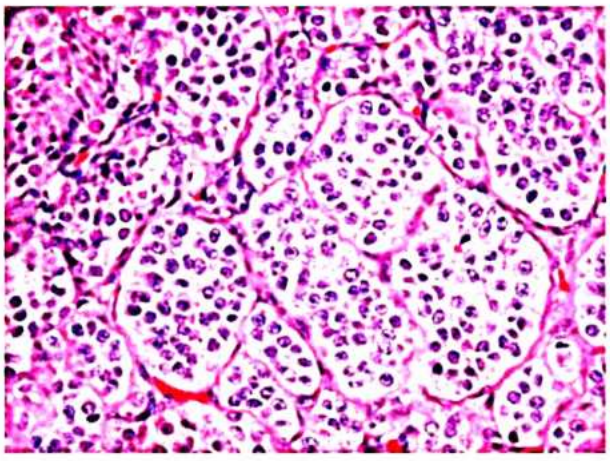
Telegram - @nextprepladdernotes

Important Information

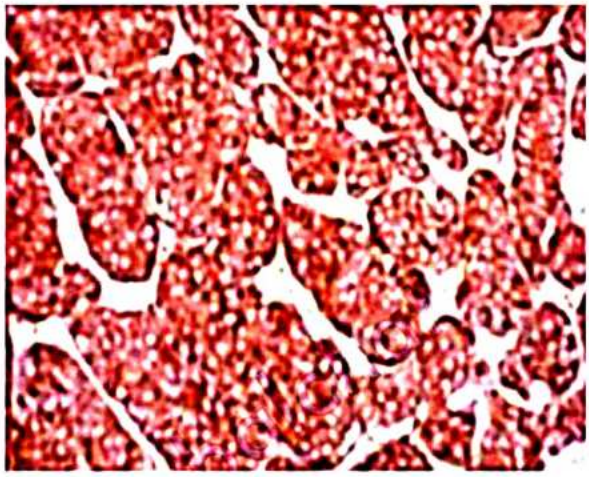
- Paraganglioma includes:
  - Urinary bladder
  - Mediastinum
  - Organ of Zuckerkandl (at Aortic bifurcation)

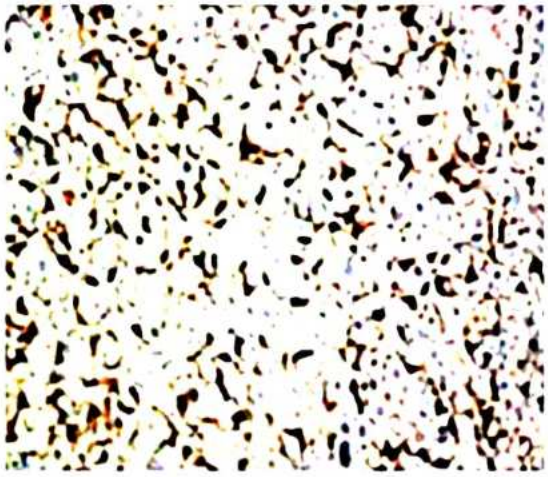
- 25% familial
- Rule of TE∃NS
  - RET (Chromosome 10)
  - EPAS 1
  - VHL (deletion of chromosome 3p)
  - NF1
  - SDH B, C, D
- Histopathology
  - Neuro endocrine tumors show nesting patterns, Zellballen pattern



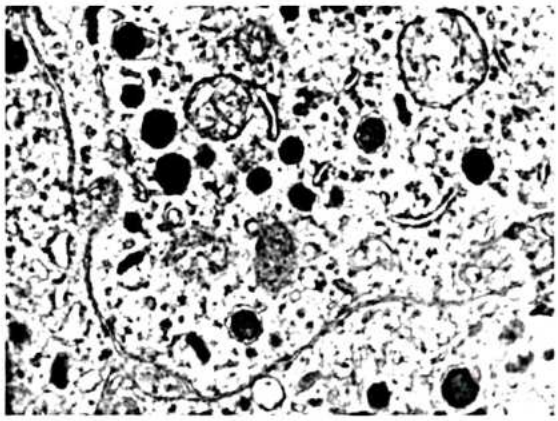


- IHC (Immuno-Histo-Chemistry)
  - Chief cells (Neuroendocrine) are surrounded by Supporting/ Sustentacular cells in the periphery.
  - Chief cells have some markers:
    - → Synaptophysin
    - → Chromogranin
    - → NSE
  - Sustentacular cells have S100 marker.
  - This presents brown color on periphery.



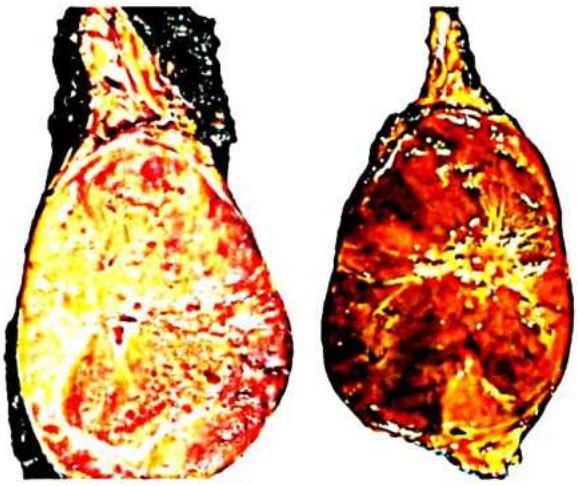


- Electron microscopy



Telegram - @nextprepladdernotes

- Dichromate reaction
  - Potassium dichromate gives black color to chromaffin cells.
  - Dichromate reaction is positive in pheochromocytoma.



Dichromaet Reaction

- Tumor markers: Metanephrines

Pituitary Gland

00:46:36

- 2 lobes (Anterior, posterior)
- Anterior lobe (SAL GCT)
  - Acidophilic cells
    - → Somatotrophs
    - → Lactotrophs
  - Basophilic cells
    - → Gonadotrophs
    - → Corticotrophs
    - → Thyrotrophs
- Posterior lobe, stores
  - Oxytocin
  - ADH

Pituitary gland tumors

- Pituitary adenoma
- Pituitary blastoma
- Pituitary carcinoma

Pituitary Adenoma

- Microadenoma (<10 mm)
  - Hormonally active
- Macroadenoma (>10 mm)
  - Compression symptoms
  - Headache

Important Information

- Lactotrophs produce prolactin
- Prolactinoma is a microadenoma

Genetics

- Pituitary carcinoma- HRAS
- Pituitary blastoma- DICER1
- Corticotroph adenoma
  - CDKNIA
  - USP8
- Somatotroph adenoma
  - PRKAR1A
  - AIP
  - GNAS
- Lactotroph adenoma
  - PRKAR1A
  - AIP

a. Somating Activating Mutations

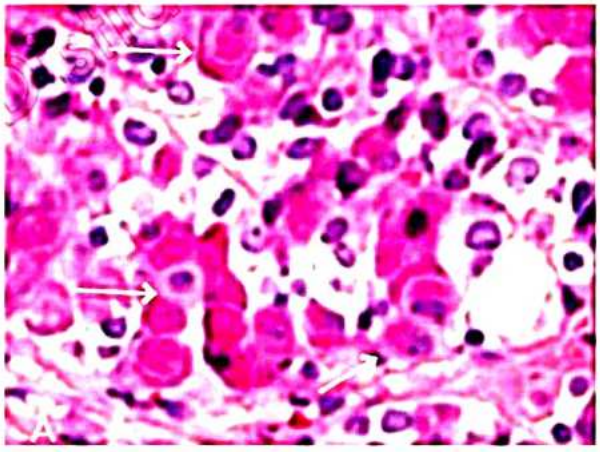
- HRAS
- USP8
- GNAS

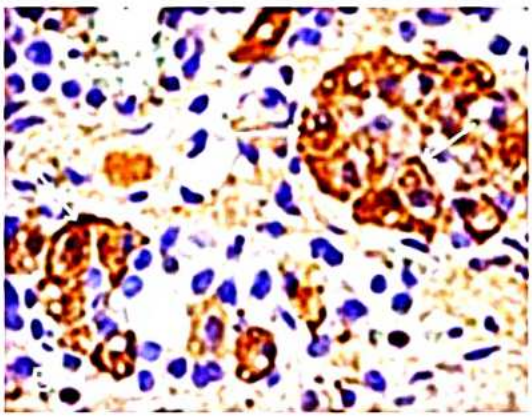
b. Germline Inactivating Mutations

- DICER1
- CDKNIA
- PRKAR1A
- AIP

ACTH adenoma

- Crook Hyaline change in pituitary gland.
- Cells become pinkish.
- Cytokeratin positive cells.





Telegram - @nextpreladdernotes

MEN syndrome

00:55:58

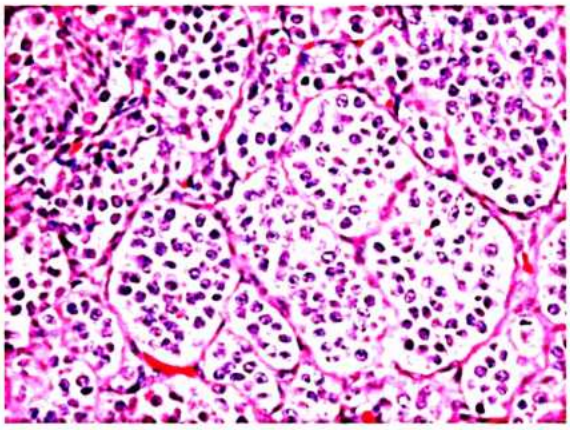
- Multiple Endocrine Neoplasia

MEN I	MEN IIa	MEN IIb	MEN IV
Wermer's syndrome	Sipple syndrome		
MENIN	RET	RET	CDKN1B
• Pituitary adenoma • Parathyroid Hyperplasia • Pancreatic tumor	• Parathyroid Hyperplasia • Medullary carcinoma thyroid • Pheochromocytoma	• Mucosal neuroma • Marfanoid habitus • Medullary carcinoma • Pheochromocytoma	Similar to MEN I • Pituitary adenoma • Parathyroid Hyperplasia • Pancreatic tumor

Telegram - @nextprepladdernotes

PREVIOUS YEAR QUESTIONS

Q. A 38-year-old woman presents to the clinic complaining of intermittent episodes of headaches and sweating. Her family history is significant for VHL gene mutation in her father. On exam, she has mild hypertension and elevated plasma Metanephrines. A non-contrast CT of the abdomen reveals attenuating 6 cm tumor in the adrenal medulla. An adrenalectomy was done and is shown in the photo. Which immunostains should be expressed?

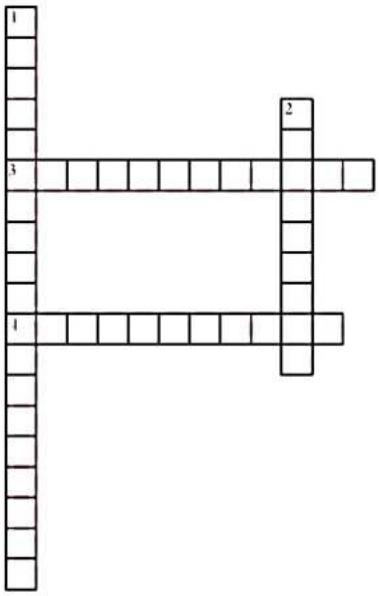


a. Inhibin
b. PAX8
c. Synaptophysin, chromogranin
d. CK

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. It is a microadenoma
4. Bleeding occurs when the bone fracture which releases ?

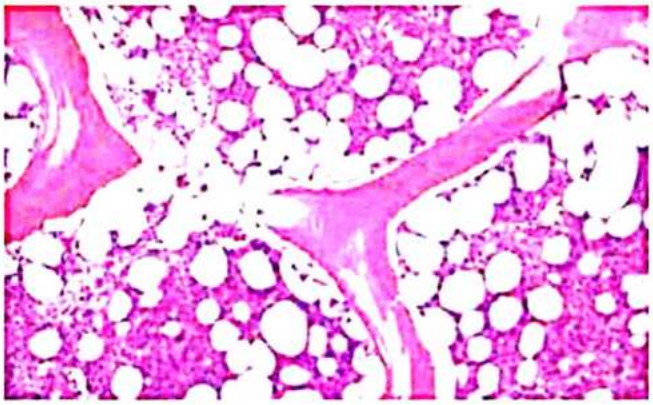
Down

1. Most common cause is Parathyroid adenoma
2. Lactotrophs produce?

Telegram - @nextprepladdernotes

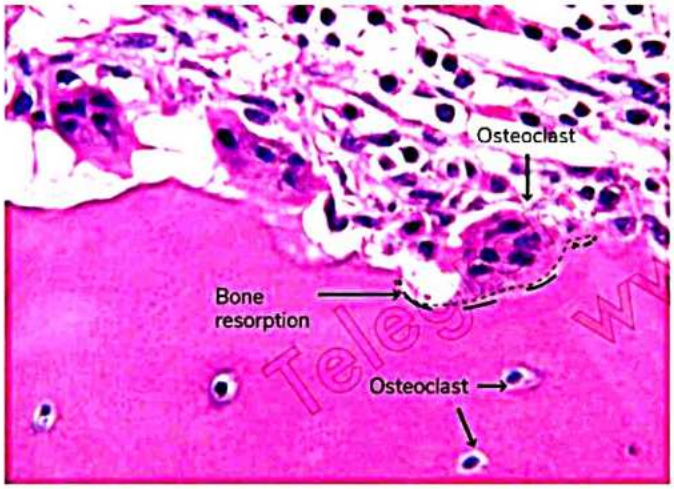
45 BONE AND SOFT TISSUE PATHOLOGY

Normal Bone and Bone Marrow



- Bone trabeculae
- Bone marrow (cells+ fat)

Bone trabeculae

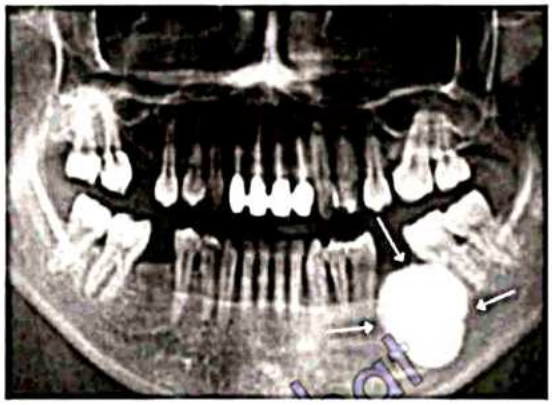


Osteoblast (makes)	Osteoclast (breaks)
It makes the bone layer by layer	Howship's Lacunae eats up the bone.
Converts into osteocytes	It is a multinucleated giant cell.

Alberg Schonberg / Marble Bone disease/ Osteopetrosis

- It is an Osteoclast dysfunction
- Carbonic anhydrase 2 defect

Bone Tumors



1. Bone Forming
2. Cartilage Forming
3. Undifferentiated
4. Indeterminate

1. Bone Forming Tumors

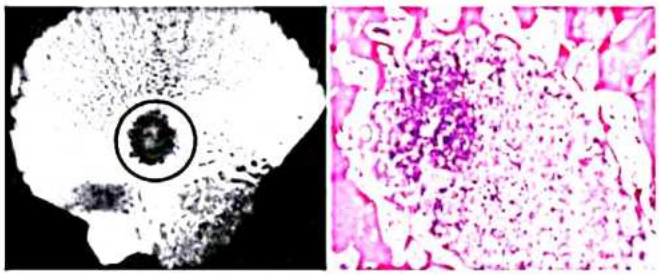
00:06:34

Osteoma:

- Facial and skull bones
  Gardner's syndrome (Multiple osteoma) → FAP + sebaceous cyst, fibromas, osteomas and supernumerary teeth.

Osteoid Osteoma And Osteoblastoma:

Common features: Radiologically
- Nidus in the center, thick cortical bone
- Microscopically- woven bone in periphery



Features	Osteoid Osteoma	Osteoblastoma
Size	Less than 2 cm	More than 2 cm
Site	Tibia	Spine
Pain	Nocturnal pain	Dull pain
On treatment	Relieved on taking aspirin	Not Relieved on taking aspirin

Telegram - @nextprepladdernotes

Osteosarcoma:

Risk factors

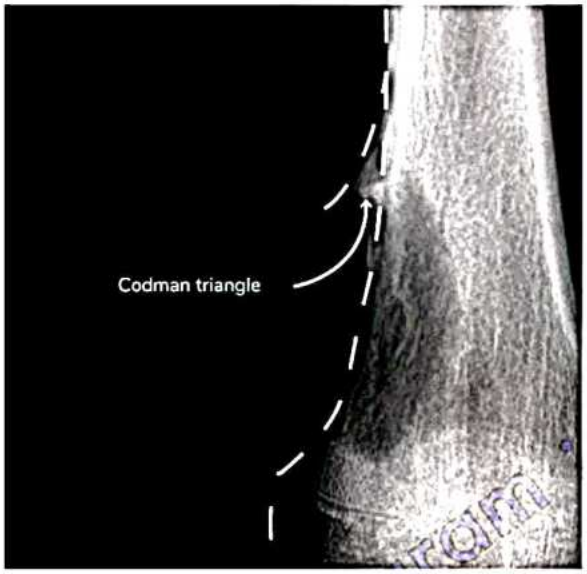
- Loss of p53, RB
- Gain of CDK4, MDM2
- History of previous bone disorder: Paget's disease

Bimodal age distribution

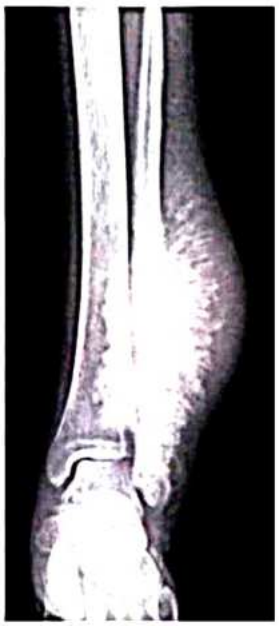
- Primary (younger age)
- Secondary (older age)

Site

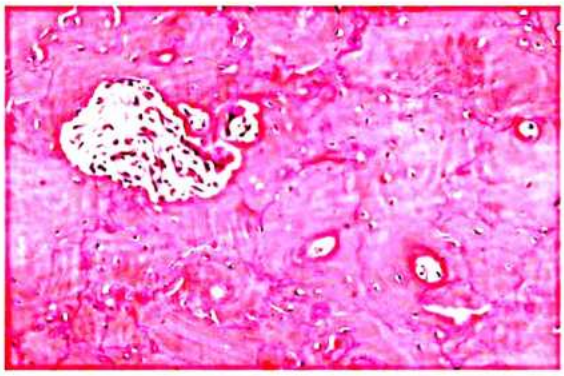
- Metaphysis
- The lower end of the femur (make codman's triangle with having periosteum and shaft of bone on two sides)



- The upper end of the tibia (sun ray or sunburst appearance)

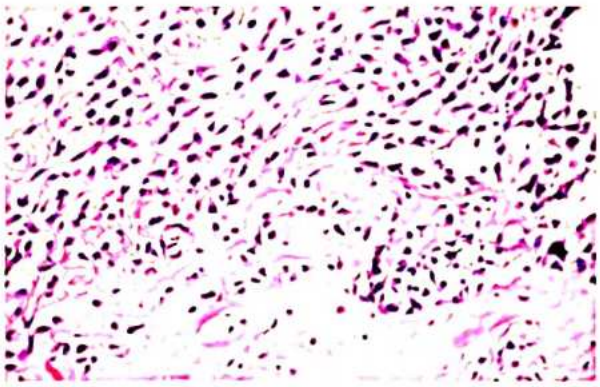


Paget's disease:



- Occur in bone, breast and vulva.
- Seems like a jigsaw puzzle or mosaic pattern.
- SQSTM gene mutation is responsible for Paget's disease.

Hallmark Of Osteosarcoma



- Osteoid is the hallmark of osteosarcoma that is thin, pink, lacy, and glossy material.

Metastasis

- Lung
- Bone to bone
- Brain

Treatment

- Radioresistant
- Surgery

2. Cartilage Forming Tumors 00:24:36

Enchondroma

Mutation

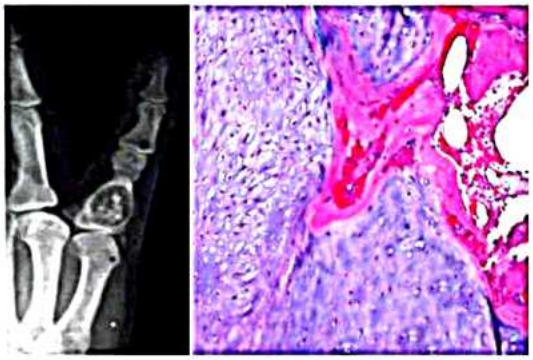
- Isocitrate dehydrogenase 1 (IDH 1)
- Isocitrate dehydrogenase 2 (IDH 2)

Syndrome

- Olliler's syndrome (only enchondromas)

Telegram - @nextprepladdernotes

- Maffucci syndrome (Enchondroma, female ovarian tumor, glioma, hemangioma)



- Location: hands and feets
- Permeation is not seen in this
- Calcification speckled present

Osteochondroma/ Exostosis

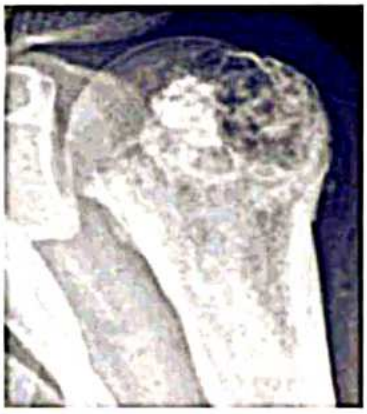
- Marrow and cortex exit out as a tumor in a mushroom shape.
- A cartilage cap is present over that tumor.

- Radiology and pathology shows size discrepancy because radiologist cannot see the cartilage cap, whereas pathologist can.

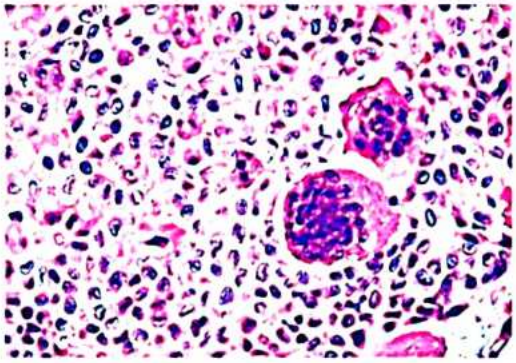
Mutation

- EXT1
- EXT2
- Gene

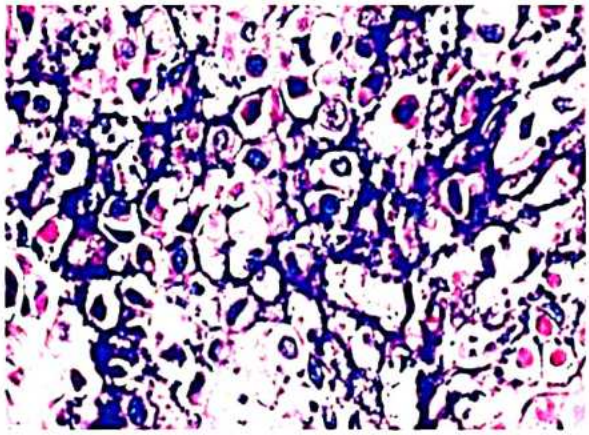
Chondroblastoma/Codman Tumor



- Epiphyseal in location

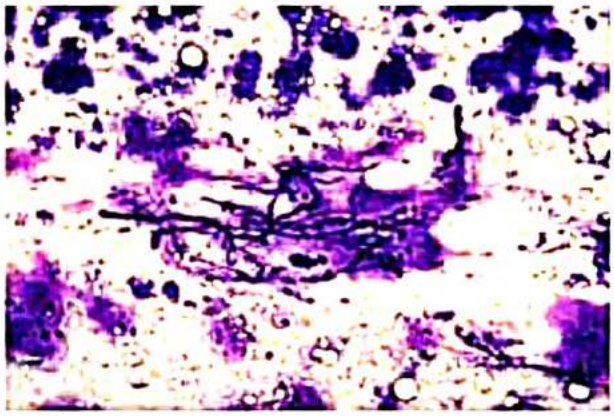


- Giant cells present
- Coffee bean nucleus appearance
- Chicken wire calcification is present



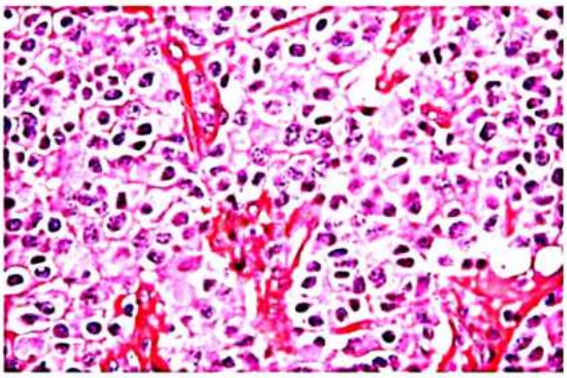
Chicken wire in pathology:

1. Chicken wire blood vessels FNAC (image): mucinous carcinoma breast

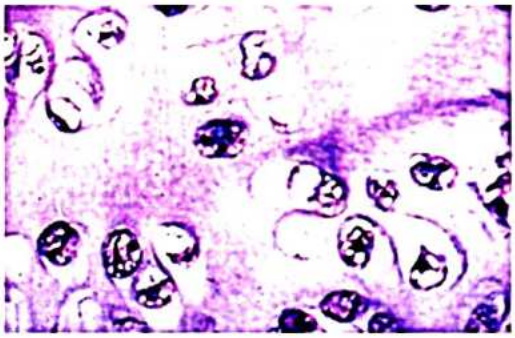


2. Chicken wire blood vessels Biopsy (image): Oligodendroglioma

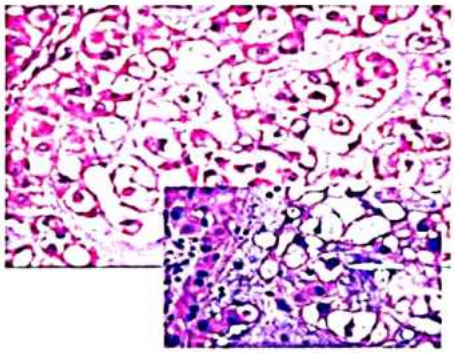
Telegram - @nextpreladdernotes



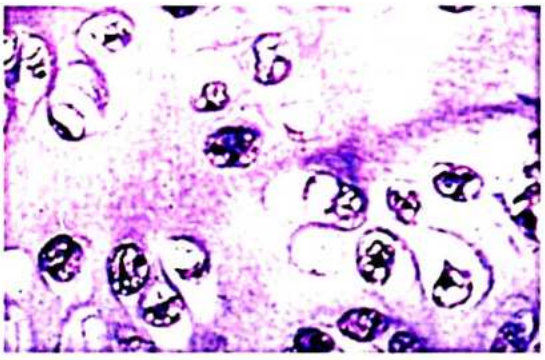
3. Chicken wire calcification (image): Chondroblastoma



4. Chicken wire fibrosis (image): alcoholic liver disease

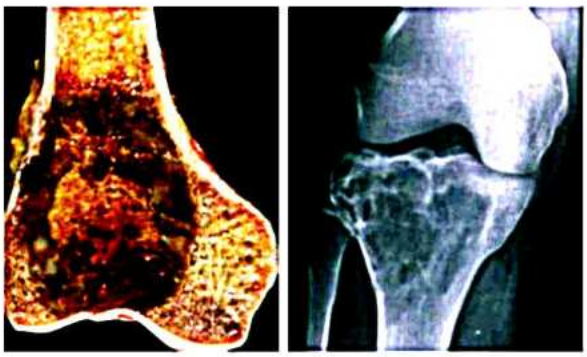


Chondrosarcoma



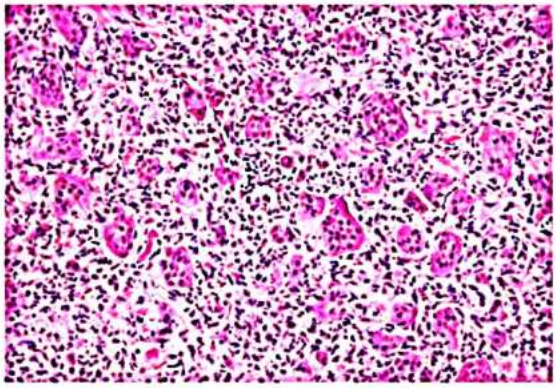
- It is metaphyseal except for clear cell chondrosarcoma that is epiphyseal.
- Occurs in the pelvis, shoulder (axial skeleton)
- Permeation is seen in this
- It is malignant cartilage (multiple cells inside single lacunae)

3. **Indeterminate**



- Locally aggressive tumors
- It is a giant cell tumor/osteoclastoma

Giant cell tumor



- Epiphyseal in location
- Occurs in 20-40 year old
- The lower end of the femur, the upper end of the tibia
- Soap bubble appearance because of hemorrhages.
- The mononuclear cell is the part of GCT
- A multinucleated giant cell is just the reaction to a tumor.
- Giant cell tumor results in activation of the RANK-RANKL pathway.
- This is cured by an anti-RANKL drug that is denosumab.

Giant cell tumor in a tendon sheath

- Hemosiderin pigment is present along with giant cells.
- CSF 1 R gene mutation.

Tumors with giant cells

- Chondro blastoma
- Osteosarcoma (cancer)
- Fibrous dysplasia
- Non-ossifying fibroma

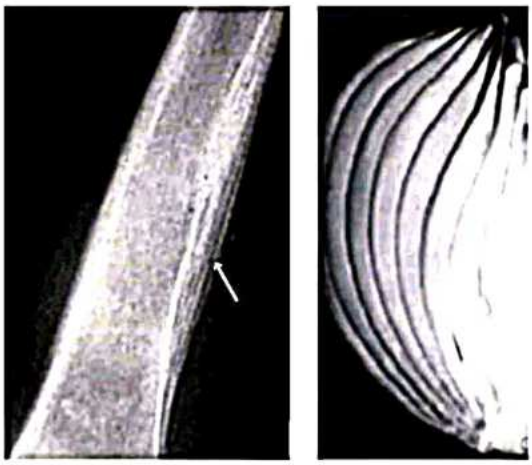
Telegram - @nextpreladdernotes

- Benign fibrous histiocytoma
- Malignant fibrous histiocytoma

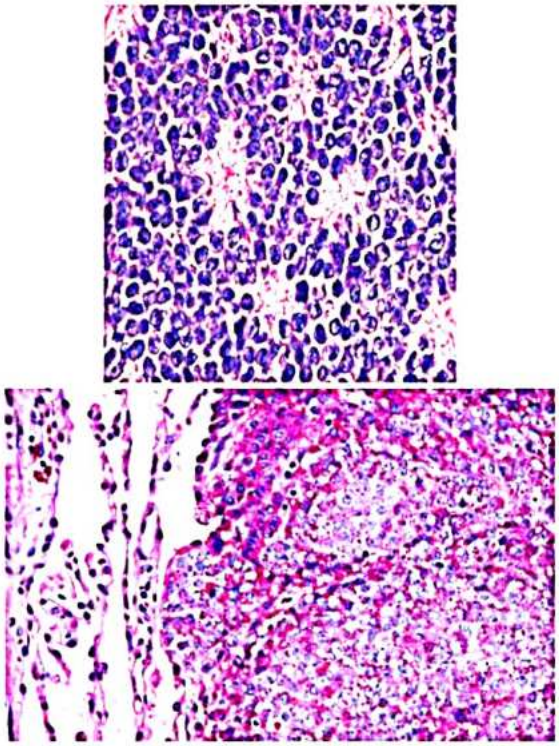
4. Undifferentiated

Ewing's Sarcoma

- Age: Adolescent
- Gender: Male
- Mutation: t(11:22) EWS-FLI 1 gene
- Clinical features: Pain and swelling in knee and fever
- Blood tests: Increase in TLC and ESR
- It mimics osteomyelitis
- Site: Diaphyseal
- X-ray: Onion peel appearance (periosteal reaction)



- Small round blue cell tumor

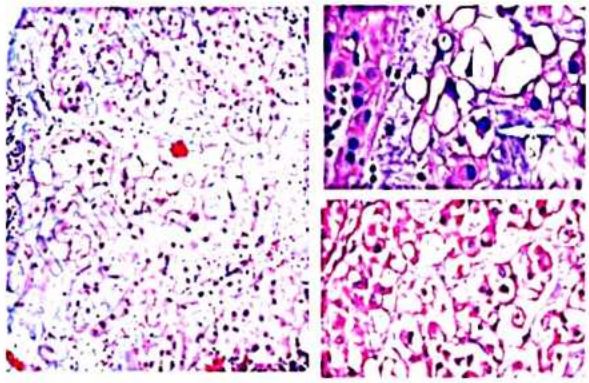


- Homer wright pseudorosette
- Stain: Pas+ (contains glycogen), diastase sensitive
- Markers: CD99/Mic 2

Location	Tumors
Epiphysis	• GCT • Chondroblastoma • Clear cell chondrosarcoma
Metaphysis	• Simple Bone Cyst • Osteosarcoma • Fibrous Dysplasia • Aneurysmal bone Cyst • OsteoChondroma
Diaphysis	• Multiple myeloma • Adamantinoma • Leukemia • Ewing's Sarcoma • Osteoid Osteoma

Other tumors

Chordoma



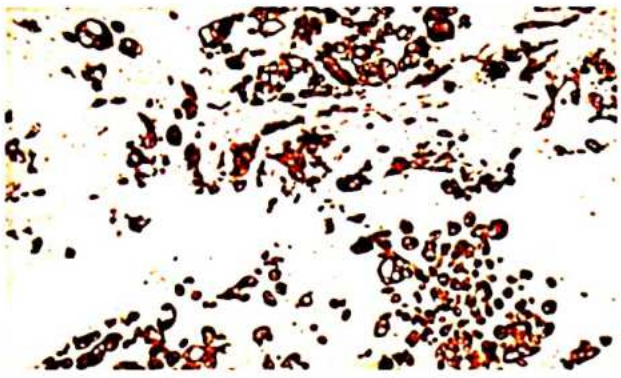
Origin: Notochordal Elements

Site-

1. Clivus
2. Sacrococcyx

Genetics: Brachyury Gene

Marker- brachyury positive



Cells: Bubbly cells or physaliferous cells

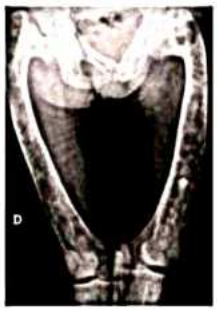
Telegram - @nextprepladdernotes

Tumor Like Conditions

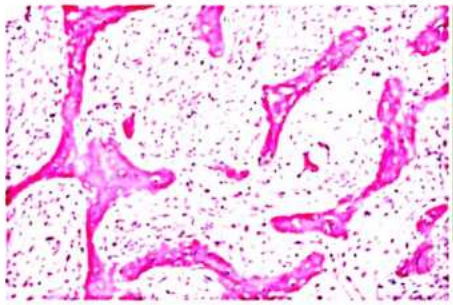
01:10:17

- Fibrous dysplasia
- Non-ossifying fibroma
- ABC

i. Fibrous Dysplasia

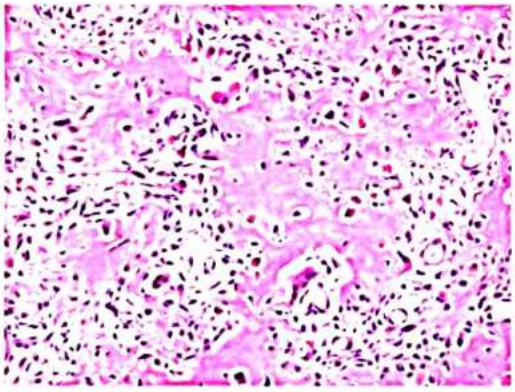


- GNAS1 mutation
- Mazabraud syndrome - Fibrous Dysplasia, Myxomo
- McCune Albright syndrome- Fibrous Dysplasia, cafe au lait spots, precocious puberty
- Shepherd crook deformity
- Chinese letter bony trabeculae appearan



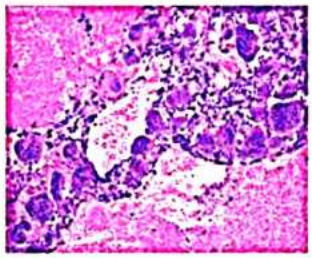
- No osteoblastic rimming

ii. Non-Ossifying Fibroma



- Chinese letter appearance
- Osteoblastic rimming

iii. Aneurysmal Bone Cyst



- Radiolucent
- Metaphyseal
- USP 6 rearrangement
- Blood channels lined by MNGC

MNEMONIC MANU

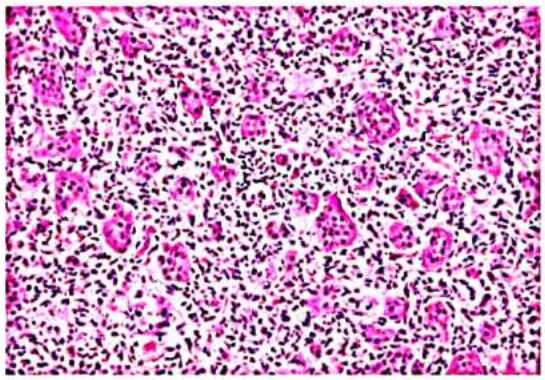
U-USP6 gene seen in Myositis Ossificans, ABC, and Nodular fasciitis.

MCQ's

Q. A large intracytoplasmic glycogen vacuole is associated with which of the following bone tumors?

a. Chondroblastoma
b. Chondrosarcoma
c. Ewing's Sarcoma
d. Leiomyosarcoma

Q. A 30-year-old woman had a 4.5 cm intramedullary mass in the epiphysis of the distal femur. The microscopic image is from the curettage specimen. Which of the following is true about this entity?



a. Lesion never demonstrates cortical destruction and soft tissue involvement
b. Neoplastic cells are osteoclast-like giant cells
c. Treatment may include targeted therapy against RANKL
d. Tumor is a high-grade malignancy

Q. MIC2 is a marker of?

a. Chondroblastoma
b. Chondrosarcoma

Telegram - @nextprepladdernotes

c. Ewing's Sarcoma
d. Leiomyosarcoma

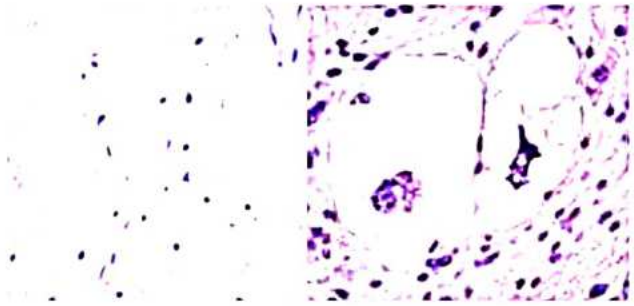
Q. What is the underlying genetic mutation in patients with McCune-Albright syndrome?
a. GNAS1
b. p53
c. MDM
d. SDH

Q. GCT bone most commonly affects?
a. Metaphysis
b. Epiphysis
c. Diaphysis
d. All are equally affected

Soft Tissue Tumors

01:22:46

Fat Tumor

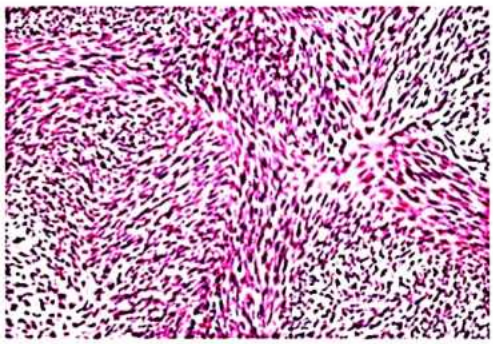


Lipoma (Benign):
- Superficial
- Nucleus at periphery

Liposarcoma (malignant):
- Deep-seated
- LipoBlast
- Indented nucleus
- Retroperitoneum

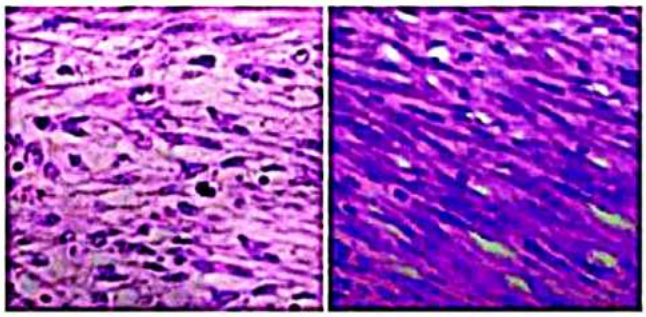
Myxoid liposarcoma (lipoblasts) shows chicken wire blood vessels.

Fibrosarcoma



- Herringbone pattern

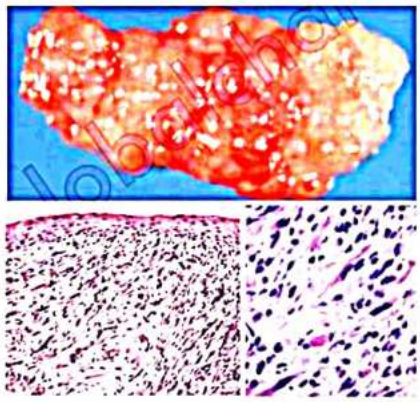
Smooth Muscle



Leiomyosarcoma (malignant): Cigar-shaped nuclei
Leiomyoma (benign): Cigar-shaped nuclei

Skeleton muscle tumor

Rhabdomyoma

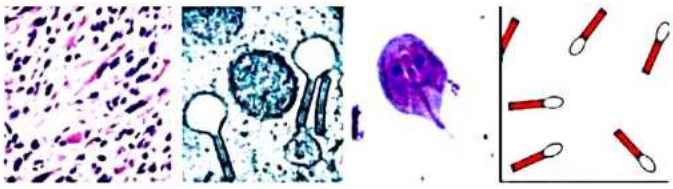


- TSC
- SPIDER cells
- PAS+

Rhabdomyosarcoma

- Sarcoma Botryoids
- Embryonal RMS
- Mucosal sites
- The cambium layer has so many cells
- Below the cambium layer, hypocellularity is there
- Strap cell
- Tennis racquet appearance
- Stain: PTAH-Phosphotungstic Acid Hematoxylin
- IHC: Desmin present, Myo D1 present, Myogenin present

Tennis Racquet



RMS | LCH | Giardia Lamblia | Clostridium Tertium

Telegram - @nextpreladdernotes

Tumor	Translocation	Genes	Extra
Ewing's sarcoma	t(11:22)	EWS-FLI	Homer Wright Pseudorosettes PAS+
Desmoplastic small round cell tumor	t(11:22)	EWS-WT1	
Extraskeletal myxoid chondrosarcoma	t(9:22)	EWS-CHN	
Synovial sarcoma	t(x:18)	SS18-SSX	Biphasic Tumor
Alveolar soft part sarcoma	t(x:17)	TFE3-ASPL	
Infantile fibrosarcoma	t(12:15)	ETV6-NTRK3	
Liposarcoma	t(12:16)		Lipoblasts present
Rhabdomyosarcoma	t(1:13) t(2:13)		
Dermatofibrosarcoma protuberance	t(17:22)	COL1	
Nodular fasciitis	t(22:17)	USP6	

Questions

Q. Find the correct match.

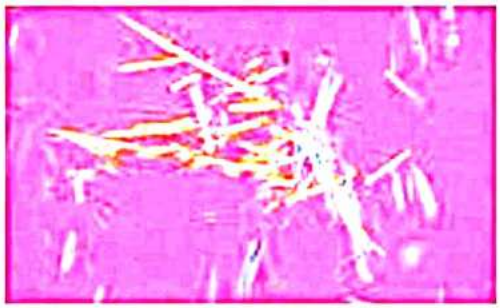
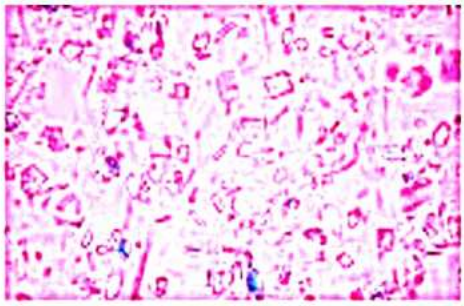
1) t(17:22)	A) ASPS
2) t(9:22)	B) Extraskeletal myxoid chondrosarcoma
3) t(12:15)	C) DFSP
4) t(x:17)	D) Infantile fibrosarcoma

Ans. 1)-C)
2)-B)
3)-D)
4)-A)

Q. Infantile Sarcoma is associated with?

a. t(11;22)
b. t(12;15)
c. t(2:13)
d. t(12:14)

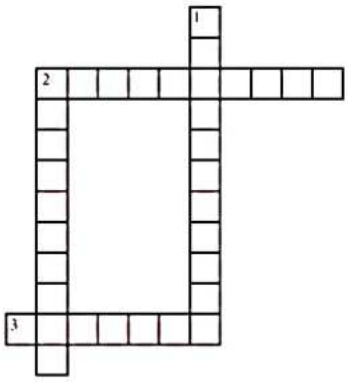
Telegram - @nextprepladdernotes

Features	Gout	Pseudogout
Crystal	Uric Acid	CPPD
Shape	Needle Like	Rhomboid
		
Birefringence	Negative	Weakly positive
Joints	1st MTP-small joints	Knee
Xray	Periarticular erosions soft Tissue swelling	Chondrocalcinosis

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. Howship's Lacunae eats up the bon
3. Facial and skull bones

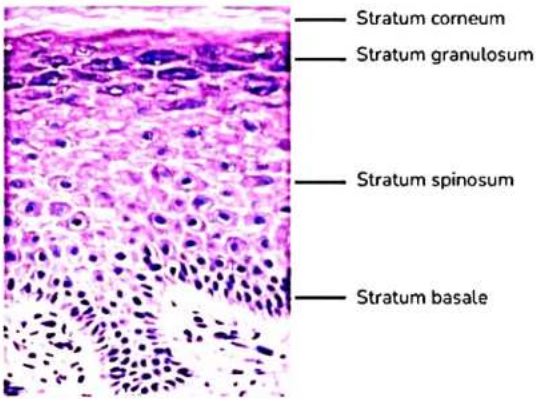
Down

1. Isocitrate dehydrogenase I
2. Makes the bone layer by layer

Telegram - @nextpreladdernotes

46 SKIN PATHOLOGY

Layers of Epidermis



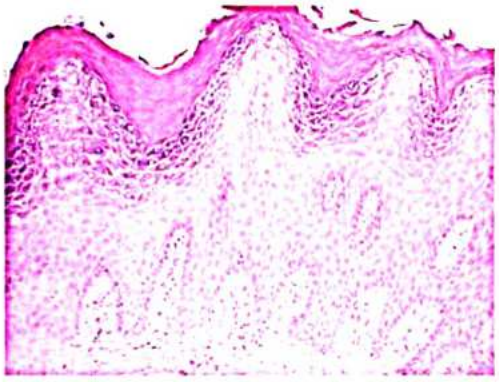
- Stratum basale: (Melanocyte and melanin)
- Stratum spinosum
- Stratum granulosum: Keratohyalin granules (keratin is produced out of these granules)
- Stratum corneum: is the topmost layer containing Keratin.
  - These are anucleated keratin (not having a nucleus)
  - When they have a nucleus, the situation is called parakeratosis (nuclei present in Stratum corneum)
  - Very importantly, this phenomenon is seen in Psoriasis.
- Stratum Lucidum - This layer is present in some parts of the body, like the palms and the soles.

Neuroendocrine cells in Skin

- Neuroendocrine cells are called Merkel cell
- Associated with Merkel cell carcinoma caused by a virus Merkel cell virus
  - Its CK7 CK 20 profile of this carcinoma is 20 negative.

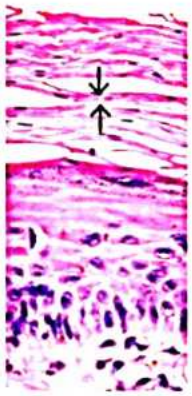
Hyperkeratosis

00:05:20



- Excess of keratin

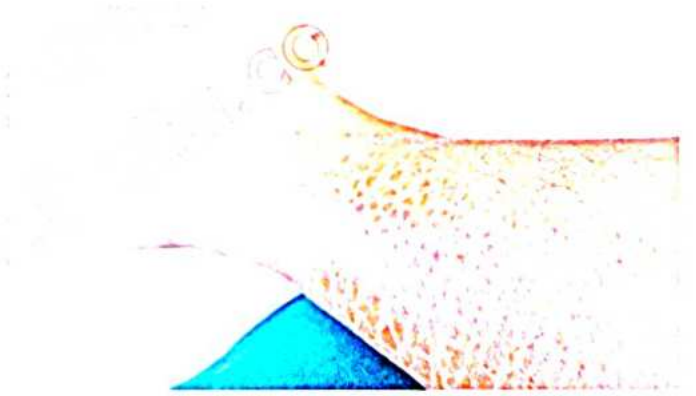
Parakeratosis



Nuclei present in S. corneum
Seen in psoriasis

Ichthyosis

00:06:45



- Clinical presentation - Fish-like scales are present on the body
  - The reason can be idiopathic, or it can be hereditary and can be acquired and associated with cancer
  - These scales have a persistent cell-to-cell adhesion, so it is not falling off and is sticking together.
- Microscopically, ichthyosis shows an increase in keratin.
- Mnemonic
  - I - Increases
  - C - Stratum corneum
  - H - Hyperkeratosis

Psoriasis

00:08:23

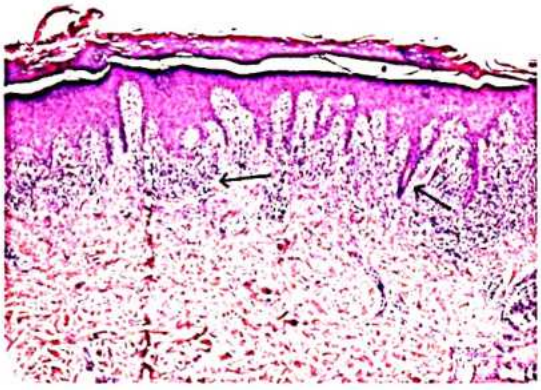
- This condition will have silvery scales
- Underneath the scales, pink plaques can be seen
- When the scales are removed, the patient shows pinpoint bleeds, known as the Auspitz sign. (MCQ)

Telegram - @nextpreladdernotes

Munro's microabscess	Pautrier's microabscess
Psoriasis	In mycosis fungoids (cutaneous TNHL)

- The patient will have Parakeratosis - retention of the nuclei
- Collection of inflammatory cells in the stratum corneum - Munro's microabscess (MCQ)
- Spongiform pustules of Kogoj - seen is stratum spinosum - a collection of inflammatory cell

Lichen Planus 00:13:08

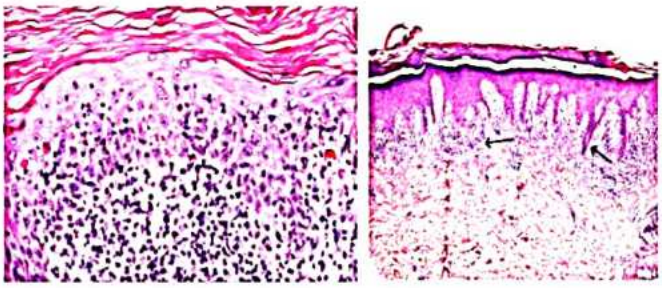


- Disease with six Ps
  - Pruritic
  - Purple
  - Planar
  - Papule
  - Plaque
  - Polygonal

Wickham Striae

- Lacy white colour lines
  - Seen due to hypeorgranulosis

Microscopy



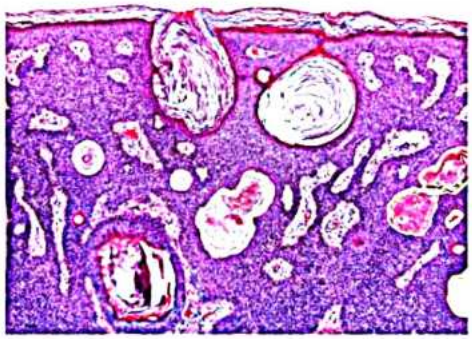
Lichen planus's microscopy

- Saw tooth of rete ridges can be seen
- Band-like lymphocytic infiltrate at the dermo-epidermal junction.
  - Colloid body / Civatte body or the cytoid body.
- Cytoid bodies are the apoptotic bodies.
- The dead cell will be removed, and the area will become white, called Max Joseph space.
- The keratin layer under microscopy looks thicker, indicating hyperkeratosis and hypergranulosis.

Seborrheic Keratosis 00:18:57

- Stuck on appearance.

Multiple stuck on the back of the trunk



- Under the microscope, keratin-filled cysts are present.
- These have been associated with FGFR3 mutations

Telegram - @nextpreladdernotes

Note:

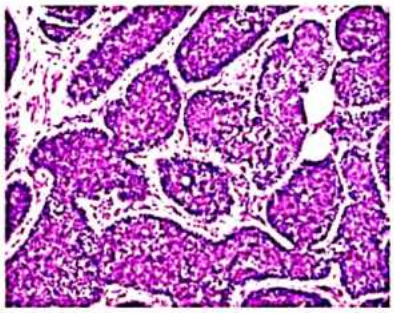
- FGFR3 is also related with
  - Seborrheic keratosis - Skin related
  - Acanthosis nigricans - Skin related
  - Achondroplasia - Orthopedics related
  - Thanatophoric dwarfism - Orthopedics related

- Leser Trelat sign
  - History - Patient with multiple Seborrheic keratoses with sudden onset
    → Associated with internal malignancy
    – GIT-related malignancy - stomach or colon cancer

Skin Appendageal Tumors 00:23:02

- The appendix of the skin is like hair, eccrine glands, apocrine glands, sebaceous glands etc.

Cylindroma 00:23:35

- Shows CYLD (Cylindromatosis) mutations
- Can come with multiple cylindromas - Brooke Spiegler syndrome
  - Present on head-turban tumor
- Jigsaw puzzle pattern is also seen under a microscope in
  - Skin appendageal tumor - Cylindroma
  - Primary biliary cirrhosis also shows Jigsaw puzzle nodules.

Pilomatrixoma 00:25:43

- Showing ghost cells
- Differential diagnosis of ghost cells
  - Pilomatrixoma
  - Coagulative necrosis
- Hair follicle tumor

Syringoma 00:26:45

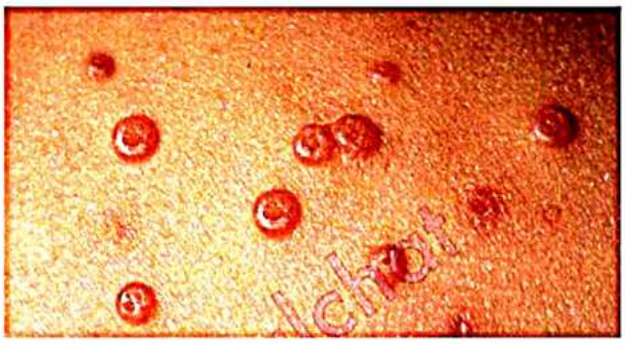
- Comma or a tadpole projections

Trichilemmomas 00:27:53

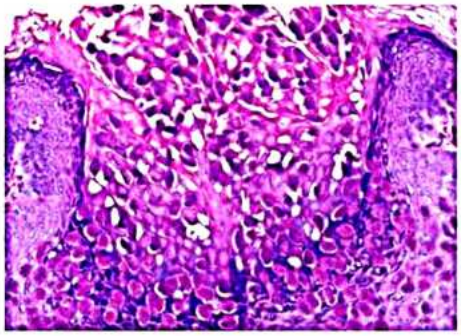
- Cowden syndrome
  - Mutation of Pten gene, chromosome 10
- Muir Torre syndrome
  - Two disorders are associated with it
    → Sebaceous adenoma (sebaceous gland tumors)
    → Keratoacanthoma

Molluscum Contagiosum 00:29:20

- It is caused by a virus called the Molluscum Contagiosum virus
- Clinical appearance
  - These patients tend to present with umbilicated papules



→ When it is pressed some cheesy curdy material comes out
  - These lesions had a classical cup shape - open from the top
    → These cups are filled with pink color bodies called HP (Henderson Patterson) bodies




HP bodies

- HP bodies are seen in molluscum and trachoma
- The molluscum bodies are Henderson Patterson Bodies
- Halberstaedter Prowazeki bodies are seen in a case of trachoma

Telegram - @nextprepladdernotes

- Stains Phloxine tartrate stain is used for Molluscum Contagiosum
  - Used for
    → HP bodies
    → Paneth cells (present in GI track)

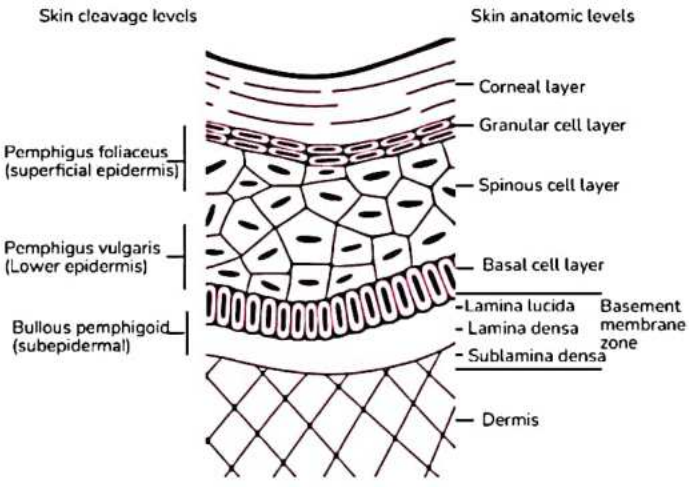
Bullous Disorders 00:34:47

- The split will result in the formation of a bulla

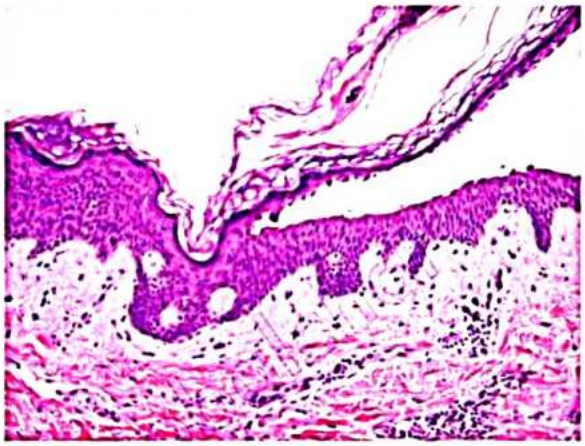
Pemphigus

- Two types
  - Pemphigus Vulgaris
  - Pemphigus foliaceus
- **Pemphigus vulgaris** - will have everything (trick)
  - Antibodies against Desmoglein (dsg) 1 and 3 both will be involved
  - Involvement will be against the skin and oral cavity
  - The lesions will be vesicles and bullae, which will rupture very easily and leads to the formation of a crust

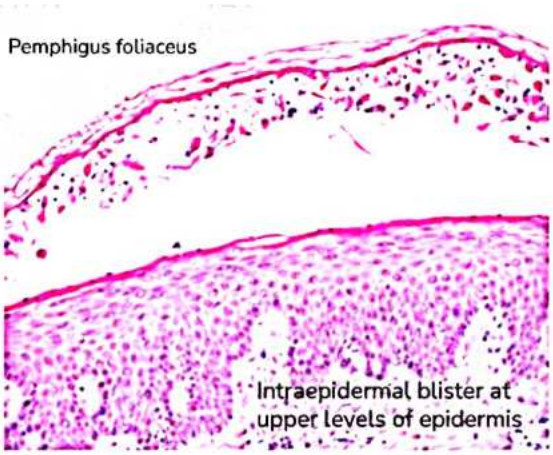
- **Pemphigus foliaceus** - only have one thing (trick)
  - Antibodies against desmoglein 1
  - Only skin is involved
- In Epidermis



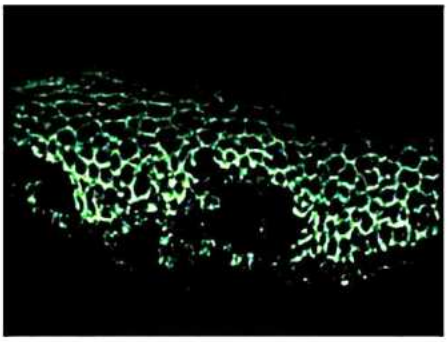
  - The stratum corneum - has the first desmoglein in the max amount
    → Thus pemphigus foliaceus will affect this layer
  - At the bottom in Stratum basale, desmoglein 3 is maximum, and desmoglein 1 is in a small amount - pemphigus vulgaris is present.
- In Pemphigus foliaceus the split will be in the first layer - the subcorneal split



- In Pemphigus Vulgaris, there is a suprabasal split



  - The layer left is the basal layer - Row of tombstone appearance (MCQ)
    → It is also seen in coagulative necrosis
- Immunofluorescence
  - In Pemphigus fishnet or reticular pattern is seen.

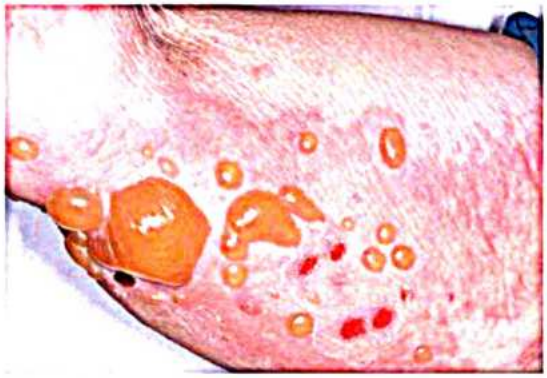


Telegram - @nextpreladdernotes

- When skin biopsy is sent to the path lab
  - Fixative 10 percent neutral buffered formalin is used.
  - But for Immunofluorescence, it is not sent in formalin because formalin is autofluorescent material.
    → It is sent in Normal saline
- **Paraneoplastic pemphigus**
  - Sometimes pemphigus can be seen in association with other syndromes.
    → Like Non-Hodgkin's lymphoma

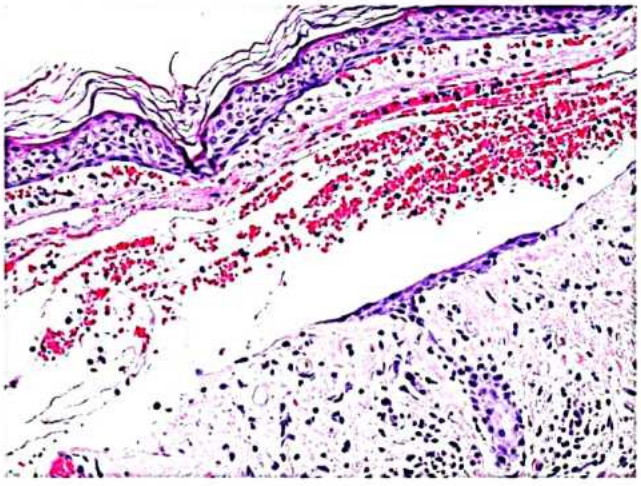
Bullous Pemphigoid 00:43:15

- In this case, bullae will be well-formed and tense

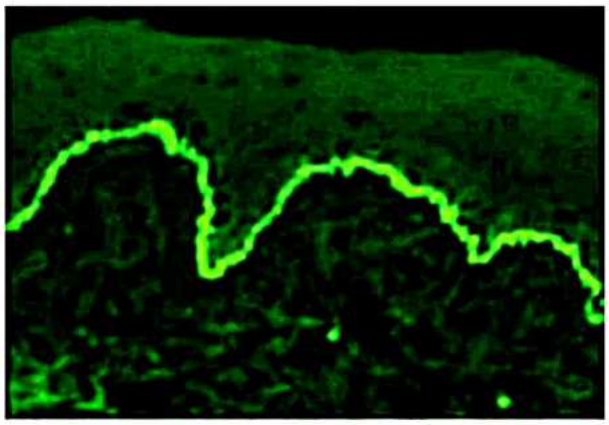


- Commonly seen in the
  - Flexor aspect of the forearm
  - Axilla
  - Groin
- Antibodies are against hemidesmosomes
  - Eg. BPAG (Bullous pemphigoid antigen)

- Cells at the bottom of the Epidermis are connected with the basement membrane below with a connection known as Hemidesmosomes
  - Eg. BPAG (Bullous pemphigoid antigen)
- A subepidermal split is seen



- Bullous cavities have orange cells called eosinophils.
- Under Immunofluorescence

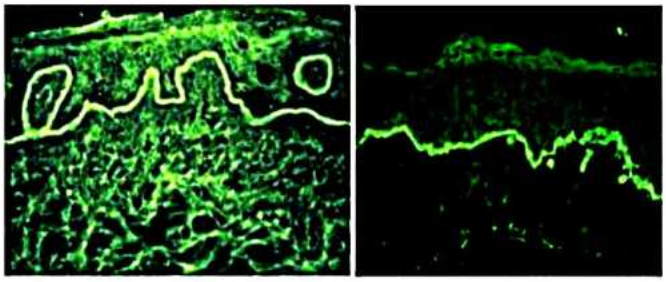


  - Linear Immunofluorescence
  - Ribbon candy appearance
- A ribbon candy appearance is seen on the roof of the bulla
  - Because the target was Bullous pemphigoid antigen

Epidermolysis Bullosa Acquisita (EBA)

- In this subepidermal split is seen
- It is because of the collagen 7 defect
- Immunofluorescence will be on the floor of the bulla

Bullous Pemphigoid vs EBA

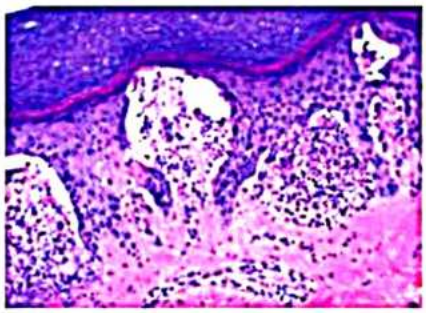


- Trick to remember
  - Pemphigoid - god (as above)
  - Epidermolysis bullosa acquisita (aqua/water is on the ground)

Dermatitis Herpetiformis 00:52:46

- It is also a subepidermal split
- Due to IgA (Immunoglobulin A) antibody.
- Associated with a disorder of the intestine called Celiac disease
  - Tip lesions (trick - Dermatitis can be remembered as Dermati-TIP)

Telegram - @nextpreladdernotes



Hailey - Hailey Disease 00:55:56

- ATP family gene defects is seen
- Neither are the cells separated nor are they together.
  - The condition is known as Dilapidated brick wall appearance
    → It shows lesions in the folds of the body like the neck, axilla, or groin of the body.

Summary

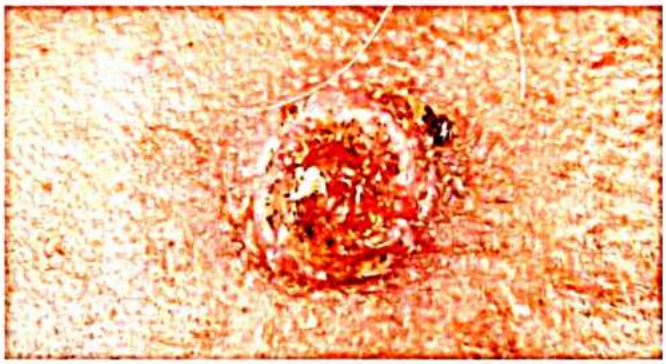
Disorder	Defect	Split	Immunofluorescence
Pemphigus Vulgaris	Dsg 1, 3	Suprabasal split (tombstone appearance)	Fishnet pattern
Pemphigus foliaceus	Dsg 1	Subcorneal split	Fishnet pattern
Bullous pemphigoid	BPAG	Subepidermal split	Ribbon candy appearance on the roof
EBA	Collagen 7	Subepidermal split	Ribbon candy appearance on the floor
Dermatitis Herpetiformis	Celiac disease - IgA antibody	Subepidermal split	Tip lesions

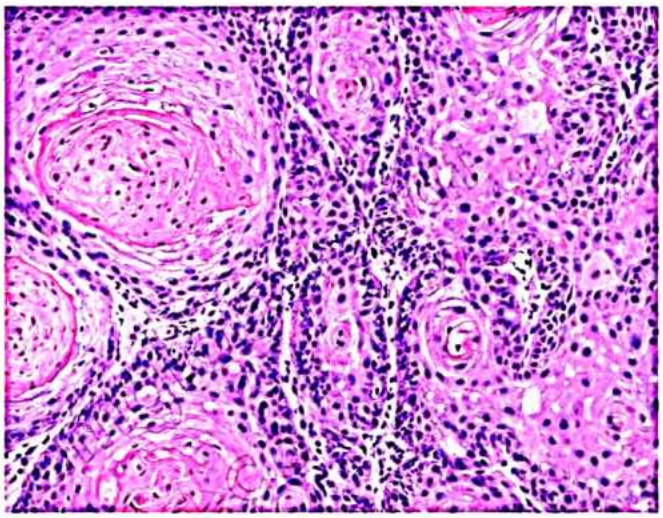
Carcinomas of Skin 00:59:42

- Three main cancers
  - Squamous cell carcinomas
  - Malignant melanoma
  - Basal cell carcinoma

Squamous Cell Carcinomas

- Present like cauliflower-like lesion





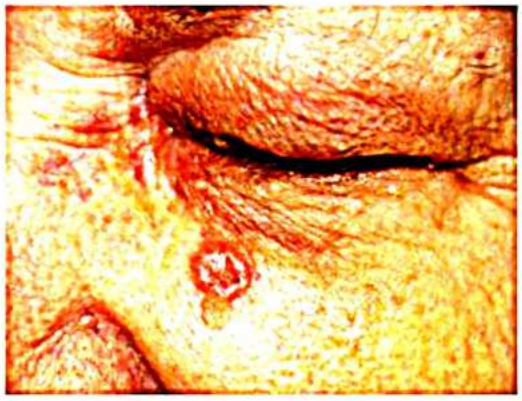
- Keratin pearls
- Based on keratin pearls Squamous cell carcinomas can be divided into
  - Well-differentiated Squamous cell carcinomas (WDSCC)
    → Keratin production is max
  - Moderately differentiated Squamous cell carcinomas (MDSCC)
  - Poorly differentiated Squamous cell carcinomas (PDSCC)
    → Keratin production is min
  - This classification is known as Broder's classification
- Risk factor
  - Radiation
  - Ultraviolet rays (sun-exposed sites are affected)
    → Can also cause actinic keratosis (premalignant condition) and from this Squamous cell carcinomas can arise.
  - Arsenic

Basal Cell Carcinoma

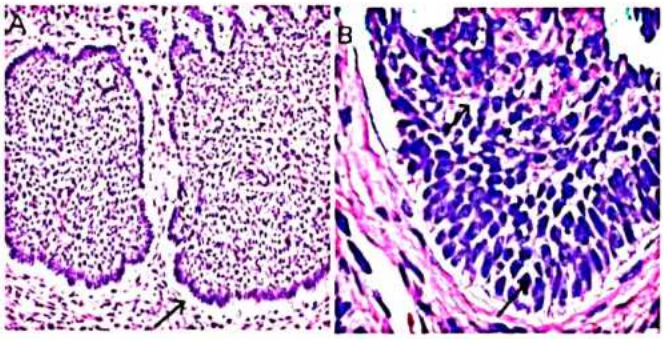
- Two syndromes associated with it
  - Gorlin syndrome
    → PTCH gene defect - SHH (sonic hedgehog) pathway is affected.

Telegram - @nextprepladdernotes

- Xeroderma pigmentosum
  → Nucleotide excision repair (NER) defect also causes basal cell carcinoma
- It tends to occur near the medial canthus



- Defined as pearly white lesions
- Rolled margins
- Associated with telangiectatic blood vessels
- Histopath:

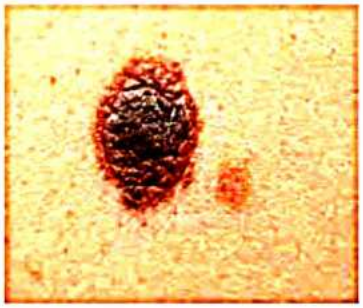


- Blue basaloid cells arranged in peripheral palisading
- Retraction artifact

Malignant Melanoma

01:08:47

- Genetics
  - P16 mutation
  - NRAS
  - CKIT mutation
  - BRAF mutation
- Looks like pigmented lesions

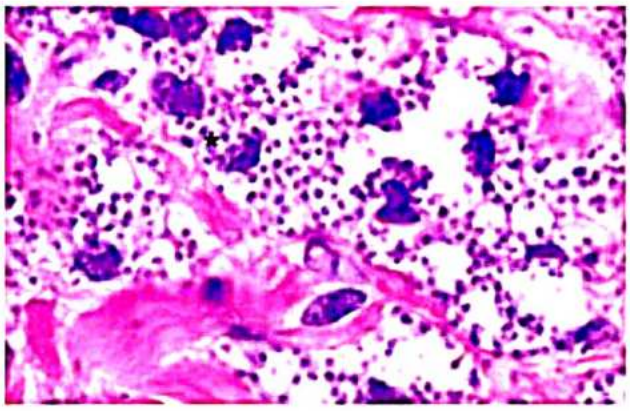


- ABCDE is seen
  - Asymmetry - in a border or the color
  - Border irregularity
  - Color variability - Hyper or hypopigmented
  - The diameter crosses 5 mm
  - Elevated
- Tumor cells will have a brown pigment called melanin
- Stains of melanin pigment
  - Masson Fontana stain
  - Schmorl stain
  - An enzyme called dopa oxidase (the best method for analyzing melanin)
  - Immunohistochemistry - HMB45 + S100 + Melan A positive

Miscellaneous

01:12:58

Cutaneous Leishmaniasis



- Leishmania is Kala Azar
  - Its skin manifestation
- Leishmania organisms or LD bodies (dot-dot appearance) are seen

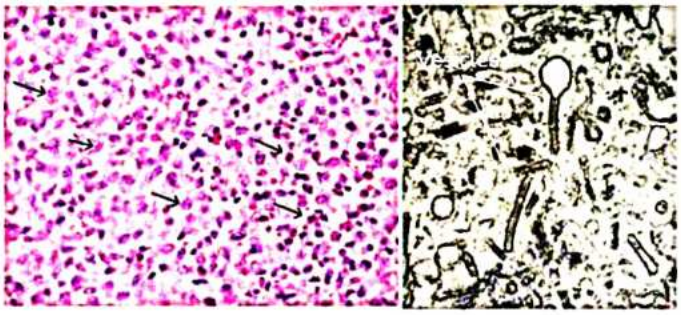
Langerhans Cells Histiocytosis

- Langerhans cell is an antigen-presenting cell in the skin
- Mnemonic - Rafael Nadal

Telegram - @nextpreladdernotes

- Raf = BRAF mutation
- Ace technique of serve = CD1a positive
- Speed of ball - >100 mph = S100 positive.
- The tennis ball has a line = coffee bean appearance
- Tennis racquet = electron microscopy shows tennis racquet appearance

- Three types - LCH
  - L - Letterer Sewe disease
    → Liver is involved
    → Lytic bony lesions
    → Seborrheic dermatitis is also seen
  - C = E - Eosinophilic granuloma
    → Lytic bone lesions
  - H - Hand Schuller Christian disease
    → Triad involved - BCDE
      - Bony lesions in a calvarial defect
      - Diabetes insipidus
      - Exophthalmos
  - L - also stands for lung or pulmonary LCH
    → Seen in smokers
- Diagnosis



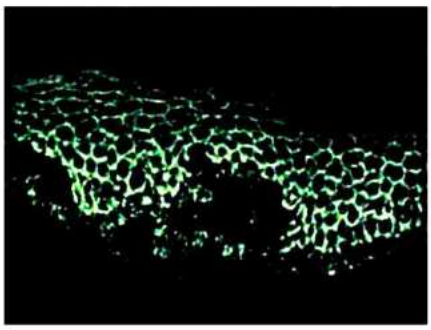
  - Light microscopy -
    → Eosinophils are seen (orange-looking cells)
    → Coffee bean nuclei
  - Immunohistochemistry (IHC)
    → CD1a, CD207 (langerin), S100 positive, HLADR positive
  - Electron microscopy
    → Gold standard technique
    → Tennis racket appearance of Birbeck granules

Hint

- A 70-year-old female with bony lytic lesion
  - Diagnosis - Multiple myeloma
- 9-year-old boy come with bony lytic lesion
  - Diagnosis - LCH is involved

MCQ 01:24:13

Q. 20-year-old female presents with blisters over the skin and in the oral cavity. Her skin biopsy shows a special pattern on immunofluorescence? Mark false statement.



A. Antibodies against desmoglein
B. Antibodies against desmoglein 3
C. Antibodies against hemidesmosomes
D. Fishnet appearance

Description:

- Pemphigus vulgaris - dsg 1 and 3 are involved
- hemidesmosomes are involved in bullous pemphigoid

Q. Tip lesions are seen in?
A. Pemphigus vulgaris
B. Pemphigus foliaceus
C. Bullous pemphigoid
D. Dermatitis herpetiformis

Description:

- Made up of IgA antibodies

Q. Ribbon candy appearance on immunofluorescence is seen in?
A. Pemphigus vulgaris
B. Bullous pemphigoid
C. Pemphigus foliaceus
D. Dermatitis herpetiformis

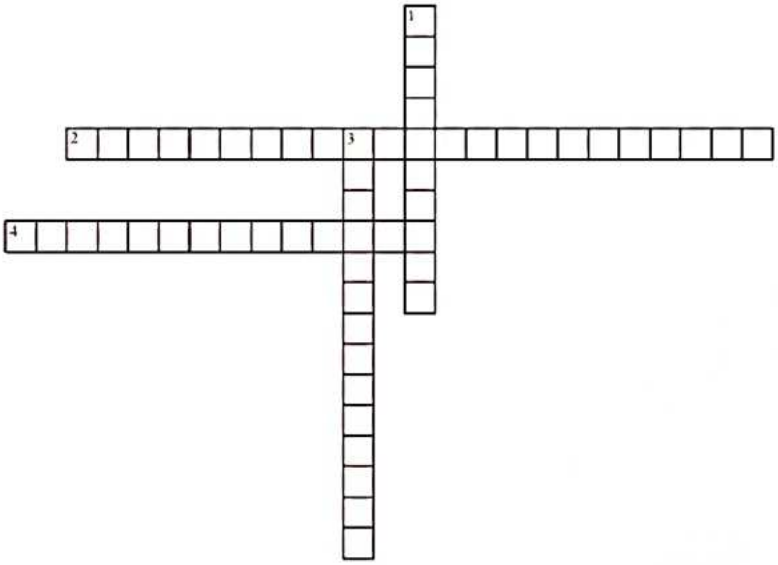
Description:

- Seen at the roof of the bulla

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. It is also a subepidermal split
4. The dead cell will be removed, and the area will become white, called ?

Down

1. Neuroendocrine cells are called
3. This layer is present in some parts of the body, like the palms and the soles

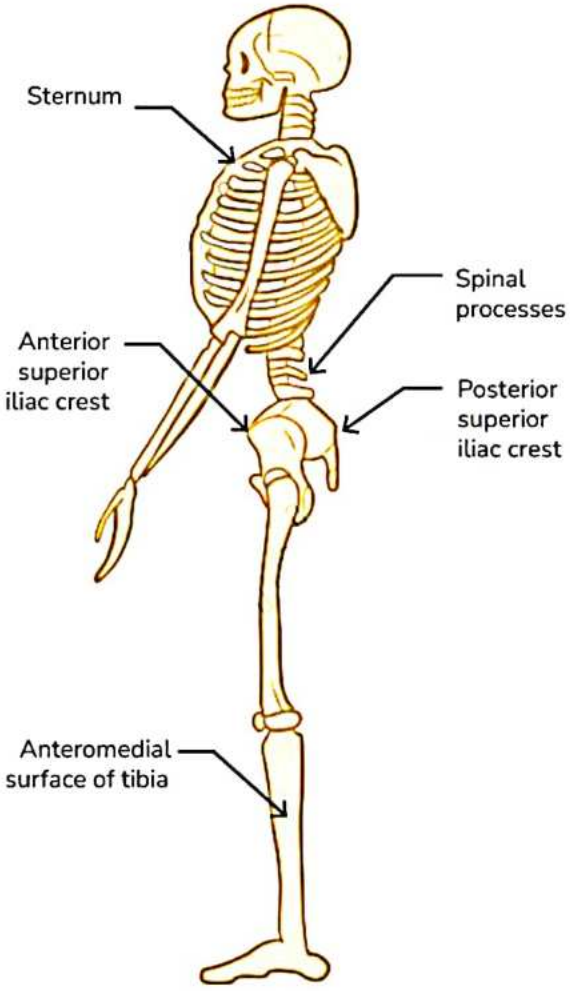
Telegram - @nextprepladdernotes

47 INTRODUCTION TO HEMATOLOGY

Haematology

- **Hematopoiesis occurs:**
  - First 1-3 months of our life, this process of making blood cells occurs in the Yolk Sac.
  - By 3 months, the liver starts taking up the entire process.
  - The liver continues but by 4 months the bone marrow of all the bones of the body starts. This process is going to occur till birth and even till puberty.
  - After Puberty, long bones of the body make up a sufficient amount of bone marrow.

Bone Marrow Basics 00:01:52

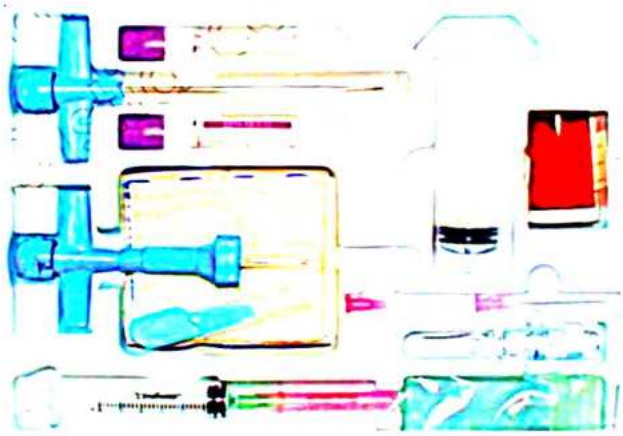


- The two main techniques: Bone Marrow Aspirate & Bone Marrow Biopsy
- The most common site preferred is PSIS (Posterior Superior Iliac Spine)
- In obese individuals, Anterior Superior Iliac Spine.
- In children: Anteromedial surface of the Tibia.

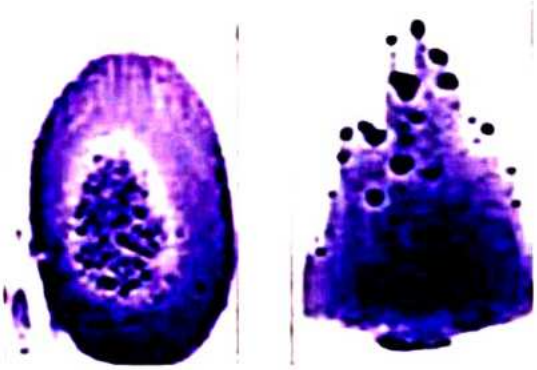
Salah and Klima Needle 00:04:50

Needle for Bone Marrow Aspirate

- Side screw: salah needle
- Longitudinal screw: klima needle



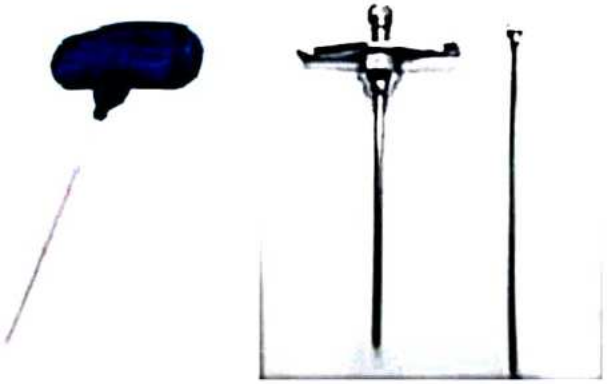
Bone Marrow Aspirate



- This is looking like any other Peripheral Smear.
- BMA: blood + bone marrow particles

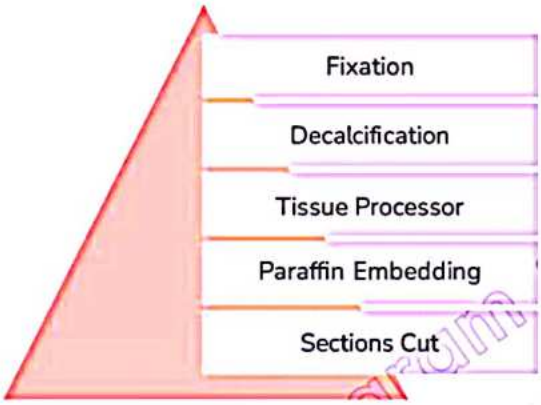
Telegram - @nextpreladdernotes

Needle for Bone Marrow Biopsy



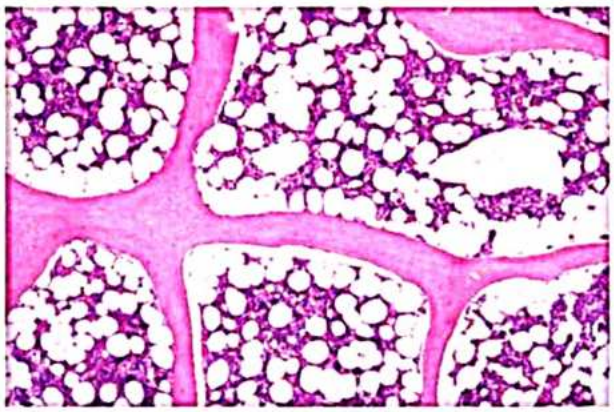
- Jamshidi Needle for Bone Marrow Biopsy and this is also known as Trephine Biopsy Needle.
- It is a T-shaped needle.

Bone Marrow Biopsy Processing 00:08:42



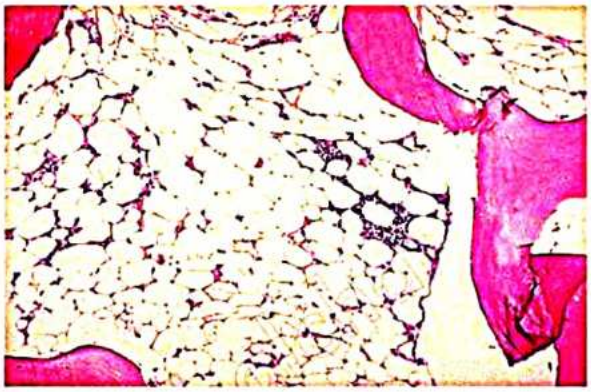
- **Fixation:**
  10% NBF (Fixative for everything in light microscopy).
- **Decalcification:**
  10-15% EDTA

Normal Bone Marrow Biopsy Sample



- The marrow has two aspects:
  - **Cells**- The cells we get in between are the cells.
    → A child would have 75% of cells and only 25% of Fat.
    → An elderly person would have 25% cells and 75% of Fats.
    → An adult has 50% of cells and 50% of fat. (1:1 ratio)

- The ideal Cellularity is 100–Age.



Dry Tap 00:15:42

- When there is no material, it is known as Dry Tap.
- Aplastic Anaemia.
- Myelofibrosis
- Hairy cell Leukaemia
- AML M7
- Myelophthisic Anaemia

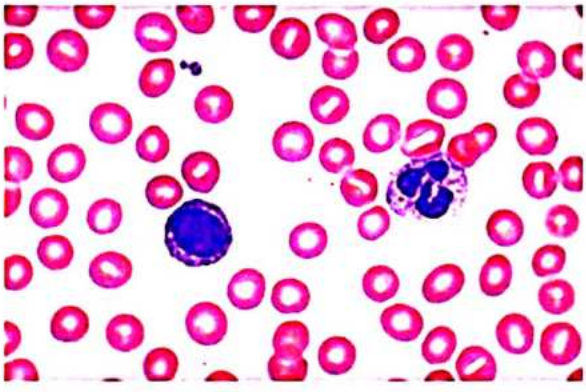
Thus, diagnosis depends on: Bone marrow biopsy

Stains

- For Peripheral Smear & Bone Marrow Aspirate: the Romanowsky Stain and it includes:
  - Giemsa Stain
  - Leishman Stain
  - Wright Stain
  - Jenner Stain
  - Field Stain

In most liquid cases, we use Giemsa or Leishman Stain.

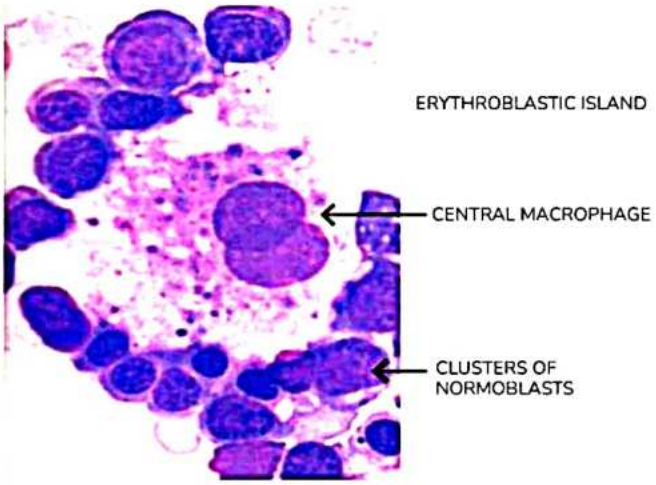
- Composition: Eosin Y (pink colour) and Methylene Blue/Azure B (blue colour)



Telegram - @nextpreladdernotes

- The pH of the Romanowsky Stain is 6.8.
- For Malaria, you have to keep a pH of 7.2

Erythropoiesis



- The Macrophage in the centre can be called the Nurse Cell because it will give all the iron to the erythroid Precursors.
- This is referred to as Erythroblastic Island.

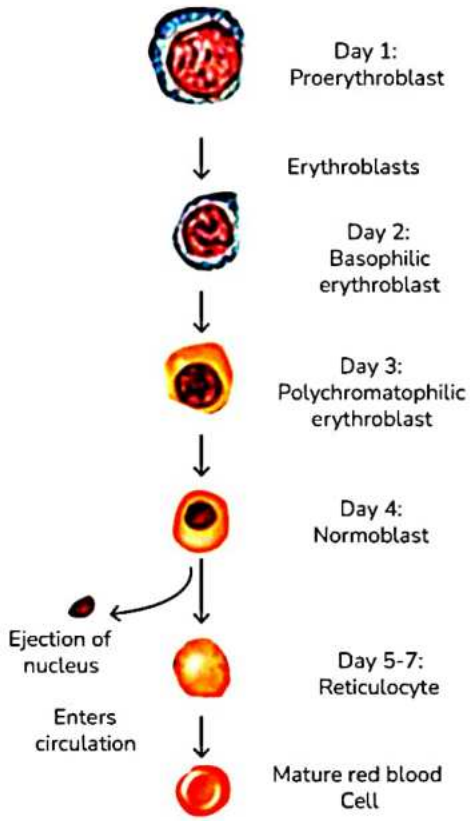
Flow Chart

00:24:27

Pronormoblast
↓
Early Normoblast (also known as Basophilic Normoblast)
↓
Intermediate Normoblast (Polychromatophilic Normoblast)
↓
Late Normoblast (Orthochromic Normoblast)
↓
Reticulocyte
↓
Erythrocytes

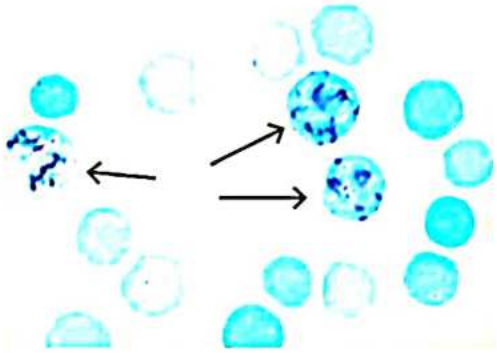
- All the blasts in the name means it is present in the Bone Marrow.
- So, the first 4 are present in the Bone Marrow.
- All the blast phases would have the nucleus.
- Red blood cells don't have a nucleus but it's the mature red blood cells which don't have the nucleus. The RBCs in the bone marrow have a nucleus.
- Erythrocytes are the Red Blood Cells.
- The first cell in the Peripheral Blood is the Reticulocyte. It is in Peripheral blood and not in Bone marrow.
- The first cell in this series which will have the nucleus is the Reticulocyte.
- As we go from top to bottom, the overall size of the cell will decrease. (Largest to Smallest)
- Similarly, when we go from top to bottom the size of the Nucleus also decreases. But the nucleus was present till the 4th stage (late Normoblast).
- The first Haemoglobin appears in the very first cell. It is only seen on an electron microscope. Haemoglobin is seen in light microscopy in the intermediate Normoblast or Polychromatophilic Normoblast.

Reticulocyte



- Reticulocyte is also referred to as Polychromatophil.

Supravital Stain



- This is a Supravital Stain.
- Eg: New Methylene Blue and Brilliant cresyl Blue

Telegram - @nextprepladdernotes

- The blue colour granules are the Reticulum or RNA. This is why the cells are called Reticulocytes.
- Supravital is Staining in a living State.
- Therefore, there is no methanol fixation.
- New Methylene Blue is the best stain we have.

Reticulocyte Count

- ADULT 0.5 TO 2%
- CORD BLOOD 2-6%

Corrected Reticulocyte Count

- CRC = RC X Hb of patients / Hb of normal person (15)
- Another formula is = RC X Hct (patients) / Hct (normal person)
- Haemoglobin X 3 = Hct (Hematocrit)
- As the normal Haemoglobin is taken at 15, Normal Hct will be 45.

Reticulocyte Production Index (RPI)

- RPI is known as Poor Man's Bone Marrow.
- RPI = CRC / Maturation Time

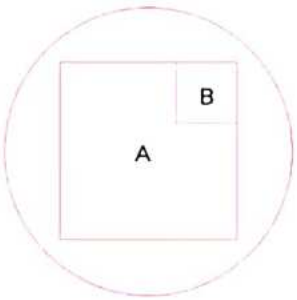
Hematocrit	Maturation Time
45	1 day
35	↑1.5 day
25	↑2 days
15	↑2.5 days

- RPI >3 ↑BM activity
- RPI 2-3
- RPI <2 ↓BM activity

- RPI >3 Good Bone Marrow Function
- RPI <2 Poor Bone Marrow Function

Miller Disc Method of Counting

- It is used for Reticulocytes counting.

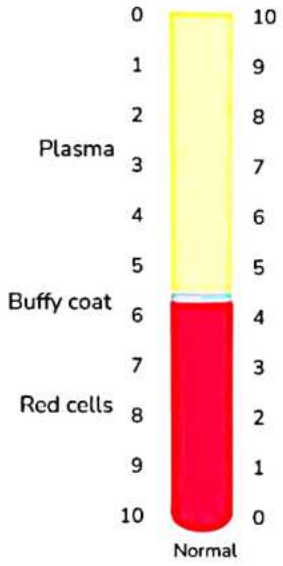


RBC Indices

00:47:38

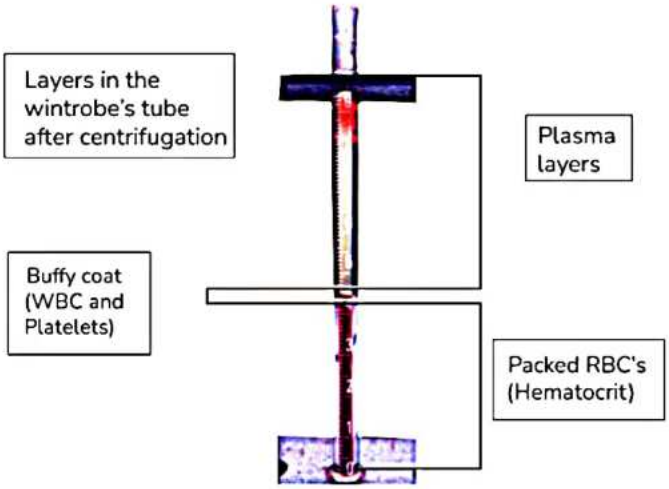
- Two very important things:
  - **PCV-**
    → Packed Cell Volume or Hematocrit (roughly 45%).
  - **ESR-**
    → RBCs settled down. So, this is known as ESR (Erythrocyte Sedimentation Rate).

Wintrobe's Tube



- The down-to-up reading is for PCV.
- The up-to-down reading is for ESR.

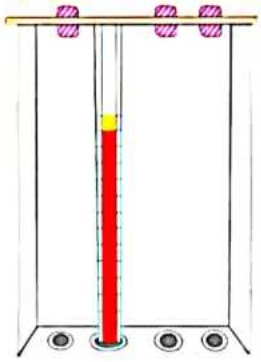
Wintrobe's Tube of Lab



- 42% is Hematocrit according to reading.
- Use of Wintrobe's Tube-
- Used for estimation on PCV/Hematocrit
- Also, used for assessing the ESR.

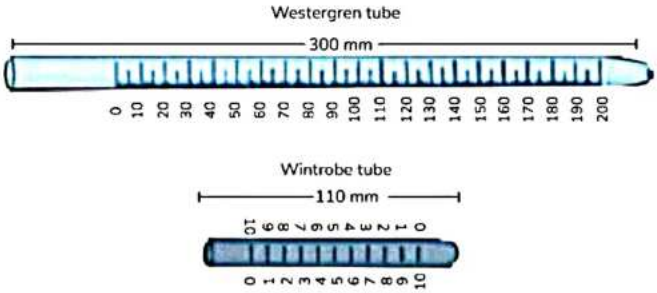
Telegram - @nextprepladdernotes

Westergren Pipette: ESR



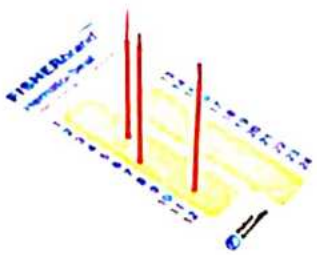
- It is only for ESR.
- It is a Pipette because it is open from both sides, unlike the test tube.

Difference Between Wintrobe's Tube & Westergren Pipette



	Wintrobe	Westergren
Bore	3 mm	2.5 mm
Graduation	Up to 100 mm	Up to 200 mm
Anticoagulant	EDTA (Lavender Vacutainer)	Tri Na Citrate (Blue)
Amount of Blood	Less amount	More amount

Micro Hematocrit



- Capillary Tube.
- It is something that we can use as a Bedside process.

Stages of ESR (Erythrocyte Sedimentation Rate - 60minutes)

- **Stage 1: Stage of Rouleaux Formation/Aggregation (10 min)-**
- **Stage 2: Stage of Sedimentation/Settling (40min)-**
- **Stage 3: Stage of Packing (10min)-**

MCV (Mean Corpuscular Volume)

- MCV measures average size of RBC.

MCH (Mean Corpuscular Haemoglobin)

- This is measuring Haemoglobin per red blood cell.
- Their normal value is 27-32 pg.

MCHC (Mean Corpuscular Haemoglobin Concentration)

- This is measuring Haemoglobin in the entire Hematocrit.
- Their normal value is 33-37 g/dL.

MCV:
<80 fl = microcytic
80-100fl = normocytic
>80 fl = macrocytic

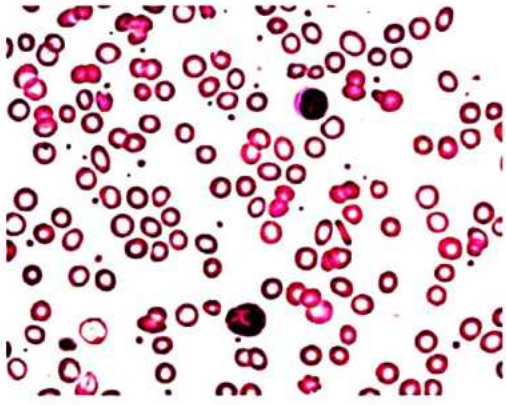
MCH(Hb):
<27pg = hypochromic
27-32pg = normochromic
32pg = hyperchromic

MCHC (Hb conc):
33-37 g/dl
Increased in heredetary spherocytosis

RDW (Red Cell Distribution Width)

- This measures a variation in the size of the Red Blood Cells.
- The variation in size of RBCs is known as Anisocytosis.
- The normal value is 11.5-14.5%.

Peripheral Smear Analysis



Telegram - @nextprepladdernotes

- Size of RBC is compared to nucleus of lypmhocyte.
- For knowing Hypochromia, central ⅓rd pallor is considered normal in RBCs.
- But suddenly we say that there is more than Central ⅓rd pallor, now it's looking wide. It will now be known as Hypochromia.

Anisocytosis

- It is the variation in the size of the Red Blood Cells.

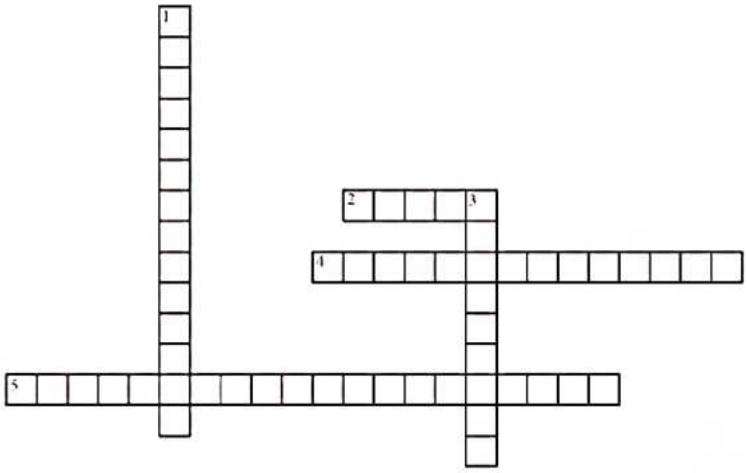
Poikilocytosis

- It is the variation in the shape of the Red Blood Cells.

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. Pink colour stain
4. It refers to Red Blood Cells production
5. This is a Jamshidi Needle for Bone Marrow Biopsy and this is also known as?

Down

1. It is the variation in the shape of the Red Blood Cells
3. The Macrophage in the centre

Telegram - @nextpreladdernotes

48 MICROCYTIC HYPOCHROMIC ANAEMIA

Mnemonic
- S - Sideroblastic anaemia
- I - Iron deficiency anaemia (M/C nutritional deficiency)
- T - Thalassemia
- A - Anaemia of chronic disease
  - It has two different types of morphologies.
    - → Normocytic/ Normochromic (more common)
    - → Microcytic/ Hypochromic

Iron Deficiency Anaemia 00:01:17

Iron Metabolism
- Site of iron absorption- Duodenum
- Form of iron in food- Fe3+
- Form of iron in absorption- Fe2+
- Food iron is 2 types;
  - Heme iron; Non Vegetarian food
  - Non heme iron; Vegetarian food
- Acidic pH: HCL, amino acids, ascorbic acid(Vit C) increases the absorption.
- Alkaline pH: Milk, phytate, tannate, tea and coffee decreases the absorption.

Iron Transportation
- TransFerrin is the transportation form of iron.
- Storage form of iron is ferritin
- Ideally, One molecule of TransFerrin binds to 6 molecules of iron.
- Practically, one molecule of Transferrin can bind to two molecules of iron.
- Normal Transferrin Saturation is 33%.
- The bonded transferrin and iron molecule gets transported to bone marrow.
- Erythroblasts in the red bone marrow make haemoglobin.
  - Erythroblasts have a lot of transferrin receptors and the molecules bind here.
- Increased transferrin receptors present on early erythroid precursors
- Late normoblasts release all of its Transferrin receptors.

Q. What is the marker of erythropoiesis?
Ans: Increase in serum levels of Transferrin receptors

Iron Absorption
Apical part

Food Fe^{3+}
↓Cyt B reductase
Fe^{2+}
↓Via DMT1 transporter
Absorbed easily into the duodenal cell

Duodenal cell

Fe^{2+}
↓via ferroportin
Enters blood vessel

Basolateral side

Fe^{2+}
↓Cerruloplasmin & Hephaestin
Fe^{3+} binds with transferrin (TF)
↓
enters bone marrow

- Ferroportin is also present in placenta and macrophages of bone marrow.
- Wherever iron is required inside the body, ferroportin is used.
- Transferrin binds with molecules of Fe3+ and goes to Erythroblasts to make haemoglobin.
- Erythroblasts have nucleus
- Transferrin receptors are present near the bone marrow and TF binds with Fe reaches bone marrow.
- The bounded molecules get engulfed and known as endosomes.
- Iron and transferrin must be dissociated by decreasing the PH.
- Endosome is acidified and the dissociation is done.
- Majority of iron is used in the production of haemoglobin.
- 90% of iron goes to mitochondria and 10% remains in the cytoplasm.

Master Regulator of Iron 00:21:07
- Hepcidin is the master regulator of iron
  - **Source**: Hepatic/ liver
  - **Function**: Inhibits iron absorption by inhibiting ferroportin
  - Inhibits release of iron from ferritin.

Causes of IDA
- Iron deficiency anaemia is the most common nutritional deficiency in our country.

Causes
- Inadequate iron diet
- High iron demand in conditions like
  - Pregnancy
  - Lactation
  - Growing child
- Increased blood loss
  - Due to parasitic infections like Hookworm(in children)

Telegram - @nextpreladdernotes

- In adults, Peptic ulcer disease
- In old person, Colon cancer
- A female with heavy menstrual bleeding.

- Site of iron absorption is duodenum: Duodenal/ intestinal disease
  - Celiac disease causes
    → Diarrhoea
    → Deficiency anaemia
    → Duodenum effect

Stages of IDA

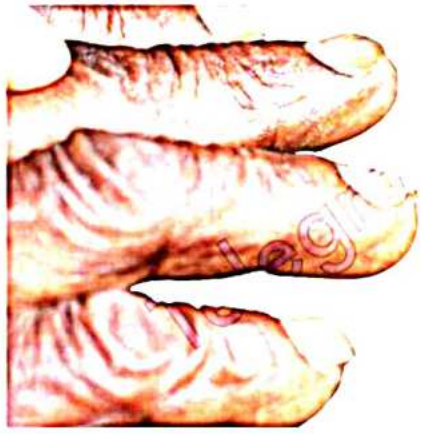
- Occurs in 3 stages

Stage-1: Stage of decreased storage.
- Decrease in ferritin.

Stage-2: Stage of iron deficiency erythropoiesis
- Iron profile is deranged.

Stage-3: Stage of iron deficiency anaemia
- It is a microcytic hypochromic anaemia.

Clinical Presentations



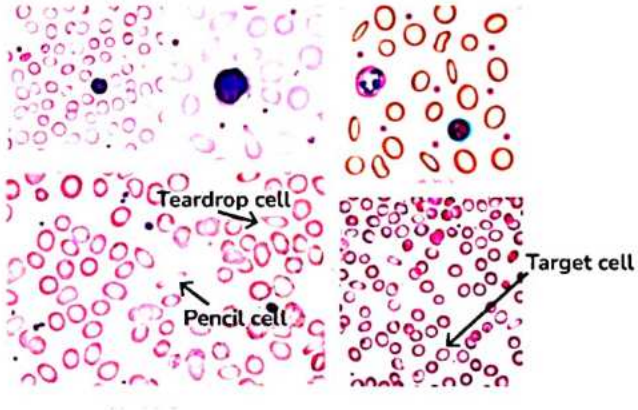
- Fatigue
- Koilonychia (Spoon shaped nails)
- Angular cheilitis/ Angular stomatitis
- In children, PICA
  - Eating chalk or mud
  - Eating non edible items
- Plummer Vinson Syndrome
  - Is a middle aged female
  - Also known as Patterson Kelly brown syndrome

Triad:
- Sidero penic dysphagia.
- Dysphagia- difficulty in eating (esophageal webs)
- Atrophic glossitis

Blood Findings

- Low Hb
- Low MCV
- Low MCH
- Low MCHC
- High RDW
  - Anisocytosis
- This is a nutritional problem.

Microscopically



- RBCs are smaller to the lymphocytes. These are microcytic cells
- RBCs show more than 1/3rd pallor. These are hypochromic
- anisopoikilocytosis seen anisocytosis.
- Pencil cells
- Sometimes, RBC hardly has any haemoglobin which looks like a ring
  - This is known as a leptocytes/ pessary cell.
  - It has a thin rim of Hb.
  - It is so severely deficient.
- Retic count increases.
  - Not in proportion to the degree of anaemia
- Bone marrow should be doing more work and making more reticulocytes for me.

Iron Studies

- Low serum iron
- Low transferrin saturation
- Low ferritin
- Increased **TIBC:** Total iron binding capacity
- **FEP:** Free erythrocyte protoporphyrin
  - These levels are increased

Normal Values

Serum iron	50-150 ug/dl; avg-100
Transferrin Saturation	15-50%(~33%)
TIBC(Fe x 3)	300 ug/dl

Telegram - @nextpreladdernotes

Bone Marrow Studies

- Micro normoblasts are seen in the bone marrow.
- Perl's/ prussian blue stain is used
  - There is no iron in the stores
- This is gold standard
  - Most specific test for testing iron deficiency.

Treatment

- Iron supplementation
- Treat the underlying cause such as
  - Blood loss
  - Diet, etc.
- **Follow up:** After blood testing and diagnosis we give the patient iron supplements.
- Ask the patient to come after 7 days/ 1 week
  - Find out reticulocyte count

Anaemia of Chronic Disease (AOCD) 00:48:33

- It is predominantly Normocytic and Normochromic anaemia.
- It can also be microcytic and hypochromic anaemia.

Pathogenesis

- Chronic indicates it is a long term disorder.
- For example, Rheumatoid arthritis
- In chronic conditions, IL 6 will be increased and acts on the liver.
  - Releases Hepcidin (master regulator)
    - → Inhibits Fe absorption
    - → Serum iron levels, transferrin saturation are decreased.
    - → Inhibits the release of Fe from ferritin.
    - → So, ferritin levels increase.
- This leads to decrease in TIBC

Profile of AOCD Varying from IDA

IDA	Feature	AOCD
Low	Serum iron	Low
Low	Transferrin saturation	Low
Low	Ferritin(bank account)	More
More	TIBC	Less

Mnemonic: TIBC

- T: Top in
- I: IDA
- B: Bottom in
- C: Chronic disease

New Parameter

- Serum transferrin receptor/log ferritin
  - >1.5 IDA; has low ferritin
  - <1.5 AOCD; has high ferritin

Treatment of AOCD

- Treat underlying causes like rheumatoid arthritis, etc.

Sideroblastic Anaemia 00:56:07

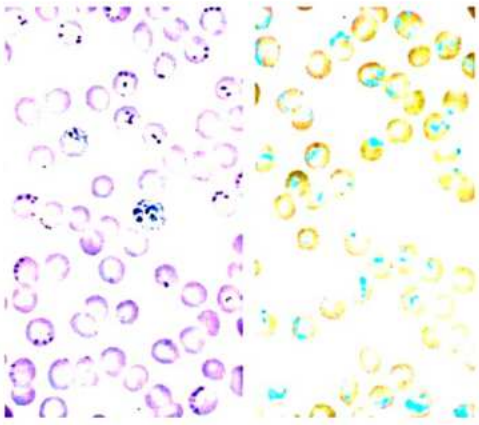
- **Sidero:** Iron
- This type is iron overload
- In Sideroblastic anaemia, iron is very high.

Heme Formation

- IIb = heme+globin
- Heme is made up of
  - Iron
  - Protoporphyrin
- Both of them fuse in the mitochondria
- Ferrochelatase, an enzyme, helps in the fusion.
- Succinyl coA forms ALA by ALA synthase
  - This forms porphobilinogen by ALA dehydratase
  - By a series of steps, it forms protoporphyrin.
- Anything other than iron goes wrong in this pathway
  - Causes Sideroblastic anaemia
- If it lacks iron, it is called iron deficiency anaemia.

Causes of Sideroblastic Anaemia

- Congenital causes
  - Due to enzyme deficiency
- Acquired causes
  - Vitamin B6 deficiency
  - Patient with lead and alcohol poisoning
    - → Alcohol causes mitochondrial poisoning

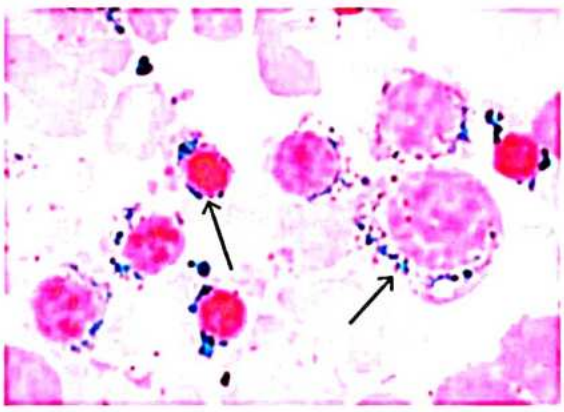


Peripheral smear

- There are a lot of Fe aggregates
- They look like bodies known as Pappenheimer bodies
  - Made up of iron
- To prove it, stain with perl's/ Prussian blue

Telegram - @nextprepladdernotes

Bone marrow



- Ringed sideroblasts are seen.
- The stain used is Perl's/ Prussian blue
- Ringed sideroblasts is said when there is atleast 5 granules of iron
  - Which covers 1/3rd of the nucleus.
- Ringed sideroblasts is seen in
  - Sideroblastic anaemia
  - Myelodysplastic syndrome
    → It shows SF3B1 mutation
    → Associated with ringed sideroblasts.

Feature	Sideroblastic Anaemia
Serum iron	Very high
Transferrin Saturation	Very high
Ferritin	Very high
TIBC	Low

Feature	IDA	AOCD	Sideroblastic Anaemia
Serum iron	Less	Less	More
Transferrin saturation	Less	Less	More
Ferritin	Less	More	More
TIBC	High	Less	Less

MCQs

Q. A 43 yr old Caucasian female complaining of reduced energy and fatigue is found to have hypochromic, microcytic anaemia. Her past medical history is significant for stable angina treated with metoprolol and aspirin. Iron supplementation is prescribed. Several weeks later, a peripheral blood smear demonstrates numerous enlarged red blood cells that appear blue on the Wright Giemsa stain. The bluish colour of these red blood cells is best explained by the presence of which of the following?

A. Haemoglobin precipitates
B. Nuclear membranes
C. Ribosomal RNA
D. Histones

Explanation

- Big blue cells on the Wright Giemsa stain are Polychromatophilic/ Reticulocytes.
- Reticulocyte is made up of Ribosomal RNA.

Q. A 38 yr old man presents to the emergency department due to severe alcohol intoxication. The patient is agitated and refuses to answer any question in regards to his medical history. The vital signs are within normal limits. The complete blood count result demonstrates haemoglobin of 11.5 g/dL, hematocrit of 39% and mean corpuscular volume of 77 um3. Using a special dye, blue- coloured spots are seen in peripheral smear along with occasional blue colour rings. What are the most likely findings on the ferritin, total iron binding capacity, and serum iron levels?

A. Ferritin: low, total iron binding capacity: low, serum iron: low
B. Ferritin: normal, total iron binding capacity: normal, serum iron: normal
C. Ferritin: high, total iron binding capacity: low, serum iron: high
D. Ferritin: high, total iron binding capacity: low, serum iron: low

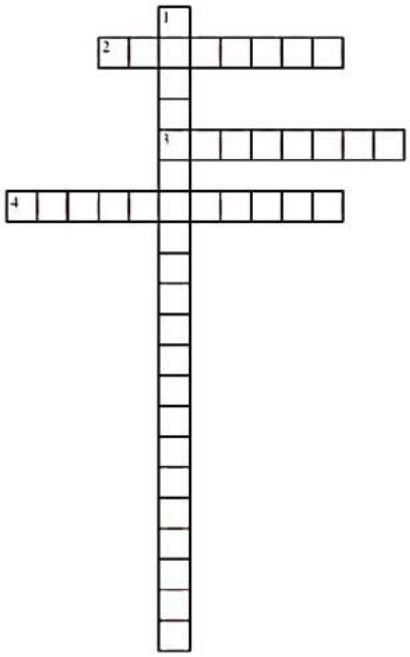
Explanation

- Normal MCV is 80-100 um3
- This condition is a microcytic condition.
- Perl's/ Prussian blue stain is used.
- They might be talking about pappenheimer bodies.
  - Iron is more in this case
- Alcohol intoxication leads to Sideroblastic Anaemia

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. It is the storage form of iron
3. Site of iron absorption
4. It is the transportation form of iron

Down

1. It is the most common nutritional deficiency in our country

Telegram - @nextpreladdernotes

49 MACROCYTIC HYPOCHROMIC ANEMIA

Associated with
- Liver disorders
- Hypothyroidism
- Megaloblastic anemia
- Chemotherapy drugs
- Mnemonic: LHMC

Megaloblastic Anemia

00:00:58

- Deficiency of Vitamin B12 and folic acid.

Vitamin B12

- Absorbed at terminal ileum.
- Absorbed only if B12 is combined with intrinsic factor (IF).
  - Intrinsic factor also called castle's factor.
    → Released by Parietal cells of the stomach.
- Vegetarian food is deficient in vitamin B12.

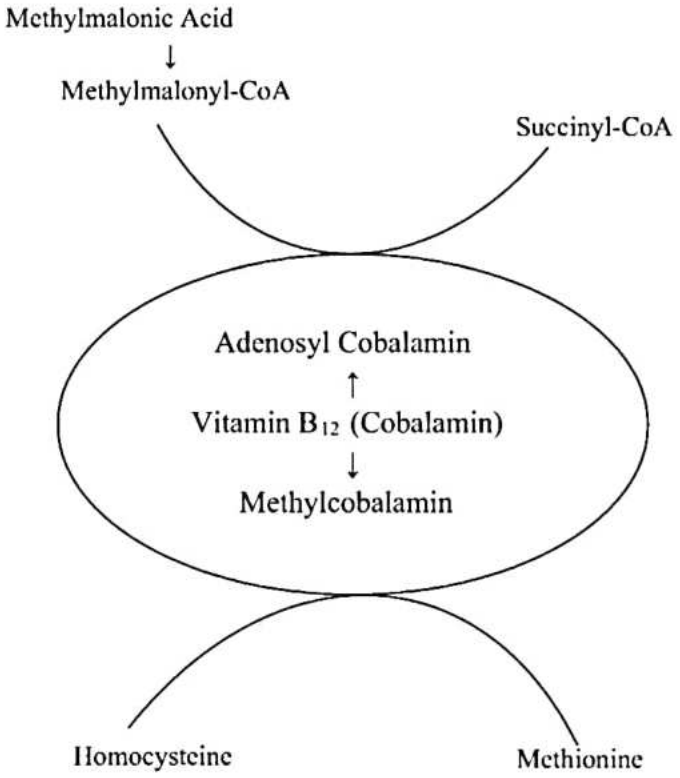
Food with B12 when ingested
↓
B12 is separated from food by peptic enzymes
↓
Enters the stomach
↓
Binds with haptocorrin - released by Salivary gland
↓
The complex enters the duodenum
↓
Haptocorrin is separated from B12 by Pancreatic enzymes
↓
B12 binds with intrinsic factor (IF)
↓
Moves to terminal ileum
↓
Terminal ileum has cubilin receptors
↓
B12-IF binds to the receptors and get absorbed
↓
Absorbed B12 is transported by transcobalamin 2

Uses of Vitamin B12

- **Thymidine production** - important for nuclear maturation.

Nuclear Cytoplasmic Asynchrony

- Patient with vitamin B12 deficiency:
  - Nuclear membrane is not matured.
  - Cytoplasm is matured.
  - N:C asynchrony.



- Vit. B12 Helps in conversion of
  - Methylmalonyl CoA to succinyl CoA
  - Homocysteine to methionine.

- Vitamin B12 deficiency doesn't convert the methylmalonyl CoA to succinyl CoA.
  - Methylmalonyl CoA levels increase whereas succinyl CoA levels decrease.
  - Succinyl CoA is important for myelin production.
  - Due to deficiency of myelin production.
  - Leads to Neurological symptoms.

- Vitamin B12 deficiency doesn't convert homocysteine to methionine.
  - Homocysteine levels increase and methionine levels decrease.
  - Increase in homocysteine levels leads to atherosclerosis.

Causes of Vitamin B12 Deficiency

- Vegetarian diet
- **Increased demand** - may be pregnancy, lactation, growing child etc.
- **Infections** - Diphyllobothrium latum

Telegram - @nextpreladdernotes

To Remember

- **Iron deficiency anemia** - Caused by Hookworm.
- **Megaloblastic anemia** - Caused by Diphyllobothrium latum.
  - Also called Fish tapeworm.

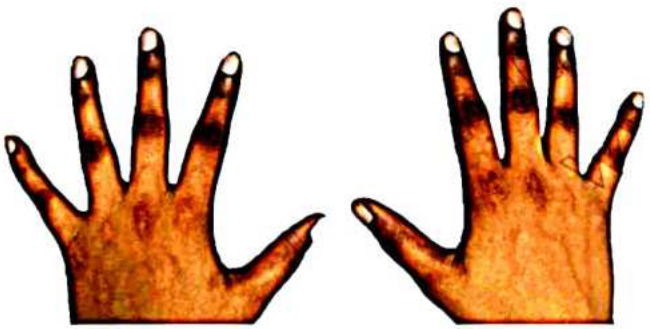
- **Gastrectomy** - no formation of intrinsic factors.
- Ileal disorders
- Malabsorption
- Pancreatic insufficiency
- Pernicious anemia

Pernicious Anemia 00:13:03

- It is an autoimmune disorder.
- 3 types of antibodies.
  - **Type 1:** Inhibits binding of B12 to intrinsic factor.
  - **Type 2:** Inhibits binding of B12-IF to iliac cubilin receptors.
  - **Type 3:** Ab against Parietal cells.
    → Intrinsic factor is not released.

Clinical Features

- Fatigue and pallor.
- **Hyperpigmentation** - mainly seen in knuckles



- **Atrophic glossitis** - Called beefy red tongue (smooth tongue).
- **Atrophic gastritis** - due to Pernicious anemia (Ab against Parietal cells).
  - Risk factor for gastric adenocarcinoma.

Clinical Feature seen only in B12 deficiency and not seen in folic acid deficiency

- Neurological manifestations.
  - Subacute combined degeneration.
  - Mnemonic: SACD.
  - Degeneration of spinal tract.
  - Both sensory and motor loss - so called combined.

RBC Indices

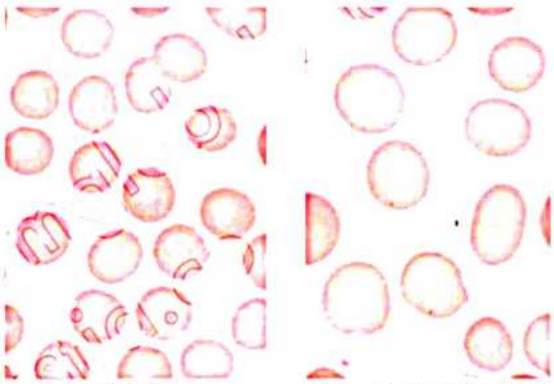
- Hemoglobin - low.
- MCV - high (> 100fl).
  - Normal - 80 to 100 fl.
- MCH - high.
- MCHC - normal.

Blood Picture

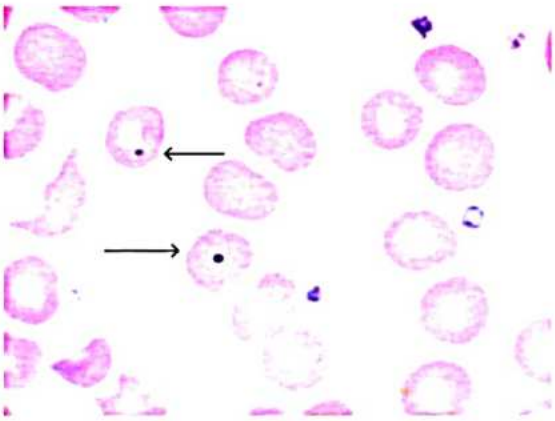
Shows problems associated with:

- RBC
- WBC
- Nuclear cytoplasmic asynchrony affects all the cells along with RBCs.

RBC Findings



- The RBCs shows the presence of
  - **Macrocytes** - large RBCs.
  - **Macroovalocytes** - large oval shaped RBCs.
  - **Howell jolly bodies** - single dot like structures on RBCs.



→ Composed of DNA or nuclear remnants.
→ Seen in Megaloblastic anemia and post splenectomy.

- **Cabot rings** are also seen.

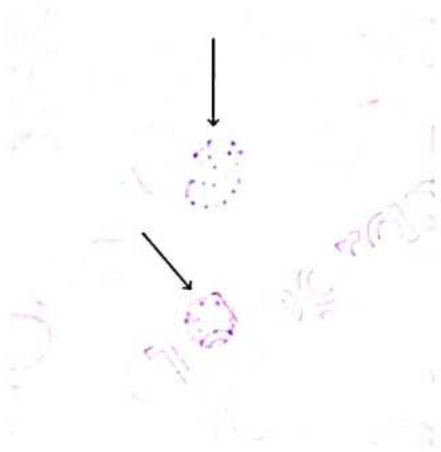
Telegram - @nextpreladdernotes

→ Composed of arginine rich mitotic spindle.
→ Can be molded in any shape.
→ Seen in 8 shaped structures.

Arginine

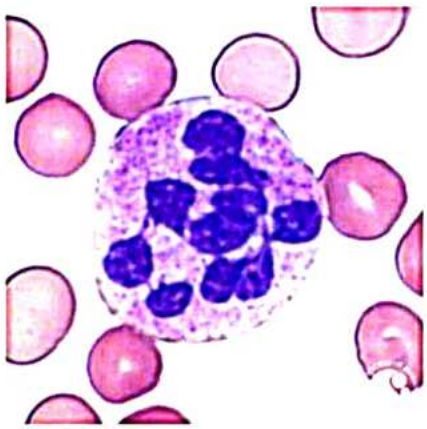
- Arginine rich mitotic spindles - Cabot rings.
- Arginine in killing mechanism - NETS.
- Arginine is the precursor of - Nitric oxide.

  - **Basophilic stippling**



Finding in WBC

Hypersegmentation of neutrophil is observed.



- A neutrophil is said be hypersegmented when:
  - 5% of the Neutrophils have 5 lobes.
  - Any 1 neutrophil has more than or equal to 6 lobes.
- The megaloblastic anemia present with pancytopenia ultimately.
  - RBC, WBC and platelets count is low.

Bone Marrow Findings

- Bone marrow hypercellular (hyperplasia).
  - It is erythroid hyperplasia as RBCs are mostly affected.
- In normal bone marrow,
  - Myeloid / erythroid ratio is **3:1**
- In erythroid hyperplasia,
  - There will be a reversal of the M / E ratio.
  - More erythrocytes are present.
  - Shows sieve like chromatin.

Biochemical Findings

- Ineffective erythropoiesis causes lysis.
  - Increases serum bilirubin and LDH.

Serum bilirubin	Increases
Serum LDH	Increases
Vitamin B12	Decreases
Folic acid	Decreases
Homocysteine	Increases
Methylmalonyl CoA	Increases
Anti IF antibody	Present (Pernicious anemia)

Schilling Test 00:30:26

- Used to find the cause of megaloblastic anemia.
- Not for the diagnosis of megaloblastic anemia.

Folate Metabolism and Deficiency

- **Site of folate absorption** - Jejunum.
- Chromosome controlling the folate metabolism - chromosome 21.
- Deficiency of folate seen in:
  - Alcoholic
  - Pregnancy
    → In some cases may cause neural tube defects.
- **Clinical Features:** Same as vitamin B12 deficiency.
  - Neurological symptoms are not seen.
- Serum folate and RBC folate is less.
- The FIGLU test is positive.
  - Urine test for folic acid deficiency.

Telegram - @nextpreladdernotes

FIGLU Test

Histidine
↓
Converts to FIGLU (Formimino glutamic acid)
↓
Uses folic acid
↓
Converts to glutamate

In folate deficiency:

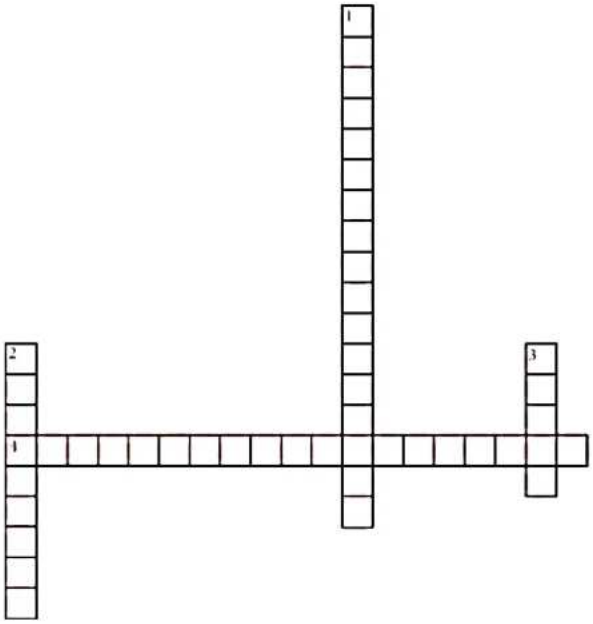
Histidine
↓
Converts to FIGLU
↓
No folate is available to convert to glutamate
↓
Therefore, FIGLU is excreted through urine

- Treatment includes vitamin B12 and folic acid supplements.

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

4. It is caused by Diphyllobothrium latum

Down

1. It is mainly seen in knuckles and interphalangeal areas
2. It is important for nuclear maturation
3. Ineffective erythropoiesis causes

Telegram - @nextpreladdernotes

50 HEMOLYTIC ANEMIAS

Two types

- IVH (Intravascular Hemolysis): Inside blood vessels
- EVH (Extravascular Hemolysis): Spleen, Liver

IVH (Intravascular Hemolysis) 00:00:58

- When a blood vessel gets damaged, Hemoglobin (Hb) comes out.
- Hb combines with Haptoglobin, this leads to reduction in the Serum Haptoglobin levels.
- Saturation of Haptoglobin makes the Hb to combine with Hemopexin, which also gets saturated.
- This leads the Hb to combine with Albumin, which causes Meth Albuminemia.
- Hb in IVH travels to all body parts, at the kidneys it gets eliminated as Hemoglobinuria.
- Kidneys also absorb Hemosiderin, which causes Hemosiderinuria.

EVH (Extravascular Hemolysis)

- Primarily seen in the spleen (splenomegaly) and liver.
- RBC breaks extravascularly, producing Heme (iron containing part).
- Heme → Biliverdin → Unconjugated Bilirubin → Uro-Stercobilinogens, pigment stones

Golden Point: Carbon monoxide (CO) is produced endogenously along with the production of Biliverdin.

Classifications

A. Intracorpuscular Defects

- **Inherited:** membrane, enzymes or hemoglobin defects
  - **Membrane defects:** Hereditary spherocytosis (HS), Hereditary elliptocytosis
  - **Enzyme defects:** G6PD deficiency, Pyruvate kinases deficiency, Hexokinase deficiency
  - **Hemoglobinopathies:** Sickle cell anemias, Thalassemias, HbC, HbD
- **Acquired:** PNH (Paroxysmal Nocturnal Hemoglobinuria)

B. Extracorpuscular Defects

- AIHA (Autoimmune Hemolytic anemia)
- MAHA (MicroAngiopathic Hemolytic anemia)-HUS, TTP, DIC which causes clotting
- Malaria (Black water fever)
- March Hemoglobinuria (capillaries in the heels of soldiers breaks down)

Extravascular Hemolysis (EVH)	Intravascular Hemolysis (IVH)
Membrane defects	PNH
Hemoglobinopathies	PCD
Enzyme defects	Enzyme defects
AIHA (warm)	MAHA(HUS, TTP, DIC)

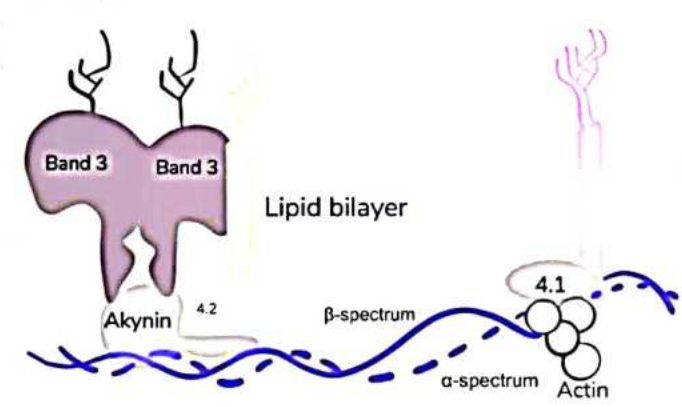
Golden Points

- Enzyme defects are seen in both Extravascular Hemolysis and Intravascular Hemolysis.
- All Intracorpuscular defects, AIHA causes Extravascular Hemolysis.

Intracorpuscular Defects

1. Inherited

a. Membrane Defects



- **Shape:** Biconcave
- Shape is maintained by Spectrin (alpha, beta)
- Head end: Ankyrin, Band 4.2, Band 3
- Tail end: Actin, Band 4.1, Glycophorin A

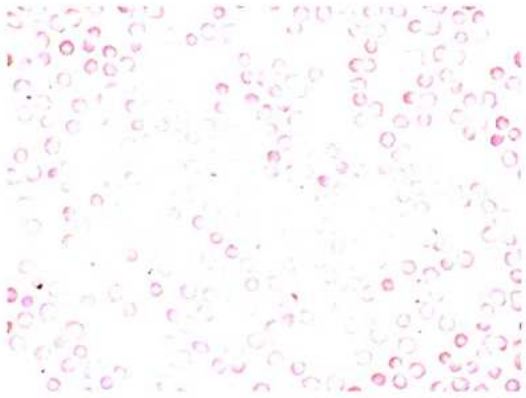
Golden Point: Glycophorin A is the abundant protein in RBC

HS (Hereditary Spherocytosis) 00:22:09

- Autosomal dominant (70%), as in Ankyrin mutation.
- Autosomal recessive, as in Spectrin mutation.

Telegram - @nextpreladdernotes

Band 3 defect

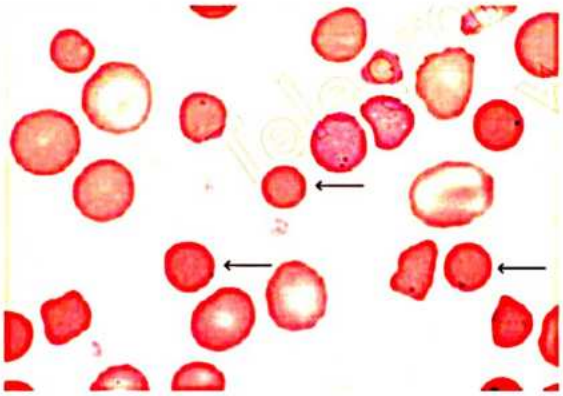


- Band 3 defect, Pincer cells/ Mushroom cells
- Inheritance of HS: Mostly Autosomal dominant (Ankyrin), rarely Autosomal recessive (Spectrin)
- Spectrin mutation is more severe

Golden Point: Recessive disorders are more severe than dominant mutations.

Pathogenesis of HS

- Cells with membrane defects pass through spleen (tiny capillaries).
- Reduction in the surface area to volume ratio leads to formation of Spherocytes.



- Micro Spherocytes are smaller than RBCs, MCV is low.
- Removal of water causes reduction of size.
- Hemoglobin concentration increases and central pallor is absent (MCHC is elevated).
- Life span reduces to 10-20 days (normal: 120 days)
- These cells are removed by spleen (EVH).
- Bone marrow increases the reticulocyte count.
- The MicroSpherocytes (small) and Reticulocytes (big) lead to variation in the size of RBC (increased RDW).

Golden Points

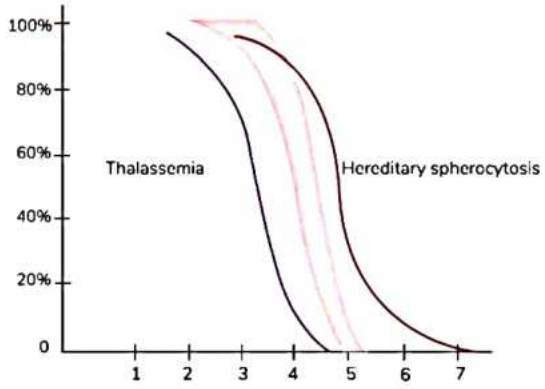
- Most common cause of Spherocytes is AIHA.
- Other causes include HS and G6PD deficiency.
- Pregnant women with spherocytes are investigated for AIHA (Coombs test).
- If Coombs test is negative, then we consider HS and G6PD deficiency.

Clinical Features of HS

- Classical triad: EVH
  - Pallor (increased RBC breakdown)
  - Jaundice (increased bilirubin levels)
  - Splenomegaly (increased RBC breakdown)
- Bile pigment gallstones are also present.

- **Parameters in HS (IMP)**
  - Reduced Hemoglobin
  - Reduced MCV
  - Variable MCH
  - Increased MCHC (water loss)
  - Increased RDW (variation in size)
  - Increased Reticulocyte count (big)

- **Screening Test for HS:**
  - Pink's Osmotic Fragility Test



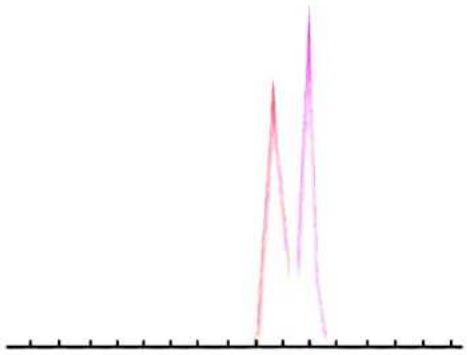
  - X-axis, concentration of NaCl
  - Y-axis, number of RBCs under gone lysis.
  - In a normal person, RBC lysis begins at 0.5% NaCl, complete at 0.3% NaCl.
  - HS patient, has high osmotic fragility (weak), graph shifts towards right.
  - RBC lysis begins at 0.7% NaCl, complete at 0.5%.
  - In thalassemia patients, graph shifts to left, they have less fragility (tough).

Confirmatory Tests for HS

- EMA (Eosin 5' Maleimide) test
  - Eosin 5' Maleimide is a dye, which binds to band 3.

Telegram - @nextprepladdernotes

- Flow cytometry is used to detect.



→ Flow cytometry gives Dot plot analysis and Histogram.

→ The peaks of the graphs of HS and control are compared.

- EKTA Cytometry
  - RBCs are subjected to stress.
  - As the cells are weak, they break.

Treatment for HS

- HS is caused due to genetic defects.
- Hence they are difficult to stop.
- Vaccination for encapsulated organisms is done.
- Spleen is removed (Splenectomy).
- In post splenectomy cases, spherocytes are still present.
- But EVH does not occur, because the spleen is removed.

Echinocytes

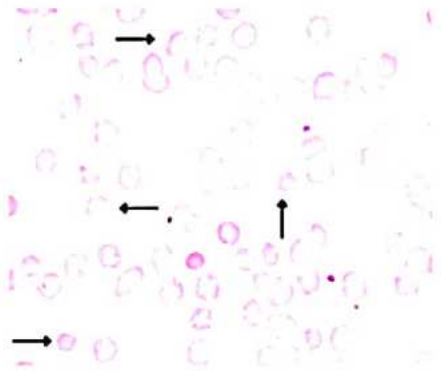
- Burr cells
- Seen in Burns, uremia, prolonged exposure to EDTA

Golden Point

- If the sample is collected from a patient and observed lately, the shapes may get blurred, due to EDTA changes.
- Hence, the time of collection of samples plays a crucial role.

Acanthocytes

- Spike/Spur cells
- Abetalipoproteinemia



b. Enzyme Defects

G6PD deficiency 00:48:55

- Inheritance: X linked recessive
- Boys are commonly affected

Precipitating Factors

- Fava beans (mediterranean region) cause Favism
- Fever
- Infection
- Drug: Antimalarial drugs

Recall

- During COVID 19 pandemic, all the healthcare workers are given Hydroxycholoquinolines as prophylaxis.
- But before administration, blood tests are taken to verify normal G6PD levels.
- Because Hydroxycholoquinolines act as a precipitating factor for G6PD deficiency.

- G6PD deficiency is an intermittent disorder, occurring in the presence of precipitating factors.
- It is primarily EVH, rarely IVH.
- It reduces in the absence of precipitating factors.
- Features of chronicity (splenomegaly and gallstones) are not seen.

Pathogenesis of G6PD Deficiency

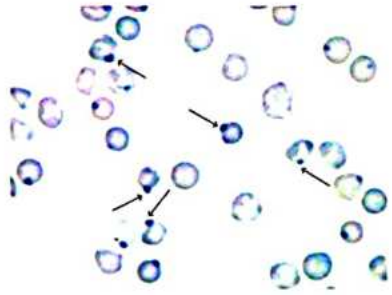
- Glucose pathway: Glucose → G6P → 6PG.
- G6PD, converts G6P to 6PG
- Glutathione reductase converts NADP into NADPH (Autofluorescent) and GSSG to GSH.
- H2O2 (free radical) conversion to H2O is also seen simultaneously.
- Hence, G6PD reduces free radical concentration, indirectly.
- Absence of G6PD
  - Do not convert G6P to 6PG
  - Conversion of NADP to NADPH does not takes place
  - Conversion of GSSG to GSH is also inhibited.
  - This inhibits conversion of Hydrogen peroxide to water.
  - This finally results in an increase in the concentration of free radicals (Oxidative stress).
- Oxidative stress causes denaturation of hemoglobin and forms Heinz bodies.
- Healthy part of the Hemoglobin is separate from the denatures part, looks like a bite cell.

Golden Points

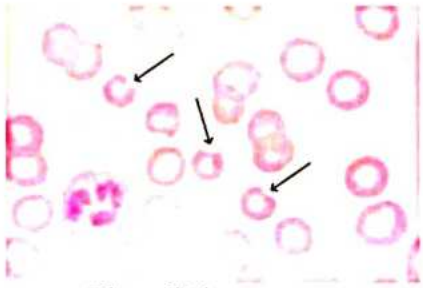
- Heinz bodies are not stained with Romanowsky family stains.

Telegram - @nextpreladdernotes

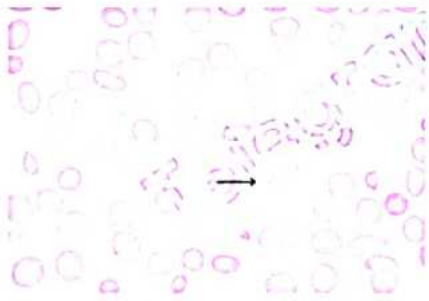
- If Romanowsky family stains are used, the bodies may not appear and it produces an illustration of a bite cell (Degmacyte).
- They are stained with Crystal violet stains.
- Spleen starts destroying RBC, producing spherocytes.
- Spherocytes are seen in AIHA, HS and G6PD deficiency.



Heinz body on crystal voilet stain



Bite cell/ degmacyte on romanowsky stain



Blister cell on romanowsky stain

Diagnosis of G6PD Deficiency

- Peripheral smear test
- Precipitating factors
  - Antimalarial drugs
  - Fava beans
  - Infections

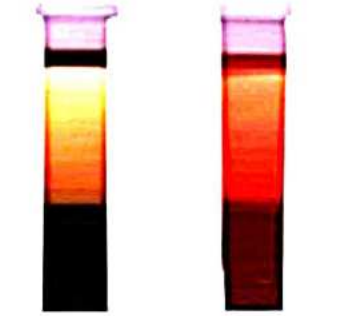
Clinical Features of G6PD Deficiency

- Intermittent episode
- Pallor
- Jaundice
- No splenomegaly and gallstones

Screening Test for G6PD Deficiency

(not done nowadays)

- **Methemoglobin reduction test**

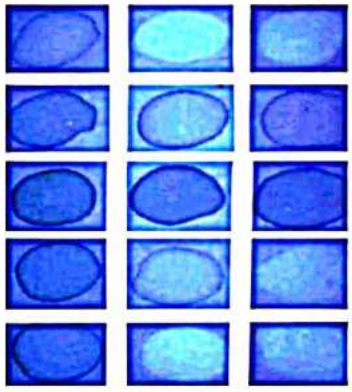


Deficient blood Normal blood

  - Sodium Nitrite converts Hb (hemoglobin) red in color to Hi (methemoglobin) brown in color.
  - Adding methylene blue should stimulate the pentose phosphate pathway, reducing methemoglobin.
  - In normal cases, G6PD reverses the conversion of methemoglobin to hemoglobin.
  - But in G6PD deficiency, methemoglobin is not converted to hemoglobin.
  - Hence brown color persists in G6PD deficiency.

To remember

- In Hemoglobinuria urine samples are collected.
- In G6PD blood samples are collected.
- **Fluorescent spot test**



  - NADPH is Autofluorescent
  - **In normal cases** - NADPH is produced, which shows fluorescence.
  - **In G6PD deficiency** - NADPH is not produced, fluorescence is not seen.

Confirmatory Tests for G6PD Deficiency

- G6PD assay (majorly used)

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2. Acquired - PNH (Paroxysmal Nocturnal Hemoglobinuria) 01:13:00

- Only acquired Intracorpuscular defect.
- Hemoglobin is eliminated in the night time through urination.

Pathogenesis of PNH

- The PIGA gene on the X chromosome develops GPI anchors.
- These anchors help in holding the molecules like
  - CD55/DAF (Decay Accelerating Factor)
  - CD59/MIRL (Membrane Inhibitor of Reactive Lysis)
  - C8 binding protein
  - Leukocyte alkaline phosphatase (inner side)

Recall: WBC chapter for Leukocyte alkaline phosphatase.

Golden Points

- CD55, CD59 are the controllers of the Complement system (induce breakdown of cells).
- At night, respiratory rates slightly reduce, which inturn reduces pH.
- Reduced pH activates the Complement system which is controlled by CD56, Cd59.
- In PNH, the PIGA gene and anchors (CD56, CD59) are absent, which leads to an uncontrolled complement system.
- This causes uncontrollable RBC breakdown.
- The broken RBCs are eliminated through urine.

Recall

- PIGA gene defect
- GPI anchors are absent
- CD55, CD59 are absent
- C8BP is absent
- LAP score reduced.
- Only acquired defect.

Golden Point

- PNH is an acquired defect, not all cells are affected.
- Dual populations of cells are present.
- Majority of cells lack CD56, Cd59.
- CD56, CD59 are present in some of the cells

Clinical Features of PNH

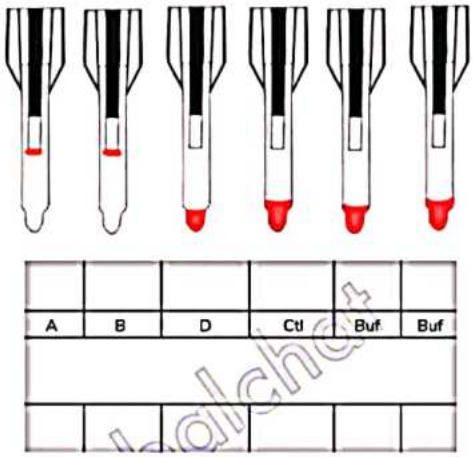
Triad: HAT

- Hemolytic anemia (IVH), CD59/MIRL is dominant
- Thrombosis, most common cause of death
  - Cerebral vein thrombosis (CVT)
  - Hepatic vein thrombosis (HVT)- Budd chiari syndrome.
- Pancytopenia/ Aplastic anemia(AA)

Screening Tests

- Ham's acidified serum test (not used nowadays)
- Sucrose lysis test (not used nowadays)
- Gel card analysis test

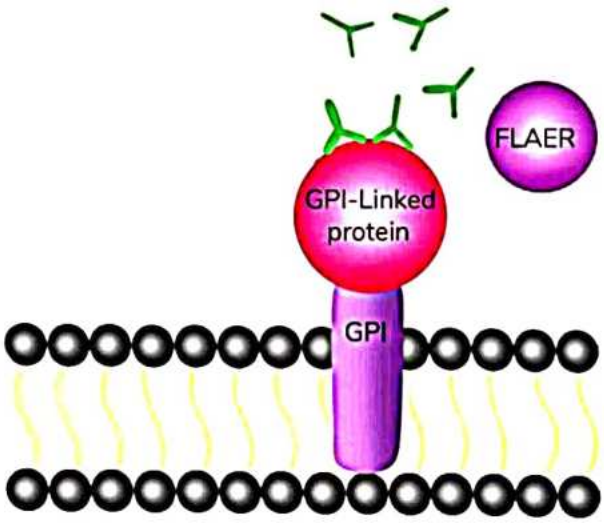
Gel Card Analysis Test



- Gel card consists of CD59, CD56 and a control
- Ag-Ab (Antigen-Antibody) reaction occurs to form a complex
- When the patient's blood is added to the gel card.
  - In a normal person: CD59, CD56 are present, the blood stops at the top, it is positive.
  - In PNH case: CD59, CD56 are absent, the blood stops at the bottom, it is negative.
- This is not a confirmatory test, because of the presence of a dual population.

Confirmatory Tests for PNH

- **Flow cytometry**
  - Detects CD59, Cd55
  - It may give dual population
- **FLAER** (Flow cytometry with Aerolysin)



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- Detects GPI anchors
- It finds the root cause of PNH

Complications of PNH

- Hemolytic anemia
- Thrombosis
- PNH is bipolar
  - Aplastic anemia
  - AML
- PNH is a pre neoplastic condition

Treatment for PNH

- Eculizumab (C5 Inhibitor)
- Hematopoietic Stem Cell Transplantation (HSCT)

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PREVIOUS YEAR QUESTIONS

Q. Most abundant protein?
Ans: Glycophorin A

Q. Most common mutation in HS?
Ans: Ankyrin

Q. Spectrin mutation is?
Ans: Autosomal recessive mutation in HS.

Q. Which protein is never mutated?
Ans: Glycophorin A

Q. What are the crises found in HS?
Ans:

- Aplastic crisis-Parvovirus B19
  Hemolytic crisis- EBV (Epstein Barr virus)

Q. Heinz bodies are seen on?
Ans

- Crystal violet staining
- Denatured Hemoglobin
- G6PD deficiency
  Heinz bodies are not seen on romanowsky stain.

Q. Bites cells are known as?
Ans: Degmacytes

Q. Light used in fluorescence testing is?
Ans: UV light

Q. Eculizumab may cause which infection?
Ans:

- Eculizumab Inhibits complement system, inhibits MAC (Membrane Attack Complex).
- This may lead to a risk of Meningococcal infection.

Q. What is the LAP score in PNH?
Ans: Absence of anchors reduces the LAP (Leukocyte alkaline phosphatase) score.

Q. Do I have to take the Meningococcal vaccine before taking Eculizumab?
Ans: Yes, the vaccine has prophylactic properties.

Q. Protein responsible for Biconcave shape?
Ans: Spectrin

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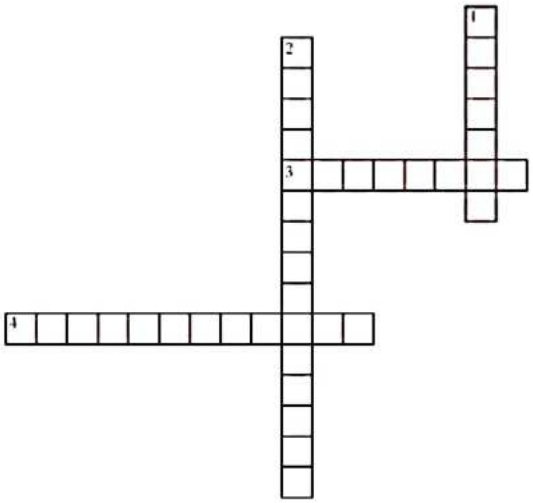
CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Protein responsible for Biconcave shape
4. Most abundant protein

Down

1. Most common mutation in HS
2. Kidneys also absorb Hb which causes?

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51 SICKLE CELL ANEMIA AND THALASSEMIA

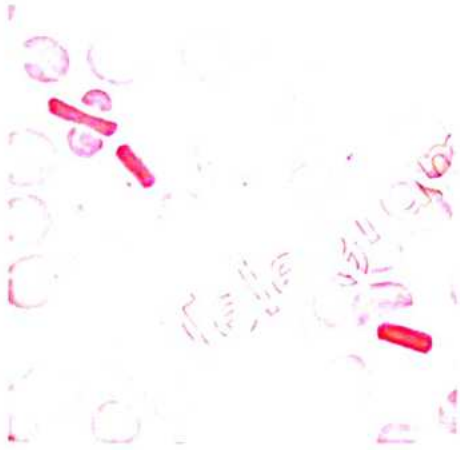
- **Hemoglobinopathies** - Sickle cell anemia and Thalassemia.
- **Normal hemoglobin**
  - Adult hemoglobin (HbA) - 95% and above - made of α 2 and β 2 chain.
  - HbA2 - Less than 3% - made of α 2 and delta 2 chain
  - Fetal hemoglobin (HbF) - made of α 2 and gamma 2 chain

1. Sickle Cell Anemia

- Mutation - Point mutation
  - Problem occurs at β 6 subunit.
  - Glutamic acid is changed to valine.
  - When glutamic acid is present - patients have HbA.
  - When changed to valine the HbA changes to sickled hemoglobin (HbS).
  - So, it is a missense point mutation.

To Remember

- At β 6 position, if glutamic acid changes to lysine.
  - It is called HbC hemoglobin disorder.



Factors of Sickle Cell Anemia

- Amount of HbS and HbA
  - HbS ∝ Sickling.

To Remember

- Normal Cell converts to Sickle cell when:
  - O2 levels are low - Hypoxia.
  - Less PH - Acidic environment.
  - Less water - Dehydration.
- **Dehydration:** More sickling.
- **Hypoxia:** More sickling.
- **Less PH:** More sickling.
- **Amount of fetal hemoglobin**
  - Fetal hemoglobin has more affinity to oxygen.
  - More oxygen - less sicking.
- **Sickle-α thalassemia**
  - In α thalassemia there is no α chain.
  - Less hemoglobin.
  - So as there is less hemoglobin, sickling is less.

Types

- **HbAA** - Normal.
- **HbAS** - Heterozygous type.
  - Sickle cell trait.
- **HbSS** - Homozygous type.
  - Sickle cell anemia.
- Sickle cell anemia is autosomal resistance disease.

Compound Heterozygous

- Contains both harmful genes.
- Like HbβS - Here both the genes are harmful.
  - β thal and Sickle cell genes are present.
- If it contains one normal and one harmful gene - heterozygous.

Pathophysiology

Regular Normal cells
↓
In the absence of oxygen and less pH
↓
The cells convert to sickle shape - This is a reversible process
↓
When there is enough oxygen and proper PH the sickle shaped cells are converted to normal again - reversible sickling
↓
When there is repeated reversible sickling it leads to irreversible sickling
↓
These sickle shaped cells are very sticky
↓
Stick to each other and forms a mass
↓
If these get stuck in the narrow blood vessels - results in vaso-occlusive crisis.

Clinical Features of Sickle Cell Anemia

- Pallor
- **Splenomegaly** - It is an extravascular hemolysis and broken by spleen.
  - Causing splenomegaly.
- **Jaundice** - RBCs are broken therefore increases bilirubin.

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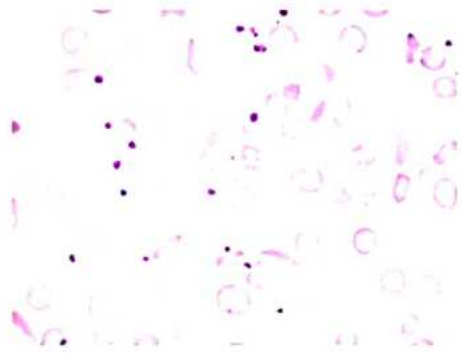
- **Vaso-occlusive crisis in different organs**
  - **Brain** - results in stroke.
  - **Heart** - results in MI.
  - **Lungs** - results in acute chest syndrome.
  - **Bones**
    - → Vertebra - Fish mouth vertebra.
    - → Femur - Avascular necrosis.
    - → Digits - Acute dactylitis.

To Remember
- Fish mouth vertebra is seen in:
  - Sickle cell anemia
  - Osteoporosis
- Fish mouth valve is seen in - Rheumatic Heart Disease (RHD).

Other Crisis of Sickle Cell Anemia
- **Aplastic crisis:** Associated with parvovirus B19 infection.
- **Hemolytic crisis:** Associated with Ebstein Bar Virus.
  - Everything is broken off.
- **Sequestration crisis:** All sickle cells are sequestered into the spleen.
  - Attacks the spleen and damages it.
  - Referred as multiple splenic infarcts.
  - Leads to decrease in spleen size - Auto splenectomy.

To Remember
- Initially the spleen gets enlarged as it is performing more functions.
- When a sequestration crisis forms - the spleen gets shrinked.
- Leads to auto splenectomy.

Diagnosis of Sickle Cell Anemia
- **Hemoglobin:** Hb levels decrease
- **Reticulated Count:** As bone marrow works more the reticulocyte count increases.
- **Bilirubin:** As it is an extravascular hemolysis the breakdown of RBCs increases bilirubin.
- **ESR:** Erythrocytes Sedimentation Rate.
  - Normal cells get packed and settle down.
  - Sickle cells do not get packed and are not settled down.
  - Hence, ESR is low.



Sickle-shaped cells
- These are also called drepanocytes.
- Only seen in sickle cell anemia.

Note: Sickle-shaped cells are seen only in sickle cell anemia but not in sickle cell trait.

a. Sickling Test 00:17:56
- Done if there are no sickle cells to know if it is sickle cell trait or not.

Sickling test

Take a clean slide
↓
Add a drop of blood of patient
↓
Add 2% sodium metabisulfite or sodium dithionite - To remove oxygen from the cells
↓
Cover the slide with coverslip - To prevent the exposure of atmospheric oxygen
↓
Seal the coverslip with paraffin wax or nail paint
↓
The cells converts to sickle shape

- This test is very primitive.
- Do NOT differentiate between sickle cell anemia and sickle cell trait.

b. Solubility Test
- Sickle cells are sticky and also have less solubility.
- It is also a primitive test.

Blood is added into the test tube
↓
Add sodium dithionite to remove oxygen
↓
Sickle cells form polymers
↓
Makes the test tube turbid
↓
Indicates less solubility

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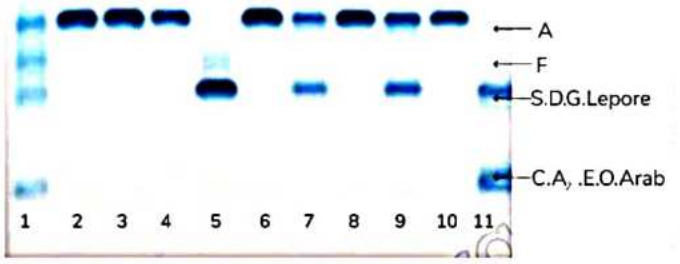
c. Hemoglobin Electrophoresis

- **2 types**
  - Cellulose acetate electrophoresis - pH 8.4 (alkaline).
  - Citrate electrophoresis - pH 6.2 (acidic).
- **Cellulose acetate electrophoresis**
  - Hemoglobin electrophoresis has 2 ends - anode and cathode.
  - Hemoglobin bands are formed.
    - → Hemoglobin H
    - → Hemoglobin A
    - → Hemoglobin F
    - → Hemoglobin S
    - → Hemoglobin A2
  - Mnemonic: HAFSA2
  - These bands are formed from anode to cathode.
  - Along with hemoglobin S, hemoglobin D can also be seen.
  - To differentiate these the other type citrate electrophoresis is used.



Cellulose acetate electrophoresis

- **Citrate electrophoresis**
  - Here the hemoglobin S and D are splitted which are formed in cellulose acetate electrophoresis.
  - With Hemoglobin A2 - C, E, O-Arab.

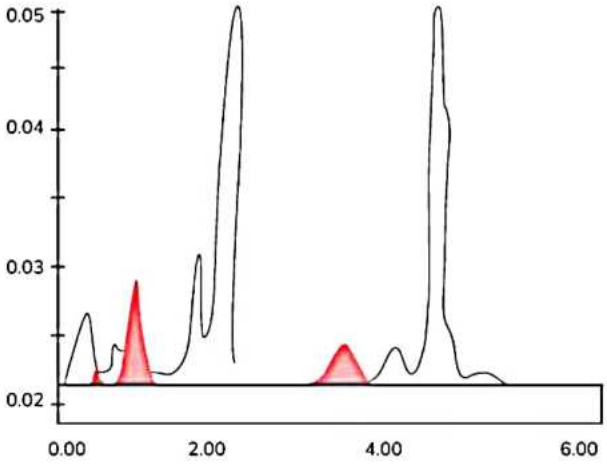
Note: Hemoglobin A2, C, E, O-Arab are present near the cathode.

Concept - Slide is taken and blood is placed at cathode

- If hemoglobin moves faster and reaches the anode - **HbA (normal).**
- If hemoglobin presents both towards anode and cathode - **HbAS (sickle cell trait).**
- If hemoglobin moves slower and stays at cathode - **IIbS (sickle cell anemia).**
- Even the electrophoresis doesn't show proper results as there are overlaps and no quantity is examined.

d. HPLC: High-Pressure Liquid Chromatography.

- Gold standard test for any type of hemoglobin disorders.



HPLC graph

- Graph shows different peaks for different hemoglobin.

Peak Name	Retention time, Min
P1 window	0.63-0.85
F window	0.98-1.20
P2 window	1.24-1.40
P3 window	1.40-1.90
A_0 window	1.90-3.10
A_2 window	3.30-3.90
D window	3.90-4.30
S window	4.30-4.70
C window	4.90-5.30

  - Peak name and retention time
- The peaks are formed based on the elution time.

Treatment of Sickle Cell Anemia 00:31:31

- As it is a hereditary disorder there is nothing more can be done.
- Hydroxyurea (to treat hypoxia)
  - Increases the fetal hemoglobin.
  - Therefore increases oxygen affinity.

2. Thalassemia 00:32:11

- Adult hemoglobin - Made of α2 and β2 chains.
  - This means there are 2 α chains and 2 β chains.

4 α genes present on chromosome 16 and 2β genes present on chromosome 11

↓

Gives 2 α chains and 2 β chains respectively

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↓

Absence of α gene - α thalassemia

Absence of β gene - β thalassemia

Note

- α thalassemia - There is α gene deletion.
- β thalassemia - There is β gene mutation.

A. β Thalassemia

- **3 types**
  - **Thal major** - always dependent on transfusion. → Called Cooley anemia.
  - **Thal intermedia**
  - **Thal minor / trait** - Heterozygous

Feature	Thal major	Thal intermedia	Thal minor / trait
β chain production	• No β chain production. • $\beta^0\beta^0$	• Little chains present.	• One β chain is normal. • Others are
Clinically	• Pallor • Jaundice • Splenomegaly	• Pallor • Jaundice • Splenomegaly	Asymptomatic
Hb	3 to 5 (more severe)	5 to 8	> 8
Iron profile	Increased	Normal	Normal
Hb electrophoresis and HPLC	Increased HbF	Both increased	HbA2 > 3.5%

- β thalassemia is also an extravascular hemolytic disease.

β Thalassemia - Genetics (Update)

- Most of them are promoter region mutation.
- Splicing mutation can also occur - most common.
  - **Splicing** - removes introns.
  - Mutation in intervening sequence - IVS1-5 G to C in India.
- Chain termination mutation.
- **Frameshift mutation:** Occurs at +8/9th codon or +41/42 codon.

To Remember: Only deletion in β thalassemia - 619 base pair deletion.

a. Pathogenesis of β Thalassemia Major

- β chains are missing.

1st possibility,

It stays alone and forms α 4 tetramers

↓

This leads to ineffective erythropoiesis

↓

Increases load on bone marrow - Erythroid hyperplasia

2nd possibility,

It combines with gamma 2 (fetal hemoglobin)

↓

This has high affinity for oxygen

↓

Less oxygen is reached to tissues

↓

Stimulates the release of erythropoietin

↓

Increases work load on bone marrow - Erythroid hyperplasia

- Therefore, in thalassemia major:
  - HbF increases
  - RBC increases but Hb decreases - Typical in thalassemia patients.
  - Iron levels increase.

As there is erythroid hyperplasia it requires some energy to work more

↓

Stimulates the GIT to absorb more Iron - Iron levels increases

↓

The patients are kept on blood transfusions

↓

Repeated blood transfusion - increases Iron levels

↓

Finally there is Iron overload

Erythroid hyperplasia increases work on other bones and organs

↓

It stimulates erythropoiesis from other bones and organs

↓

This erythropoiesis from other bones - leads to Crew Cut Skull

↓

The erythropoiesis from other organs - Extra medullary Hematopoiesis

↓

Crew cut appearance of skull

Chipmunk facies

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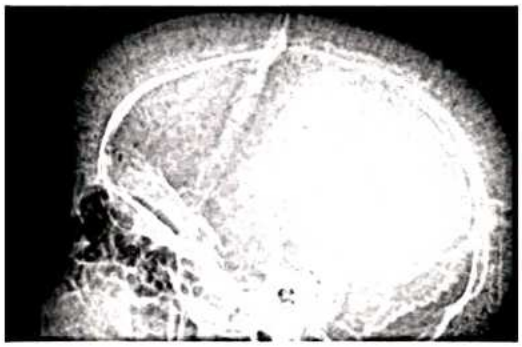
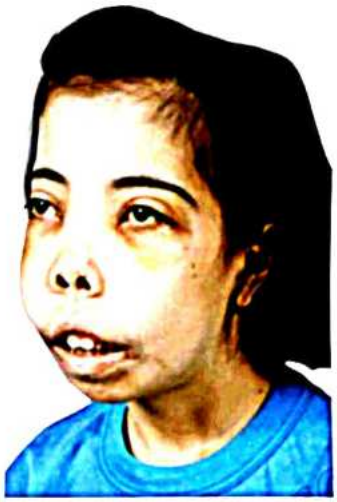


Crew cut appearance of skull

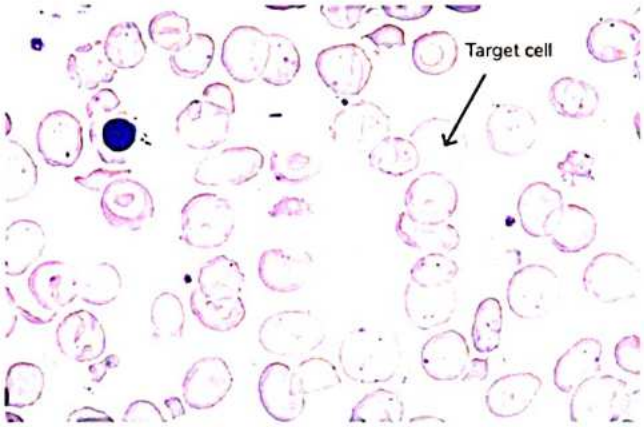
- This is also called Hair on End appearance.
- Seen in both thalassemia and sickle cell anemia.
- This is an indication of erythropoiesis in other bones.



Chipmunk facies

- Maxillary Prominence is present.

Peripheral Smear Finding of β Thalassemia Major



Peripheral Smear

- It is a type of microcytic hypochromic anemia.
- Nucleated RBCs are present.
- Target cell or codocyte is also present.

- **Confirmatory test** - HPLC.
  - Shows high HbF.

Note: Globin gene sequencing is the best technique.

b. β Thalassemia Minor/Trait

- Microcytic hypochromic anemia.
- Asymptomatic.
- The condition is confused with iron deficiency anemia.

Feature	Iron Deficiency Anemia	β Thalassemia Minor
Type	Microcytic hypochromic anemia	Microcytic hypochromic anemia
RBC	Less	More
RDW (size variance)	More	Normal
Mentzer index: MCV/RBC	> 13	< 13
HbA2 - confirmatory test	<3.5%	> 3.5%

Nestroft Test

- One of the screening tests for β thalassemia.
- It is the Naked Eye Single Tube RBC Osmotic Fragility.
- Thalassemia cells are always tough.

Method

2 test tubes are taken - patient blood and normal person's blood is added in each

↓

5 ml of 0.35% normal saline is added to both - Hypotonic solution

↓

The hypotonic saline starts entering the RBCs

↓

A white paper with black line is placed behind both the tubes

↓

The RBCs of normal person blood breaks due to hypotonic saline - the black line is visible

↓

Thalassemia cells do not break - the line is not visible

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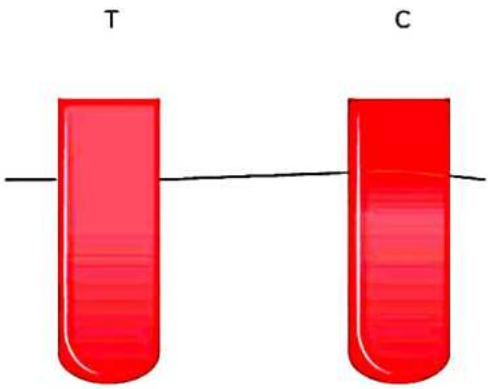
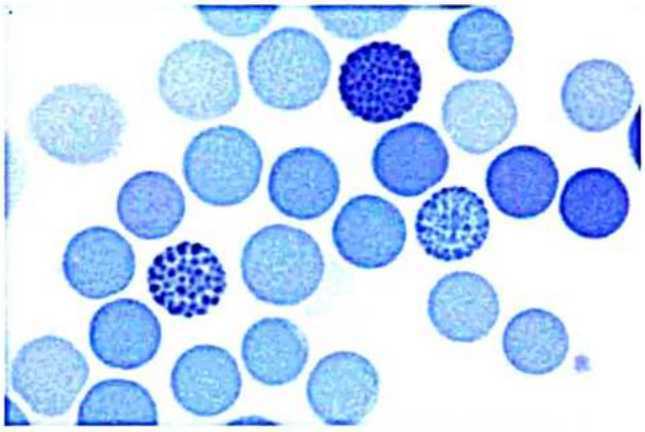


- Mnemonic: ThALESSemia
  - A - absent lines
  - LESS - Mentzer index is < 13.

B. α Thalassemia

- α chains are missing - due to α gene deletion.

α Gene Detection	State
AA/AA - All 4 are present	Normal
AA/A- 1 α gene is deleted	Asymptomatic
AA/- - 2 α genes are deleted	α thalassemia trait
A-/- - 3 α genes are deleted	• Formation of β 4 tetramers • Shows golf ball inclusions • HbH disease
All 4 α genes are deleted (- -/- -)	• Hb Barts disease • Results in intrauterine death. • Gamma 4 tetramers are formed • Mnemonic: Barbaad fetus



Golf ball inclusions

- Mnemonic: **He** is a **Boy** playing **Golf** at **3 AM**
  - **He** - HbH
  - **Boy** - β 4 tetramers
  - **Golf** - Golf ball inclusions
  - **3 AM** - 3 α gene deletion

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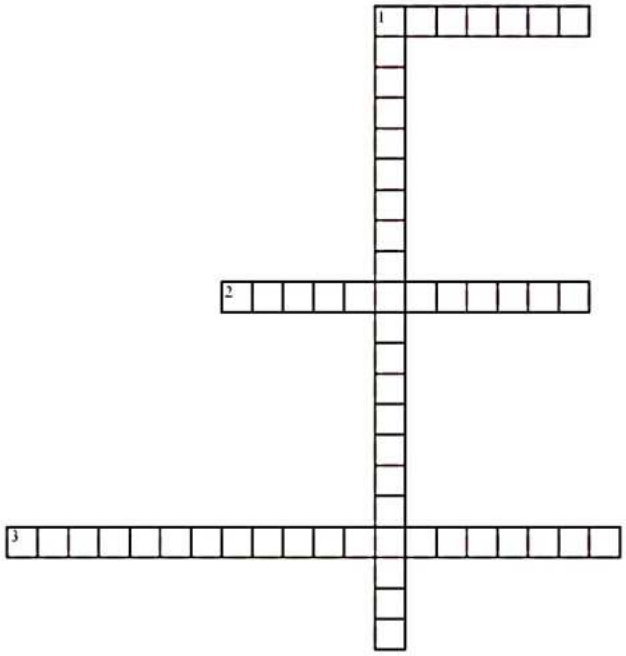
CROSS WORD PUZZLES

Crossword Puzzle



Across

1. Normal Cell converts to Sickle cell when O2 levels are low
2. It is an extravascular hemolysis and broken by spleen
3. β thal and Sickle cell genes are present

Down

1. At β 6 position, if glutamic acid changes to lysine

Telegram - @nextpreladdernotes

52 AUTO IMMUNE HEMOLYTIC ANEMIA

- It is an AutoImmune Extracorpuscular defect
- **Two types** of AIHA
  - Warm AIHA (37°C) - IgG
  - Cold AIHA (4°C) - IgM/ IgG

Warm AIHA 00:00:30

- **Temperature:** 37°C
- **Antibody:** IgG
- IgG attaches to **P-Antigen** on the RBC
- IgG is an incomplete antibody (no bridge formation)
- So these IgG are engulfed by the spleen
- This results in Extravascular hemolysis (EVH)

To Remember

- Warm - Garam - IgG
- **Temperature:** 37°C
- P-Antigen
- Extravascular hemolysis (EVH)
- **Seen in**
  - CLL (If both CLL + Warm AIHA = Evan's syndrome)
  - SLE

Cold AIHA 00:00:45

- Two types
  - IgM - Cold agglutinin disease
  - IgG - Paroxysmal cold haemoglobinuria.

IgM Cold AIHA

- **Temperature:** 4°C
- **Antibody: IgM**
- IgM **I-Antigen** attached to the RBC
- **Other name:** Cold agglutinin disease

Mnemonic

- **M** - IgM
- **I** - I Antigen
- **C** - Cold AIHA
- IgM can activate complement system, break of all red blood cells so it can cause intravascular hemolysis (IVH)
- This doesn't happen, our body has CD55, Cd59

Explanation

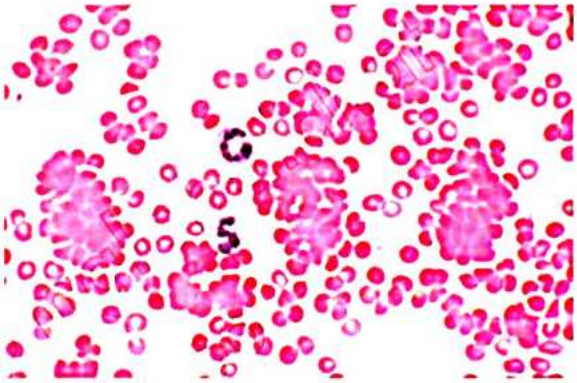
- The red blood cells got coated with C_{3b}
- Stopped by CD55, Cd59
- Then C_{3b} changes into C_{3d}
- C_{3d} means liver has taken the cells
- Then the RBCs are destroyed by Liver
- Leads to extravascular hemolysis (EVH)

To Remember

- All the AIHA has EVH.
- Only IgG cold AIHA has IVH.

IgG Cold AIHA

- This IgG antibody is given a special name - Donath Landsteiner antibody.
- This will result in IVH.
- **Other name:** Paroxysmal cold haemoglobinuria



To Remember:

- In Paroxysmal cold haemoglobinuria the RBCs will be broken down.
- Artificial temperatures are created using Frozen slides.

Hypoproliferative Anemias 00:13:13

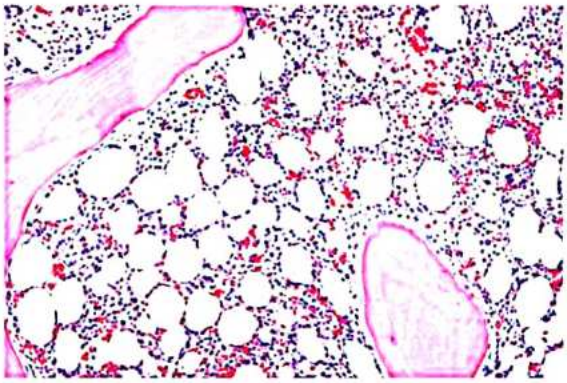
- Anemia due to bone marrow defects
- Specifically aplastic anemia

Aplastic Anemia 00:13:37

- **Causes**
  - Idiopathic (most common)
  - Drugs
  - Toxins
  - Chemicals
  - Viral Infections (Non A Non B Non G hepatitis)
  - Genetic syndrome
    - → Fanconi anemia (DNA repair defect) - Imp
    - → Dyskeratosis congenita (short telomere)
    - → Shwachman diamond syndrome (pancreatic defect)
- **Peripheral Smear**
  - Pancytopenia
- **Bone marrow aspirate**
  - Dry tap (as there are no cells)

Telegram - @nextpreladdernotes

- **Bone marrow biopsy** (investigation of choice)
  - Reduced cells
  - Increased fats

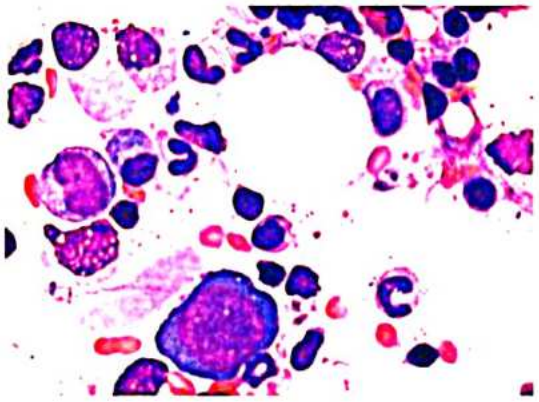


To Remember

- No Splenomegaly is seen in Aplastic Anemia.
- Only Bone marrow is involved, but not spleen.
- **Criteria for Severe Aplastic Anemia (SAA)**
  - Bone marrow cellularity is <25% + Any two of the three,
    - → **WBC** - Neutrophils <500/μl
    - → **Platelets** - Count <20,000/μl
    - → **RBC** - CRC (Corrected retic count) <1%

PRCA (Pure Red Cell Aplasia) 00:19:32

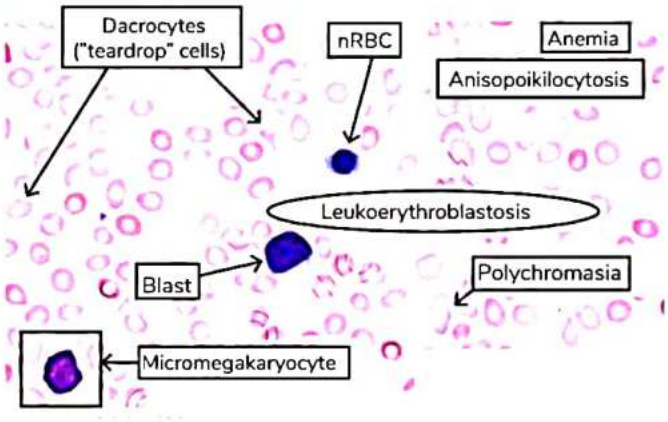
- Only red blood cells are affected
- **Causes**
  - **Parvovirus B19 (IMP)**
    - → Can get attached to **P-Antigen** of RBC
    - → Dog ear projections are seen in erythroblast



  - **Thymoma** - Can be seen in,
    - → PRCA
    - → MG (Myasthenia gravis)
  - **Large Granular Lymphocytic Leukemia (LGLL)**

Myelophthisic Anemia

- Space occupying is seen in bone marrow
- **Causes**
  - Metastasis
  - Granuloma
- This space occupying may lead to removal of RBCs and WBCs from the bone marrow
- **Leukoerythroblastic blood picture** (peripheral smear)
  - WBC Precursor
  - RBC Precursor



Peripheral smear of Myelophthisic Anemia

- Shows Leukoerythroblastic blood picture
- **"Tear drop"** cells are also seen (Dacrocytes).

Telegram - @nextprepladdernotes

PREVIOUS YEAR QUESTIONS

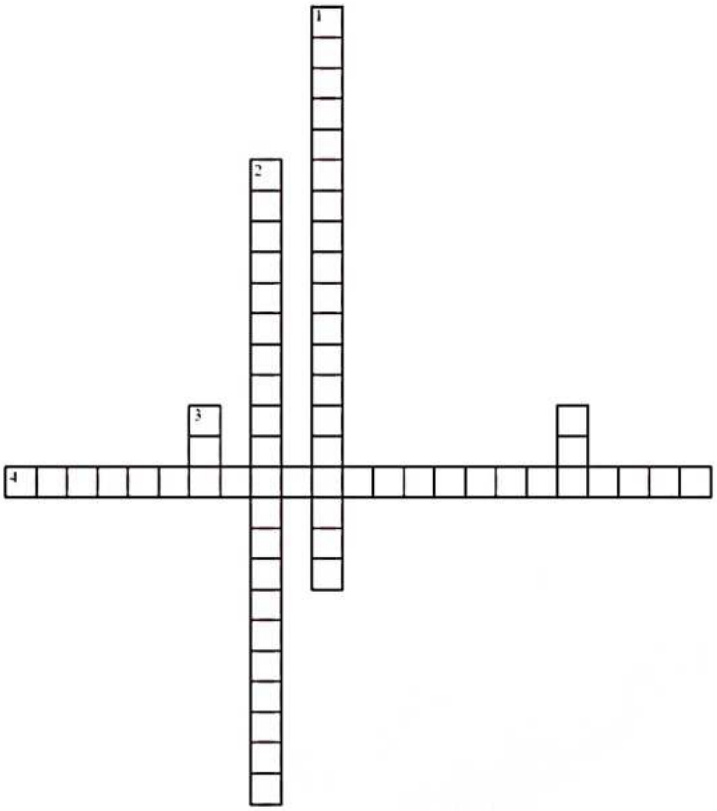
Q. Which of the AIHAs is associated with Malignancy?

Ans: IgM - Cold agglutinin disease
Mnemonic: M for Malignancy, M for Mycoplasma, M for IgM.

Telegram - @nextpreladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

4. Anemia due to bone marrow defects

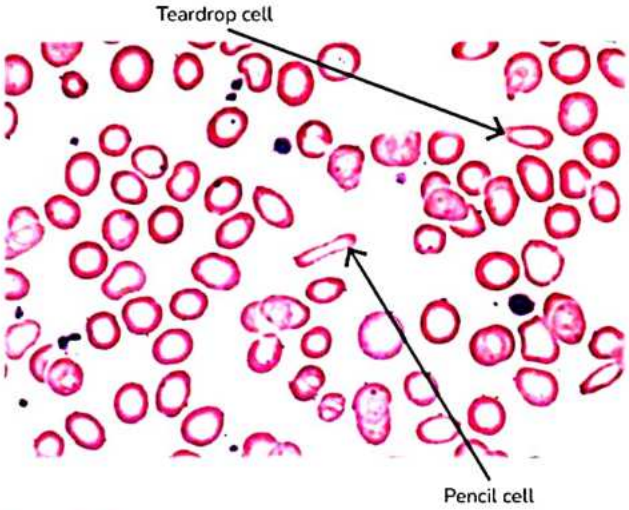
Down

1. Space occupying is seen in bone marrow
2. AIHAs is associated with Malignancy
3. Number of Cold AIHA present

Telegram - @nextpreladdernotes

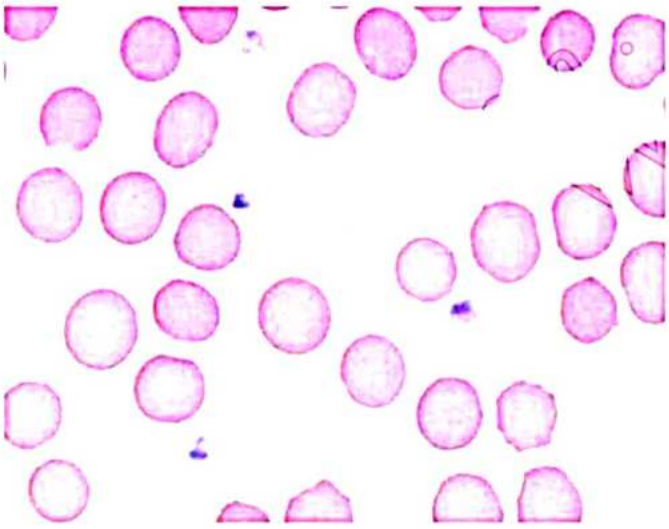
53 RBC SHAPES AND INCLUSIONS

Pencil cells



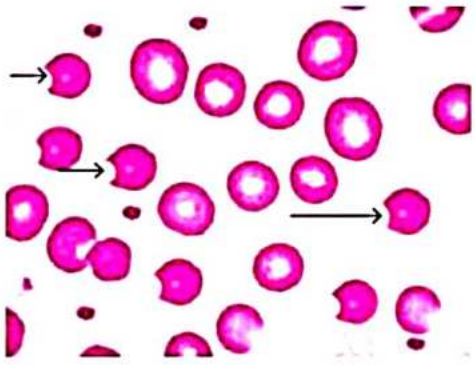
- Pencil cells
- Seen in IDA (Iron Deficiency Anemia)

Spherocytes



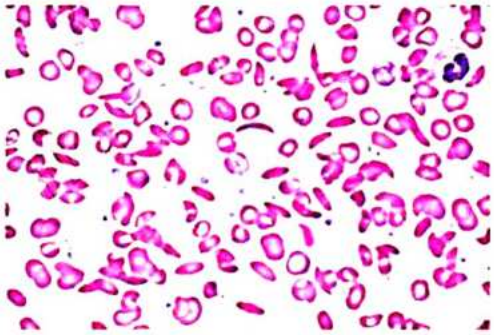
- Smaller than RBC
- No central pallor
- Most common cause: AIHA (Autoimmune hemolytic anemia)
- Other causes: Hereditary spherocytosis and G6PD deficiency

Degmacytes



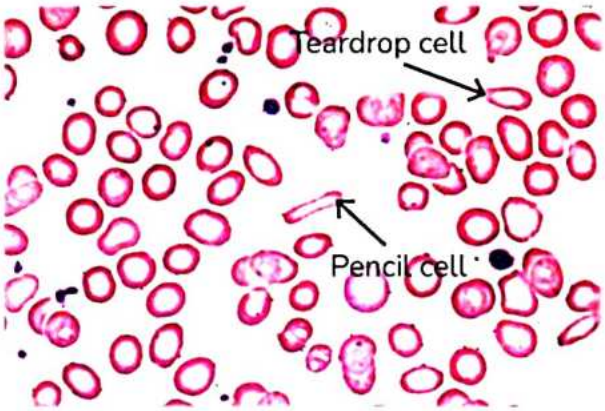
- Bite cells
- Seen in G6PD deficiency

Drepanocytes



- Sickle cells
- Sickle cell anemia

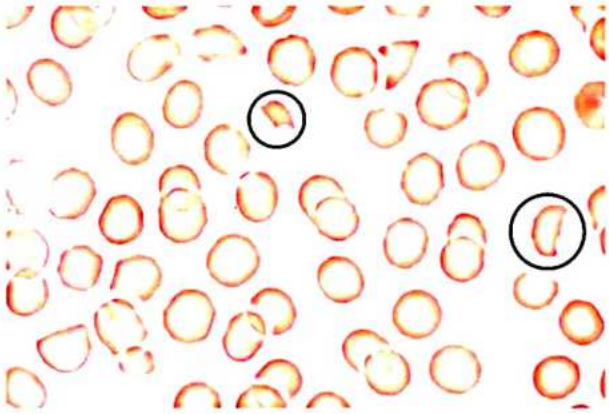
Dacrocytes



Telegram - @nextprepladdernotes

- Tear drop cells
- Seen in Myelofibrosis, Myelopthisic anemia

Schistocytes



- Fragmented cells
- Seen in MAHA (MicroAngiopathic Hemolytic anemia)- HUS, TTP

Acanthocytes

- Spike/Spur cells
- Abetalipoproteinemia

Echinocytes

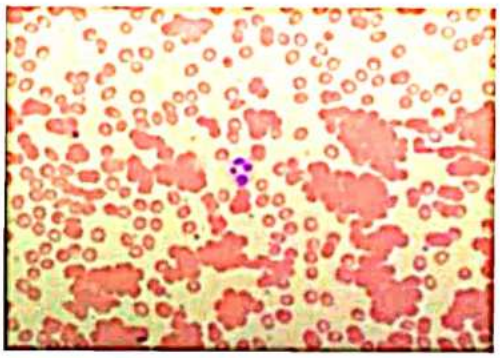
- Burr cells
- Seen in Burns, uremia, prolonged exposure to EDTA

Codocytes

- Target cells
- Seen in Thalassemia, SCA, HbC, HbE

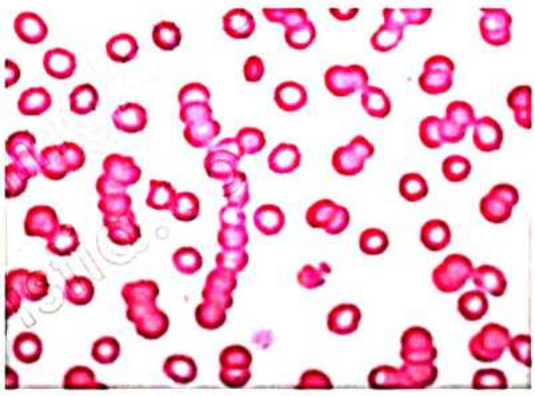
Acanthocyte, echinocyte & codocyte

Agglutination of RBCs 00:04:32



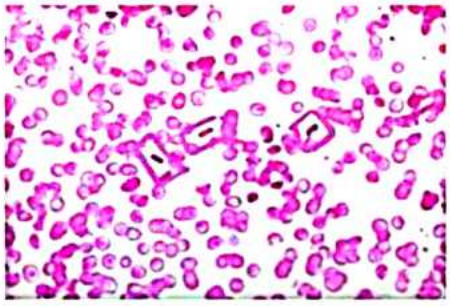
- Seen in CAD (Cold agglutinin disease)
- Occur in 4°C
- IgM antibodies are found

Rouleaux formation 00:05:02



- Stack of coins
- Seen in WBC disorder, Multiple myeloma

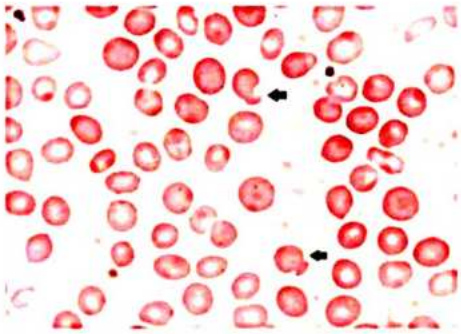
Crystal form 00:05:32



Telegram - @nextprepladdernotes

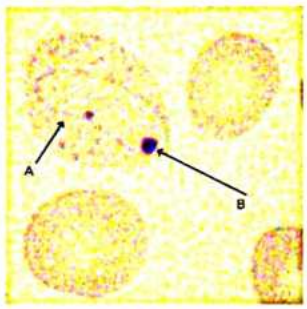
- Seen in HbC disease (Glutamic acid changes to Lysine)

Pincer cells

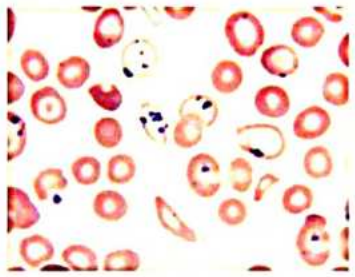


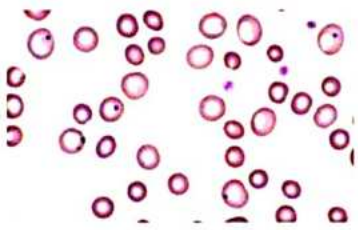
- Mushroom/Pincer cells
- Band 3 defect

Cabot ring



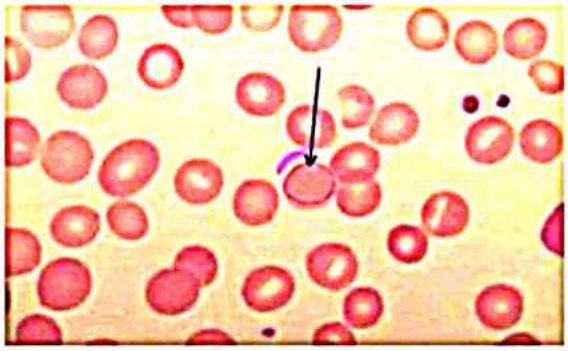
- Figure of eight or round appearance
- Made up of mitotic spindle (rich in Arginine)
- Seen in Megaloblastic anemia.





Howell Jolly bodies, Pappen Heimer bodies, Heinz bodies

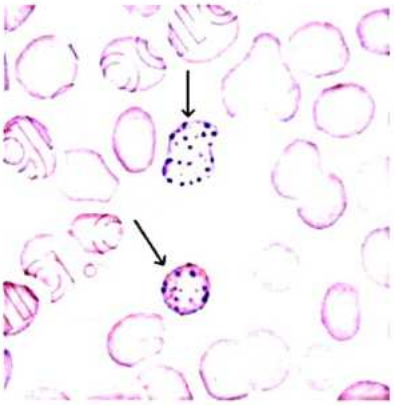
Refer Table 53.1



- The Cabot ring has spindles and can be shown in any shape.

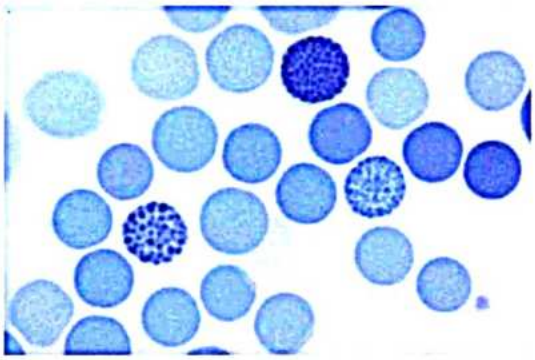
To Remember

Basophilic stippling 00:10:02



- Two types
  - Fine: Thalassemia
  - Coarse: Lead poisoning, 5' nucleotide deficiency

Beta-4 Tetramers 00:10:58



- HbH disease
- Golf Ball inclusion
- 3-alpha gene deletion
- Stain: New methylene blue

Telegram - @nextpreladdernotes

	Howell Jolly bodies	Pappen Heimer bodies	Heinz bodies
Stain	Romanowsky family	Romanowsky family	Crystal violet
Number of dots	Single	Multiple (Iron)	Denatured Hemoglobin
Seen in	• Megaloblastic anemia • Post splenectomy	Sideroblastic anemia	G6PD deficiency

Telegram - @nextpreladdernotes

PREVIOUS YEAR QUESTIONS

Q. Formula for calculating reticulocyte production index? (JIPMER May 2019)

A. Retic X patient hematocrit 4/5
B. Corrected reticulocyte count
C. Reticulocyte percentage X RBC count
D. Reticulocyte counted X100/no. of red cells

Q. Which of the following interfere with iron absorption? (FMGE June 2019)

A. Vitamin C
B. Phytates
C. Oxalate
D. Myoglobin

Q. Which of the following findings are there in iron deficiency anemia? (AIIMS Nov 2019)

A. ↑ TIBC, ↓ Ferritin, ↓ Transferrin saturation
B. ↑ TIBC, ↑ Ferritin, ↓ Transferrin saturation
C. ↓ TIBC, ↓ Ferritin, ↓ Transferrin saturation
D. ↓ TIBC, ↓ Ferritin, ↑ Transferrin saturation

Q. Which types of anemia is seen in patients of rheumatoid arthritis? (FMGE Dec 2017)

A. Normocytic and Hypochromic anemia
B. Microcytic and Hypochromic anemia
C. Normocytic and normochromic anemia
D. Macrocytic anemia

Q. Eosin-5-maleamide flow cytometry is used for diagnosis of (JIPMER May 2018)

A. G6PD
B. Hereditary spherocytosis
C. Sickle cell anemia
D. Alpha thalassemia

Q. Blister cells are seen in? (JIPMER Nov 2017)

A. Thalassemia
B. Chronic liver disease
C. Sickle cell anemia
D. G6PD disease

Q. Which of the following is true about G6PD deficiency? (AIIMS June 2020)

A. Resistant to hemolysis in hypotonic saline
B. Spectrin is involved in pathogenesis
C. Presence of spherical cells may be seen
D. It causes chronic hemolysis

Q. A boy after playing football complaining fatigue and abdominal pain. He also had a history of hand swelling in past. On ultrasonography, h has shrunken spleen. What is the likely diagnosis of this patient? (NEET – Jan - 2020)

A. Sickle cell anemia
B. Iron pancreatitis
C. Acute pancreatitis
D. Intermittent porphyria

Q. An 18 years old patient's hemogram shows Hb 12 g%, RBC count of 6 million, decreased MCV (56), decreased MCH (29) AND RDW OF 14. What is the most probable diagnosis? (JIPMER – Nov - 2017)

A. Iron deficient stores
B. Folate deficiency
C. Beta thalassemia trait
D. Normal lab parameters

Q. A 30 years old women came with complaints of easy fatigability, exertional dyspnea and weight loss. She also has a complaint of frequent fall. Physical examination revealed there was b/L decreases in vibration sense. Her hemoglobin levels were 8.2g%. she was treated with folate. Her anemia improved but neurological symptoms worsened. Which of the following is the most probable reason for the condition? (NEET - Jan - 2020)

A. Folate not absorbed
B. Folic acid deficiency unmasked pyridoxin deficiency
C. Deficiency of folate reductase in CNS
D. Folate therapy cause rapid use of B12 stores aggravating symptom

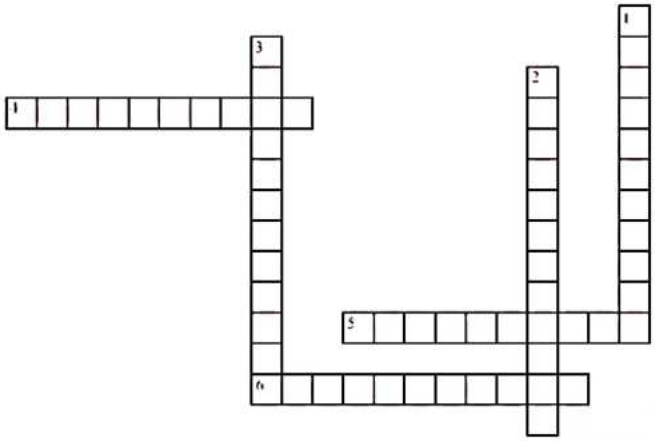
Q. All the following are criteria for diagnosing severe aplastic anemia except? (JIPMER – Nov – 2017)

A. Bone marrow cellularity < 25%
B. Reticulocyte < 60,000/mm^3
C. Platelet < 20,000/mm^3
D. Absolute neutrophil count < 1500/mm^3

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

4. Tear drop cells
5. Dog Bite cells
6. All cells are packed, round

Down

1. Burr cells
2. Sickle cells3Fragmented cells

Telegram - @nextpreladdernotes

54 BASICS OF WBC

- Pluripotent Stem Cell (CD 34+)
  ↓
- Multipotent Stem Cell
  ↓

Common Myeloid Progenitor	Common Lymphoid Progenitor
It gives rise to 1. Colony Forming Unit G/M (CFU G/M) G: Granulocytes M: Monocytes 2. Colony Forming Unit M/E (CFU M/E) M: Megakaryocytes E: Erythroid	It gives rise to T-lymphocytes, B-lymphocytes, and Natural Killer Lymphocytes (NK Cells).

Granulocytes have neutrophils, basophils, and eosinophils.

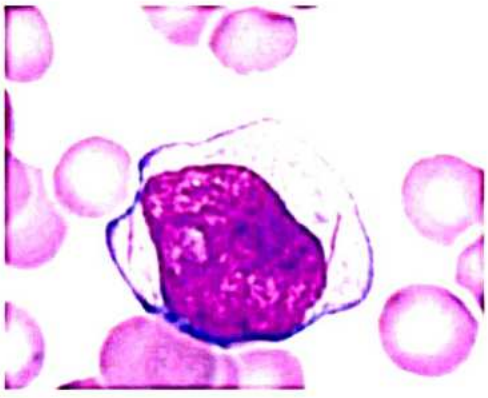
Markers: 00:04:40

- T-lymphocytes: CD 1 to 8, Cd28
- B-lymphocytes: CD10, CD19 to 23, Cd40
- NK cells: CD16, Cd56
- Myeloid cells (Granulocytes): MPO, CD13, CD33
- Monocytes: CD11, CD14, Cd64+
- Megakaryocytes: CD41,CD42, CD61+
- Erythroid: CD71 (Transferrin receptor)

Myelopoiesis

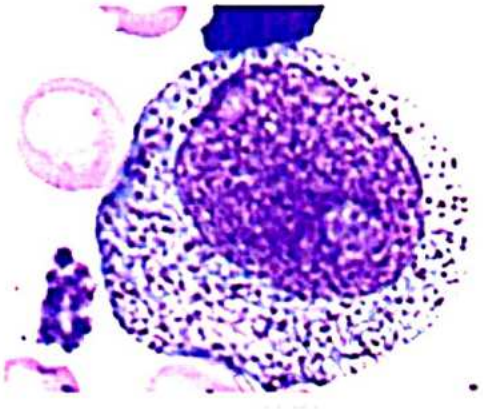
- Myeloblast gives rise to Granulocytes (N/B/E)
- Monoblast gives rise to Monocytes
- Lymphoblast gives rise to Lymphocytes

Myeloblast (It Has Auer Rods)



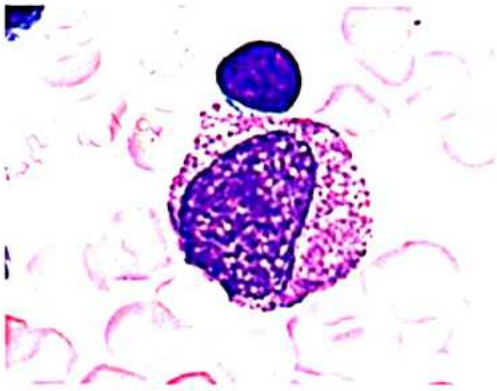
↓

Promyelocyte (Biggest cell)



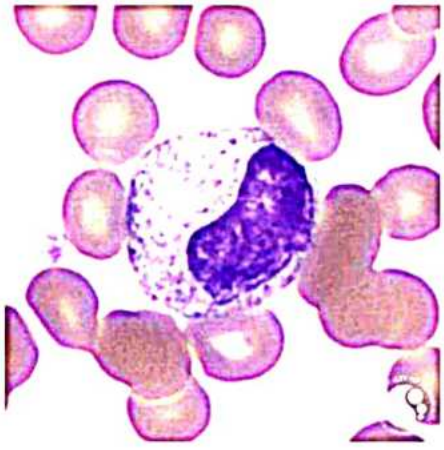
↓

Myelocyte (D shaped nucleus)



↓

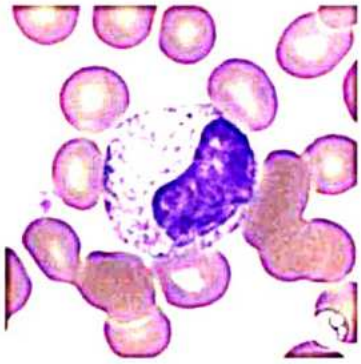
Metamyelocyte (Indented nucleus)



↓

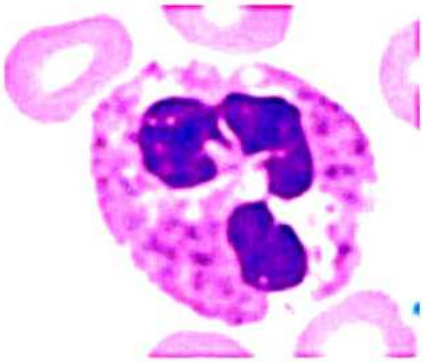
Band/stab Form

Telegram - @nextpreladdernotes

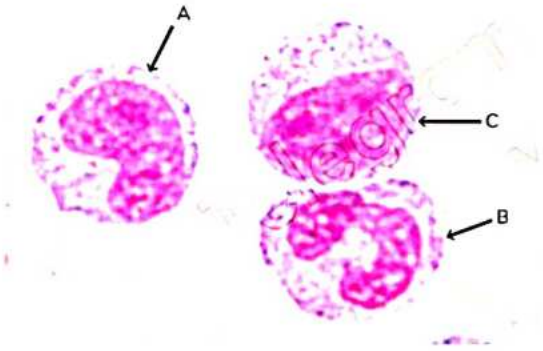


↓

Neutrophils/basophils/eosinophils.



Myelocytes decide whether there will be the formation of neutrophils, basophils, or eosinophils.



Refer Table 54.1

Q. Identify the Blast.

Ans: Myeloblast (Auer rods present)

Cell count: 00:23:32

Cell		Absolute Count
Never	Neutrophil	2000-7000/mm3
Let	Lymphocyte	1000-3000/mm3
Monkeys	Monocyte	200-1000/mm3
Eat	Eosinophil	50-500/mm3
Banana's	Basophil	0-100/mm3

Identification:

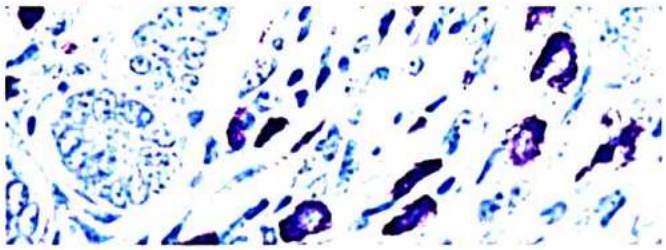
Neutrophil
- 3-5 nuclear lobes
- Seen in Acute inflammation, Bacterial infections

Lymphocyte
- Seen in Viral infections, Chronic inflammation, Typhoid, TB

Monocyte
- Kidney shaped/reniform Nucleus
- Seen in Malaria, JMML in children (Juvenile myelomonocytic leukaemia), CMML in adults (chronic myelomonocytic leukaemia)

Eosinophil
- Orange-red granules
- Bilobed Nucleus - spectacles/ headphone
- Seen in Allergies and Parasitic Infections

Basophil
- Blue-Black Granules
- CML (Chronic Myelogenous Leukaemia), PV (Polycythemia vera)



- Special Stain of basophils and mast cells is Toluidine blue (Metachromatic stain). It shows two colours, blue and purple.

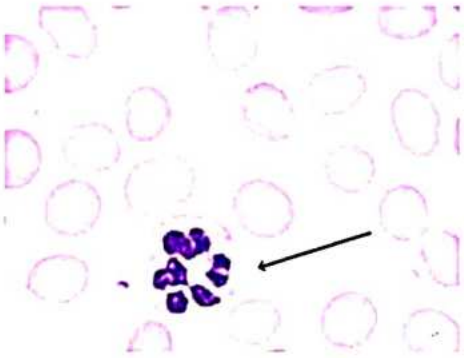
Diseases of WBC 00:34:37

- Non-neoplastic
- Neoplastic

Telegram - @nextpreladdernotes

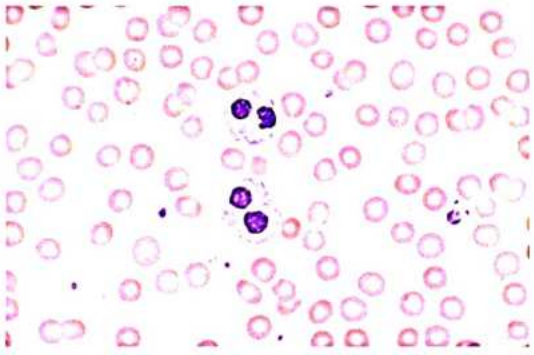
Wbc Abnormalities: Non-neoplastic

1. Hypersegmented neutrophil



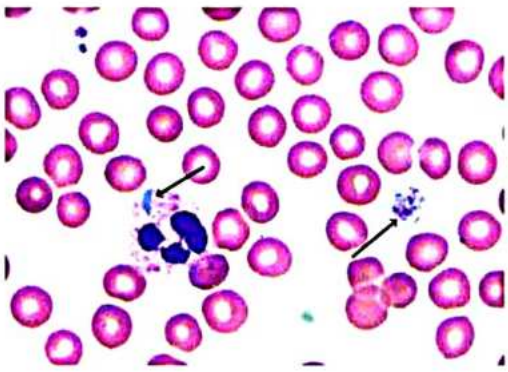
- Nuclear lobes equal to or more than 5
- Causes Megaloblastic anaemia

2. May hegglin anomaly (MYH9 g)



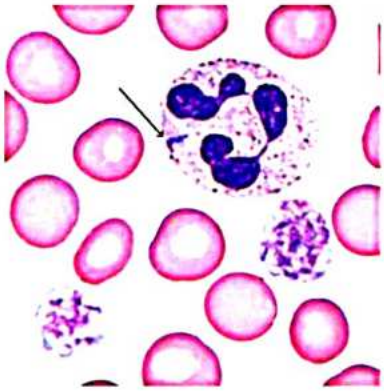
- It causes giant platelets, low platelet count, Inclusions in granulocytes ("Dohle body like" Inclusion)

3. Hyposegmented neutrophil



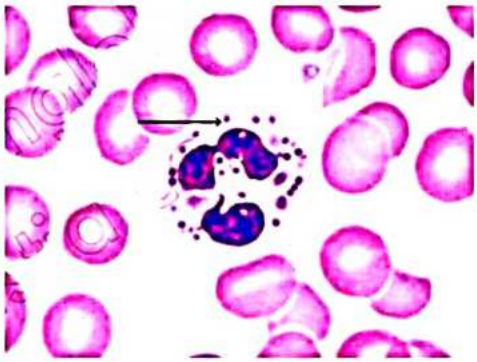
- Referred to as Pelger huet anomaly
- If present supplementary to other syndromes like Myelodysplastic syndrome, it is referred to as Pseudo-pelger huet anomaly

Dohle bodies



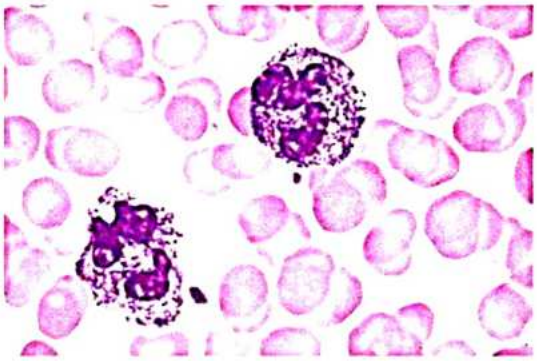
- Seen in patients with severe sepsis
- They are formed by dilated endoplasmic reticulum organelles.

4. Large cytoplasmic granules



- Chediak-Higashi syndrome (LYST gene defect)

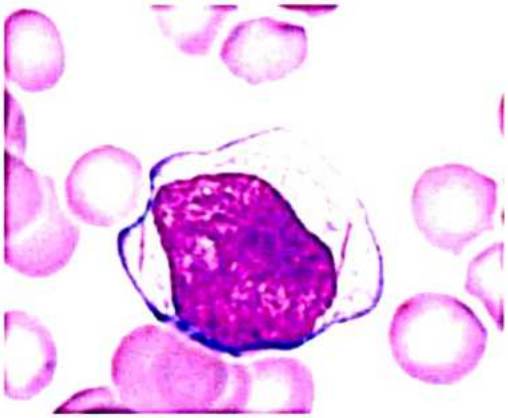
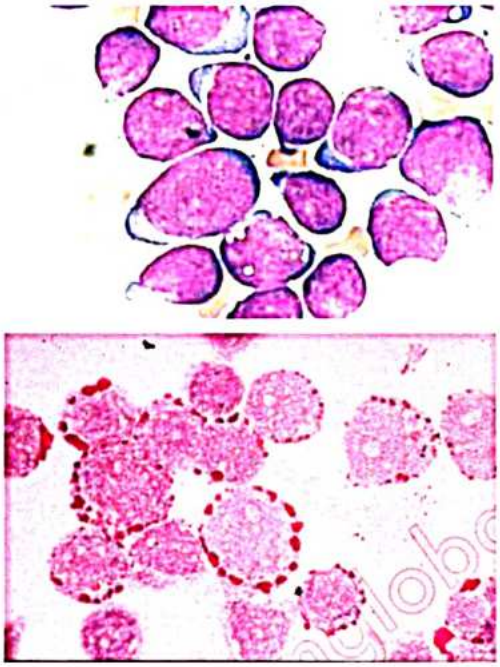
5. Alder Reily anomaly



- Large cytoplasmic granules
- Associated with Mucopolysaccharidoses

Telegram - @nextpreladdernotes

Table 54.1

Myeloblast	Lymphoblast	Monoblast
		
• Auer rods (Lysosomes present) • MPO • Sudan black B • CAE(Chloroacetate Esterase)	• No Auer rods • PAS (block positivity)/ Dot blot positivity) • Acid phosphatase for T-lymphoblasts	• Non Specific Esterase

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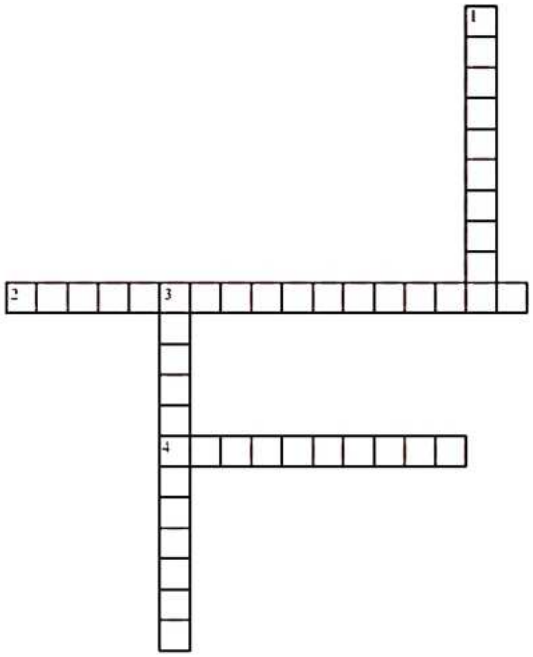
CROSS WORD PUZZLES

Crossword Puzzle



Across

2. Causes giant platelets, low platelet count, Inclusions in granulocytes
4. This cell has an absolute count of 1000-3000/mm3

Down

1. Has 3-5 nuclear lobes and is seen in Acute inflammation, Bacterial infections
3. Myeloblast gives rise to _____

Telegram - @nextpreladdernotes

55 LEUKEMIA AND LYMPHOMA PART 1

Difference between leukemia and lymphoma

Leukemia	Lymphoma
Blood cancer	Lymph node cancer
Inside the blood	Inside the lymph node

- Leukemia can become lymphoma
- Lymphoma can become leukemia
- Terminology depends on site

Leukemia

- **Acute Leukemia**
  - ALL - acute lymphoblastic leukemia
  - AML - acute myeloid leukemia
- **Chronic Leukemia**
  - CLL - chronic lymphocytic leukemia
  - CML - chronic myeloid leukemia
- Acute leukemia: blast cells > 20%
- Chronic leukemia: blast cell < 20% - almost negligible

Lymphoblast

- Special stain- PAS block positivity
- T lymphoblast - acid phosphatase
- No auer rod

Acute leukemia

- Blasts count:
  - WHO: > 20%
  - FAB: > 30%
- Blasts could be in the bone marrow and/or in the peripheral blood
- Aleukemic leukemia - blast count is more than 20%
  - Blasts only in the bone marrow
  - Not in the peripheral blood

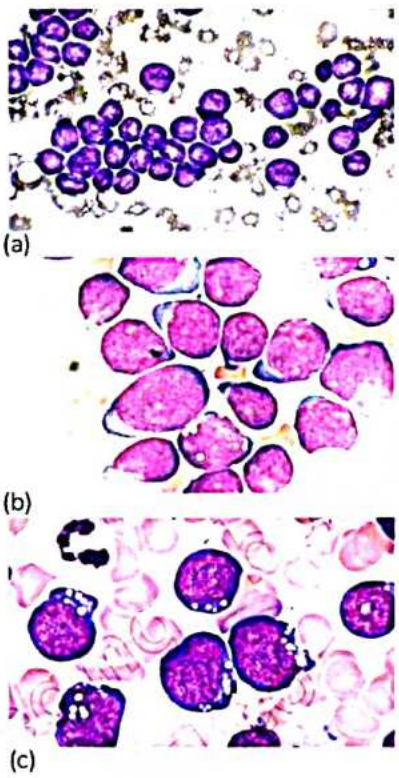
ALL - Acute Lymphoblastic Leukemia

- Most common leukemia in children
- Most common in Down's syndrome (overall)

Classification

- Lymphoblasts ≥ 20%

ALL - FAB classification



1. **ALL L1 - Homogenous blasts**
   Most common - ALL L1 - best prognosis
2. **ALL L2 - Heterogenous blasts**
3. **ALL L3 - cytoplasmic vacuolations**
   - They contain fat
   - Oil Red O Positive (fat)
   - Least common - ALL L3 - worst prognosis

Update - ALL L3 is similar to Burkitt's lymphoma

- Hand mirror shaped cells - variation of lymphoblast

ALL-WHO classification 00:13:54

1. **B - ALL** (B for bachas - children)
   - PAX5 - loss of function mutation
   - E2A
   - EBF
   - Good prognosis
2. **T - ALL** (T for Teenagers, T for tissues)
   - Tissue infiltration
     → **CNS**
     → **Mediastinum**
     → **Testes**
   - NOTCH mutation
   - Poor prognosis

PAX5 GENE - for B Cell Development
Loss of function mutation - lymphoblast do not mature → lymphoblastic leukemia

Telegram - @nextpreladdernotes

ALL - Genetics

Good prognosis	Bad prognosis
• Hyperdiploidy	• Hypodiploidy
• Trisomy 4,7,10	• t(9:22)
• t(12:21)	• MLL1 gene

Important Information

Tip to remember (anything in 3 - good)
- Hyperdiploidy (more than 2)
- Trisomy 4,7,10 (4, 4+3, 7+3)
- t(12:21) (1+2=3, 2+1=3)

t(9:22) - Bcr-Abl fusion 00:20:53
- Philadelphia chromosome

Tip to remember - **LMN** - ALL, CML, CNL

- If Bcr-Abl fusion protein weighs
  - 190 kD - ALL
  - 210 kD - CML
  - 230 kD - CNL
- Lesser the weight of Bcr- Abl fusion protein → more is the tyrosine kinase activity → bad prognosis
  - ALL - bad prognosis- for t(9:22)

ALL - clinical features
- Commonly seen in children
- Lymphoblast is proliferating so much → other lineages are suppressed
  - Anemia
    → Fatigue
    → Tiredness
    → Pallor
  - Due to depletion in neutrophil, eosinophil, basophils, monocytes
    → Recurrent infections
  - Thrombocytopenia
    → Bleeding manifestations
  - Organ involvement - (T-ALL - tissue infiltration)
    → Hepatosplenomegaly

ALL - Diagnosis
- Peripheral blood & Bone marrow > 20%
- **Stains of lymphoblasts**
  - PAS positive + Dot, blot or block positivity
  - T-ALL - acid phosphatase
- **Flow cytometry - diagnosis of choice**

Prognostic factors

Good	Feature	Bad
2-10 years	AGE	<1years, >10 years
female	GENDER	Male - testicular involvement
	RACE	blacks
L1	FAB	L3
B-ALL	WHO	T-ALL
Hyperdiploidy Trisomies - 4,7,10 t(12:21)	GENETICS	Hypodiploidy t(9:22) MLL1

Q. OIL RED O positivity is seen with which of the following?
a. ALL L1
b. ALL L2
c. ALL L3
d. All of the above

Q. 14-year-old boy with chest discomfort, shows normal bilateral lung field on chest X-ray and mediastinal widening chest CT scan. Mediastinal mass measures 11cm. Biopsy from the mediastinal mass shows cells of lymphoid lineage. The cells are positive for TdT,CD2,CD7, further molecular studies show NOTCH mutation. Which of the following is the most likely diagnosis
a. Neurogenic tumors
b. B-ALL
c. T-ALL
d. Thymoma

Q. A 5 year old boy with a 1 month history of loss of appetite, fatigability, unexplained irritability, and intermittent low grade fever. Patient's mother also says he also often has bone pain, especially in his lower limbs. Physical examination is significant for the presence of generalized pallor, splenomegaly and generalized lymphadenopathy. His lower extremities are tender to palpation. But there is no joint swelling, warmth, or erythema. Laboratory findings are significant for a hemoglobin of 8.0 g/dl, a total white blood cell count 61,900/mm3, and a platelet count of 90,000/mm3. A peripheral smear shows the presence of atypical lymphocytes. Bone Marrow biopsy is performed which demonstrates 30 % of the bone marrow cells as a homogenous population of lymphocytes. Immunophenotyping confirms the diagnosis of acute

Telegram - @nextpreladdernotes

lymphoblastic leukemia. Which of the following chromosomal abnormalities is associated with an unfavorable prognosis in this patient?

a. Trisomy 4
b. Trisomy 10
c. Hyperdiploidy
d. t(9:22)

Answer - D t(9:22) has bad prognosis

AML - Acute Myeloid Leukemia

- Blasts ≥ 20%
- All the myeloid lineage cells are **myeloperoxidase positive**

AML - FAB classification 00:34:30

- AML M0 - AML M7

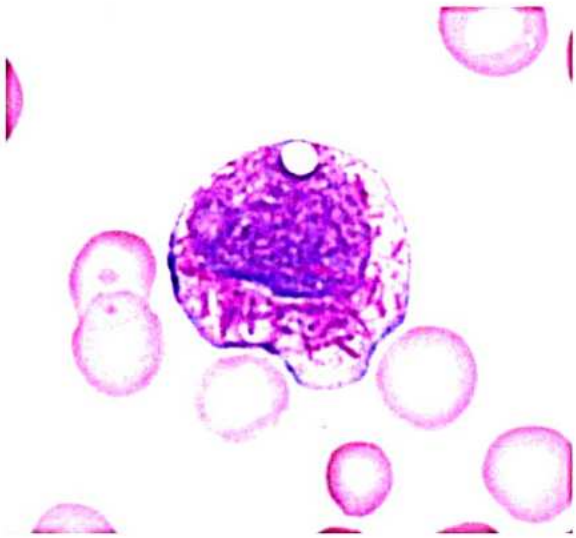
1. **AML M0 - AML undifferentiated**
   - Myeloperoxidase - **MPO - negative**
2. **AML M1 - AML without maturation**
3. **AML M2 - AML with maturation**
   - Most common
   - t(8:21) RUNX1-RUNX1T1 fusion

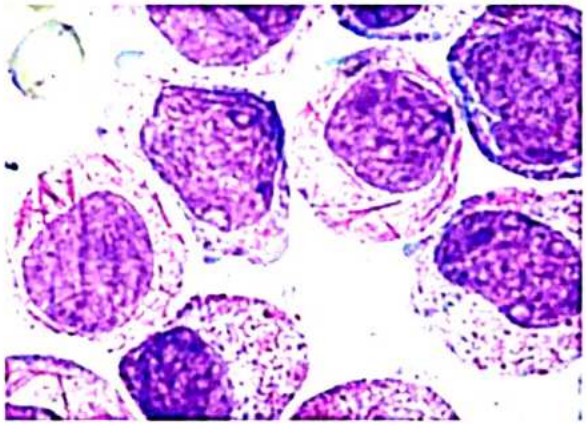
Important Information

Tip to remember -

- AML M1 - little brother - without maturation
- AML M2 - big brother - with maturation

4. **AML M3 - APML - acute promyelocytic leukemia**
   - t(15:17) - PML-RARA fusion
   - RARA - retinoic acid receptor alpha
     → ↑ Promyelocyte: Criss-Cross Auer Rods - Faggot Cell





FAGGOT CELL - AML-M3
→ Contain procoagulant material
→ Only leukemia that can cause DIC - disseminated intravascular

AML - M3 - treatment
→ **No chemotherapy:** because if the cells are killed → procoagulant material come out → **DIC**
→ treatment: ATRA - all trans retinoic acid +As2O3 - arsenic trioxide
- **DOC** - diagnosis of choice: AMLM3: **FISH**
  → To detect Chromosomal anomaly t(15:17)

5. **AML - M4 - acute myelomonocytic leukemia**
   - Myeloblasts and monoblasts
   - inv 16
   - CBF-MYH fusion
     → CBF - Core binding factor

   Tip to remember
   - 2 type of cells
   - 2x2 = 4 AML - M4
   - 4x2 = 16 inv 16

6. **AML M5 - acute monoblastic leukemia**
   - Only blasts - monoblasts

AML M4 and AML-M5 shows Tissue infiltration (monoblasts - tissue infiltration)
- **Gums - gum hypertrophy**
- **Skin - leukemia cutis**

7. **AML M6: pure erythroid leukemia** - DI Guglielmo Disease
8. **AML M7 - Acute megakaryoblastic leukemia**
   - Least common
   - Most common in Down's syndrome < 3 years
   - Bone Marrow aspiration - Gives a dry tap
     → Due to Fibrosis in bone marrow

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Down's syndrome

- Most common hematological abnormality: TAM - transient abnormal myelopoiesis
  - Resolve within 6- 7 months (transient)
- Most common leukemia (overall): ALL
- Most common leukemia if child < 3 years: AML-M7

AML - WHO classification

I - AML with recurrent genetic abnormalities

- t(8:21) - RUNX1-RUNXIT1 (M2)
- t(15:17) - PML-RARA (M3)
- inv 16 - CBF-MYH (M4), with eosinophilia
- t(1:22)
- T(6:9), with basophilia
- AML with NPM mutation
- AML with CEBPA mutation

II - AML therapy associated

- Alkylating agents
- Topoisomerase

III - AML associated with MDS (myelodysplastic syndrome)

- **At least > 50 % of cells show myelodysplasia**
  → In 2 or more than 2 lineages

IV - AML - NOS - not otherwise specified

- Similar to FAB except AML-M3
- AML MO- M7 except M3

V - AML associated with Down's syndrome - AML M7

Chloroma

- Not a benign tumor
- Myeloblastoma / Chloroma / Granulocytic Sarcoma
- Site: AML in soft tissue - **orbit → proptosis**
  - AML M2
  - AML M5
- Microscopically
  - Myeloblasts
  - ARBISKOV Cells - atypical monocytes / macrophages
- Markers
  - MPO +
  - CD 43
  - CD 45
  - Lysozyme +

AML - diagnosis and stains

- Blasts ≥ 20%
- Myeloblasts - **Auer Rods**
- **DOC of AML - flow cytometry**
- **Except for AML M3 - FISH**
  - To look for t(15:17) - PML-RARA
- **AML - STAINS**
  - PAS - peroxidase - AML M3,6,7
  - MPO - myeloperoxidase - myeloblast
  - CAE - chloroacetic esterase - myeloblast
  - NSE - non specific esterase - monoblasts
- AML M0: MPO -ve
- AML M1: MPO +, CAE +
- AML M2: MPO +, CAE +
- AML M3: PAS +
- AML M4: MPO +, CAE +, NSE +
- AML M5: MPO +, CAE +, NSE+
- AML M6: PAS +
- AML M7: PAS +
- Which is the AML that show dual esterase positive
  - AML M4 - has both the blasts , so shows dual esterase positivity

AML - Prognosis

- **Good prognosis**
  - M2 - t(8:21)
  - M3 - t(15:17)
  - M4 - inv 16

- AML - blasts ≥ 20%
- Except **t (8:21), t(15:17) or inv 16**
- This is enough for diagnosis - **Blast count not considered**

Provisional category - AML with mutation RUNX1

Q. Highest concentration of auer rods is noted in?

a. AML M2
b. AML M3
c. AML M4
d. AML M5

Ans. B. AML M3 - FAGGOT CELLS - acute promyelocytic leukemia - criss cross auer rods

Q. A 15 year old boy presented with a history of bleeding gums, subconjunctival bleed and purpuric rash. Investigation revealed the following results. HB- 6.4 mg/dl ; TLC- 26,500/mm3 . platelet 35000/mm3. Prothrombin time 20 sec with a control of 13 sec, partial thromboplastin time - 50 sec. Peripheral smear is suggestive of acute myeloblastic leukemia. Which of the following is the most likely?

a. AML M2
b. AML M3
c. AML M4
d. AML M5

Answer - AML M3 - associated with DIC

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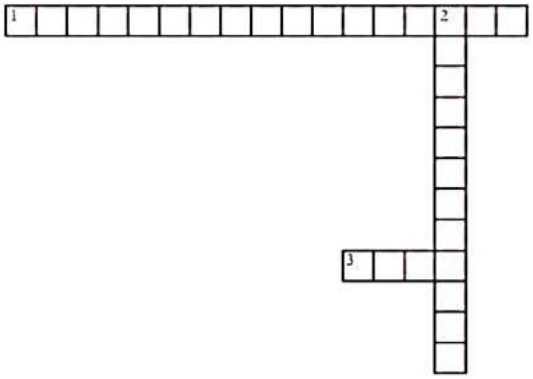
CROSS WORD PUZZLES

Crossword Puzzle



Across

1. Blast count is more than 20% and blasts only in the bone marrow
3. Retinoic acid receptor alpha

Down

2. First cell in lineage

Telegram - @nextpreladdernotes

56 LEUKEMIA AND LYMPHOMA PART 2

Chronic Lymphocytic Leukemia

This is the Leukemia of Exceptions.

- This is the only leukemia which is not associated with radiation.
- It is a deletion of 13q
- It is also the most common Leukemia seen in elderly.
- It is the most common Leukemia in the west. (Western Population)

CLL- Clinical Features

- The earliest finding is a little vague because it is Fatigue.
- Shows Lymphadenopathy (LAP)
- Shows Hepatosplenomegaly (HSM)
- Shows Organ involvement

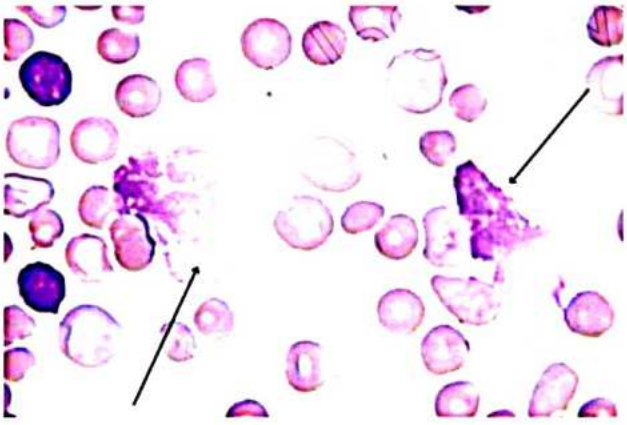
CLL- Diagnostic Criteria 00:02:52

There are a total of three things and all three criteria need to be met.

1. Absolute Lymphocyte Count, it will be more than 5000/mm³.
2. Bone Marrow: The Lymphocyte would be more than 30%.
3. CD 5+ & CD 23+ (Dual Positivity): Technique used is Flow Cytometry (FCM).

There is a new marker:

- CD 200 +: Positive in Chronic Lymphocytic Leukemia.



Smudge cells: parachute/basket cell
Smearing artefact: due to loss of vimentin, referred as cytoskeletal theory

CLL- Genetics

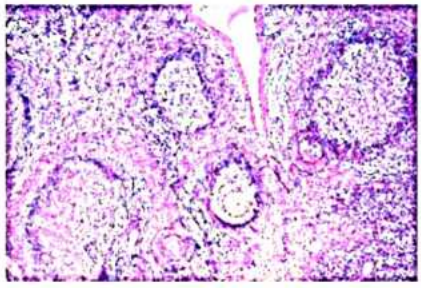
- The most common genetic: Deletion 13q & Deletion 17p can be there.
- Trisomy of Chromosome 12.

Chr 13q: The deleted part is microRNA 15 & 16.

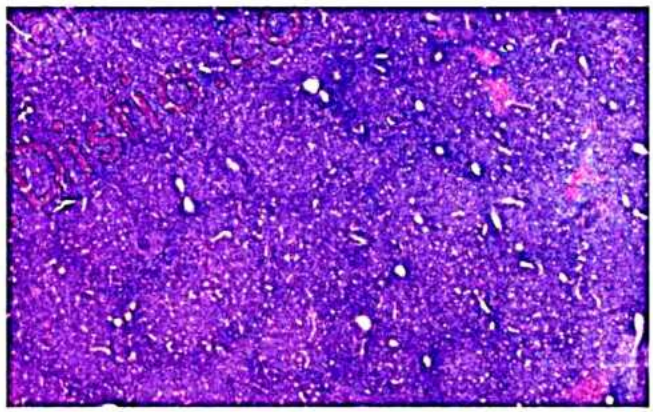
- Also, there is a bruton Tyrosine Kinase Mutation that can be noted.

CLL- Transformation 00:11:21

1. Evan's syndrome: aiha + cll+ itp
2. Richter syndrome: cll/sll changing tp dlbcl



Normal Lymph node



CLL shows pseudofollicular pattern

CLL- Prognostic Pattern

Good	Poor
Post GC B cell	Naive B cell
MicroRNA 15 & 16	CD 38
	Notch
	Zap70
	B2 Microglobulin

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CLL- Staging

Two stagings are there:

- **Binet Staging:**
  - A - <3 areas of Lymphadenopathy[1]
  - B - >3 areas of Lymphadinopathy[1]
  - C - Anema (<10g/dl) ± thrombocytopenia (<100x10⁹/l)

- **Rai Staging:**
  - 0 - Lymphocytosis in peripheral bone marrow only
  - I / II - Lymphadenopathy[1] ± Hepatosplenomegaly
  - III / IV - Anemia (<11 g/dl) ± thrombocytopenia (<100* 10⁹/l)

MCQ

Q. One of the following leukemia almost never develops after radiation:

A. AML
B. ALL
C. CLL
D. CML

Q. A 72-year-old man as a known case of CLL presents with new findings of 3cn cervical lymph nodes. On FNAC the Lymph node shows appearance or large pleomorphic cells with prominent nucleoli. Numerous mitosis also noted. Which of the following transformations correlate best with the mentioned above?

A. CLL progression
B. CLL with evan's Syndrome
C. Richter's Syndrome
D. Prolymphocytic Leukemia

Chronic Myeloid Leukemia

CML- Genetics

t(9:22)- Philadelphia.

There is a fusion of BCR and ABL. And due to this there is an increased Tyrosine Kinase activity, known as CLL.

Treatment:

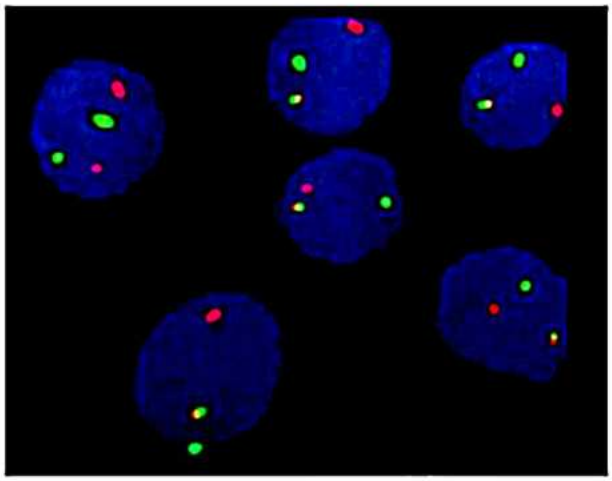
Imatinib, a tyrosine kinase inhibitor

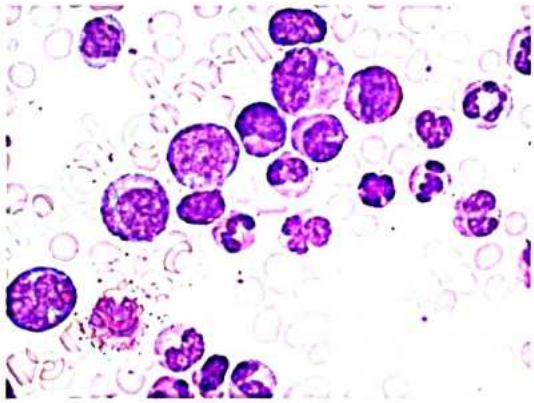
BCR-ABL Fusion

ALL- 190 kD
CML- 210 kD
CNL- 230 kD

- This is because of the rule that, lesser the weight of fusion protein greater is the Tyrosine kinase activity. Weight and Tyrosine kinase activity are inversely proportional. And ALL has the least weight here, that is why it got the worst prognosis and maximum Tyrosine kinase activity.

Translocation (9:22) Fish Test





CML shows

1. Garden party appearance
2. Pseudo Gaucher cells

CML- Clinical Features

- Dragging sensation in the abdomen.
- Massive Splenomegaly (something pulling abdomen down).
- Peripheral smear, and we will see a shift to the left and Basophils. Also, the CML is suspected.

CML- Phases

CML has three phases known as CAB (Chronic, Accelerated and Blast Crisis).

Chronic:

<10% blast

Accelarated:

Additional cytogenegics: i17q, trisomy 8
10-19%Basophils > 20%
Spleen
TLC increased or decreased
Platelet increased or decreased
} non responsive to treatment

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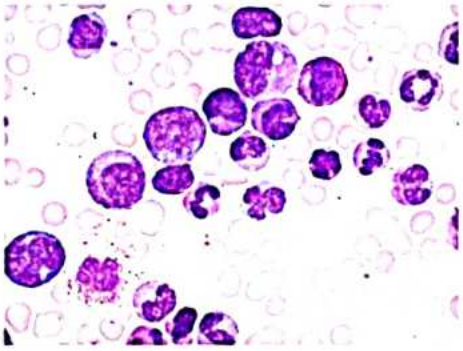
Blast crisis:
>/= 20% blast
Extramedullary blast proliferation
AML>>ALL

If it's a Myeloblast it's AML and if it's a Lymphoblast it's ALL. But more chances are that CML will go into AML.



CLL	CML
Convent Girl Appearance	College Girl / Garden Party Appearance

Treatment

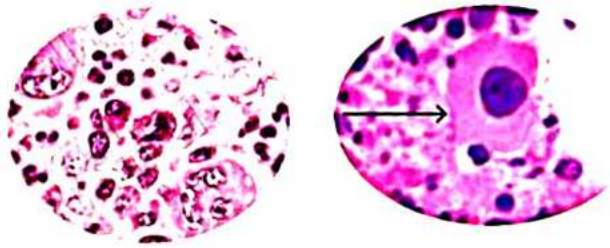
- Imatinib is used for – CML
- GIST (Gastrointestinal Stomach Tumor)
- Whenever we want to do Tyrosine Kinase inhibition, we use imatinib.

CML	Characteristics	Leukemoid Reaction (Leukemia Like- not a real Leukemia)
t (9:22)	Cause	Ph – & Sepsis +
Massive Spleen	Spleen	–
High	TLC	High but not as high as CML
> 20% Acc	Basophils	–
Decreased	LAP score	Increased

LAP/NAP Score

Leucocyte alkaline phosphate
Neutrophil alkaline phosphate
The normal LAP score is 40-100. The situations where LAP score will be increased or decreased are:

- **Increased LAP (LAKES, PONDS):**
  - Leukemoid Reaction
  - Pregnancy
  - OCP
  - Neutrophilia / Sepsis
  - DOWN'S SYNDROME
- **Decreased LAP:**
  - CML
  - PNH



So, CML tends to show dwarf Megakaryocytes.

MCQ

Q. There is a 49-year male who comes with complaints suggesting Acute febrile illness. Peripheral Smear is taken which revealed hyperleukocytosis, left shift in Granulocytes, increase in Basophils and Platelets is highly suggestive of?

A. AML
B. ALL
C. CML
D. CLL

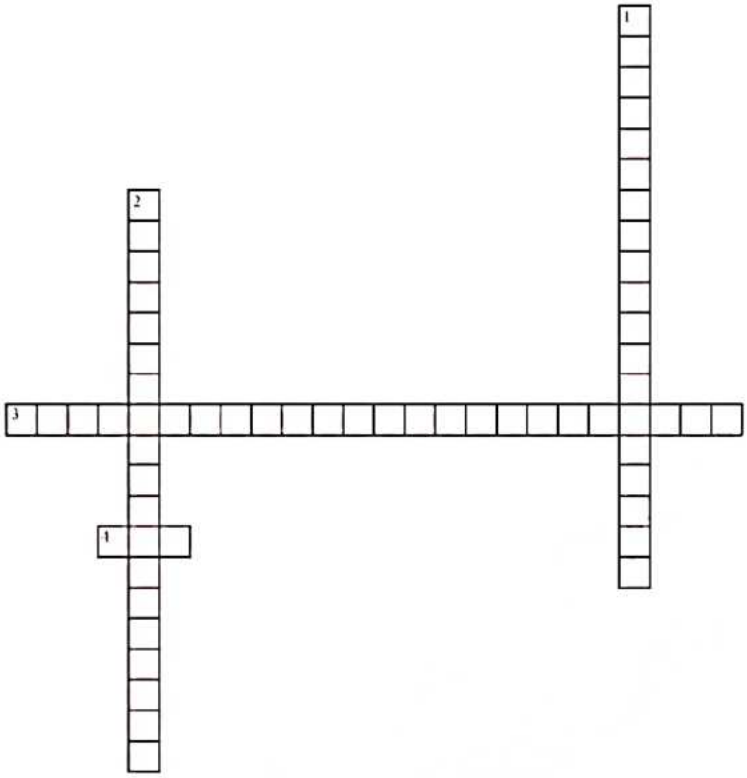
Q. Which of the following statements mentioned below regarding CML accelerated phase is incorrect?

A. Shows blasts 10-19%
B. Shows Basophils 10-19%
C. Persistent Splenomegaly, non-responsive to therapy
D. Persistent thrombocytosis, non responsive to therapy

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. When CLL becomes a lymphoma it is known as?
4. Leukemia that almost never develops after radiation

Down

1. Full form of AP
2. Theory suggests that if Vimentin is there, cells feel strong & if it is not there, cells feel weakened

Telegram - @nextpreladdernotes

57 LEUKEMIA AND LYMPHOMA PART 3

Lymphoma

Difference between Hodgkin's and Non- Hodgkin's Lymphoma

Hodgkin's Lymphoma	Non-hodgkin's Lymphoma
LN group (Cervical, Axillary, Inguinal)	LN group (Messenteric, Waldeyer's Ring)
Extra Nodal Involvement - (Very uncommon)	Extra Nodal Involvement - It tends to go outside the Lymph Nodes.
Spread - Contiguous Spread without skipping.	Spread - non Contiguous Spread
Bimodal Age Distribution	Elderly

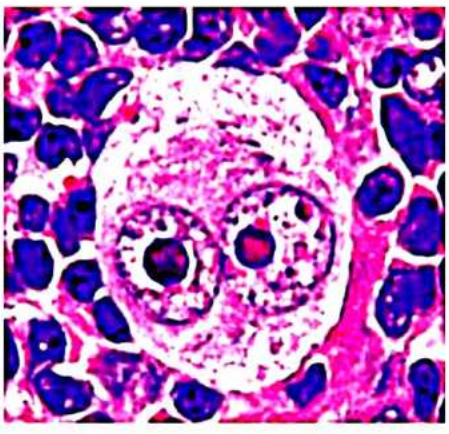
- Most common site of ExtraNodal Lymphoma is the Stomach.
- The most common site of ExtraNodal Lymphoma in HIV positive patients: CNS Lymphoma

Hodgkin's Lymphoma 00:05:47

Cell of Origin:
- It is a Post Germinal B Cell.

Pathogenesis:
- EBV has LMP 1 Gene it will increase the levels of NF KB, resulting in Hodgkin's Lymphoma.
  - Not all Hodgkin's Lymphoma occurs due to the EBV virus.
  - Chromosome 2 copy gain, increase in the Rel Gene.
  - This gene also increases the NFKB.

Characteristic Cell:
- It is the Reed - Sternberg Cell



- Owl Eye Appearance
- 45 microns in size, the eyes are 7 micron in size called Mirror Image Nuclei.
- The Pink dots are the Prominent Pink Nucleoli.
- There is another Owl Eye which is blue in color is seen in the CytomegaloVirus,

Markers:
- CD 15
- CD 30 - Most Sensitive
- PAX 5 - Most Specific (B cell origin Tumor)

Clinical Features:
- Pel Ebstein Fever (Waxing & Waning / Intermittent)
- Weight Loss
- Painless Lymphadenopathy (LAP)
- Pain on Alcohol Consumption (Paraneoplastic Syndrome)

Types of Hodgkin's Lymphoma

These are of two types:

1. **Classical Hodgkin's Lymphoma**
   - It is associated with EBV
   - RS cells are CD 15+, PAX 5 + and CD 30+
2. **Non-Classical Hodgkin's Lymphoma:**
   - It is not associated with EBV
   - RS cells in this are CD 20+, CD 45+, EMA+, Bcl 6+

Types of Classical Hodgkin's Lymphoma

Refer Table 57.1

Diagram 00:18:27
- lacunar cells are a formalin fixation artefact as the collagen bands retract in 10% neutral buffered formalin
  - formaling artefact onwards

Types of Non-Classical HL

Treatment
- ABVD
  - Adriamycin
  - Bleomycin
  - Vincristine / Vinblastine
  - Dacarbazine

Staging:

Ann Arbor Staging

Staging is a greater predictor of prognosis than histological grade. (Staging >>> histological)

Telegram - @nextpreladdernotes

- Stage I- Single LN region. (One group of Lymph node affected)
- Stage II- at least 2 groups/ region affected on same side of Diaphragm
- Stage III- LN on both sides of Diaphragm are affected. Sometimes 3s is used which means splenic involvement.
- Stage IV- Distant Sites
- Each of these stages can be A & B as well, such as 1a,1b, 2a, 2b and more.
- A - No B symptoms.
- B - B cell symptoms (Fever, Sweats, weight loss)

MCQ

Q. 40-year-old patient with cervical and Inguinal Lymphadenopathy. He is also complaining of coughing and significant weight loss since the past 4 months. Excision biopsy of the cervical lymph nodes reveal large atypical cells, with mirror image nuclei against a background of Eosinophils and plasma cells. The large atypical cells are positive for CD 15, CD 30, and negative for CD 3 AND CD 45. Which of the following is the most likely diagnosis?

a. HL - Mixed Cellularity
b. HL - Nodular Sclerosis
c. HL - Lymphocyte Rich
d. NLPHL

Q. 40 year old patient with cervical and Inguinal Lymphadenopathy. Mediastinal mass is also noted. He is also complaining of coughing and significant weight loss since the past 4 months. Excision biopsy of the cervical lymph nodes reveal large atypical cells showing a characteristic clearing around them. The lymph node also shows collagen bands running across. The large atypical cells are positive for CD 15, CD 30, and negative for CD 3 AND CD 45. Which of the following is the most likely diagnosis?

a. HL - Mixed Cellularity
b. HL - Nodular Sclerosis
c. HL - Lymphocyte Rich
d. NLPHL

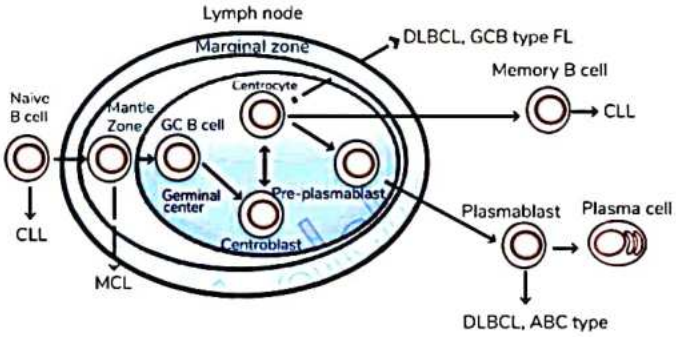
Non-Hodgkin's Lymphoma

- The non-hodgkin's Lymphoma takes their origin either from B or From T cells.
- They are also divided into two:

1. B-NHL- These are 7.
2. T-NHL- These are 2.

B- NHL

a. Follicular Lymphoma
b. Diffuse Large B cells Lymphoma } (from Germinal Center)
c. Burkitt's Lymphoma
d. CLL/SLL (Naive B cells)
e. Mantle Cell Lymphoma (Mantle zone)
f. Marginal zone Lymphoma/ Maltoma (Marginal zone)
g. Hairy Cell Leukemia (Memory B cell)



Lymph Node

- ORIGIN OF LYMPHOMAS
- Naive B cell- CLL/SLL
- Mantle cell zone (unchallenged B cell)- Mantle cell lymphoma
- Germinal Centre- Follicular lymphoma, Burkitt's lymphoma, Diffuse Large B cell Lymphoma
- Post Germinal centre B cell- Hodgkin's lymphoma
- Marginal zone (memory cells)- MArginal Zone lymphoma/ MALToma
- Memory B cells- Hairy cell leukemia
- Plasma cells- Multiple Myeloma

Note - Refer to main videos for menmonics and tricks

Genetics

- **Chromosome 8** - t(8:22), t(8:14), t(2:8)
- Chromosome 8 has c myc - menominc- t(8: even number)
- **Chromosome mnemonic- 11 and 18 at the margins,** t(11:18), Marginal zone Lymphoma or Maltoma.
- **Chromosome mnemonic- 14 and 18 are teenagers- best friends- friendly lymphoma,** Follicular Lymphoma.
- **Chromosome 11 & 14,** t(11:14), this is seen in Mantle Cell Lymphoma. This also shows positivity formantle mental dumb, SOX 11+.

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	CD 5	CD 23	CD 200
CLL / SLL	+	+	+
MCL	+	–	–
MZL	–	–	–

Follicular Lymphoma 00:46:08

- Common indolent Lymphoma
- It has a very good Prognosis.
- Translocation: t(14:18)
- **Lymph Node Biopsy:**
  - Centrocytes- They have a cleaved Nucleus. It looks like the Buttock cell.
  - Centroblasts- They have a non cleaved Nucleus.
- **Bone Marrow Involvement**: Paralrabecular Spread.

Markers:

- Bcl2 +, CD 19, CD 20 +

Diffuse Large B cell Lymphoma 00:49:48

- It is the most common NHL in the world.

Genetics-

- It shows Bcl 6 gene rearrangements.

Transformation

- Maybe it was a FL or CLL /SLL which has transformed into DLBCL
- SLL tranformed into DLBCL is known as richter syndrome is known as Richter's Syndrome.
- It has two varieties:
  - **Immunodeficiency associated**: It is associated with HIV+
  - **Primary Effusion Lymphoma**: It is the Human Herpesvirus 8 +
  - B & T markers are Negative here, CD 30 and CD 38 are positive.

HHV8 +:

- Kaposi Sarcoma
- Primary Effusion Lymphoma (PEL)
- Castleman Disease

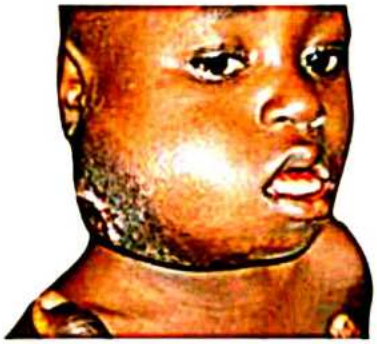
Burkitt's Lymphoma

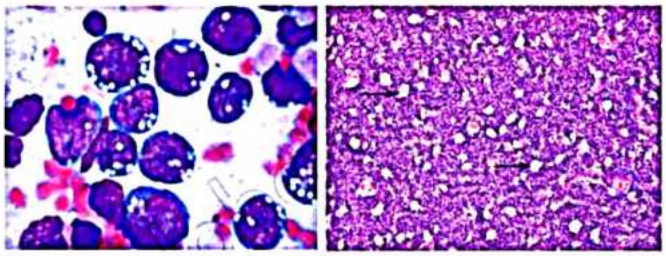
Genetics-

- t(8:even number), t(8:14), t(8:22).
- The 8 simplifies C myc amplification.
- It is the most commonly associated with Tumor Lysis Syndrome.

Types:

- **Endemic-** all the cases are associated with EBV.
- An African child with Jaw Swelling.





  - **Non-Endemic/ Sporadic**: In this EBV can be present in 20-30%. The site is Ileocaecal mass (GIT Involvement).
  - **Immunodeficiency**: In this EBV can be present in 30-50%. Any site of the body can be affected if the patient is HIV+.
- The EBV involvement is 100%.
- On FNAC & biopsy:
- In FNAC, there are vacuolations present (Oil red O+).
- In Biopsy, It shows the famous Starry Sky appearance. (Sky is the tumor cells & stars are tingible body macrophage)
- The special stain of Burkitt's Lymphoma is the Ki67 / MIB 1 - 100%. Ki67 is a Proliferation tumor. So, when Proliferation is 100%, it's a highly Proliferative tumor.

Ki67 / MIB 1 –

100%	Burkitt's Lymphoma
< 14%	Luminal A Breast Cancer.
> 14%	Luminal B breast Cancer.

Mantle Cell Lymphoma 01:03:38

Genetics:

- It has Translocation (11:14)

Markers:

- Cyclin D1, CD 5+, CD 23/ 200 –

New Marker:

- SOX 11

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When the Mantle Cell Lymphoma occurs in the GIT, it can result in Polyps.

- Lymphomatoid Polyposis.
- It's a classical case of Mantle Cell Lymphoma when CD 5+ and CD 23, CD 200 –

Marginal Zone Lymphoma / Maltoma

Types:

- Translocation t(11:18)
- API - MLT (Maltoma) gene fusion occurs.
- H Pylori causes Maltoma.
- It could also be associated with Sjogran's Syndrome or Hashimoto's Disease.

Markers:

- CD 5 –
- CD 23 –
- CD 200 –

Treatment:

- For H pylori, the treatment shall be antibiotics. But for others it shall be Chemotherapy as others Lymphoma.

Note- Refer to main videos for tips and tricks to remember hairy cell leukemia

Hairy Cell Leukemia

Gender

- M/C in Boys

Mutation

- BRAF V_{600} E Mutation

Clinical Features-

- Massive Splenomegaly
- Red Pulp of Spleen is affected
- All leukemia and lymphomas go to white pulp of the spleen.
- All leukemia and lymphomas involve white pulp of the spleen, except Hairy cell leukemia and Hepatosplenic lymphoma which involves Red pulp of the spleen.

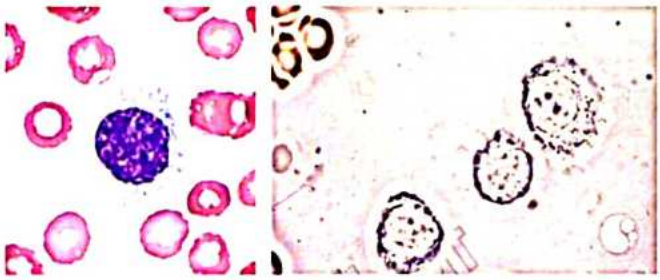
CBC: Pancytopenia with Monocytopenia

Infections: Mycobacterium Avium Complex

Bone Marrow Aspirate:

Dry tap.

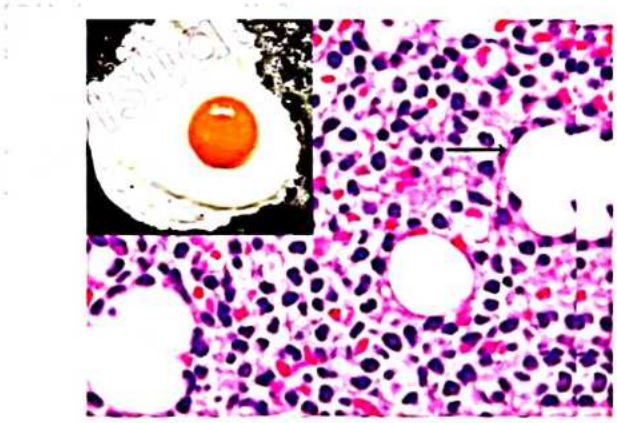
- A lot of hairy cells on it.
- Releases PDGF, TGF or all other factors which cause fibrosis of marrow.
- These are the factors which cause Myelofibrosis leads to Pancytopenia with Monocytopenia.

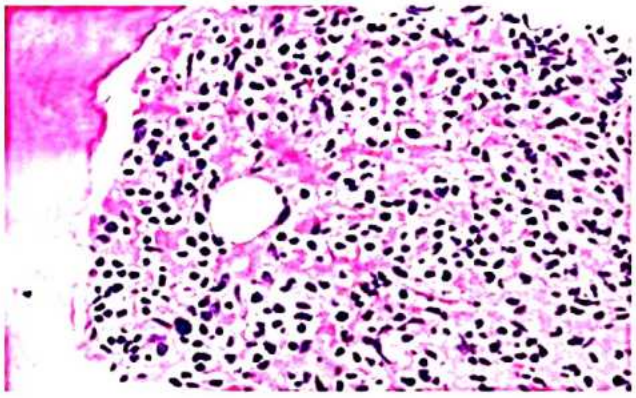


- Hairy cells are best seen in phase contrast microscopy

Diagnosis:

- Bone Marrow Biopsy
- On bone marrow Biopsy: Fried egg Appearance.





- There is a nucleus and there is a white area around it.

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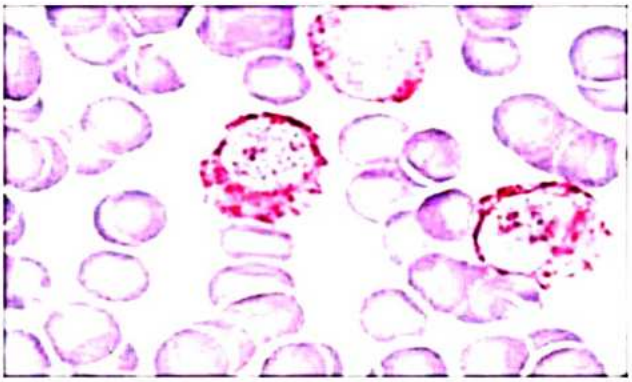
Special Stain-

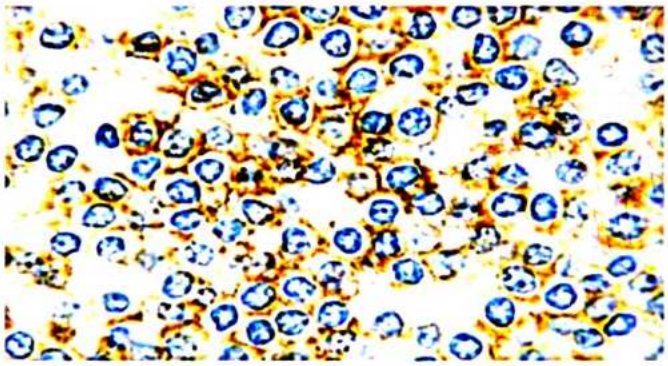
- The Stain is TRAP (Tartrate Resistant Acid Phosphatase).



- The color with TRAP is Red.

Markers-

- CD 11c, CD 25, CD 103
- Annexin A1
- DBA 44
- All of these markers are done on Immunohistochemistry. The color in immunohistochemistry is brown.



- Annexin V → Apoptosis
- Annexin A1 → Hairy Cell Leukemia

T- NHL

Mycosis Fungoides:

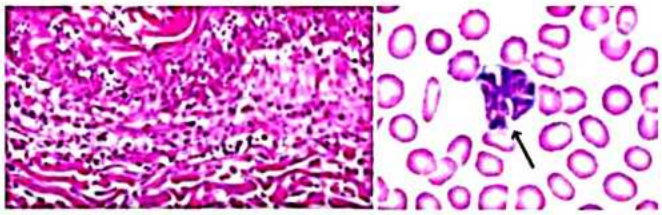
- It is a Cutaneous T- NHL.

Clinical Features:

- Erythema, Pruritus

Biopsy: Paurtrier's Microabscess.

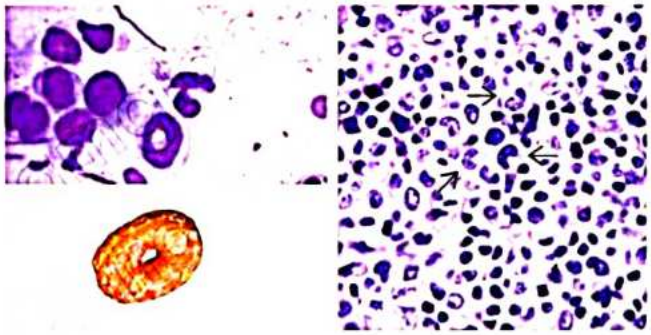
- This is known as Epidermotropism.
- Tumor cells goes into blood vessels: Sezary syndrome
- This is known to have Cerebriform Nucleus.



- Paurtrier's Microabscess → Mycosis Fungoides
- Munro's Microabscess → Psoriasis

Anaplastic Large Cell Lymphoma:

- ALK Gene
- CD 30+
- This tumor microscopically shows two cells:
  - Doughnut cell
  - Hallmark cell- Kidney shaped cells



CD 30+ →

- Embryonal Carcinoma Testis
- Hodgkin's Lymphoma (Reed Sternberg Cell)
- Non Hodgkin's Lymphoma (Primary Effusion Lymphoma & Anaplastic Large Cell Lymphoma)

MCQ

Q. HIV-positivee patients present with pleural and pericardial effusion. Pleural tap reveals atypical lymphoid cells associated with positivity for CD 30 AND CD 38. However B and T cell markers are not expressed. Further Positivity for HHV 8 antigen is seen

a. DLBCL
b. PEL
c. Kaposi Sarcoma
d. Follicular Lymphoma

Q. Ki67 is nearly 100% in which of the following Lymphoma?

a. DLBCL
b. Burkitt's Lymphoma
c. Mantle Cell Lymphoma
d. Follicular Lymphoma

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Q. Which of the following is not seen in Burkitt's Lymphoma?
a. t(8:22)
b. t(8:14)
c. t(2:8)
d. t(11:18)

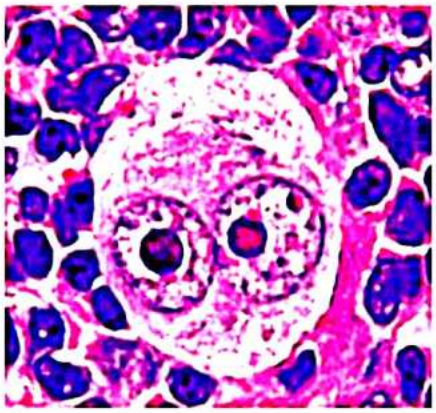
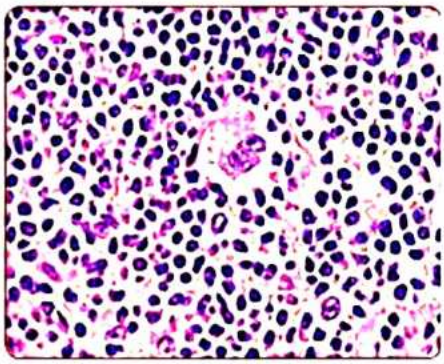
Q. A 60 year old male patient has been fatigued for the past 4 months. On physical examination massive Splenomegaly is noted, however no Lymphadenopathy seen. Lab studies show hemoglobin 8g/dl, MCV 90/fl, TLC 1600/mm³ and platelet count 49000 mm³. The most likely diagnosis is Hairy Cell Leukemia. Mark the following statement which is incorrect:
a. Most commonly involves Red pulp of spleen
b. Patients shows atypical Mycobacterial infection most commonly
c. Hairy cells are best seen on phase contrast microscopy
d. Bone marrow aspirate shows fried egg Appearance

Q. Which of the following involved Red pulp of the Spleen?
a. Follicular Lymphoma
b. Hepatosplenic Lymphoma
c. Hairy cell Lymphoma
d. Both B and C

Q. Which is the Lymphoma that presents with Cutaneous lesions?
a. Mycosis Fungoides
b. Anaplastic Lymphoma
c. HL
d. Burkitt's Lymphoma

Telegram - @nextpreladdernotes

Table 57.1

HL	RS cell	Extra	Prognosis
Classical Hodgkin's Lymphoma	CD 15, 30 and PAX 5 	EBV +	
Nodular Sclerosis (Most common worldwide)	Lacunar Cells 	Collagen Bands Mediastinal Involvement EBV +/–	Best in Classical Hodgkin's Lymphoma
Mixed Cellularity (Most common in India)	Classical RS cells (Classical Owl Eye)	Polymorphous background (Eosinophils, Plasma cells, Lymphocytes) Most common with EBV Most common with HIV (strongest association with HIV) Most common with B Cell symptoms	
Lymphocyte Rich	Mononuclear cells	Shows single eye (Single mononuclear RS cell)	
Lymphocyte Depleted	Mummified RS cells	Immunodeficiency associated (associated with HIV)	Worst Prognosis

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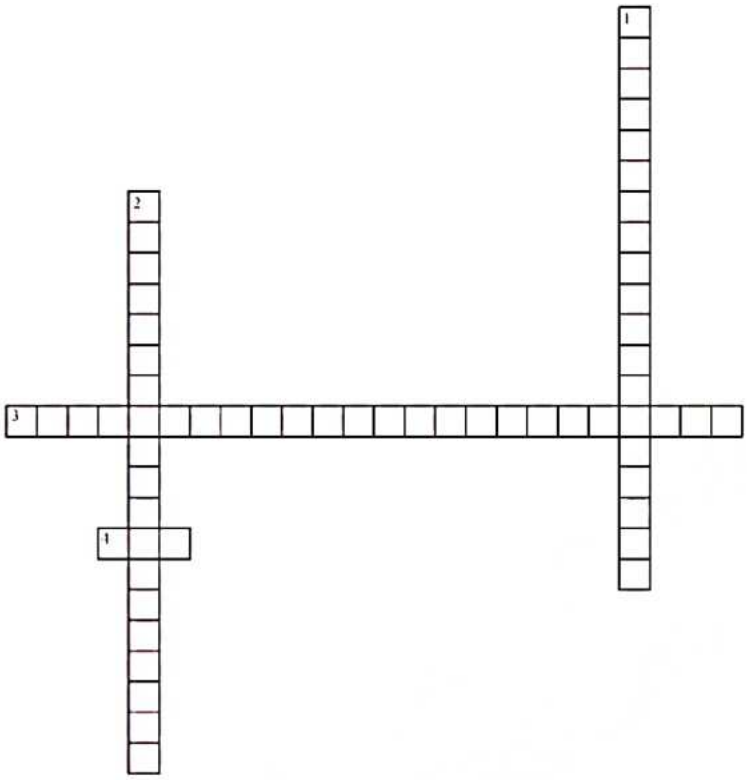
CROSS WORD PUZZLES

Crossword Puzzle



Across

3. When CLL becomes a lymphoma it is known as?
4. Leukemia that almost never develops after radiation

Down

1. Full form of AP
2. Theory suggests that if Vimentin is there, cells feel strong & if it is not there, cells feel weakened

Telegram - @nextpreladdernotes

58 CHRONIC MYELOPROLIFERATIVE DISORDER AND MYELODYSPLASTIC SYNDROMES

1. Polycythemia vera
2. Chronic Myelogenous Leukaemia
3. Essential thrombasthenia
4. Primary myelofibrosis
   - All are present with panmyelosis,
   - CML shows t(9:22) translocation known as Philadelphia chromosome and is only present in CML. All others are Philadelphia chromosome negative.

Polycythemia vera 00:02:41

Major Criteria

- Increase in haemoglobin
- In males > or = 16.5 gm
- In females > or = 16 gm.
- Panmyelosis
- JAK2V617F mutation (causes problem on codon 617)

Minor Criteria

- Erythropoietin levels are normal or suboptimal.

Clinical Features

1. Increase in RBC:
   - Hyperviscosity
     Features: Red Plethoric Face, Headaches, hypertension, vision disturbances
   - Thrombotic episodes
     Hepatic Vein Thrombosis: Budd Chiari Syndrome
     Cerebral Vein Thrombosis: Dangerous

2. Increase in WBC:
   - Increased Mast Cell & Basophils
   - Hot Water Bath causes mast cell degranulation and histamine is released, thus causing Aquagenic pruritus.

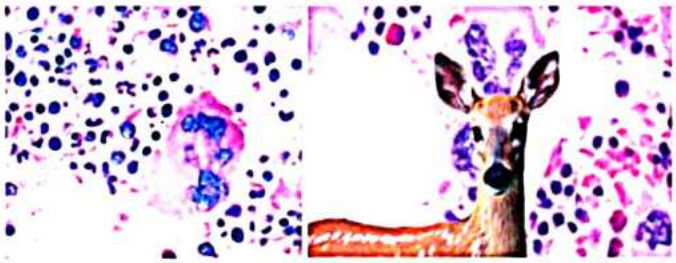
3. Increase in Platelets:
   - Erythromelalgia
   - When platelets go through tiny capillaries (fingers, hands, toes, etc), they start forming aggregates that cause burning sensation in hands and feet.

Polymerase chain reaction→single-base primer extension for known locus (JAK2V617F).

Essential Thrombasthenia 00:14:19

Major criteria

I. Platelet count more than 4.5 lakhs/mm^3
II. Hyperlobated megakaryocyte or staghorn megakaryocyte.



(I) Bcr-Abl negative
(ii) JAK2V617F/MPL/CALR mutation

Minor Criteria

- Rule out reactive thrombocytosis (Example: infection, Iron deficiency anaemia)

All 4 major criteria or 3 major + minor criteria

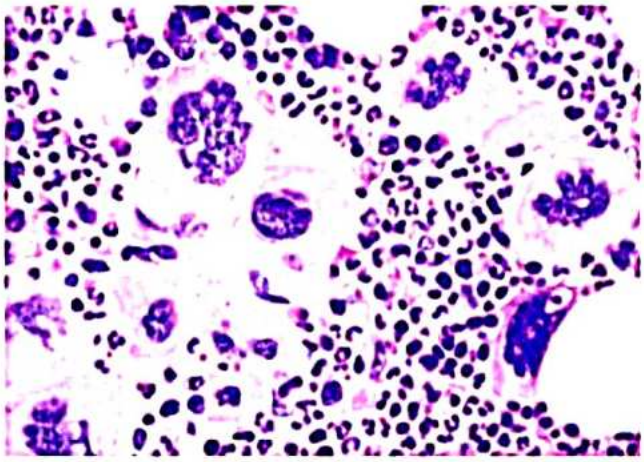
Clinical Features

- Erythromelalgia (Burning and throbbing sensation in hands and feet)

Primary Myelofibrosis 00:20:28

- Bone Marrow Fibrosis
- It is a megakaryocyte defect
- It releases a lot of mediators like PDGF, TGF beta, FGF which cause fibrosis
- Most fibrogenic cytokine: TGF beta
- Teardrop RBCs/ Dacrocytes seen
- Leukoerythroblastic picture

Megakaryocyte in primary myelofibrosis



Bone marrow biopsy

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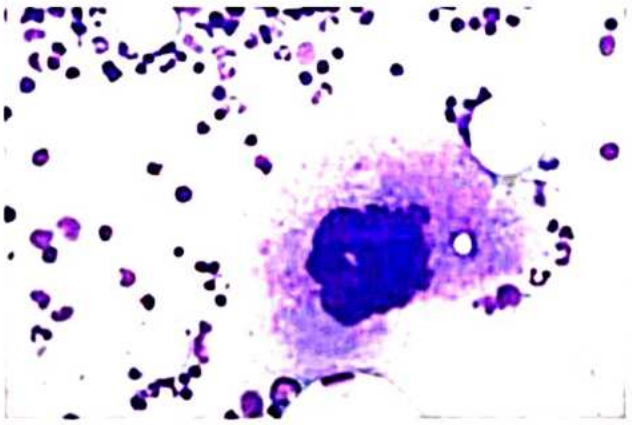


Bone marrow Aspirate

- Cloud-like Megakaryocyte

Fibrosis in primary myelofibrosis

- On putting up with reticulin stains, black fibres appear.
- Dilated sinusoids

Splenomegaly is seen in primary myelofibrosis patients.

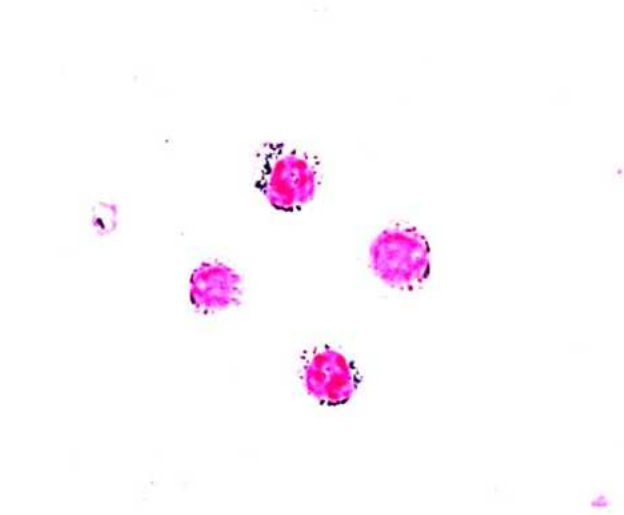
Myelodysplastic Syndrome (MDS) 00:28:52

- It is a precancerous stage
- Dysplasia is seen in RBC, WBC, and Platelets.

Genetics

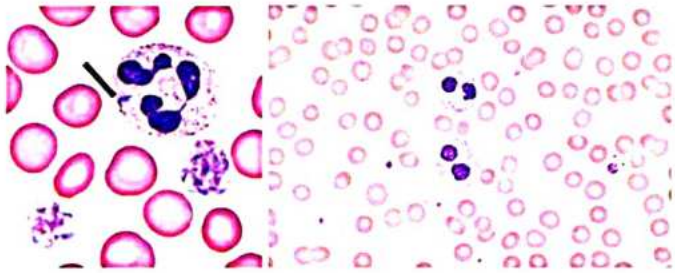
- Adults: del 5q (elderly)
- Children: Monosomy 7

Dysplasia in RBC



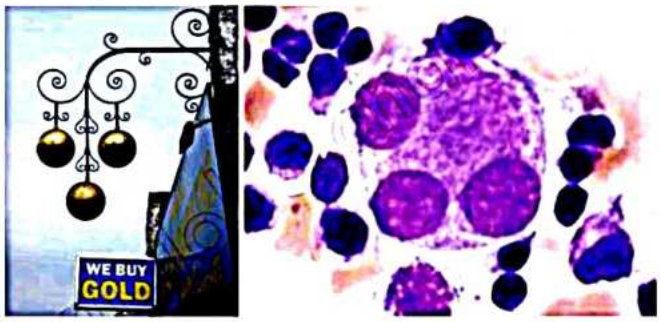
- Prussian Blue or Perl's Stain (Fe)
- Ringed Sideroblasts

Dysplasia in WBC



- It shows Pelger Huet Anomaly (MDS) and Dohle Bodies

Dysplasia in Platelets



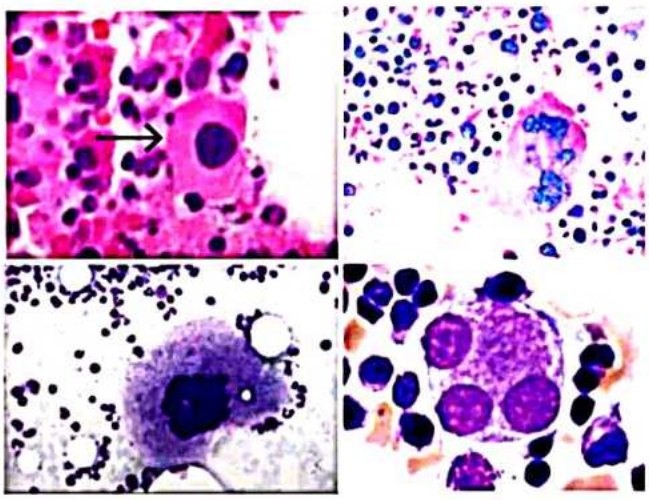
- Pawn Ball Megakaryocyte (3 metallic ball appearance)

Category	Blasts in BM	Extra
MDS with single lineage dysplasia	Less than 5%	Present in any one of RBC, WBC, or Platelets.
MDS with multi lineage dysplasia	Less than 5%	Present in two or more than 2 lineages
MDS-ringed sideroblasts	Less than 5%	More than or equal to 15% ringed sideroblasts*
MDS with isolated del 5q	Elderly	
MDS with excess blasts 1	Between 5 to 9%	
MDS with excess blasts 2	Between 10 to 19%	
MDS unclassifiable		
Refractory cytopenia of childhood	Monosomy 7	

Telegram - @nextpreladdernotes

*If MDS is with SF3B1 mutation then even 5% of ringed sideroblasts will be the cut-off.

Summary

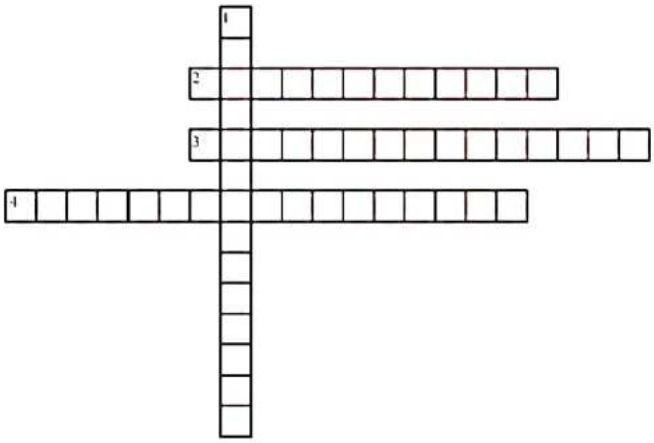


a. Dwarf Megakaryocyte
b. Staghorn Megakaryocyte
c. Cloud-like Megakaryocyte
d. Pawn ball Megakaryocyte

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. Visible in in primary myelofibrosis patients
3. Burning and throbbing sensation in hands and feet
4. Hot Water Bath causes mast cell degranulation and histamine is released, thus causing _____

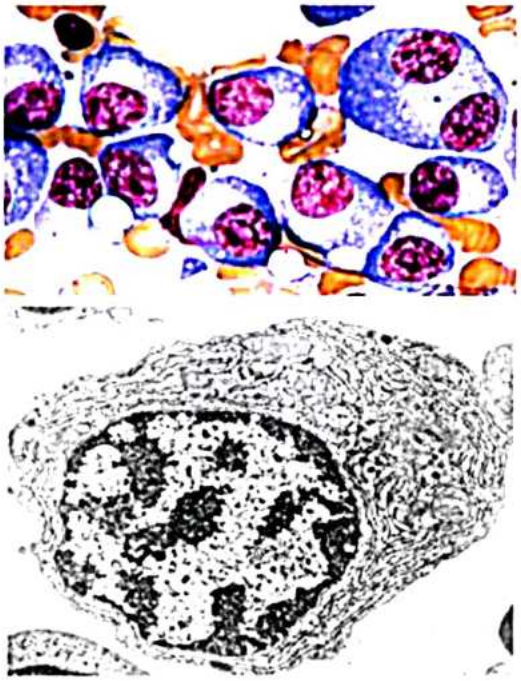
Down

1. Clinical feature of Polycythemia vera that involve Red Plethoric Face, Headaches, HTN, vision disturbances

Telegram - @nextprepladdernotes

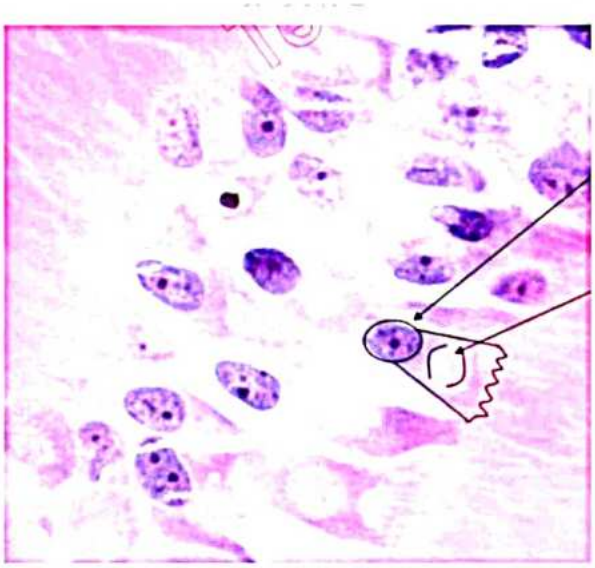
59 PLASMA CELL DYSCRASIA

Normal plasma cell?



- Where is the nucleus?
  - Eccentric nucleus with cartwheel chromatin
  - Perinuclear halo/Hof
  - Reason for perinuclear halo is golgi apparatus

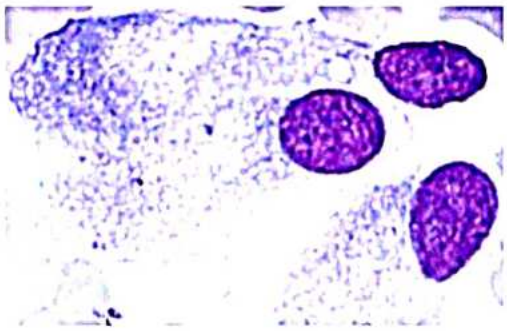
Osteoblasts



Similar to a plasma cell

- Eccentric nucleus on one side
- Paranucleur Hoff/ halo

Identify the image



Disorders

- Multiple myeloma
- Smouldering multiple myeloma
- Monoclonal gammopathy of undetermined significance (MGUS)
- Plasma cell leukaemia
- Waldenstrom's macroglobulinemia
- Heavy chain disorder

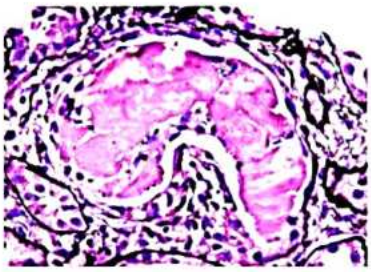
Multiple Myeloma - Genetics 00:05:26

- t(11;14) (also seen in mantle cell lymphoma)
- Del 13q (also seen in CLL)
- ORIGIN: Post-Germinal Centre B cell

Pathogenesis

Increased Plasma cells
↓
Increased Ig (monoclonal)
↓
Produces Kappa or lambda light chains
↓
Causing light chain excess → Urine→Bence Jones Proteins
↓
Myeloma kidney is formed (A amyloid L light chain type)
↓
CKD with 2°hyperparathyroidism
Increased calcium

- Normal Ig will produce (k & λ) light chains (60:40 ratio)



Myeloma kidney

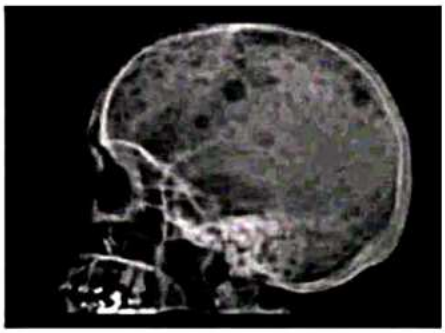
Telegram - @nextpreladdernotes

- Cast formed known as myeloma cast: fractured cast with breaks and cuts
- Bence jones proteinuria- made up of a light chain

Interleukin

- IL 6 → Plasma cell proliferation → responsible for activating the RANKL pathway → osteoclastic activity → Bony Lytic Lesions (exam question)
- Plasma cells releasing DKK1 (DICKKOPF 1) blocks osteoblast production.
- No bone formation → Alkaline phosphatase level will be normal.
- Elevation of calcium, but ALP is normal.

Clinical Features

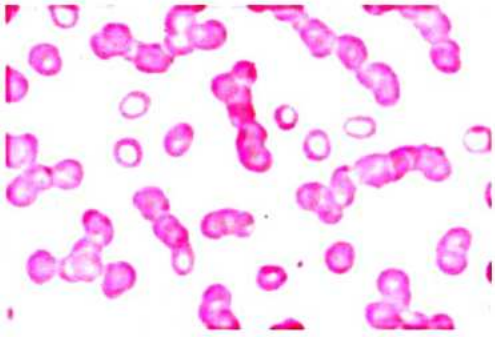


- Elderly patients will have bony lytic lesions
- Skull: Salt and pepper (also seen in hyperparathyroidism)
- Vertebrae: low back pains

Criteria

- Plasma cells >10% - in the bone marrow only, there is no plasma cell in blood vessels
- IG >3 gm/dl — most common involved is IgG
- Myeloma defining event
  - **C** - calcium levels elevated (>11 gm/dl)
  - **R** - renal insufficiency (s.creatinine level > 2 mg/dl)
  - **A** - Anaemia (Hb <10gm)
  - **B** - Bony lytic lesions
  - **S** - Plasma cell >60%
  - **Li** - light chains ratio - involved / uninvolved > 100
  - **M** - MRI atleast 1 lesion of > 5 mm is seen

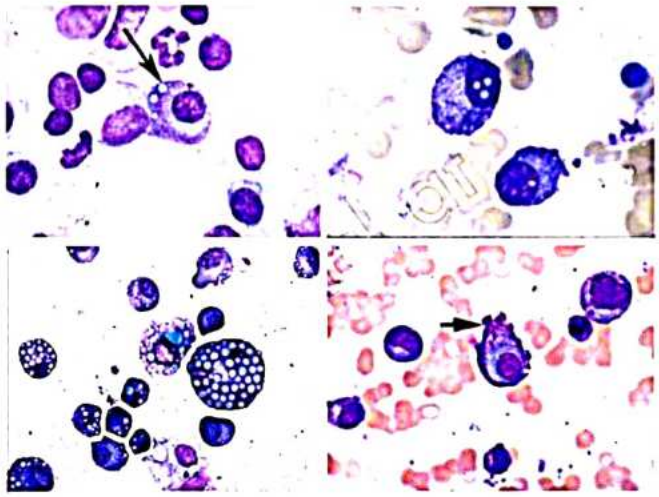
Multiple myeloma blood findings: 00:21:06



Rouleaux Formation

- **RBCs** are spread out & not sticking to each other because of negative charge called Zeta potential
- **Multiple myeloma will** have lots and lots of antibodies (immunoglobulin) covering RBCs Eliminating negative charges
- There is a **loss of Zeta potential,** RBCs will be stuck with each other forming rouleaux

Bone Marrow Aspirate Finding:



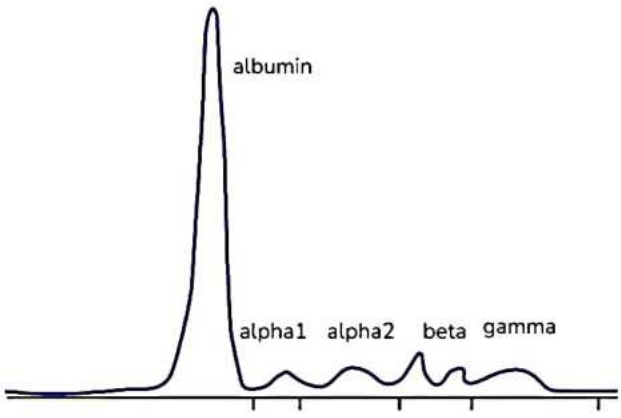
i. Russell Body: intra cytoplasmic inclusion
ii. Dutcher Body: intranuclear inclusion
iii. MOTT/ mulberry cell
iv. Flame Cell - IgA type multiple myeloma
The composition of all these findings is Antibodies (Ig)

Bone Marrow Biopsy

- Sheets of plasma cell

Refer Table 59.1

Serum Protein Electrophoresis (Spep)

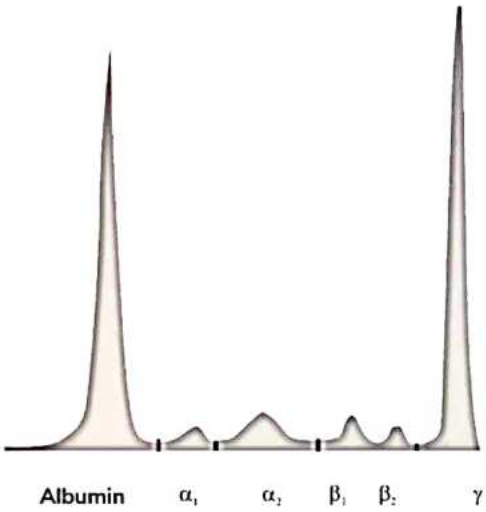


Telegram - @nextpreladdernotes

Samples: blood/urine (antibodies are present in both)

- Normal blood sample is made up of Albumin and three folded finger-like structure
  - Alpha 1 proteins (alpha 1 antitrypsin)
  - Alpha 2 protein(alpha 2 macroglobulin)
  - Beta (proteins)
  - Gamma antibodies (immunoglobulins)

The gamma (Ig) will increase and show a spike



- An M spike - monoclonal or myeloma spike
- Most common - IgG

Report

- Immunofixation - will tell the type of immunoglobulin

Treatment of Multiple Myeloma

- Lenalidomide
- Dexamethasone
- Bortezomib

Targeted Therapy -

- Anti CD38- Daratumumab

Smouldering Multiple Myeloma - Criteria 00:35:12

- Plasma cell > 10%
- Ig > 3gm/dl
- There is no myeloma-defining event

Monoclonal Gammopathy of Undetermined Significance - Criteria (MGUS)

- Plasma Cell <10 %
- Ig <3 gm/dl
- No MDE

Feature Table

Features	MGUS	SMOULDERING MM	MM
Plasma Cell	Negative	> 10%	> 10%
Ig	Negative	> 3 gm	> 3 gm
MDE	Negative	Negative	MDE is present (Slim Crab)

Plasma Cell Leukaemia - Criteria

- Plasma cells are present in bone marrow
- **Plasma cells>20% of peripheral blood**

Waldenstrom's Macroglobulinemia (IgM)

- Also known as **LPL** - Lymphoplasmacytic lymphoma
- **Cells** - Lymphocytes, plasma cells and mast cells
- **Genetics** -
  - Deletion of 6 chromosomes (most common)
  - MYD 88 mutation
- **Clinical Features**
  - IgM increase:
    → hyperviscosity:
    - Headache
    - visual disturbance
    - haemorrhage (cerebral, Retinal)

Heavy Chain Disease

Alpha chain disease - Seligmann Disease

- Stomach - H. Pylori
- Small intestine - IPSID

Mu chain Disease

- Cytoplasmic vacuoles
- CLL
- BJP - Benes Jones proteinuria

Gamma Chain Disease - Franklin Disease

- Palatal Edema
- Waldeyer's Ring

MCQs

Q. The proliferation and survival of myeloma cells depend on which of the following cytokines?

a. IL-1
b. IL-6
c. IL-2
d. IL-5

Telegram - @nextpreladdernotes

Q. What are large, homogenous cytoplasmic inclusions in plasma cells called?

a) Dutcher bodies
b) Councilman bodies
c) Russell bodies
d) Mallory hyaline bodies

- Councilman bodies– hepatitis CV
- Mallory hyaline bodies- ALD

Q. What causes the M spike in Waldenstrom's macroglobulinemia?

a. IgM
b. IgG
c. IgA
d. IgD

Q. Is malignancy associated with Waldenstrom's macroglobulinemia?

a. Mycosis fungicides
b. Smouldering myeloma
c. Primary effusion lymphoma
d. Lymphoplasmacytic lymphoma

- Smouldering myeloma– Multiple Myeloma
- Primary effusion lymphoma– a type of DLBCL
- Lymphoplasmacytic lymphoma — LPL

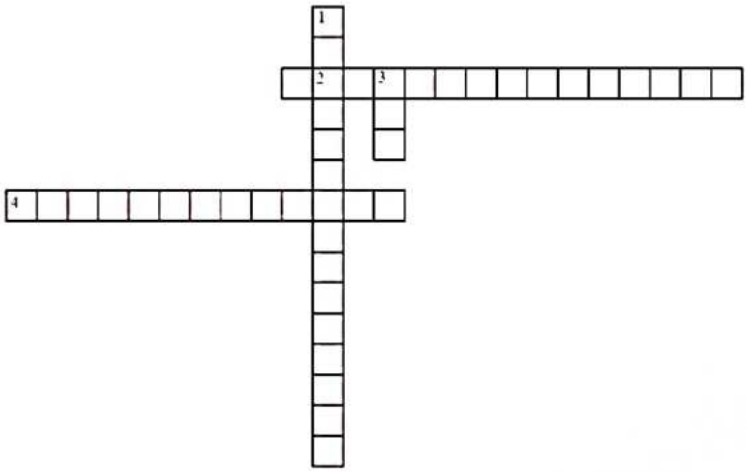
Table 59.1

	Parental markers		Self markers		
Cells	**CD 45 (Leukocyte common antigen)**	**CD 19 (B cell marker)**	**CD 38**	**CD 138**	**Ig**
Normal plasma cells	Positive	Positive	Positive	Positive	Both Kappa and lambda Polyclonal antibodies
Malignant plasma cell CD 56 +/- Syndecan1 + / CD 138	Negative	Negative	Positive	Positive	Monoclonal Either kappa or lambda

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

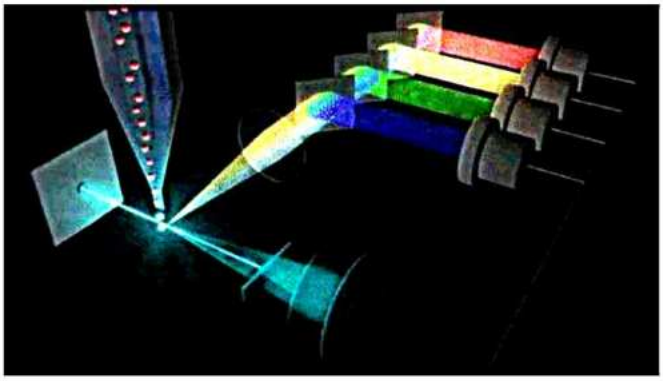
2. The whitish area around the nucleus
4. large homogenous cytoplasmic inclusions in plasma cells

Down

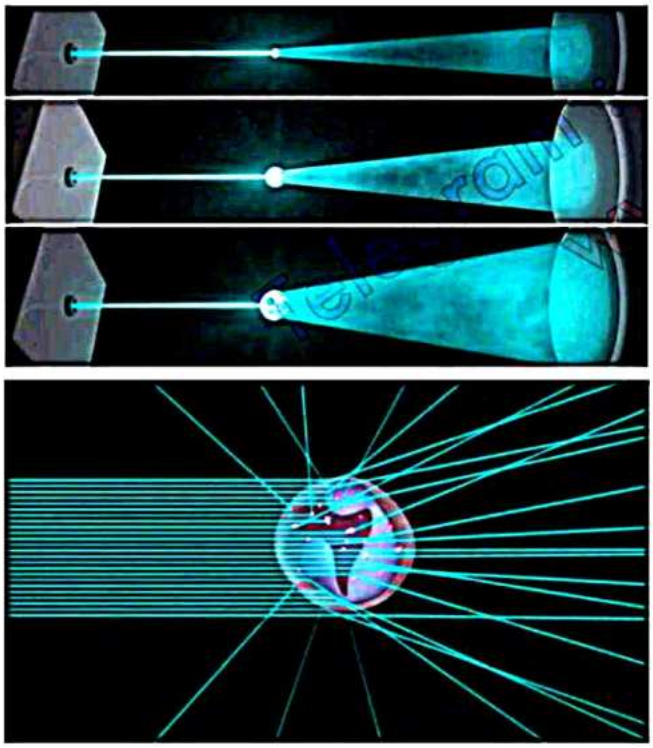
1. Something like cartwheel chromatin
3. What causes the M spike in Waldenstrom's macroglobulinemia?

Telegram - @nextprepladdernotes

60 FLOW CYTOMETRY



1. Samples: Samples can be blood, bone marrow aspirate, FNAC, body fluids
2. Dyes: FITC (Fluoro Iso Thio Cyanate)
3. Hydrodynamic focusing: Sheath fluid is added to sample to produce single light that is known as Hydrodynamic focusing. Sample + marker & dye + sheath fluid
4. Light: Blue coloured laser light of wavelength 488 nm falls on the cells.



- Once the laser light comes, some part of the light goes forward (forward scatter) and some part of it goes sidewards (side scatter).
- If the cell is small then the light will scatter less and if the cell is bigger in size, the light that will fall on it will scatter more.
- Forward scatter is indicative of size of cells i.e., larger the size of the cell, larger the forward scatter.
- Side scatter depends on the complexity or granularity of the cells i.e., more the granularity of the cell, more will be side scatter.

Flow cytometry is a FACS (Fluorescence activated cell sorting) machine.

Lineage	Markers		
Blasts	TdT, HLA DR, CD 34		
LCA (Leukocyte common antigen)	CD 45		
T Cells	CD3, CD 1-8, 28		
B cells	CD19, CD10, CD 19-23, CD40		
NK cells	CD 16,56		
Myeloid cells	MPO, CD13, CD33		
Monocytic lineage	CD11, CD14, CD64		
Megakaryocytic lineage	CD41, CD42, CD61		
CLL/SLL	CD5+	CD23+	CD200+
MCL	CD5+	CD23-	CD200-
MZL	CD5-	CD23-	CD200-
Erythroid cells	CD 71 (TRANSFERRIN Receptor)		
PNH	CD 55- CD59-		
Glanzmann thrombasthenia	(Gp 2a 3a defect)		
Plasma cells (normal)	CD45+ CD 19+ CD38 + CD138+		
Multiple myeloma (cancer)	CD45-CD19-CD38+ CD138 +		

Telegram - @nextprepladdernotes

Questions:

Q. Diagnosis?
CD34+/HLA DR+/MPO+
Ans. AML

Q. Diagnosis?
CD34+/TdT+/CD3+/CD7+
Ans. T-ALL

Q. Diagnosis?
Tdt+/HLA DR+/CD10+/CD19+
Ans. B-ALL

Q. Diagnosis?
CD 34+/HLA DR+/MPO+/CD 7+
Ans. AML

Q. Diagnosis?
HLA DR+/TdT+/MPO+/CD19+
Ans. Biphenotypic Leuk

Types Of Graphs in flow Cytometry

- Dot Plot Analysis
- Histograms

1. Dot Plot Analysis

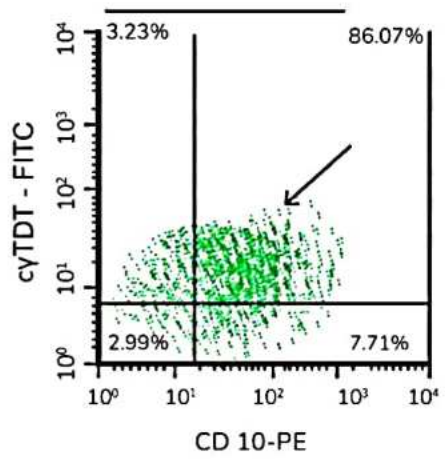
X- Y+ | Y+ X+

y↑ X- Y- | X+ Y-

x→

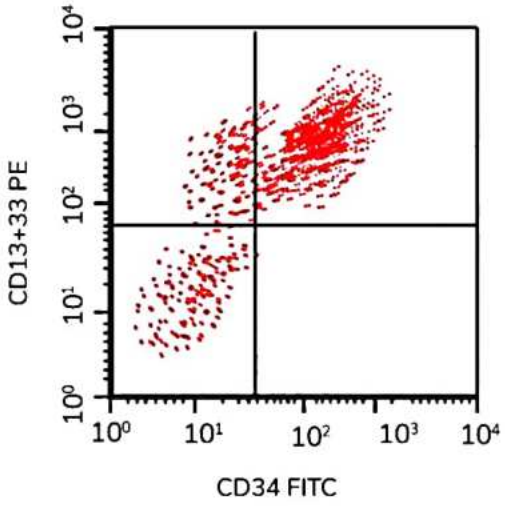
MCQ's

Q. Dot plot analysis?



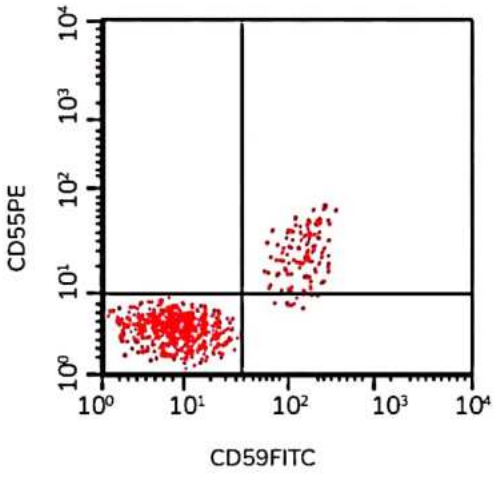
a. **B-ALL**
b. T-ALL
c. CLL
d. AML

Q. Dot plot analysis?



a. B-ALL
b. T-ALL
c. CLL
d. AML

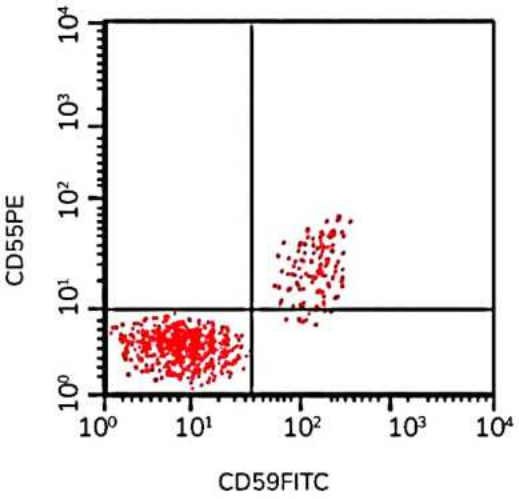
Q. Analysis?



a. B-ALL
b. Treg cells
c. Hyper IgM
d. PNH

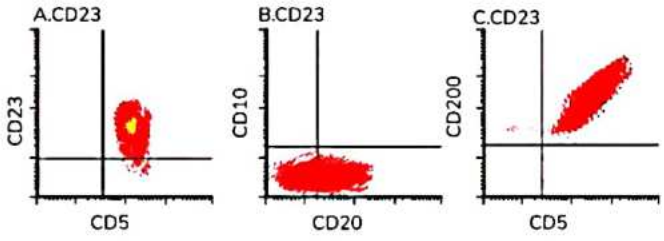
Telegram - @nextprepladdernotes

Q. Analysis?



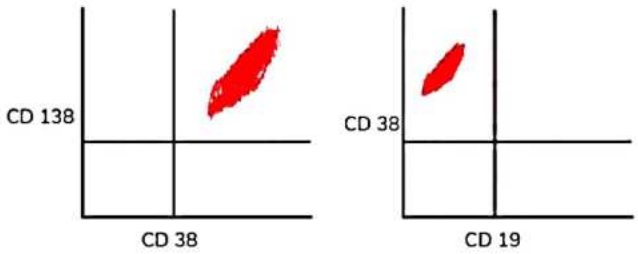
a. B-ALL
b. Treg cells
c. Hyper IgM
d. Glanzmann thrombasthenia

Q. Dot plot analysis?



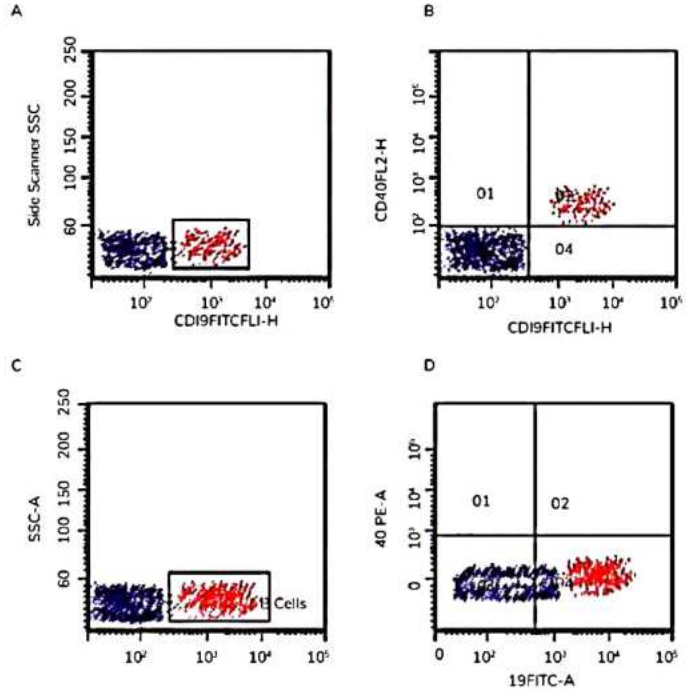
a. MZL
b. MCL
c. CLL
d. dAML

Q. Analysis?



a. Normal Plasma Cell
b. Malignant Plasma Cell

Q. Analysis?



a. B-ALL
b. Treg cells
c. Hyper IgM
d. d BTK gene defect

Telegram - @nextpreladdernotes

PREVIOUS YEAR QUESTIONS

Q. A 50yr old child presents with gum bleeding and fatigue. His PBS shows marked leukocytosis with 70% cells showing MPO positivity. Diagnosis? (FMGE 2020)

A. AML
B. ALL
C. CLL
D. CML

Q. A 4 yr old child presents with the development of fever, petechial spots and complaint of fatigue. He is also having presence of pallor, hepato-splenomegaly as well as tenderness. The clinical situation descried above is most correctly associated with which of the following? (FMGE 2020)

A. AML
B. ALL
C. CLL
D. CML

Q. A patient presented with headache & fever. His investigations revealed Hb-16g/dL, TLC of 21000/μL, platelet count of 350,000. His DLC showed neutrophils (25%), lymphocyte (20%), metamyelocytes & myelocytes 40% and eosinophils 5%. Which of the following is the next best investigation for this patient? (AIIMS 2017)

A. JAK 2 mutation
B. EPO level
C. Philadelphia chromosome
D. Bone marrow biopsy

Q. Tumor cells in Chronic Lymphocytic Leukemia or Small Lymphoblastic Lymphoma (CLL/SLL) arise from which of the following? (AIIMS 2017)

A. Mature B-cell
B. Naive B-cell
C. Centrocytes of Germinal center
D. Progenitor B-cell

Q. Which of the following is incorrect statement about nodular lymphocyte predominant Hodgkin's lymphoma; NLPHL is? (INICET Nov 2020)

A. EBV negative
B. CO 15/30 negative
C. CD 20+
D. Poor prognosis compared to classical variant

Q. Which of the following is the least likely cause of a bone marrow showing a dry tap? (INICET Nov 2020)

A. Hairy cell leukemia
B. Myelodysplastic syndrome
C. Follicular lymphoma
D. Acute megakaryocytic leukemia

Q. A 5 years old boy came with a clinical presentation of cervical lymphadenopathy. Microscopic picture of lymph node biopsy shows starry sky appearance. Which of the following translocation is unlikely to be seen in this condition? (JIPMER May 2019)

A. t (2;8)
B. t(8;22)
C. t (8;14)
D. t (11;18)

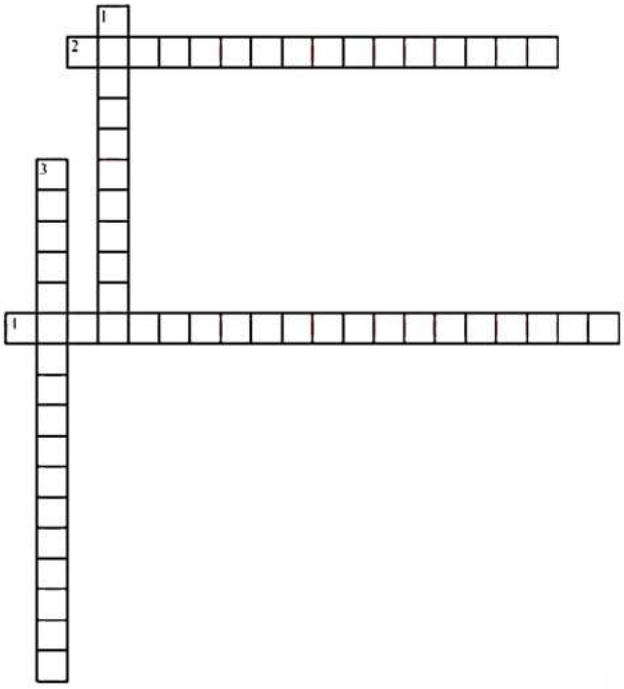
Q. True regarding hairy cell leukemia is? (JIPMER Dec 2019)

A. Characterized by mild splenomegaly
B. Pancytopenia is the characteristic finding
C. Monocytosis seen
D. Hairy cells are TRAP negative

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. What is - HLADR−/TdT+/MPO+/CD19+
4. Sheath fluid is added to sample to produce single light that is known as _____

Down

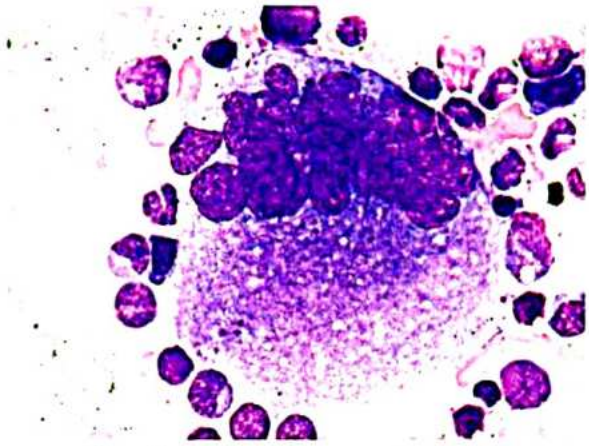
1. This depends on the complexity or granularity of the cells
3. M416, CD41, CD42, CD61 are markers of which lineage?

Telegram - @nextprepladdernotes

61 PLATELET PART -1 (COAGULATION AND BLEEDING DISORDERS)

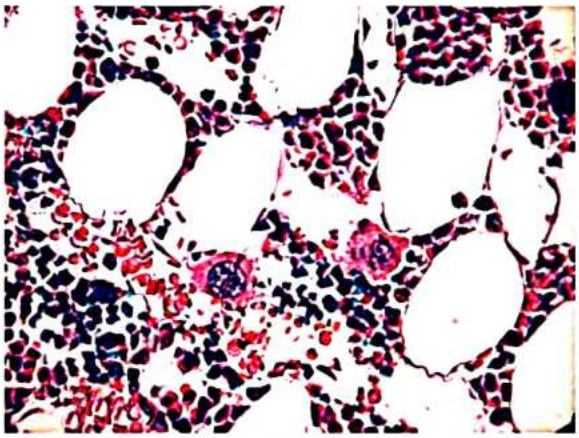
Platelets

- **Normal count** - 1.5 to 4.5 lakhs / mm³.
- **Normal life span** - 7 to 10 days.
- **Normal size** - 3 to 4 microns.
- **Precursor** - Megakaryocyte present in bone marrow.



Megakaryocyte bone marrow aspiration

- Megakaryocyte of bone marrow is multinucleated.
- Nucleus divides by endomitosis.



bone marrow biopsy- Megakaryocyte

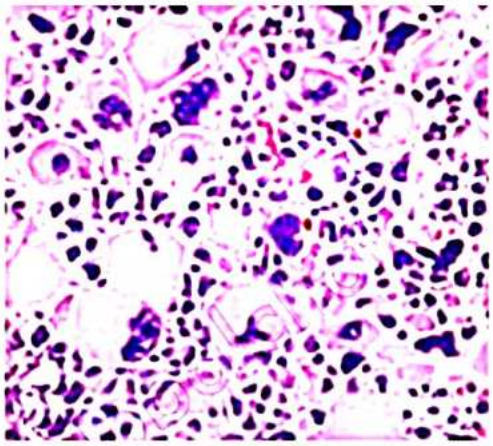
- Megakaryocytes are present near the sinusoids - perisinusoidal location.

Important Information

- Topography of megakaryocytes on biopsy - perisinusoidal.

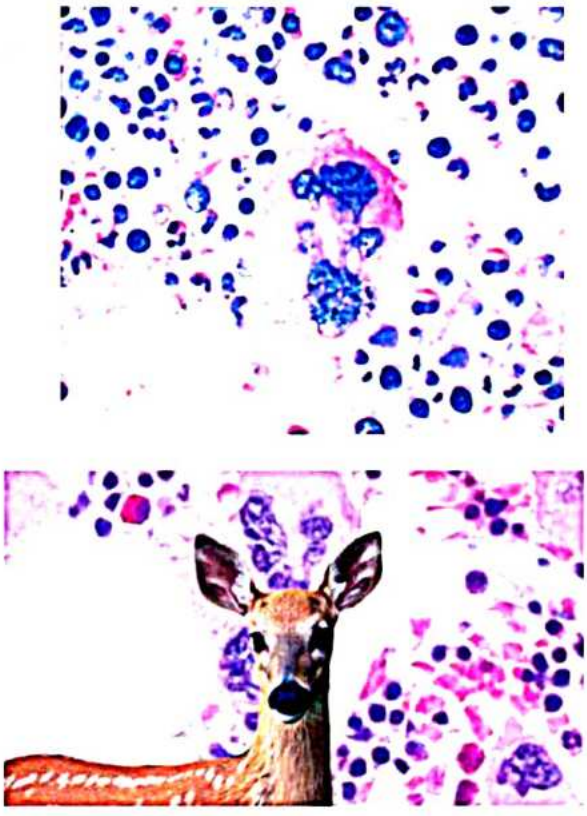
Defects in Megakaryocytes

- **Dwarf Megakaryocyte**
  - Seen in CML.



dwarf megakaryocyte

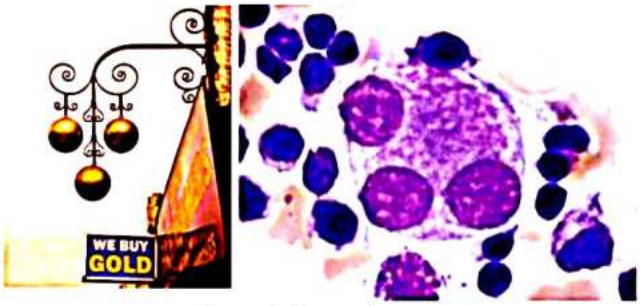
- **Staghorn megakaryocyte**
  - Seen in ET - Essential Thrombocythemia.



Staghorn megakaryocyte

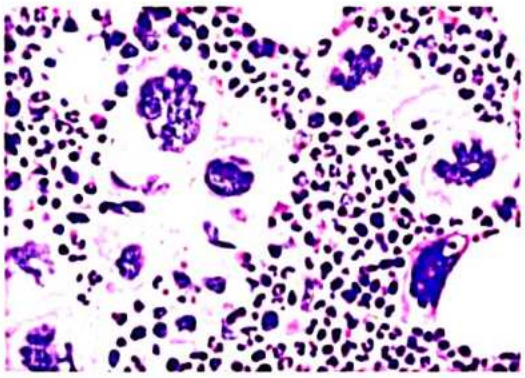
Telegram - @nextpreladdernotes

- **Pawn ball megakaryocyte**
  - Seen in myelodysplastic syndrome (MDS).



Pawn ball megakaryocyte

- **Cloud like megakaryocyte**
  - Seen in myelofibrosis.


Cloud like megakaryocyte

Clot Formation 00:06:34

- Both platelets and clotting factors combined form a clot.
- **3 steps:**
  - Platelet adhesion
  - Platelet secretion
  - Platelet aggregation

Platelet Adhesion

- First step in clot formation.
- **Includes:**
  - Gp1b/9
  - Von Willebrand factor

Platelet Secretion

- **Platelets has:**
  - **Alpha granules:** Forms
    - → P - selectin
    - → PF4 - platelet factor 4
    - → PDGF - platelet derived growth factor
    - → Factor 5, 8
    - → Fibrinogen
    - → Mnemonic: all P things and all Factors.
  - **Delta granules (dense granules):** Gives
    - → Adenosine diphosphate
    - → Adenosine triphosphate
    - → Serotonin
    - → Serum calcium
    - → Epinephrine

Platelet Aggregation

- Aggregation between Gp2b/3a and fibrinogen.

The Platelets contains Gp2b/3a on their surface
↓
Two platelets with Gp2b/3a are attached with each other by Fibrinogen
↓
All Platelets gets aggregated

Activation of clotting factors

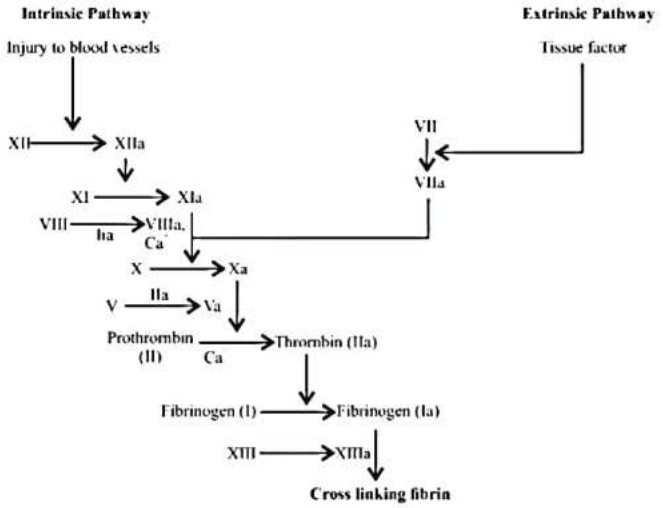
- All the three steps form the primary hemostatic plug.
- So the clotting pathway should be activated.
  - This occurs by flipping of phosphatidylserine.
  - Gives negative charge to the platelets inside the body.
  - This negative charge activates the clotting pathway.

Scott syndrome:
Defect in flipping of phosphatidylserine.

- This may lead to defects in apoptosis and clotting.

Clotting Pathway 00:19:46

- **Factor 13** - Fibrinogen stabilizing factor.
  - Also called the Laki Lorand factor.
  - It stabilizes Strengthens the blood clot.



- Vitamin K dependent factors - 2,7,9,10.

Telegram - @nextpreladdernotes

Tests

- **Bleeding Time (BT):** Measure of the Platelets.
  - Normal - 2 to 9 minutes.
  - Increased BT - defects in Platelets.
- **Clotting Time (CT):** Time required for clot formation.
  - Normal - 8 to 15 minutes.
- **Prothrombin Time (PT):** Measures extrinsic pathway and common pathway.
  - Normal PT - 11 to 16 seconds
  - INR is calculated here.
  - INR = PT of patient / PT of normal person.
  - Hence the test is also called PT/INR.

Blood sample is collected
↓
Thromboplastin is added

- Thromboplastin is derived from the brains of humans or rats.
- **Activated Partial Thromboplastin Time (APTT):** Measures the intrinsic pathway and common pathway.
  - Factors measured - 10,11,9,8,10,5,2,1.
  - Normal APTT - 25 to 30 seconds.

Blood is collected
↓
Silica or Glass beads or kaolin are added to create negative charge

- Mostly kaolin is used these days.
- **Thrombin Time (TT):** Measure of fibrinogen.
  - Elevated TT - afibrinogenemia or hypofibrinogenemia.

Coagulation Tests

- PT, APTT and TT are included under coagulation studies.
- Blood is collected in a light blue vacutainer.
  - Made up of plastic and not glass because,
    - → Glass has a negative charge.
    - → It clots the blood soon.
  - The container contains 3.2% trisodium citrate.
- Tests should be done at room temperature.
- Test should be done within 2 hours of blood collection.

Factor 13 Deficiency

- Patient presents with an umbilical cord stump bleeding.
- When tested all the BT, CT, PT, APTT and TT will be normal.
- Because these tests don't measure factor 13.
- Therefore, the test for factor 13 is urea clot solubility test.
- **Urea clot solubility test:** Measures factor 13.
  - **Weak clot** - solubilizes fast.
  - **Strong clot** - doesn't solubilize.
  - Urea is added to the clot.
    - → **If factor 13 normal** - no clot solubility.
    - → **If factor 13 deficiency** - clot is soluble.
      - – Presents as umbilical cord stump bleeding.

Special Tests

- Mixing studies
- Platelet aggregometry
- TEG

Bleeding Disorders

00:43:57

These are due to:

- **Vascular defects:** Includes
  - Scurvy
  - HSP - Henoch schonlien purpura.
  - Amyloidosis
  - Osler-weber-rendous syndrome also called hereditary hemorrhagic telangiectasia
    - → Runs in family
    - → Internal bleeding occurs
    - → Vessels are dilated
    - → TGF beta mutation occurs.
  - Vascular defects - diagnosis of exclusion.

- **Platelet defects:** Could be
  - Qualitative defect
  - Quantitative defect
- Coagulation factors defects

Platelet Defects	Coagulation Defects
• Superficial bleeds like o Petechiae o Purpura o Mucosal bleeds	• Deep tissue bleeds like o Intracranial bleeds - most dangerous o Joint bleeds - most common.

Platelet Defects

00:55:14

I) Quantitative Defect

Idiopathic / immune Thrombocytopenia purpura (ITP)

- Type of quantitative platelet defect.
- Platelet count decreases.

Platelets has Gp1b/9 and Gp2b/3a on its surface
↓
IgG binds to either of them
↓
Forms opsonin
↓
Taken up by the spleen and destroyed

- **Blood test shows**
  - Less Platelets
  - High bleeding time

- **Platelet smear shows**
  - Giant platelets-because megakaryocyte hyperplasia takes place in bone marrow.

- **Only** parameter elevated - Bleeding Time.

Telegram - @nextpreladdernotes

Features	Acute ITP	Chronic ITP
Age	Child	Adult
History of viral infection	Present	Absent
Platelet count	< 50,000	Very low platelet count
Resolution	Normal within 6 months	> 6 months
Treatment	Supportive treatment	Steroids (ist line) If not responding: spleenectomy can be done

ii) Qualitative Defect

Defect	Disease
Gp1b/9	Bernard soulier syndrome (shows big sized platelets)
Von willebrand factor	Von willebrand disease
Alpha granules	Gray platelet syndrome (aged platelets)
Delta granules	Hermansky pudlak syndrome
Gp2b/3a	Glanzmann thrombasthenia
Fibrinogen	Afibrinogenemia

Important Information

- Also observed in occulo cutaneous albinism
  - Lack of pigments in:
    - → Eyes
    - → Skin
  - Delta granules defect of platelets.
- Chediak higashi syndrome also shows occulo cutaneous albinism
  - Lysosomal gene defect

iii) Heparin Induced Thrombocytopenia

Heparin

- Thrombin action is inhibited by antithrombin 3.
- Heparin increases the action of antithrombin 3.
- Therefore, it prevents the blood clot.
- In HIT - Heparin binds to PF4 (formed by alpha granules).
- Forms a complex-leading to antibody formation against Heparin-PF4 complex.
- IgG antibodies bind to the complex - it is an opsonin.
- Entire platelet is destroyed by the spleen.
- Results in Thrombocytopenia.
- Sometimes also called HITT - Heparin Induced thrombotic Thrombocytopenia.
  - Heparin also activates the platelets.
  - Results in thrombus formation.
    - → More fibrinogen is converted to fibrin.
    - → Leads to decrease in fibrinogen.
    - → Hypofibrinogenemia

Important Information

- Less fibrinogen causes:
  - Increase in thrombin Time.
- Thrombin Time increases in both:
  - HITT
  - Afibrinogenemia
- Both these diseases are differentiated by the reptilase test.
  - HITT - reptilase is normal.
  - Afibrinogenemia - reptilase is elevated.

Clotting Factors Defects

Hemophilia

- **Hemophilia A:** Factor 8 deficiency
- **Hemophilia B:** Factor 9 deficiency (Christmas factor).
  - Also called Christmas disease.
- **Hemophilia C:** Factor 11 deficiency
- **Parahemophilia:** Factor 5 deficiency
- **Pseudohemophilia:** Seen with Von Willebrand disease.
  - It is a fake hemophilia.

Important Information

- Hemophilia A and B belong to - X linked recessive group.
- More in males than females.

Hemophilia A	Hemophilia B
• Factor 8 deficiency. • Intrinsic pathway defect. • APTT is elevated. • BT, PT, TT are normal. • Factor 8 assay is done to detect factor 8 deficiency.	• Factor 9 deficiency. • Intrinsic pathway defect. • APTT is elevated. • BT, PT, TT are normal. • Factor 9 assay is done to detect factor 9 deficiency.

- Usually a case will be presented with a male with joint pain and swelling - hemarthrosis.

Treatment	
Hemophilia A	**Hemophilia B**
• It is a factor 8 deficiency • Cryoprecipitate is given. ○ Rich in factor 8 and von willebrand factor. • Recombinant factor 8 - Humate.	• It is factor 9 deficiency • Fresh frozen plasma (FFP) is given.

- Hemophilia C is autosomal recessive (AR).

Telegram - @nextpreladdernotes

Von Willebrand Disease

- Involves both platelet and coagulation factor defects.

Von Willebrand Factor

- **Source**
  - Megakaryocytes
  - Wibel - palate bodies of epithelial cells
  - Liver
- Usually monomers gradually become multimers.
  - These multimers are broken by Adam TS.

- **Function**
  - Carrier of factor 8.
  - Half life of factor 8 - 2.4 hours.
  - Half life of VWF - factor 8 - 24 hours.
- VWF deficiency indirectly causes factor 8 deficiency.
  - Looks like hemophilia A.
  - But it's not true hemophilia.
  - Therefore, it is called pseudo hemophilia.
  - The factor 8 alone will have a half life of 2.4 hours.
  - This is why all tests should be done within 2 hours.

- **Other function** - binds to Gp1b/9
- Helps in platelet Adhesion.

- **Von willebrand disease causes**
  - **Platelet adhesion defect** - BT is elevated.
  - **Factor 8 defect** - APTT is elevated.
    → Intrinsic pathway defect.
  - **Classical features of VWD** - BT high, PT normal, APTT high.
- Clinically has more superficial bleeds than deep tissue bleeds.
- **3 types**
  - Type 1
  - Type 2
  - Type 3

VWD type 1	VWD type 2	VWD type 3
• Most common. • Mild deficiency in VWF. • Quantitative defect. • Autosomal dominant.	• Autosomal dominant. • Qualitative defect. **Types** • **2a** - decrease in binding between VWF and Gp1b/9. • **2b** - increase in binding between VWF and Gp1b/9. • 2M • 2N	• Most severe. • Zero VWF. • Autosomal recessive. • Quantitative defect.

- **Treatment** - Cryoprecipitate is given.

Important Information

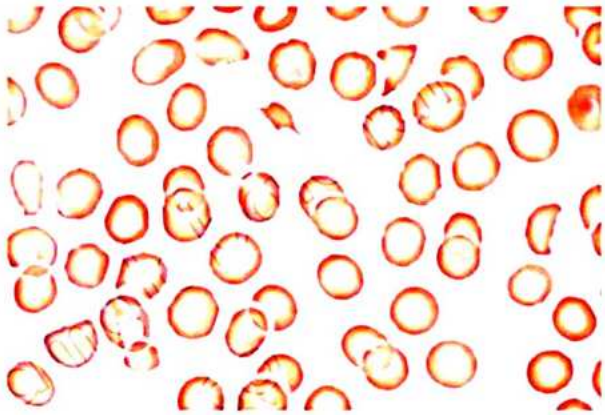
- Cryoprecipitate is given in both VWD and hemophilia.

Thrombotic Microangiopathy

2 types,

Macroangiopathic Hemolytic Anemia

- Large blood vessels are involved.
- In prosthetic valve patients the RBCs collide to the valve and may break.
- Called fragmentation of RBCs.
  - Schistocytes.



Schistocytes

Microangiopathic Hemolytic Anemia

- Small blood vessels are involved.
- **Includes**
  - HUS - Hemolytic Uremic Syndrome.
  - TTP
  - DIS
- Schistocytes are seen.

A. Hemolytic Uremic Syndrome (HUS)

2 types:

- **Typical** - Associated with E-coli or shigella.
  - Strain of E-coli - O157:H7.
  - Starts with diarrhea or dysentery.
- **Atypical** - Factor H/I/CD46 deficiency.
  - These are regulators of the complement system.
  - No control over the complement system in this type of disease.
- Overall, both lead to endothelial injury - blood clot.
- Forms thrombus.
- **Features**
  - Hemolytic anemia - forms Schistocytes.
  - Thrombocytopenia - platelets are Decreased.
    → BT is elevated.
  - Renal involvement is present.
  - Mnemonic: RAT.

Telegram - @nextpreladdernotes

B. Thrombotic Thrombocytopenic Purpura (TTP)

- Clots are formed.
- Platelets are Decreased - BT increases.
- **Defect** - Adam TS 13 (breaks multimers of VWF).
- Long multiple multimers of VWF are produced.
- **Patient presents with**
  - Hemolytic anemia - forms Schistocytes.
  - Thrombocytopenia - platelets are Decreased.
  - Renal involvement is present.
  - Fever
  - Neurological involvement.

C. Disseminated Intravascular Coagulation (DIS)

- Also called consumptive coagulopathy.
- **Risk factors**
  - Pregnancy
  - Meningococcemia
  - AMLM3 (only leukemia associated with DIS).
- There are lots of clots present in the vessels throughout the body.
- All the clotting factors and platelets get consumed.
  - Decrease in clotting factors and platelets.
  - BT, PT and APTT are elevated.
- When too much clot is formed the body breaks the clot.
  - Fibrin (clot) - Broken into fibrin degraded products (FDP).
  - Cross linked fibrin (stable clot) - Broken into D - Dimers.
- **Tests**
  - FDP - elevated levels.
  - D - dimer - elevated levels.
    → Based on a latex agglutination test.
- Underlying cause should be treated.

Disease	Platelet Count	BT	PT	APTT
Bernard soulier syndrome - qualitative	Normal	Elevated	Normal	Normal
Glanzmann thrombasthenia - qualitative	Normal	Elevated	Normal	Normal
ITP	Low	Elevated	Normal	Normal
Hemophilia A - factor 8 defect	Normal	Normal	Normal	Elevated
Hemophilia B - Factor 9 defect	Normal	Normal	Normal	Elevated
HUS / TTP	Low	Elevated	Normal	Normal
DIC	Low	Elevated	Elevated	Elevated
VWD - platelet and factor 8 defect	Normal	Elevated	Normal	Elevated

- **Defect in Platelets** - problem in bleeding time.
- **Defect with clotting factors** - problem in APTT and PT.

Telegram - @nextpreladdernotes

62 PLATELETS PART-II (MIXING STUDIES)

Special Tests

- Mixing studies
- Platelet aggregometry
- TEG

Mixing Studies 00:02:28

- Particularly done in conditions with high APTT.
  - BT, PT - normal
  - This is due to 2 reasons:
    - → **Factor deficiency:** Ex: Factor 8 deficiency (hemophilia).
    - → **More antibodies:** Anti phospholipid antibody syndrome (APLA).

Antibodies in APLA

- Anti beta2 glycoprotein antibody.
- Anticardiolipin antibody.
- Lupus anticoagulant (LAC).
- Mixing studies helps in identifying whether the APTT is raised due to Factor 8 deficiency or high antibody count.

Procedure of Mixing Studies - To identify Factor Deficiency

Patient sample is taken - assuming having Factor 8 deficiency
↓
Mixed with normal person's fresh frozen plasma - contains all factors
↓
Patient sample has high APTT
↓
After Mixing both samples - APTT is measured
↓
If APTT is normal - it is factor deficiency

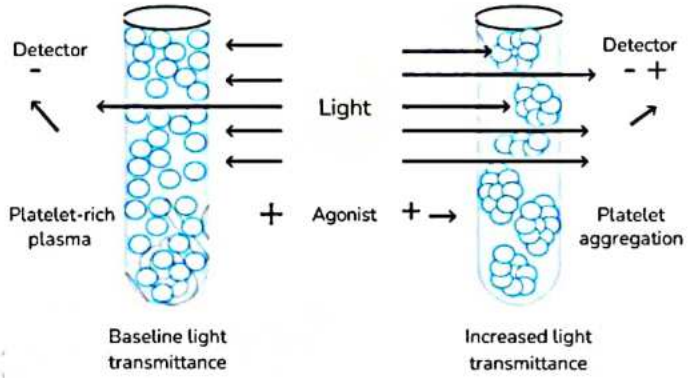
Procedure of Mixing Studies - To identify Presence if antibodies

Patient sample is taken - assuming having APLA
↓
Mixed with normal person's fresh frozen plasma - contains all factors
↓
Patient sample has high APTT
↓
After Mixing both samples - APTT is measured
↓
The antibodies in the patient sample doesn't let the factors work in both the samples
↓
This shows high APTT even after Mixing studies

- **After Mixing studies**
  - **Normal APTT** - Factor deficiency.
  - **High APTT** - More antibodies present.

Platelet Aggregometry 00:09:23

Principle

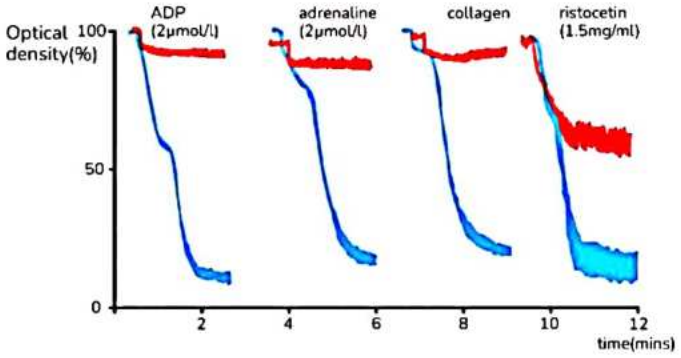


- Works on principle of increased light transmittance.

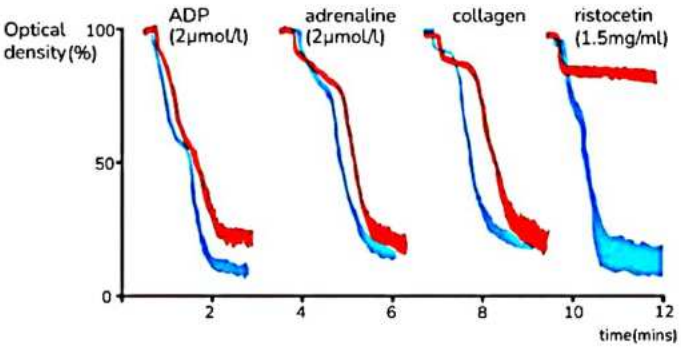
Platelet rich plasma is taken
↓
Light is passed through it
↓
As the cells are dispersed through the plasma - light doesn't transmit
↓
To the plasma ristocetin, ADP, epinephrine and collagen are added
↓
This forms clots
↓
Light is passed

- **Ristocetin**-increase the binding of gp1b/9 and von willebrand factor.
  - Test for platelet adhesion.
- **ADP, epinephrine and collagen**-increase the binding of gp2b/3a.
  - Test for platelet aggregation.

Telegram - @nextpreladdernotes



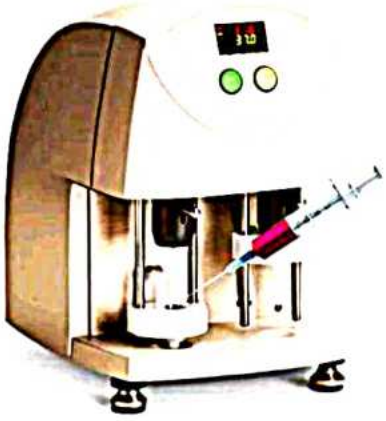
- Platelet aggregation defect.
  - Indicates Glanzmann thrombasthenia.



- **Defect in**
  - **Gp1b/9 -** Bernard-Soulier syndrome (or)
  - **Von willebrand factor -** Von willebrand disease.

Thromboelastography (TEG) 00:17:10

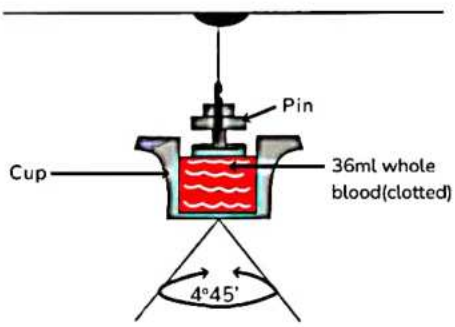
- Used in surgery, emergency departments and intensive care units.
- It's a small machine which can be kept on the bedside.
- Gives the results within 30 to 60 minutes.

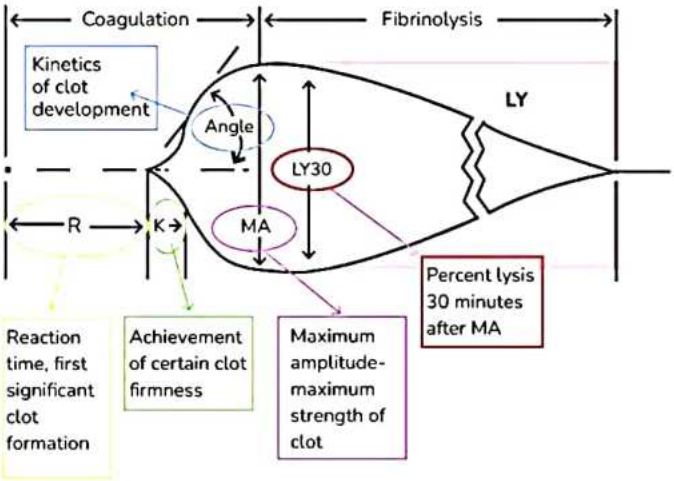


Thromboelastography (TEG) machine

- Measures platelets, clotting factors.

Machine has a cup in which blood is placed
↓
A pin or needle is present on the top
↓
The machine slightly moves the cup
↓
If any clot is forming it surrounds the pin
↓
More clot pulls the pin towards it
↓
Machine senses the changes in pain
↓
Gives the results of platelet, clotting factors

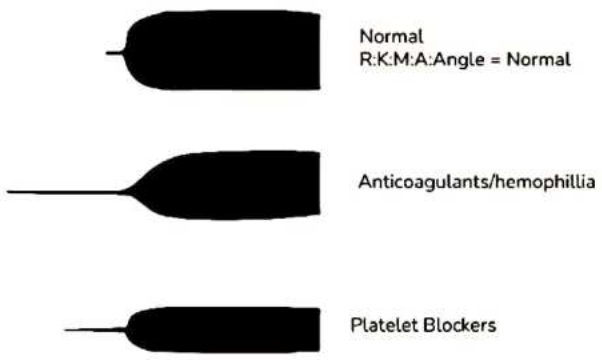




- **It has 5 stages**
  - **R -** Reaction time - 4 to 8 minutes.
  - **K -** K time, clot firmness - 1 to 4 minutes.
  - **Alpha angle -** uphill clot kinetics - 47 to 74 degrees.
  - **MA -** maximum amplitude; clot stability; 55 to 73 mm.
  - **LY30 -** Lysis 30 minutes after MA

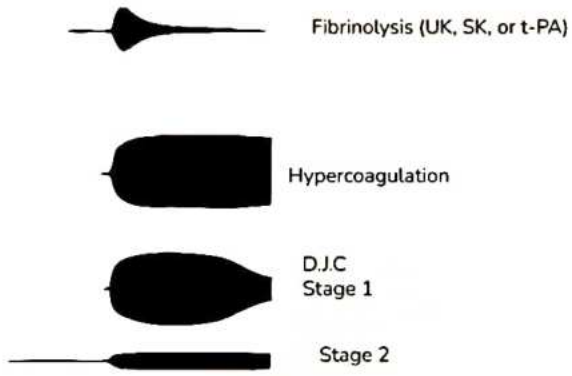
Telegram - @nextprepladdernotes

Reaction Time	
Definition	Time taken to start forming the clot.
Normal Value	5 to 10 minutes or 4 to 8 minutes.
Problem with	High R-time indicates a problem with clotting factors.
Treatment	Fresh frozen plasma.
	K Time - Clot firmness
Definition	Time until the clot reaches a fixed strength.
Normal value	1 to 3 or 4 minutes - very short period.
Problem with	K time affected - Fibrinogen defect.
Treatment	Cryoprecipitate.
	Alpha Angle - Clot Kinetics
Definition	Speed of fibrin accumulation.
Normal value	47 to 74 degrees.
Problem with	Fibrinogen
Treatment	Cryoprecipitate
	Maximum Amplitude
Definition	Highest vertical amplitude of TEG.
Normal value	55 - 75 mm.
Problem with	• 80% - platelets • 20% - clotting factors
Treatment	Platelets
	Lysis at 30 minutes
Definition	Percentage of amplitude reduction 30 minutes after maximum amplitude.
Normal value	0-8%
Problem with	More Lysis of clot - excess fibrinolysis.
Treatment	Tranexamic acid



Graph - TEG

- **The 2nd image** - has a long tail.
  - Elevated Reaction time.
  - Less clotting factors.
  - Due to hemophilia or anticoagulants.
- **3rd image** - maximum amplitude is decreased.
  - Primarily measure of platelets.
  - Due to use of platelet blockers.



Graph - TEG

- **1st image** - LY30 elevated - case of fibrinolysis.
- Case of disseminated Intravascular coagulation.
  - Lots of clots are formed.
  - **Hypercoagulable state** - stage 1.
    → R time and k time is less.
    → Maximum Amplitude is more.
  - **Consumptive coagulopathy** - stage 2.
    → Hypo-coagulable.
    → R time and k time increases.
    → Maximum amplitude decreases.

MCQs 00:37:14

Q. Match the defects with their respective diseases

A. Gp1b/9 defect	1. Hermansky pudlak syndrome
B. Gp2b/3a defect	2. Grey platelet syndrome
C. Alpha granule defect	3. Glanzmann thrombasthenia
D. Delta granule defect	4. Bernard soulier syndrome

Ans.

- Gp1b/9 defect - Bernard-soulier syndrome
- Gp2b/3a defect - Glanzmann thrombasthenia
- Alpha granule defect - Grey platelet syndrome
- Delta granule defect - Hermansky-pudlak syndrome
  - Associated with oculocutaneous albinism.

Telegram - @nextprepladdernotes

Q. Mark the following statements about PT true or false
A. Done within 2 hours
B. Done at room temperature
C. Activation with kaolin
D. Normal value is 12 to 16 seconds

Ans.
- A - true
- B - true
- C - false (activated with thromboplastin but not kaolin)
- D - true

Q. Thromboelastography is a measure of?
A. Clot strengthening
B. Clotting factors
C. Fibrinolysis
D. Interaction between platelets and clotting factors
E. All of the above

Ans. All of the above

Telegram - @nextprepladdernotes

PREVIOUS YEAR QUESTIONS

Q. In a platelet poor plasma sample, calcium and tissue thromboplastin is added. This is used to assess which of the following pathway? (AIIMS 2017)

A. Extrinsic
B. Intrinsic
C. Fibrinolytic
D. Common

Q. Investigation to distinguish between pregnancy acquired hemophilia A and lupus anticoagulant? (JIPMER 2019)

A. Factor 8 assay
B. dRVVT test
C. VWF assay
D. aPTT

Q. True for Von-Willebrand disease? (FMGE - Jun - 2018)

A. Normal PTT
B. Decreased platelets
C. Normal PT
D. Normal BT

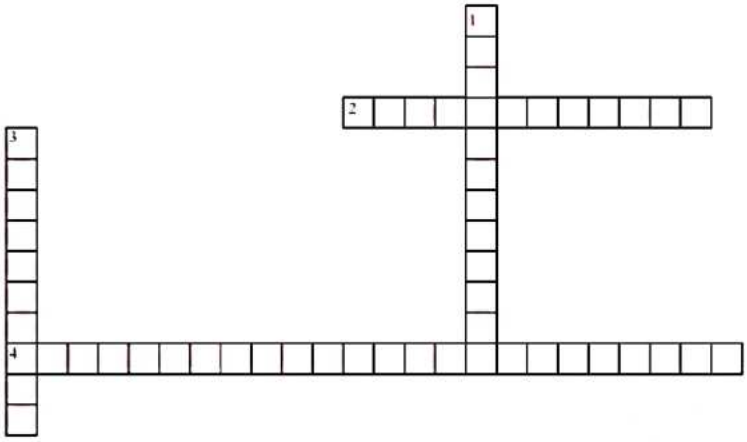
Q. Which among the following laboratory investigation is best to reveal bleeding in disseminated intravascular coagulation? (AIIMS - May - 2018)

A. Increased PT
B. Increased aPTT
C. Decreased fibrinogen
D. Increased FDPs

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. Time taken to form the clot.
4. Used in surgery, emergency departments and intensive care units

Down

1. Time until the clot reaches a fixed strength
3. Increase the binding of gp1b/9 and von willebrand factor

Telegram - @nextpreladdernotes

63 BLOOD BANKING AND TRANSFUSION MEDICINE PART-1

Blood Group Genetics 00:00:37

- The bloo d grouping system that we follow (A, B system) was given by Landsteiner.
- H gene is present in Chromosome 19
- A, B, and O = Chromosome 9
- Rh gene = Chromosome 1
- H gene is going to be the fucosyltransferase.
  - It makes an H substance and is added to A blood group like fucosyltransferase.
  - If it adds the fucosyl molecule to N-acetyl galactosamine, it makes the A blood group.
  - If it adds the fucosyl molecule to Galactose, it makes the B blood group.
  - If it is added to both N-acetyl galactosamine and Galactose, it will be an AB blood group.
  - If it is not added to any of them, it makes the O blood group.
- The maximum conversion is happening in AB. And in the O blood group, no conversion is happening.
- So, maximum H substance = O
- Least H substance = AB

Testing:-

- For testing of H substance, Ulex lectin = anti-H antibodies are required.
- Maximum H substance = O and Least H substance = AB
- O>A2>B>A2B>A1>A1B

Refer Table 63.1

- In each of these groups, H antigen is common.
- If all of them have H antigen, none of them will have antibodies.

Bombay Blood group/Bhende ET AL

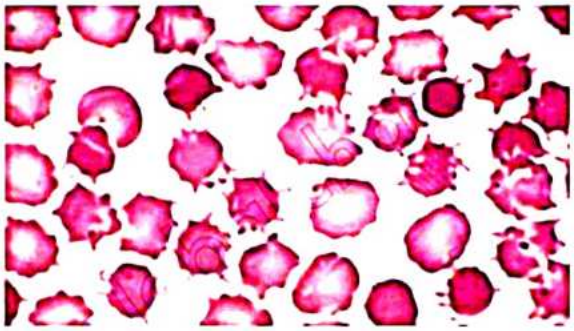
- It was identified by a group of scientists called Bhende ET AL.
- Bombay Blood group is when a person doesn't have A antigen, B antigen, or any other antigen.
  - Bomnay blood group does not have A,B,H antigen. In turn it has anti A, anti B and anti H antibodies
- AB = Universal recipient
- O = Universal Donor (No antigen)
- Safest blood group for transfusion in emergency = O
- Safest plasma for transfusion in emergency= AB

Lewis Blood Group

- Lewis Antigen are there
- Igm antibodies are present.
- RBC does not synthesize Lewis Antigen.
- It is adsorbed from plasma.

Kell blood group

- Kell phenotype deficiency is known as Mcleod phenotype.
- Mcleod phenotype is also referred to as Neuro/acanthosis syndrome
- Acanthocytes are Red blood cells with spikes



Duffy Antigen

- It is under the control of the DARC gene.
- If RBC has Duffy Antigen, it is used for Plasmodium vivax and Plasmodium knowlesi.
- If patients have Duffy antigen negative, Plasmodium vivax, and knowlesi cannot enter.
- This means there will be protection against Plasmodium vivax and knowlesi.

P antigen And I Antigen 00:14:31

- P antigen: Parvovirus and warm AIHA
- I antigen: Cold AIHA

Rh Antigen

- Chromosome 1
- C, D, E Genes. D antigen is very common.
- This is given as positive or negative.

ABO	Features	Rh
9	Gene	1
A, B, AB, O	Expression	+/-
Saliva, sweat, semen	Present in	Not present
IgM	Antibody	IgG

- Rh ag can cross the placenta.

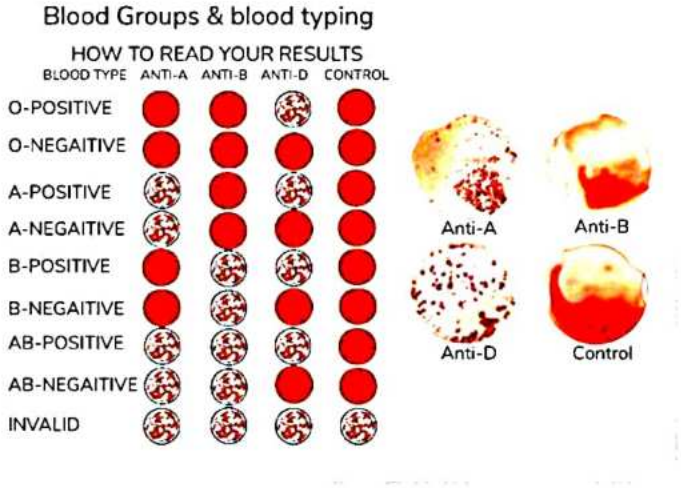
Telegram - @nextpreladdernotes

Blood grouping methods 00:18:54

- Slide method
- Tube method
- Gel card method
- ELISA
- Automated

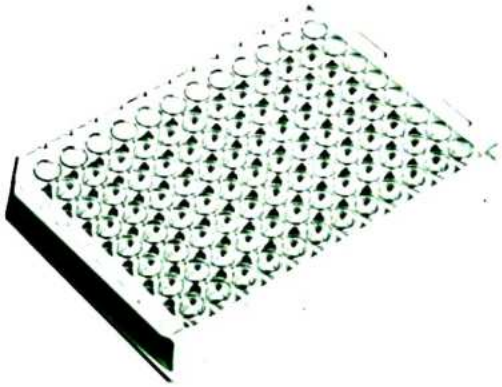
Color of antisera

- Mnemonic - BYG
- Blue - Anti A
- Yellow - Anti B
- Gray - Anti D - Rh antigen +/-
- For example, the patient is A+, it means
  - He has antigen A
  - So when antibody A will be added to it, a reaction will occur.



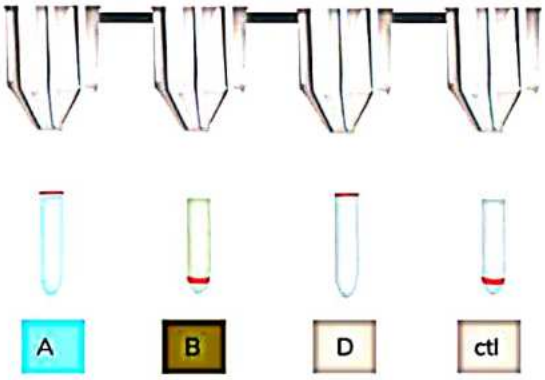
- Control - only the blood sample with normal saline.
- It means control should be clean; if it clots, the result will be invalid. It will not be a reliable sample to evaluate.

ELISA plate 00:25:05



- Total wells are 12x8 = 96 Wells (PYQs)

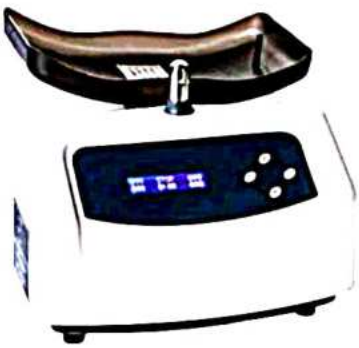
Gel card



- If you get a line on top - Positive blood group
- If you get line Neeche (bottom) - Negative

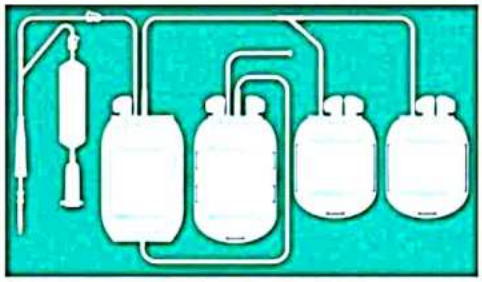
Blood donation

- Age - 18-65 years.
- Blood donation day - 1st October (voluntary blood donation day)
- Weight - minimum weight > 45 kg
  - 45-55 kg = 350 ml blood (49 ml anticoagulant)
  - > 55 kg = 450 ml blood (63 ml anticoagulant)
  - Females can also donate blood but not in the bleeding phase of the menstrual cycle.
  - Machine:
  - Biomixer



→ It displays weight.
→ It has an auto clamp, which means it will automatically close the tube as soon as the set limit is achieved.
→ It is needed for mixing the blood with anticoagulants.

- Hemoglobin > 12.5 gm



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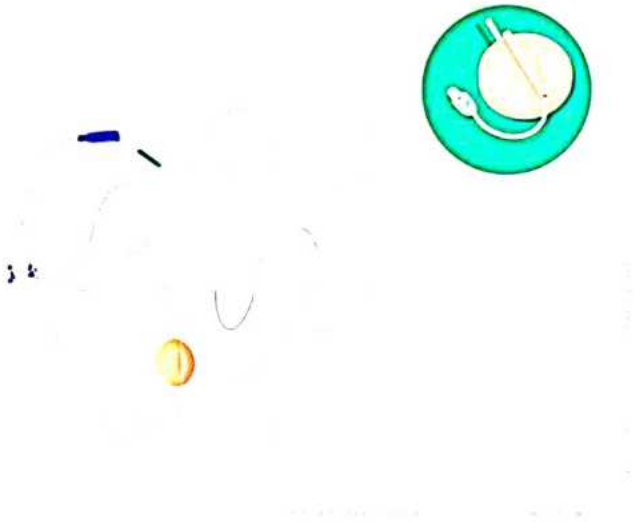
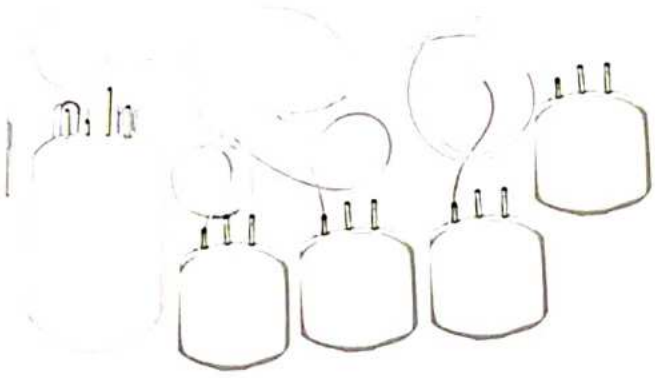
- Needle - 18-19 gauge needle
- With a needle, a micropore filter (170 microns) is attached.
- The first 15-30 ml blood goes in little bag, known as PDB - Pre donation bag. Used because-
  - So, all contaminated blood is collected separately.
- These initially contaminated blood will be used for testing for transfusion transmission infections.
- Now, the blood will go to the Mother bag, which will have Whole blood.
- Next, the blood is split into three components. All these three bags are referred to as component bags.
  - Packed red blood cells
  - Platelet-rich plasma
  - Fresh frozen plasma
- From the mother bag, two tubes go: TAB bag - Top And B ottom bag.
- When all the tubings are on the top - TAT bag - top & top bag.



  - WBC causes two problems.
    - → They release Cytokines (Interleukins), which will lead to fever.
    - → They can pose a risk for CMV transmission.
    - → So, it is required to filter the WBC out.
  - Therefore, the filter is present in a center known as Leucoreduced RBC.



- All these bags are known as Penta bags - pediatric blood transfusion.
- Tips to identify platelets from plasma bags
  - Platelets bag has swirling
    - → When the bag is turned upside down, the platelets start moving in a snaky type of movement.

Preservatives / Anticoagulants 00:47:15

- 350 ml blood - 49 ml of anticoagulants are added, and in 450 ml blood - 63 ml of anticoagulants is added
- Acid citrate dextrose (ACD)
- Citrate phosphate dextrose (CPD)
- Citrate phosphate dextrose Adenine (CPD-A)
- Citrate phosphate Saline adenine glucose mannitol (CP SAGM)
- Citrate is a calcium chelator
  - Remove all calcium and clot formation will not be there
- Phosphate acts as a buffer
- Dextrose or glucose is added for some nutrition to the cell.
- Adenine is added to give some energy to cells (ATP)
- Mannitol is added to prevent lysis of rbc's
- When ACD or CPD is used, blood can be kept for 3 weeks or 21 days.
- CPD-A - 5 weeks or 35 days
  - This is the most commonly used anticoagulant
- CPSAGM - 6 weeks or 42 days
  - Not commonly used because this bag is expensive

- Note -
  - Mnemonic - Number of letters including (-) is the number of weeks the blood will last if an anticoagulant is added

- All these time limit is for only red blood cell component

Platelet agitators

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- If the machine is working, then the shelf life of platelets rich plasma is proper 5 days and if it stops it comes to only 1-day shelf life (AIIMS).
- If the fresh frozen plasma melts or thawing occurs, then the shelf life is one day.

Summary

Component	Temperature	Shelf
Whole blood / PRBC	2-6 degree celsius	21 d (ACD/CPD) 35 d (CPDA) 42 d (SAGM)
Platelets rich plasma	20-24 degree celsius	5 days with agitation
Random Donor platelets (1 RDP = 5000 - 10000/ mm^3 platelets)		
Single donor platelets (collected from a single person) (1 SDP = 30k - 50k/ mm^3 platelets)		
Fresh frozen plasma • Poor in factor 5 and factor 8	Less than - 30 degrees celsius	1-year
Cryoprecipitate • It has factor 8, vWf, factor 1, factor 13		

Washed RBC

- It is given in case of isolated IgA deficiency (PYQ)

Screening for TTI (Transfusion Transmitted Infection)

- For india, we test for five things which include
  - HIV - Type 1 and 2
  - HBV
  - HCV
  - Malaria
  - Syphilis
- These tests are mandatory, and without testing, giving blood is a medical offense.

Transfusion Protocols

01:02:44

- WITHIN 30MINS after removal from the refrigerator
- RBC - should be completed within 4 hours
- If it is platelet-rich plasma (PRP)/Fresh frozen plasma (FFP)- within 20mins, it should be transfused.
  - FFP should be thawed/melted before giving it to the patient.
- Needle-18-19 gauge
- Micropore filter size - 170 MICRONS

Massive Blood Transfusion (MBT)

- Whatever the blood volume of the patient, if that is replaced by more than one time of that volume within 24 hours, it is called a massive blood transfusion.
- Or 50 percent of the total blood volume is replaced in 4 hours
- The ratio of RBC:PRP: FFP = 1:1:1; this is because if too much blood is added, the blood gets diluted too much, called dilutional coagulopathy.

Complications of MBT

- Metabolic alkalosis
  - When a lot of citrates is gone in the blood, there is a lot of bicarbonate production, which will happen as compensation.
    → Because of these bicarbonates, an element of metabolic alkalosis will happen.
- **Citrate toxicity** due to too much citrate anticoagulant
  - The function of citrate is calcium chelation - citrate pulls apart all calcium
  - Hypocalcemia
    → This will cause tingling and numbness in the patient.
- There is so much lysis in the blood, causing hyperkalemia meaning RBC lysis
  - Potassium level increase
  - hypocalcemia
- These patients can get Dilutional coagulopathy because the factors get diluted when so many blood products are given.
  - Due to it, patients can have bleeding, one of the most common causes of death.
- Initial hyperglycemia followed by hypoglycemia
  - SAGM (saline, adenine, glucose, mannitol) is going in the patient, elevating the glucose spike of the patient.
  - As soon as the glucose spikes, the insulin will come up, and the patient's glucose level will decrease.

Transfusion Reactions

- It can either be immediate or delayed.
- Immediate
- occurring within 24 hours
  - allergies/ anaphylaxis
  - Febrile non-hemolytic transfusion reaction (FNHTR)
  - Febrile hemolytic transfusion reaction (FHTR)
    → Hemolytic - some kind of mismatch
    - Most commonly occur due to clerical error.
    - Minor blood group problem

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→ RBC lysis will occur
  - Type II hypersensitivity reaction
- Delayed - after 24 hours
  - Eg. Antigen-antibody mismatch
  - Patient will also come with fever

Febrile non-hemolytic transfusion reaction (FNHTR)
- Patient will have a fever and no hemolysis is happening
- This will occur due to massive **cytokine release**.
  - From WBC
- How to prevent it?
  - Remove white blood cells from RBC
    → Leucoreduced RBC is given to the patient
      - WBC filter is reduced by a filter

Transfusion Related Acute Lung Injury (TRALI)
- It is happening within 6 hours
- It is related to
  - ARD (Acute /Adult Respiratory Distress Syndrome)
  - Hyaline membranes forming in the lungs
- In TRALI, the patient will have non-cardiogenic pulmonary Edema (NCPE)
- This is an **immune mechanism**
  - Anti-HLA antibody in the donor, which will react with the HLA antigen of the recipient.
  - The antibody is present in plasma; therefore, it is the Donor plasma that is creating a problem
    → Maximally associated with fresh frozen plasma

TRALI Vs. TACO
- Transfusion Related Acute Lung Injury (TRALI)
  - Associated with anti-HLA antibody
- Transfusion-associated circulatory overload (TACO)
  - This occurs due to **Preexisting comorbidities**
    → e.g.,
      - congestive heart failure
      - Pregnancy in the 3rd trimester
- Parameters to differentiate between the two
  - Blood pressure (BP)
  - Jugular venous pressure (JVP)
  - B-type natriuretic peptide (BNP)
- In TRALI
  - BP will be low sometimes
  - All these parameters will be **normal** all the time
- In TACO
  - BP, JVP, and BNP are **elevated**
- TACO can come as a delayed disorder

Delayed Transfusion Reactions
Delayed Hemolytic Transfusion Reaction
- Mismatch of minor blood groups like Kell, Duffy, Lewis, etc.

Post Transfusion Purpura
- Anti-platelet antibodies are forming
  - Happens after 7-10 days
- These antibodies are against the GP 3a receptors

GVHD
- Transfusion-associated Graft versus host disease (GvHD)
- Mechanism
  - Donor and recipient, and there is an immunity discrepancy between the two.
    → Donor - immunocompetent
    → Recipient - Immunocompromised
  - Donors will attack the poor recipient
- Organs damaged
  - SIL organs
    → Skin
      - Rash
    → Intestine
      - Diarrhoea
    → Liver
      - Jaundice
- Prevention
  - Irradiated blood is given by a procedure known as γ radiation
    → Immunocompetent T cells are removed

Infections
- There is a risk of infection due to blood contamination
- RBC - 2 -6 degree celsius
- PRP - 20-24 degree celsius
- FFP - < -30 degree celsius
- Max risk of contamination is with PRP because they are at quite a high temperature of 20 - 24 degree Celsius.
- Yersinia enterocolitica infection can be seen
- E.coli infection, and Pseudomonas can also be seen.

What to do when a transfusion reaction occurs?
1. Stop the blood transfusion
2. Three samples are to be collected
   a. Blood
      → One blood sample will come in an EDTA vial
        - Used for BG/CM (blood grouping - cross matching)
      → One blood sample in a plain red vial
        - Biochemical test is done to find out about the hemolysis part of it.
   b. Urine
      → Test for hemoglobinurea/ Hemolysis

Autologous Blood Transfusion
- Recent update in the Robin's 10 the edition

Telegram - @nextprepladdernotes

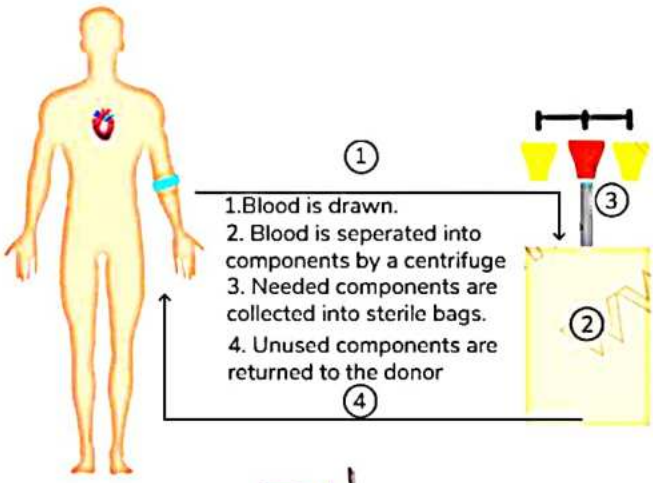
- Autologous - giving blood to myself
  - No risk of HIV, HBS or HCV
  - No risk of allergies or infection
  - No risk of GVHD or PTP

Preoperative autologous blood donation (PAD)
- Most common
- Blood is collected way before elective surgery.

Acute Normovolemic Hemodilution (ANH)
- Anesthesia ke baad (Mnemonic)
  - Given anesthesia, and before surgery, one unit of blood is taken.
  - The blood is replaced with Normovolemic samples like crystalloid or colloid (artificial blood).

Cell salvage
- Blood from the surgical drain is filtered
  - Tissue, debris, and fats are removed to get pure blood
- Very expensive and complex process

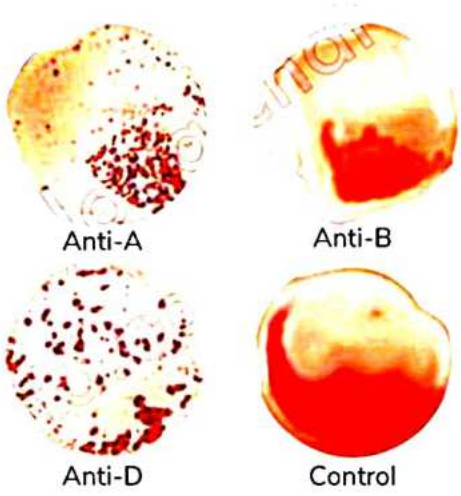
Apheresis Machine 01:39:36



- Platelet/plasma Apheresis Machine
- The blood is separated into
  - RBC
  - Platelets
  - Fresh frozen plasma
- Separated because whatever component is needed is kept,
- And then the other parts of the blood are returned to the donor.
  - Eg. Platelet Apheresis - blood bank has kept the platelets
  - Plasma Apheresis - Plasma was taken to procure antibodies.

MCQ

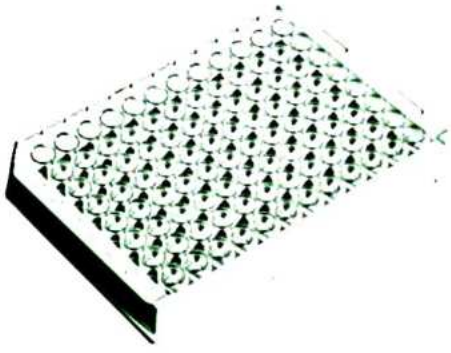
Q. Diagnose the blood group.



a. A positive
b. B positive
c. AB positive
d. O positive

- Control is clean
- Anti-A and Anti-D have Agglutination, and anti-B does not; thus, it is A positive

Q. Name the technique of blood grouping as shown in the image below.

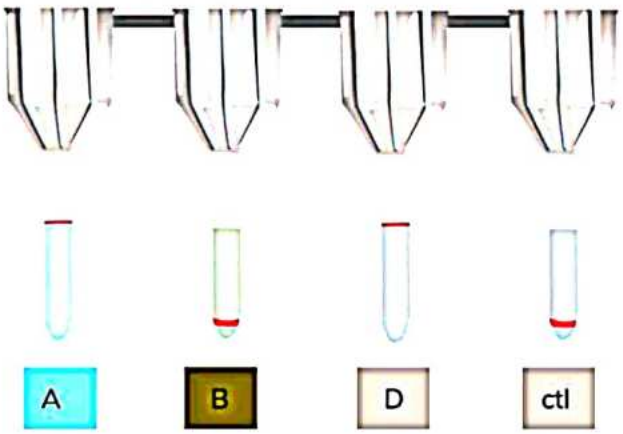


Telegram - @nextprepladdernotes

a. ELISA for blood grouping
b. Gel card for blood grouping
c. Slide test for blood grouping
d. Tube test for blood grouping

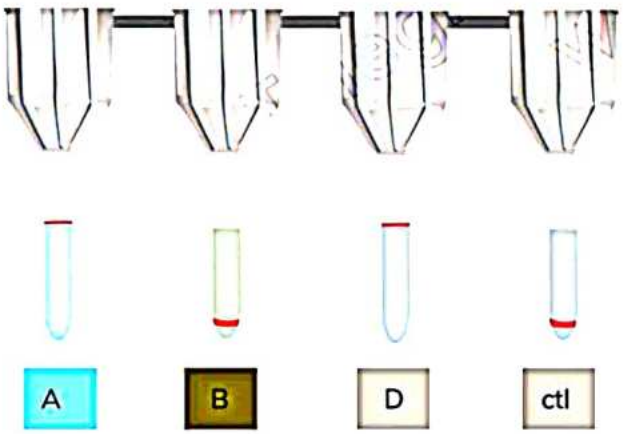
- Total 96 wells - 12x8

Q. Name the technique of blood grouping as shown in the image below.



a. ELISA for blood grouping
b. Gel card for blood grouping
c. Slide test for blood grouping
d. Tube test for blood grouping

Q. Diagnose the blood group.



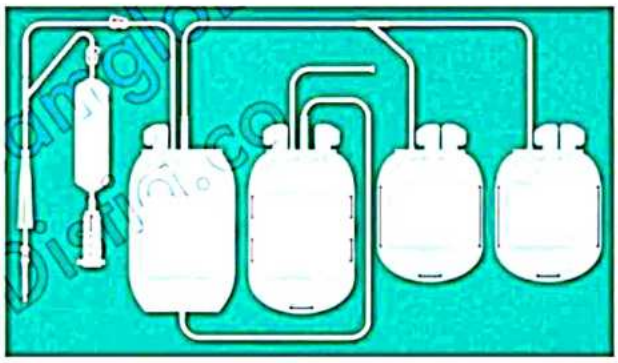
a. A positive
b. B positive
c. AB positive
d. O positive

- Mnemonic - TOP - POsitive, NEeche - Negative.

Q. Match the images with the correct option?

a. Apheresis machine
b. Biomixer
c. Gelcard
d. Crossmatch

Q. Match the images with the correct option.



a. Penta transfusion
b. Leukoreduced RBC
c. Platelet agitator
d. TAB
e. TAT

- Tubing at top and bottom.

Q. Match the images with the correct option?

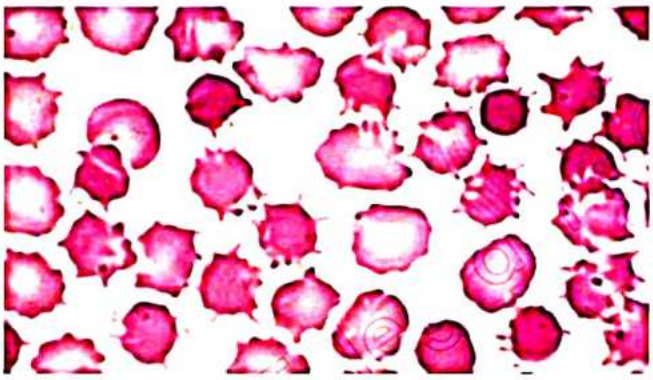
Telegram - @nextprepladdernotes

a. Penta transfusion
b. Leukoreduced RBC
c. Platelet agitator
d. TAB
e. TAT

Q. Which cells agglutinate most strongly with Ulex europaeus lectin?
a. O and A2
b. A1 and A2
c. O and A1B
d. B and A2B

- Max H substance - O > A2 > B > A2B > A1B

Q. The following peripheral smear finding is noted in a patient suffering from involuntary movements, including jerking motions, particularly of the arms and legs. Muscle tensing in the face and throat is also noted. On doing genetic analysis, which of the following blood group system dysfunction best correlates with the condition mentioned above?

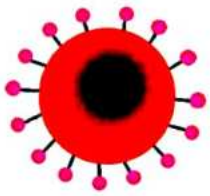
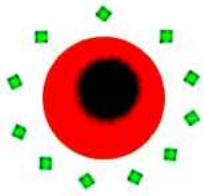
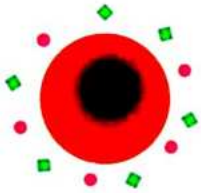
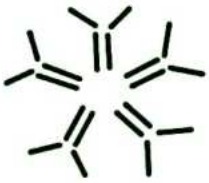
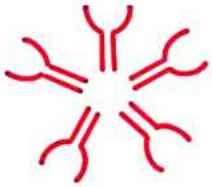


a. Kell
b. Duffy antigen
c. Rh system
d. Lewis system

- Image - Spur/Acanthocytes
- Neuro-acanthosis syndrome - also known as Mcleod phenotype - Kell system of blood group

Telegram - @nextpreladdernotes

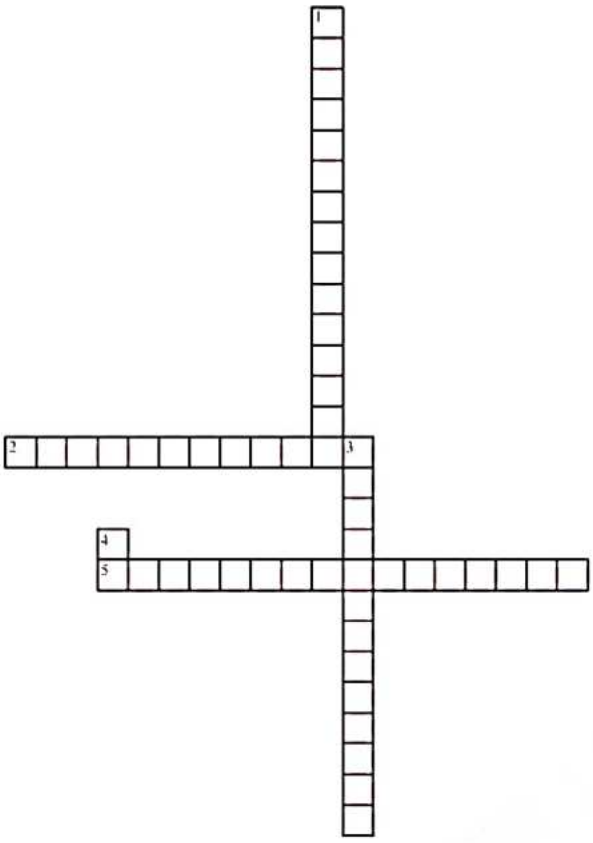
Table 63.1

	Group A	Group B	Group AB	Group O
Red blood cell type	A	B	AB	O
				
Antibodies in plasma	Anti-B	Anti-A	None	Anti-A and Anti-B
				
Antigens in red blood cells	A antigen	B antigen	A and B antigen	None
				

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. It is under the control of the DARC gene

5. It was identified by a group of scientists called Bhende ETAL

Down

1. Kell phenotype deficiency is known as?

3. Mcleod phenotype is also referred to as

4 Universal recipient

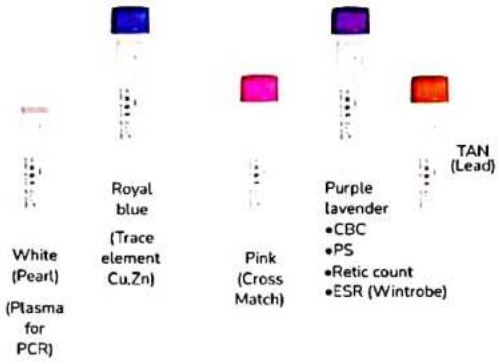
Telegram - @nextprepladdernotes

64 BLOOD BANKING & TRANSFUSION MEDICINE PART-2

Anticoagulant Added Vacuatiners 00:00:20

1. EDTA Vacutainers

- Contain powder form of EDTA (not the liquid form)
- Di potassium EDTA (K2 EDTA) is the best of EDTA
- The colors include



  - White
  - Royal blue
  - Pink
  - Purple/Lavender
  - Tan
- Mnemonic: WHITE princess belonging to a ROYAL family needs a sunTAN and her favorite colors are PINK and PURPLE.
- The tests in Purple include routine tests
  - CBC
  - Peripheral smear
  - Reticulocyte count
  - ESR(Wintrobe)
  - HbA1c
- The tests in Pink include
  - Crossmatching for blood bank
- The tests in Royal blue include
  - Tests for trace elements (Cu,Zn,etc.)
- The tests in White include
  - Plasma for polymerization chain reaction (PCR)
- The tests in Tan include
  - For Lead in cases of lead poisoning
- After collection of sample, it should be mixed for 8 times(EDTA-E for EIGHT)

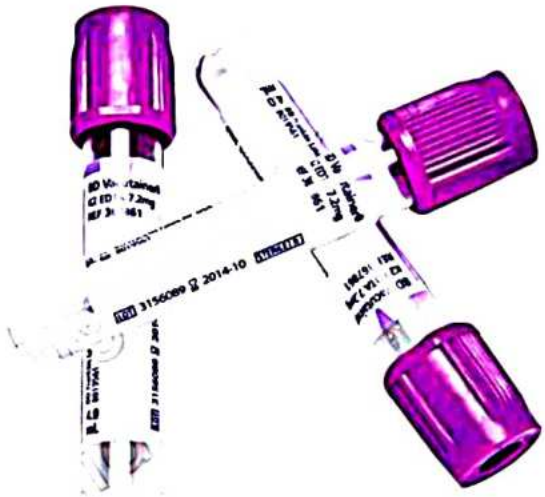
Q. Which of the following have EDTA as anticoagulant 00:04:46

1. Lavender top vial
2. Pink top vial
3. White top vial
4. Tan top vial

a. 1,2
b. 1,2,3
c. 1,2,4
d. 1,2,3,4

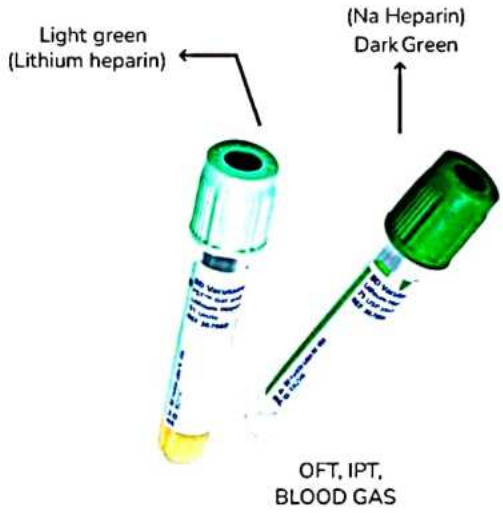
Q. Most preferred anticoagulant in Purple vacutainer is

a. K2 EDTA
b. K3 EDTA
c. No2 EDTA
d. Any of the above



2. Heparin Vacutainers 00:05:38

- These are green containers



  - Light green
    → Contains Lithium heparin (Light for Lithium)
  - Dark green
    → Contains Sodium heparin

Telegram - @nextprepladdernotes

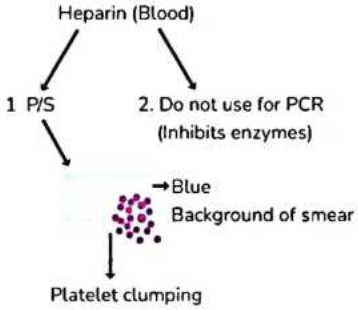
- These are used for
  - Osmotic fragility testing
  - Immunophenotyping(CD markers)
  - Blood gas(ABG)

Q. Most preferred anticoagulant in green vacutainer is? 00:07:13

a. K2 EDTA
b. K3 EDTA
c. Heparin
d. Sodium fluoride

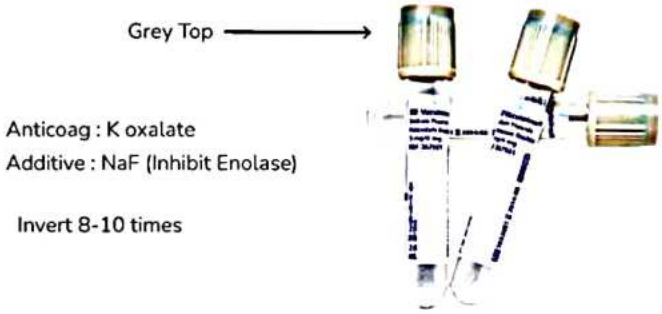
Points to Remember

- Heparin vacutainers are not used for peripheral smear as it makes the background of the smear dark blue



- Platelet clumping takes place and yields inappropriate results
- Heparin inhibits enzymes (Shouldn't be used for PCR, only White EDTA vial is used)

3. Grey Top Vacutiners 00:08:43

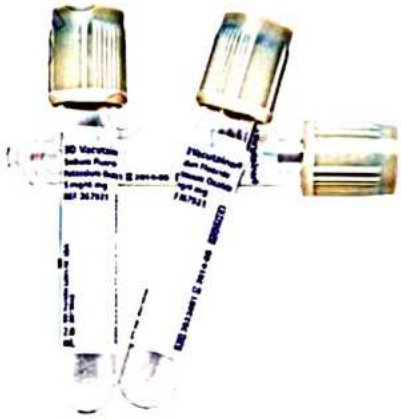


- Gray top for Glucose estimators
  - FBS
  - PPBS
  - RBS
  - GTT

Note: For HbA1c, as it estimates glycated hemoglobin rather than glucose alone, Purple/lavender vial is used.

- Anticoagulant used is Potassium Oxalate
- Additive is Sodium fluoride (NaF)
  - Added to inhibit Enolase which ultimately inhibits glycolysis.
- After sample collection, it should be inverted for 8-10 times.

Q. Most preferred anticoagulant in gray container is
a. K2 EDTA
b. NaF
c. Potassium Oxalate
d. Heparin



4. Light Blue Top Vacutainer 00:12:27

- Anticoagulant used is Trisodium Citrate
- 3.2% Trisodium Citrate is present
- Inverted for 3-4 times
- Anticoagulant: Blood ratio
  - 1:9
    → Coagulation studies(PT, aPTT)
  - 1:4
    → ESR (Westergren)

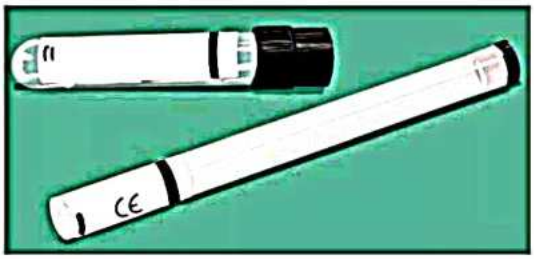
Telegram - @nextpreladdernotes

To Remember

- ESR(Wintrobe): Purple Vial (EDTA)
- ESR(Westergren): Light blue Vial (Trisodium citrate)
- Automated ESR: Black vial (Trisodium citrate)
- For platelet function test, Citrate, Theophylline, Adenosine and Dipyridamole are additionally added to LIght blue vial

5. Black Top Vacutainer

00:15:12

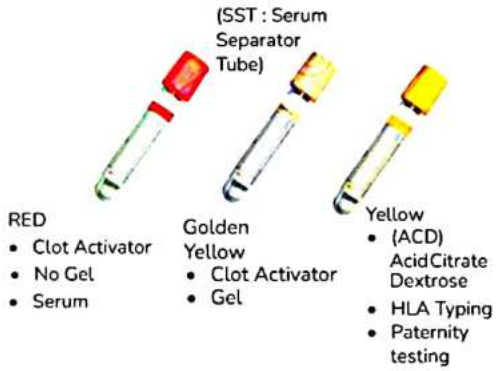


- Anticoagulant is Trisodium Citrate
- Used for Automated ESR test

Coagulating Vacutainers

00:17:41

- Used to separate serum (Serum separator tubes)
- Preferred for hormone profile tests like
  - Serum T3,T4,TSH
  - HCG
  - Prolactin,LH,FSH,Testosterone
  - KFT
  - LFT
- Inverted for 5-6 times
- Coagulating vacutainers are:



1. Red Vacutainer

00:19:36

- Clotting time is 60 mins
- Clot activator is added
- Gel is not added

2. Golden Yellow Vacutainer

- Clotting time is 30 mins
- Clot activator is added
- Gel is added that makes clot faster

3. Bright Yellow Vacutainer

- Contains Acid citrate dextrose(ACD)
- Used for HLA typing and Paternity testing

4. Orange Vacutainer

- Contains Thrombin based activator gel
- Clotting time is 5 mins only
- Inverted for 5-6 times

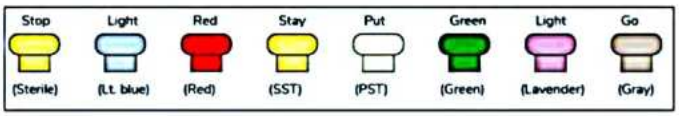
Q. Which of the following Vacutainers reduces the clotting time to 5 minutes

00:22:02

a. Light blue
b. Green
c. Orange
d. White

Order of Blood Collection

00:22:16



- Mnemonic: Stop Light is Red Stay Green Light says Go
- Samples are put into the tubes in the following order if all tubes are involved
  - **Sterile** - for cultures (Light Yellow)
  - **Light blue** - for coagulation studies
  - **Red** - for serum profiles
  - **Serum separating tubes (Yellow)** - for serum profiles
  - **Green** - for ABG
  - **Lavender** - Routine tests (CBC)
  - **Gray** - for Glucose

Telegram - @nextprepladdernotes

PREVIOUS YEAR QUESTIONS

Q. In a platelet poor plasma sample, calcium and tissue thromboplastin is added. This is used to assess which of the following pathway? (AIIMS 2017)

A. Extrinsic
B. Intrinsic
C. Fibrinolytic
D. Common

Q. Investigation to distinguish between pregnancy acquired hemophilia A and lupus anticoagulant? (JIPMER 2019)

A. Factor 8 assay
B. dRVVT test
C. VWF assay
D. aPTT

Q. True for Von-Willebrand disease? (FMGE - Jun - 2018)

A. Normal PTT
B. Decreased platelets
C. Normal PT
D. Normal BT

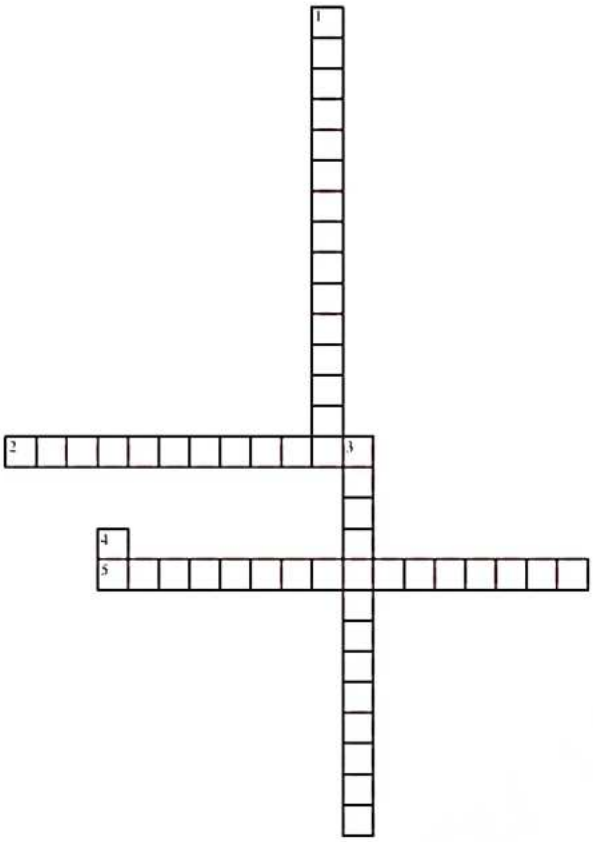
Q. Which among the following laboratory investigation is best to reveal bleeding in disseminated intravascular coagulation? (AIIMS - May - 2018)

A. Increased PT
B. Increased aPTT
C. Decreased fibrinogen
D. Increased FDPs

Telegram - @nextpreladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. It is under the control of the DARC gene

5. It was identified by a group of scientists called Bhende ET AL

Down

1. Kell phenotype deficiency is known as?

3. Mcleod phenotype is also referred to as

4 Universal recipient

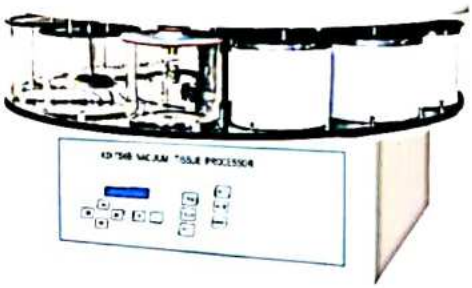
Telegram - @nextprepladdernotes

65 TISSUE PROCESSING

Steps Involved in Tissue Processing

00:00:02

Fixation



- **Fixatives**
  - 10% NBF (Neutral Buffered Formalin), for Light microscope
  - 2-2.5% Glutaraldehyde, for Electron microscope
  - Carnoy's fixatives, for Karyotyping
    → Methanol:GAA :: 3:1
  - Bouin's fixative, for testicular biopsies

Important Information

- Bouin's fixative is used for testicular biopsy, with suspicion of infertility.
- It contain picric acid.
- Picric acid is most preferred for male reproductive cells.
- All the cancerous biopsies are done in formalin.

  - Zenker's fixative, for bone marrow biopsy
  - B5 fixative, for bone marrow biopsy

Note: For Gout, alcohol or normal saline is used to wash out uric acid crystals.

Dehydration

- Remove water by using alcohol or acetone.

Clearing

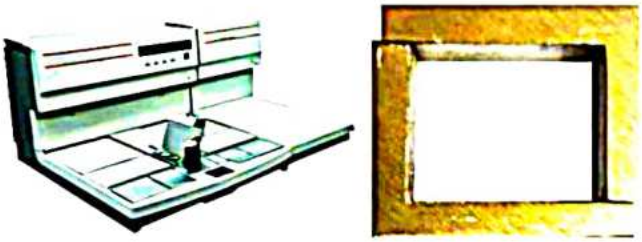
- Clean the tissues, using xylene.
- Histopathology processor is of 2 types (Closed and Open).
- All the steps (Fixation-Embedding) are done inside the machine.

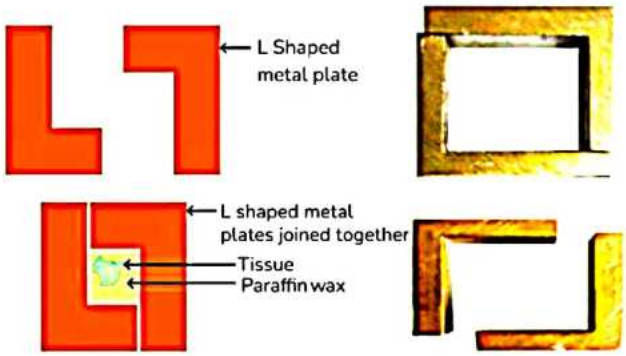
Impregnation

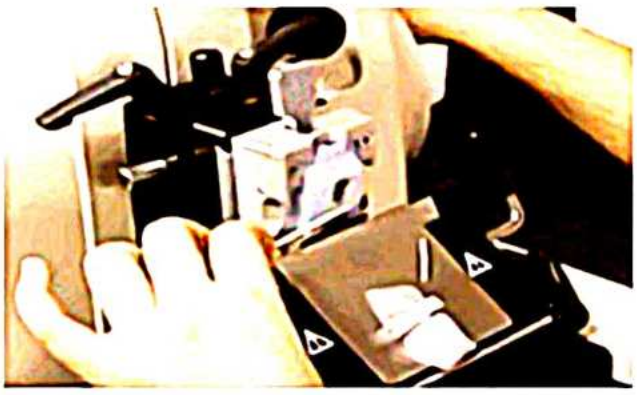
- Paraffin wax is applied.

Telegram - @nextprepladdernotes

Embedding and Blocking







- 2 L-shaped molds are fitted together
- Leukhart's mold is known as the embedding mold.
- Tissue for biopsy is placed in the mold
- Wax is filled all over the mold
- This creates a paraffin block of tissue for biopsy.

Section cutting

- The machine is rotated and the paraffin block is cut layer wise.

- The one cut slide tissue is placed in a water bath.
- This prevents the irregularities of the slice.
- The straight layer is then picked up on a slide.

Routine staining

Telegram - @nextpreladdernotes

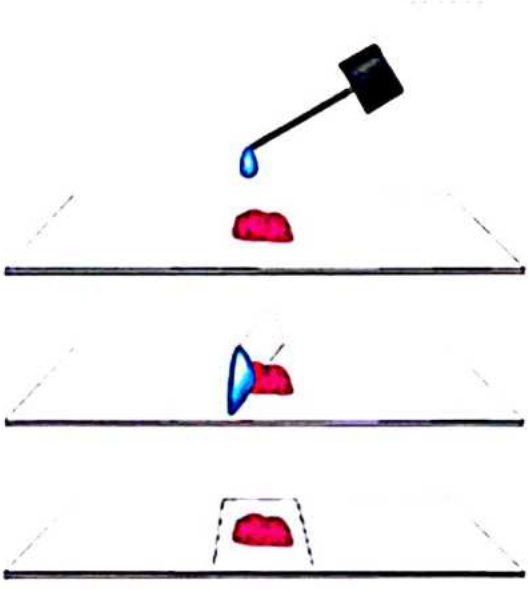
- Colors of pathology
  - Pink- Eosin
  - Blue-Hematoxylin
- Source of Hematoxylin
  - Heartwood extracts of the logwood tree.
  - Hematoxylin campechianum.
- In olden days, Hematoxylin was used as a dye for clothes.

Coplin's jar

- Made up of plastic or glass.
- Eosin or Hematoxylin solutions are filled.
- Tissues for biopsy is dipped in the jar.

Mountants

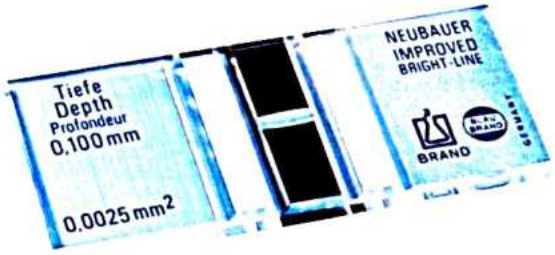
- DPX (Distrene Dibutyl phthalate xylene)
- Canada Balsam

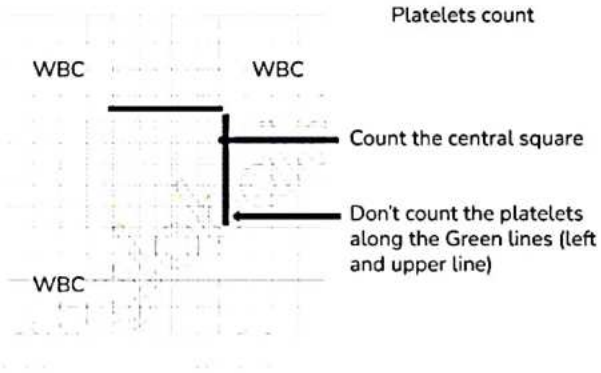


- Used to cover the slide.
- Prevent stains from drying.
- DPX (Distrene Dibutyl phthalate Xylene).
- Olden days, Canada Balsam.

TIP: Formalin can be used in any biopsy

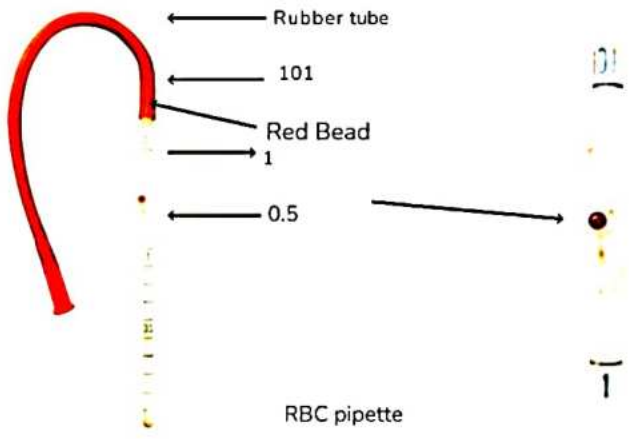
Improved Neubauer Chamber



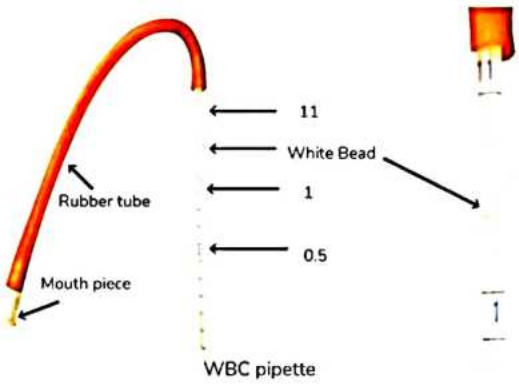


- Count fluids
  - Blood cells
  - CSF
  - Semen
- Grids are divided into:
  - WBC, 4 big squares (peripheral)
  - RBC, in the central grid
    → 4 small squares in the periphery
    → 1 central square
  - Platelets, entire central grid
    → Except the green line

Pipettes



Telegram - @nextprepladdernotes



- Bead is used to mix the blood and fluid.
- Platelet diluting fluid- Rees & Ecker fluid.

Telegram - @nextprepladdernotes

PREVIOUS YEAR QUESTIONS

Q. Arrange the following steps of histopathology processing in the correct sequence

a. Fixation
b. Clearing
c. Embedding
d. Dehydration

Ans: a-d-b-c

Q. Match the fixative with target specimen

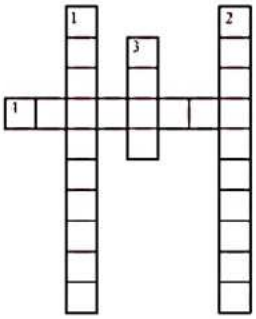
a. Bouin's fixative	1. Colon biopsy
b. NBF	2. BM samples
c. Glutaraldehyde	3. Renal biopsy for EM
d. Carnoy fixative	4. Testicular biopsy
e. Zenker's fixative	5. Karyotyping

Ans: a-4, b-1, c-3, d-5, e-2

Telegram - @nextprepladdernotes

CROSS WORD PUZZLES

Crossword Puzzle



Across

4.It can be used in any biopsy

Down

1. It is most preferred for male reproductive cells
2. It is used to cover the slide and prevent stains from drying
3. It is used to mix the blood and fluid