# RBC:

biconcave, disk-like (Spectrin protein maintains RBC shape)

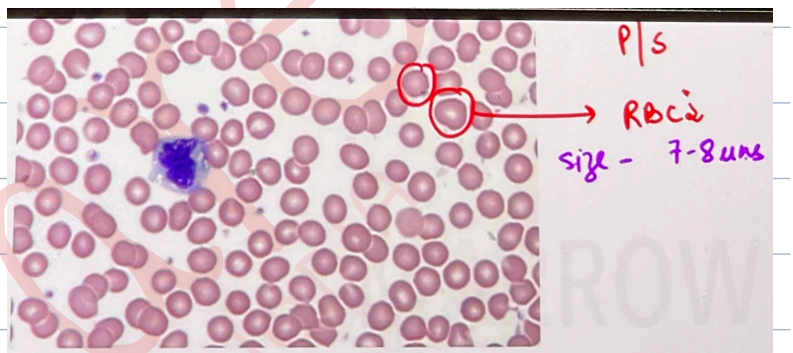
→ Normal size: 7-8 μm

[In peripheral smear (P/s), to check if RBC size is correct, compare it to a lymphocyte nucleus; both of them have similar size]

→ Lifespan: 120 days

→ enucleated

→ In normal RBCs ⇒ central 1/3 pallor




Hemopoietic stem cell

↓

Common myeloid Progenitor

↓

RBC series

Proerythroblast → Hb production starts (but not seen on microscope)

(Basophilic) Early Normoblast →

(Polychromatic) Intermediate Normoblast → Hb is first seen on microscope.

(Orthochromatic) Late Normoblast →

Reticulocyte →

↓ 1-2 days

RBC →

cell size ↓

nucleus size ↓

No NUCLEUS

# Reticulocyte :

→ immediate precursor of RBC

→ first precursor with no nucleus

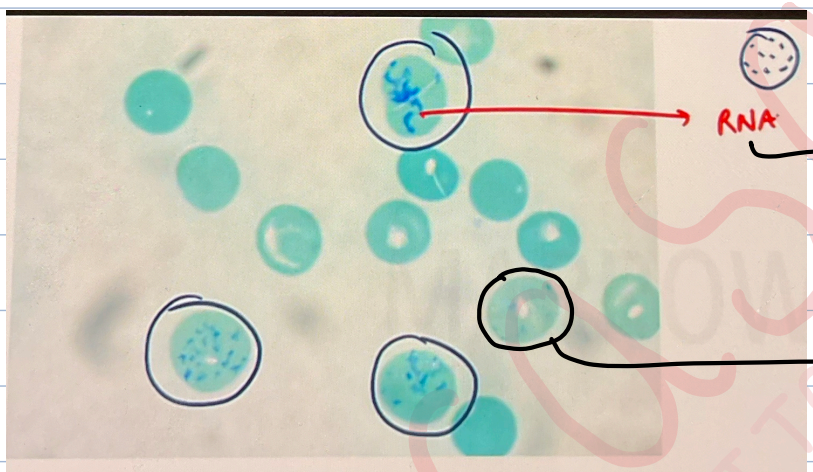
→ normal retic. count : 0.5 – 1.5 %

→ Special stain : – Supravital Stain *

- Brilliant Cresyl blue
- New methylene blue (best)

* (vital ⇒ living)

∴ supravital ⇒ stains the living state / structure of a cell




→ presence of reticulum of RNA
∴ called reticulocyte.

→ normal mature RBCs

## Increased Retic. Count (Reticulocytosis)

→ acute & chronic blood loss

→ hemolytic anemia

→ to see response to treatment in Fe or Vit. B12 deficiency anemia

## Decreased Retic. Count (Reticulocytopenia)

→ Bone marrow suppression

→ Aplastic Anemia

→ Megaloblastic anemia.

→ Leukemia

→ Metastasis

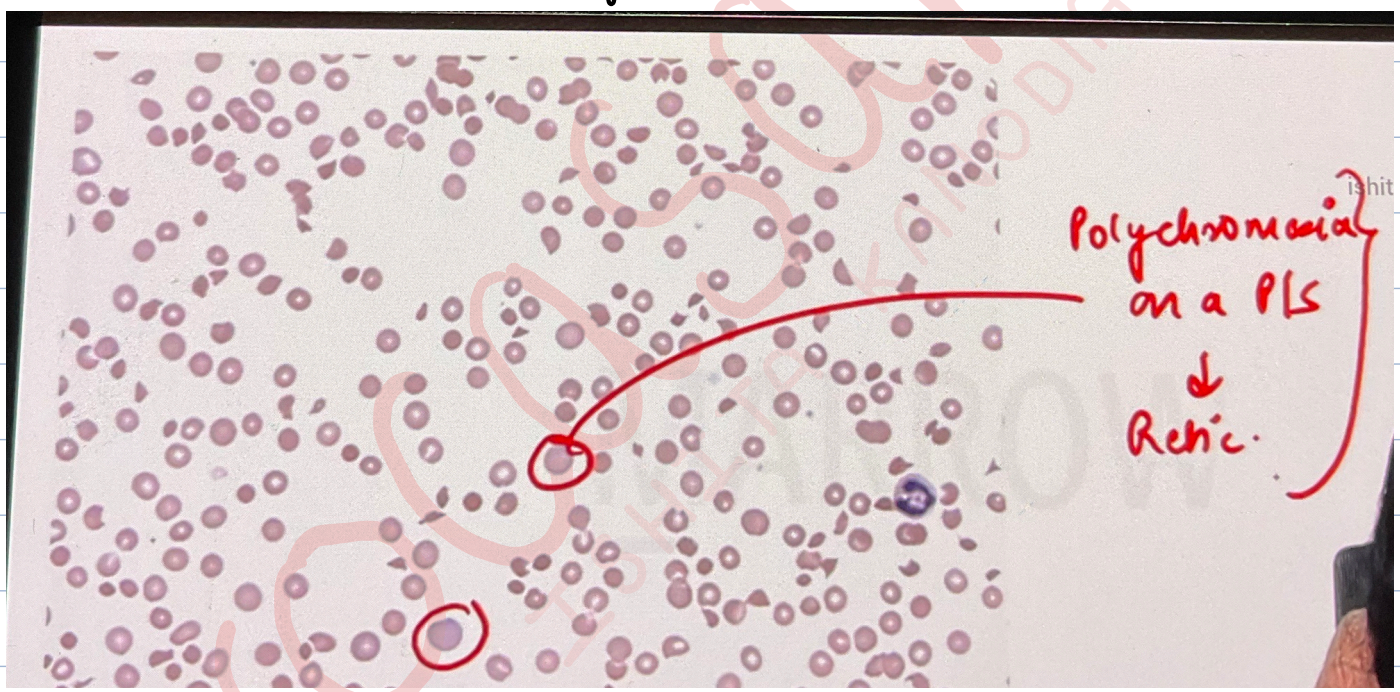
→ Renal failure

**Corrected Retic. Count (CRC)** : = Retic. % × $\frac{\text{patients Hb or HCV}}{\text{normal Hb or HCV}}$

[• HCV = Hb % × 3]

**Reticulocyte Production Index [RPI]** : = $\frac{\text{CRC}}{\text{Maturation time (acc. to PCV)}}$ → normally 1-2 days

| PCV | Maturation time |
|---|---|
| 45 | 1 day |
| 35 | 1.5 |
| 25 | 2 |
| 15 | 2.5 days |



# RBC Indices: ← MCV, MCH, MCHC, RDW

## MCV (Mean Corpuscular Volume): tells about size/volume of RBC

Normal MCV: 82-96 fL [80-100] [78-94 $um^3$]

- **Microcytic anemia:** MCV < 80 fL
  - S – sideroblastic anemia & lead poisoning
  - I – Iron deficiency anemia
  - T – Thalassemia
  - A – anemia of chronic diseases
- **Normocytic anemia:** MCV: 80 – 100 fL
  - Aplastic anemia
  - anemia of chronic diseases
  - hemolytic anemia
  - renal disease
- **Macrocytic anemia:** MCV > 100 fL.

Lady Hardinge medical College
- L – Liver disease
- H – hypothyroidism
- M – megaloblastic anemia (due to B12/folate deficiency)
- C – cytotoxic drugs

- $MCV = \frac{PCV}{RBC\ count}$

## MCH [Mean Corpuscular Hb]: average volume of Hb in a single RBC

Normal MCH: 27 – 32 pg

- $MCH = \frac{Hb}{RBC\ count}$

- Normochromic: MCH = 27 - 32 pg
- Hypochromic: MCH < 27 pg

## MCHC [Mean Corpuscular Hb. Conc.]:

→ average Hb in a given volume of packed red cells

- $MCHC = \frac{MCH}{MCV}$

Normal MCHC: 33 - 37 g/dL or %

Increased MCHC:

→ hereditary spherocytosis

{ variation in size: anisocytosis
variation in shape: poikilocytosis }

Normal MCHC:

→ megaloblastic anemia due to vit. B12 deficiency

## RDW [Red Cell Distribution Width]:

Normal RDW: 11.5 - 14.5 %

→ indicates the coefficient of variation of red cell size (degree of anisocytosis)

→ helps to differentiate Fe deficiency Anemia from thalassemia.

→ in Fe deficiency anemia: RDW increases
in thalassemia: RDW is almost normal.

# Anemia



**Definition:** A group of disorders with Hb concentration of blood below normal range:

In adult males: < 13 g/dL

In adult females: < 11.5

In newborns: < 15

At 3 months of age: < 9.5

→ Low RBC count (< 4 million/μL) is usually associated with low Hb in anemia.

**Grading of Anemia:** Mild: 8-10 g/dL

Moderate: 6-8

Severe: < 6

**Classification:** etiological (whitby's) classification: (Based on causes)

| Type | Example |
|---|---|
| Deficiency Anaemias | (i) Iron deficiency anaemia<br>(ii) Megaloblastic anaemia (pernicious anaemia) due to Vit. B12 deficiency<br>(iii) Megaloblastic anaemia due to folic acid deficiency<br>(iv) Protein & vit. C deficiency |
| Blood loss anaemia (Haemorrhagic anaemia) | (i) Acute post-haemorrhagic anemia in accidents<br>(ii) Chronic post-haemorrhagic anaemia |
| Haemolytic anaemias | Hereditary:<br>(i) Thalassemia<br>(ii) Sickle cell anaemia<br>(iii) Hereditary spherocytosis<br>(iv) G6PD deficiency<br><br>Acquired:<br>(i) due to direct toxic effects (malaria, snake venom)<br>(ii) splenomegaly<br>(iii) paroxysmal nocturnal haemoglobinuria |
| Aplastic Anaemia | due to failure of bone marrow to produce RBCs. |
| Anaemia due to chronic diseases | tuberculosis, malignancies, chronic infections, chronic lung diseases, etc. |

## Morphological (Wintrobe's) Classification: (Based on MCV & MCHC)

| Type | MCV | MCHC | Example |
|---|---|---|---|
| Normocytic Normochromic | normal ($78-94\ \mu m^3$) | normal (30 - 38%) | • acute post-haemorrhagic anaemia<br>• chronic post-haemorrhagic anaemia<br>• thalassemia |
| Microcytic Hypochromic | reduced ($<78\ \mu m^3$) | reduced (< 30%) | • iron deficiency anaemia<br>• chronic post-haemorrhagic anaemia<br>• thalassemia |
| Macrocytic normochromic | increased ($>94\ \mu m^3$) | normal (30 - 38%) | • megaloblastic (pernicious) anaemia (vit. B12 deficiency)<br>• megaloblastic anaemia (folic acid deficiency) |

**General Clinical Features of Anaemia:** anaemic hypoxia
(due to decreased $O_2$ carrying capacity of blood due to reduced Hb)

→ generalised muscular weakness (due to muscle hypoxia)
→ pallorness of skin & mucous membrane (due to deficiency of red colour Hb in blood)
→ respiratory symptoms - breathlessness, increased rate & force of respiration (due to compensatory stimulation of respiratory centre)
→ cardiovascular manifestations - tachycardia, palpitation (compensatory mechanism to increase cardiac output)
→ increased basal metabolic rate
→ lethargy, headache, faintness, drowsiness (CNS manifestations)

**Daily iron requirement:** Adult males: 5-10 mg/day
Adult females: 20 mg/day
Pregnant & lactating women: 40 mg/day

## Characteristic features of iron deficiency anaemia:

→ Koilonychia (nails become dry, soft & spoon-shaped)
→ Atrophic glossitis (tongue becomes angry red)
→ Angular stomatitis (mouth)
→ Plummer – Vinson Syndrome – oesophagus may develop membranous webs at postcricoid area leading to dysphagia
→ RBCs are micracytic, hypochromic
→ anisocytosis, poikilocytosis
→ serum iron (< 50 mg %) [Normal = 60–160 mg %]
→ low serum ferritin

## Characteristic features of megaloblastic anaemia:

→ RBCs are macrocytic
→ MCV > 94 $um^3$ → MCH increases = 50pg (normal = 28–32 pg).
→ MCHC usual normal (30–38 %) [because both MCV & MCH increase]
→ Increased serum bilirubin = > 1mg/dL (normal = 0.2 – 0.8 mg/dL)

# Anemias: decrease in Hb conc. or RBC count or both.

- HEMOLYTIC
- ACUTE & CHRONIC BLOOD LOSS
- HYPOPROLIFERATIVE (aplastic anemia)
- DEFICIENCY

WHO criteria for anemia are Hb levels below:

- 13 g/dL in men
- 12 g/dL in women
- 11 g/dL in pregnant women

## Hypoproliferative Anemias:

- Aplastic anemia
- Pure red cell aplasia (PRCA)
- Myelophthisic anemia

### Aplastic Anemia: Immune-mediated destruction of HSC due to activation of CD8+ T cells which release TNF & INF-γ causing overexpression of FAS on bone marrow stem cells ⟹ Apoptosis

→ decreased – Hb, – TLC, – Platelet, – Retic. count

CAUSES OF APLASTIC ANEMIA:

**Inherited** (mostly due to dysregulation of telomerase leading to early shortening of telomere)

→ Fanconi's Anemia – AR – FANC – A, B, C.....
(DNA repair defect)

→ Diamond – Shwachman Syndrome

→ Dyskeratosis congenita
(short telomeres)

→ Diamond – Blackfan Anemia

[AR = autosomal recessive]

**Acquired**

→ drugs (cytotoxic / chemotherapeutic)

→ Chemicals (benzene)

→ Virus (Hep. B, C ; HIV ; Parvovirus B19)

→ pregnancy

## CLINICAL PRESENTATION:

- pallor, fatigue, headache, rash skin, dizziness,
- increased risk of infections
- bleeding tendency

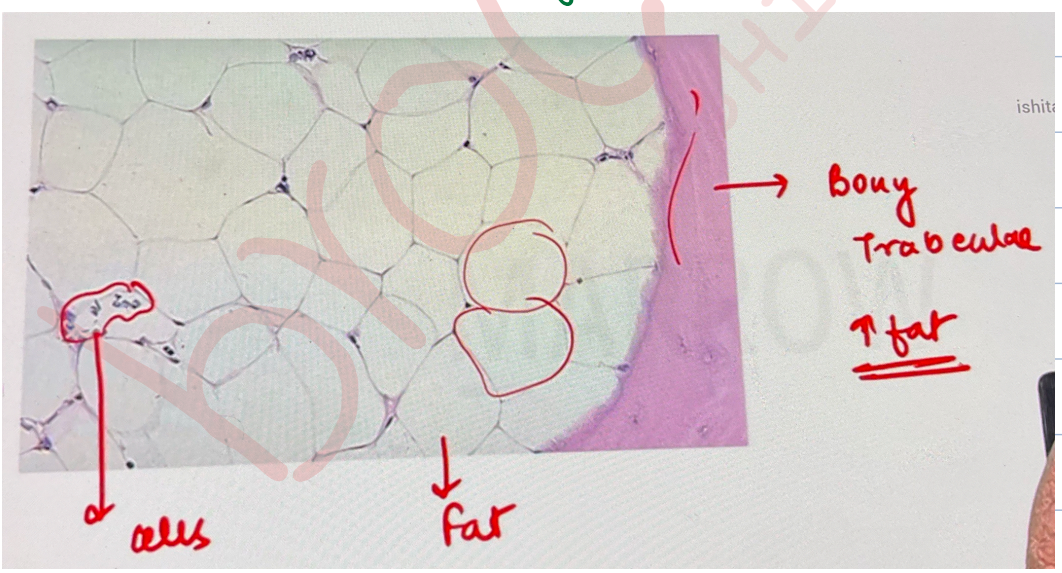
- In aplastic anemia, SPLENOMEGALY is NEVER PRESENT.

## LAB DIAGNOSIS:

Hb
TLC
Platelet
Retic. count
} ↓

- RDW ↑

→ Peripheral smear ⇒ normocytic normochromic (macrocytic) with pancytopenia
→ Bone Marrow Aspirate (BMA) ⇒ dry tap.
→ Bone Marrow biopsy (Investigation of choice) ⇒ increased fat
⇒ decreased cellularity
→ Biochemical & Radiological Tests




Normal cellularity on bone marrow biopsy = 100 − Age of patient (%)

## Other Causes of Dry Tap on BMA:

→ aplastic anemia
→ myelofibrosis
→ hairy cell leukemia
→ AML - M7
→ Myelophthisic Anemia

## TREATMENT:

→ Stem cell transplantation
→ GM-CSF

## SEVERE APLASTIC ANEMIA: [CRITERIA]

i) Bone marrow cellularity < 25 %
ii) Any two of the following — Platelet count < 20,000 / $\mu^3$
— Corrected Retic. Count < 1 %
— Absolute Neutrophil Count < 500 / $\mu^3$ (ANC)

## VERY SEVERE APLASTIC ANEMIA:

i) Bone marrow cellularity < 25 %
ii) Any two of the following — Platelet count < 20,000 / $\mu^3$
— Corrected Retic. Count < 1 %
— Absolute Neutrophil Count < 200 / $\mu^3$ (ANC)

# Pure Red Cell Aplasia [PRCA]:

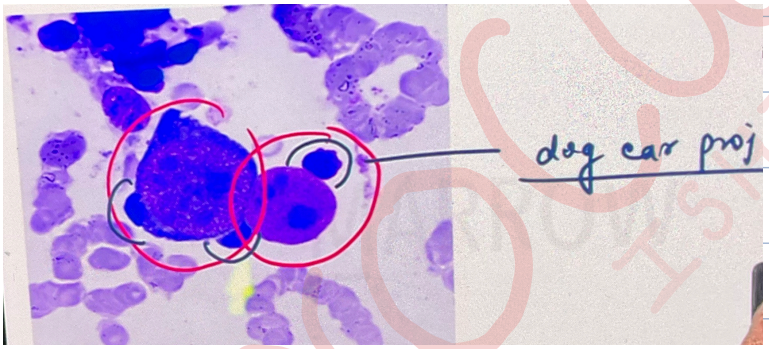
→ decrease in — erythroid precursors
— Hb
— Retic. count

## CAUSES:

### INHERITED

→ diamond blackfan syndrome

- In Parvo virus B 19, characteristic feature: DOG EAR ERYTHROID PRECURSORS are seen.

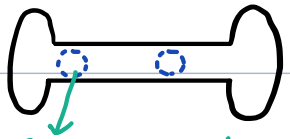



### ACQUIRED

→ Parvovirus B 19
→ Thymoma
→ Large granular lymphocytic leukemia
→ B cell disorders

# Myelopthisic Anemia:

→ Anemia caused by a space-occupying lesions in bone marrow.



space occupying lesions in a bone

Eg:
- Metastatic Cancer
- Granulomatous lesion

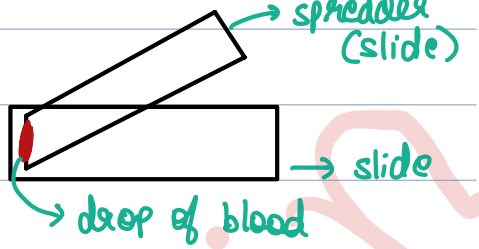
[P/S = peripheral smear]

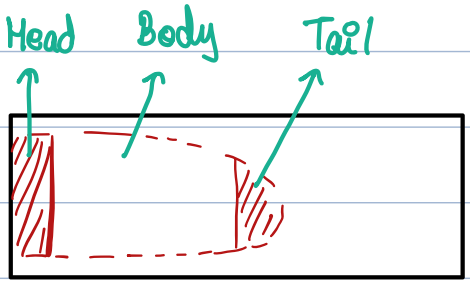
→ RBC profile: Tear drop cell / Dacrocytes

→ P/S: Leukoerythroblastic blood picture

# Peripheral Smear [P/s] Examination:

- Tongue-shaped Smear
  - Head
  - Body
  - Tail

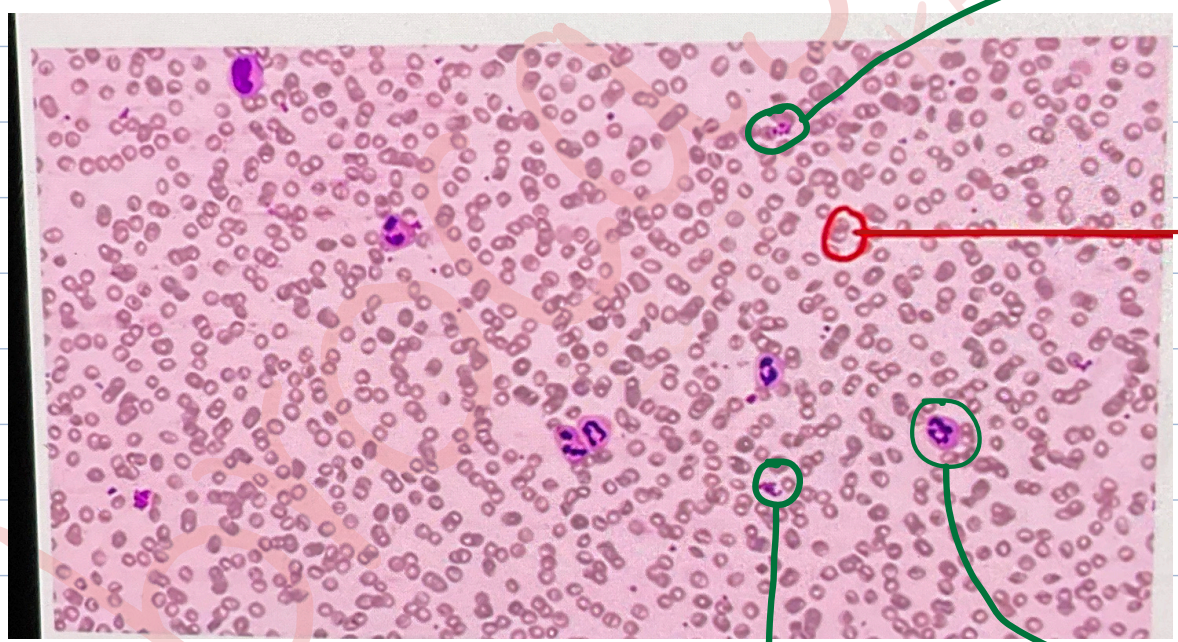


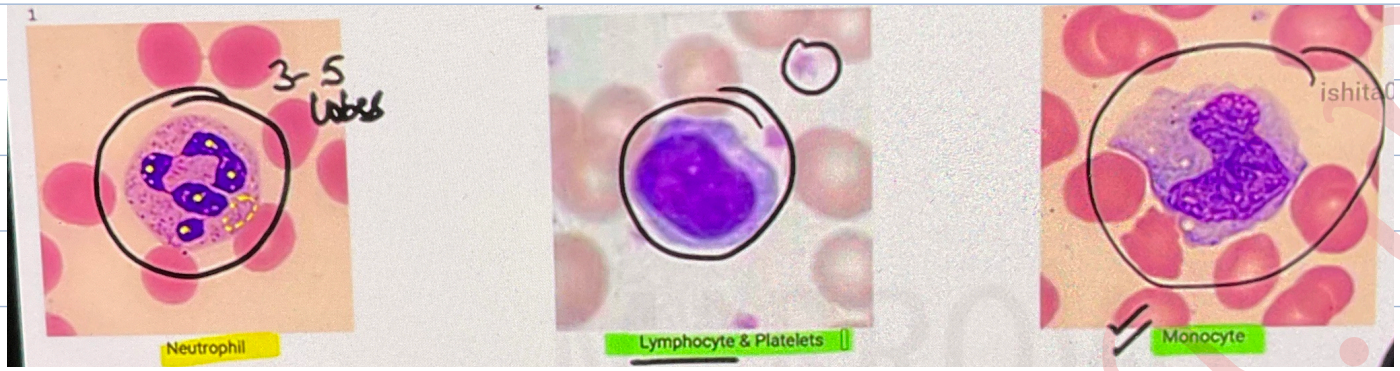



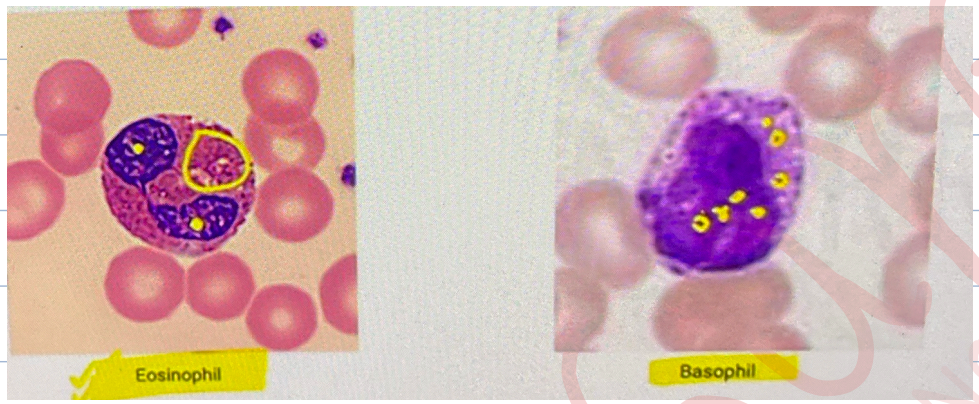

**Stain:** Romanowsky Stains
- Leishman
- Geimsa
- Wright
- Jenner

Methylene blue (basic dye)

Eosin Y (acidic)






## RBC Abnormalities on P/s:

| P/s finding | Condition |
|---|---|
| • Microcytic Hypochromic (mcv < 80) | S – sideroblastic anemia; lead poisoning<br>I – iron deficiency anemia<br>T – Thalassemia<br>A – Anemia of chronic diseases |
| • Macrocytic Anemia (mcv > 100) | L – Liver diseases<br>H – Hypothyroidism<br>M – megaloblastic anemia due to B12/ folate deficiency<br>C – cytotoxic drugs |
| • Pencil cells | Iron deficiency anemia |
| • Bite cells | G6PD deficiency |

| P/s finding | Condition |
|---|---|
| • Spherocytes  | i. Hereditary spherocytosis<br>ii. Autoimmune hemolytic Anemia (AIHA)<br>iii. Blood transfusion<br>iv. Burns |
| • Burr cell / Echinocyte (Blunt projections) →  | – CRF<br>– Uraemia<br>– Liver diseases |
| • Spur cell / acanthocyte* | A β Lipoproteinemia |
| • Sickle cell ** | Sickle cell anemia |
| • Target cell / codocyte  | i. Thalassemia<br>ii. Liver disease<br>iii. Fe deficiency anemia |

* → sharp projections 

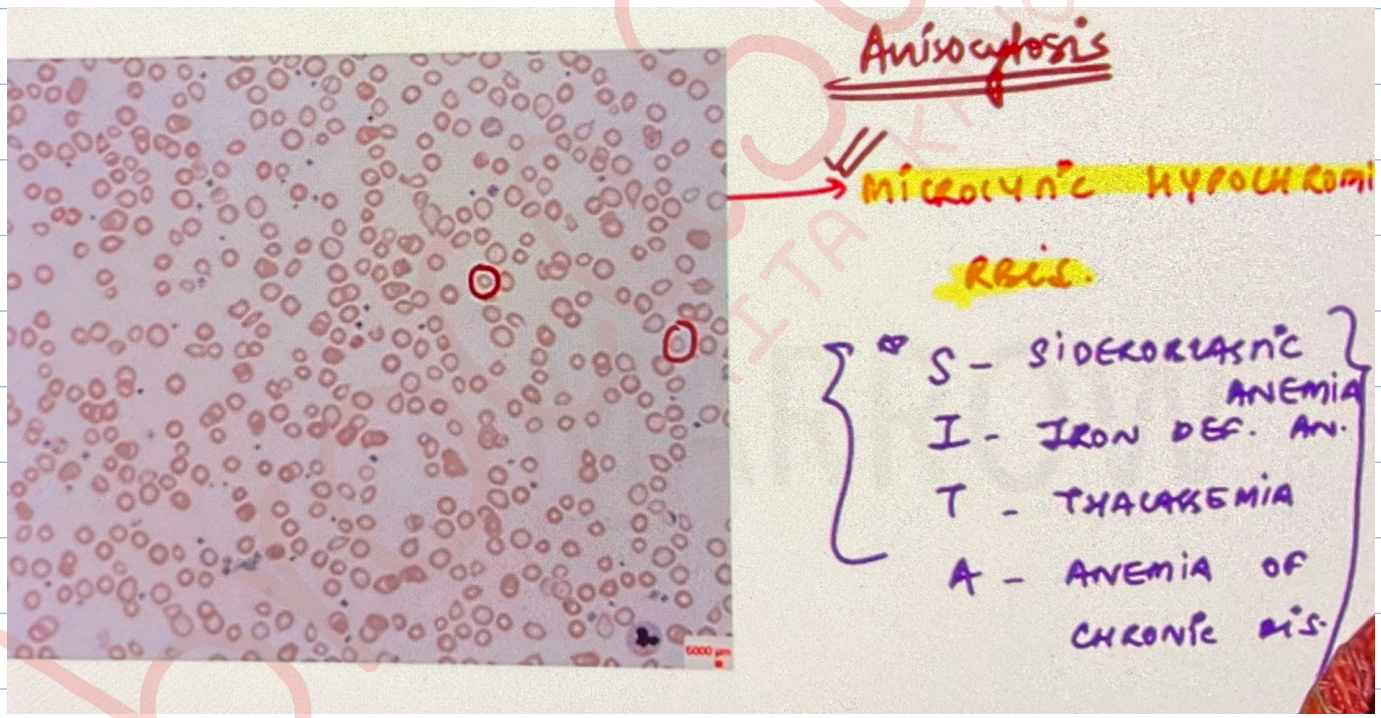
** 

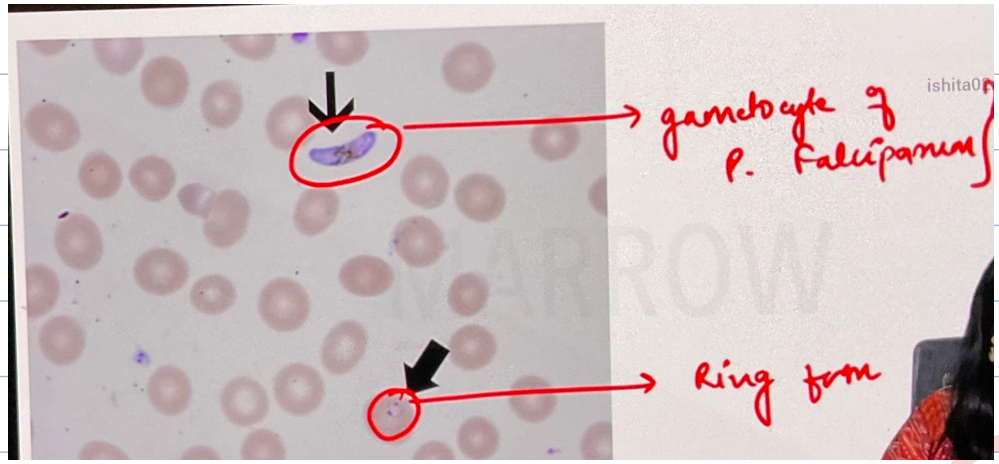
| P/s finding | Condition |
|---|---|
| • Schistocyte / Helmet Cell / Fragment Red cell  | – microangiopathic hemolytic anemia (MAHA) → HUS, TTP, DIC<br>– Prosthetic cardiac valves<br>– Mechanical disruption of RBCs |
| • Tear drop cell / dacrocyte  | i. Myelofibrosis<br>ii. Myelo-displastic syndrome (MDS)<br>iii. myelophthisic anemia<br>iv. Aplastic anemia |

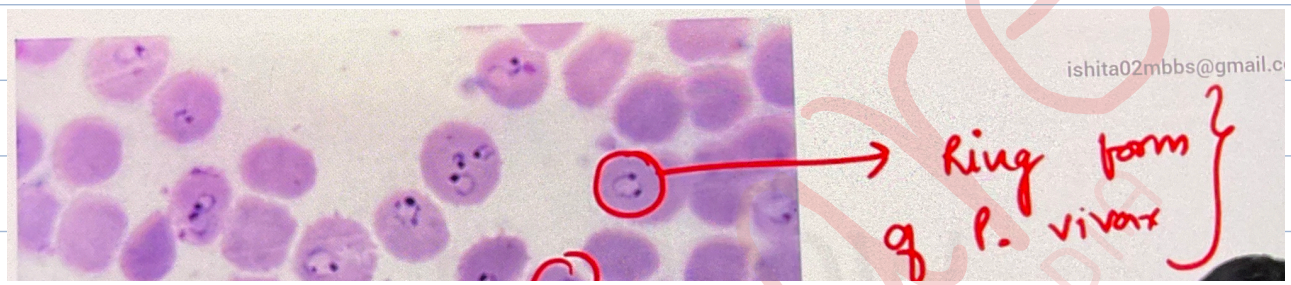
| P/s finding | Condition |
|---|---|
| • Heinz bodies (denatured Hb) | G6PD deficiency |
| • Howell jolly bodies (Remnant of nucleus)  | – Asplenia<br>– Megaloblastic anemia |
| • Pappenheimer bodies (composed of Fe)  | Sideroblastic anemia |
| • Cabot Ring   | Megaloblastic anemia |
| • Rouleaux  | Multiple myeloma |
| • Polychromasia (pink + purple) [reticulocyte] | Hemolytic anemia |
| • Basophilic stippling   | – Sideroblastic anemia (Coarse)<br>– Thalassemia (fine)<br>– Megaloblastic anemia (fine) |
|  → (slit-like spaces) | |
| • Stomatocytes | Hereditary somatocytes |

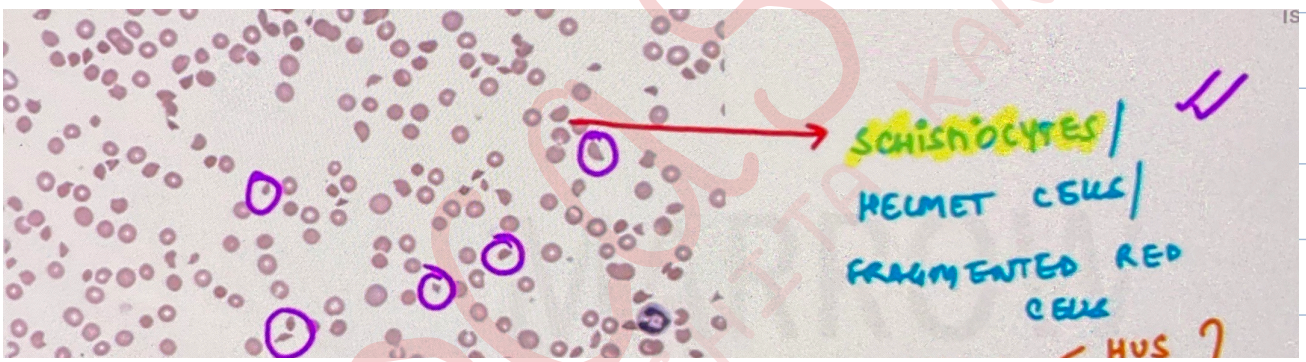
# WBC Abnormalities on P/s:

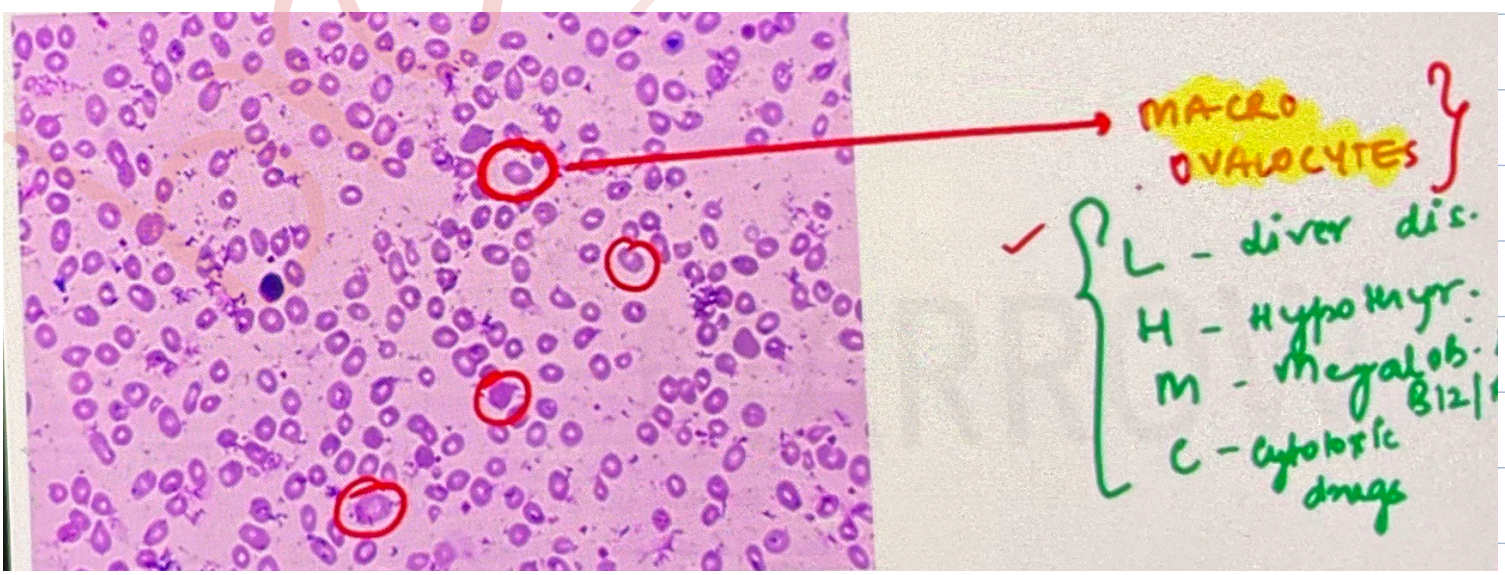
| P/s finding | Condition |
|---|---|
| • Hypersegmented neutrophil (more than 5 lobes) | Megaloblastic anemia |
| • Bilobed neutrophil | MDS |
| • Toxic granules  | Sepsis |
| • Dohle bodies (patches of dilated E.R. | Sepsis |

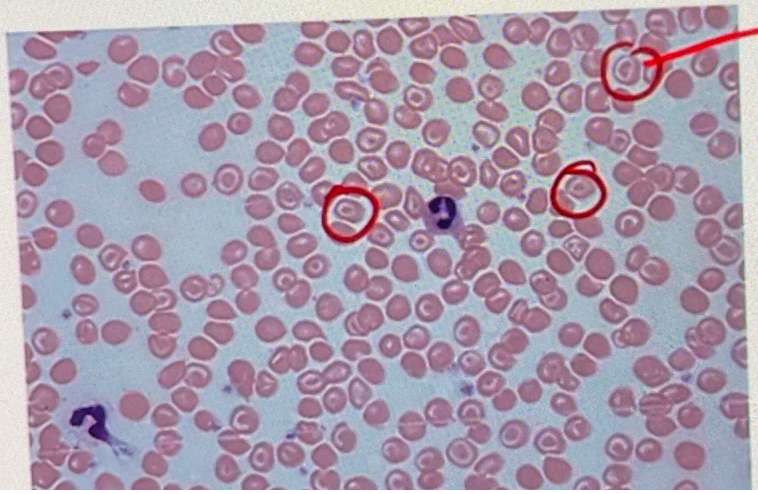




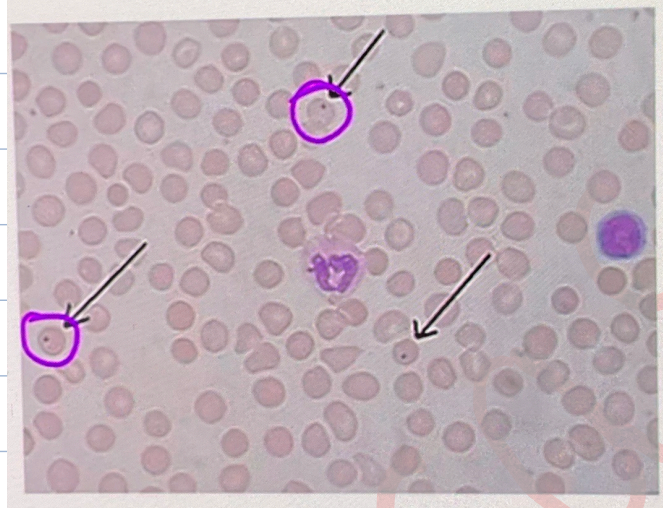





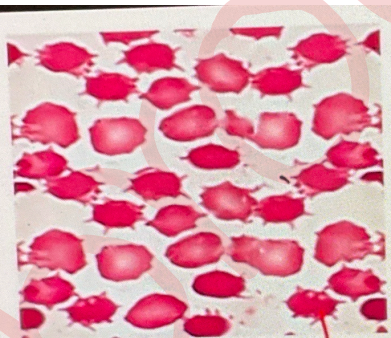
TARGET CELLS (LOOKS LIKE A TARGET)

1. THALASSEMIA
2. LIVER DISEASE
3. MEGALOBLASTIC ANEMIA



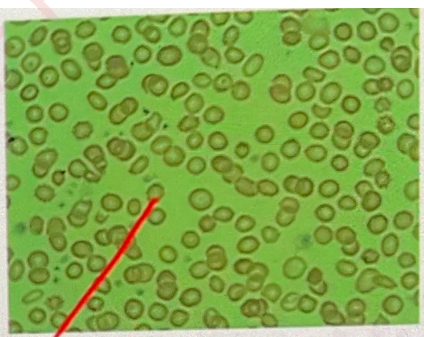
HOWELL JOLLY BODIES (NUCLEAR REMNANT)

- ★ POST SPLENECTOMY
- ★ MEGALOBLASTIC Anemia
- ★ THALASSEMIA



ACANTHOCYTE (CELLS WITH POINTED SPICULES)

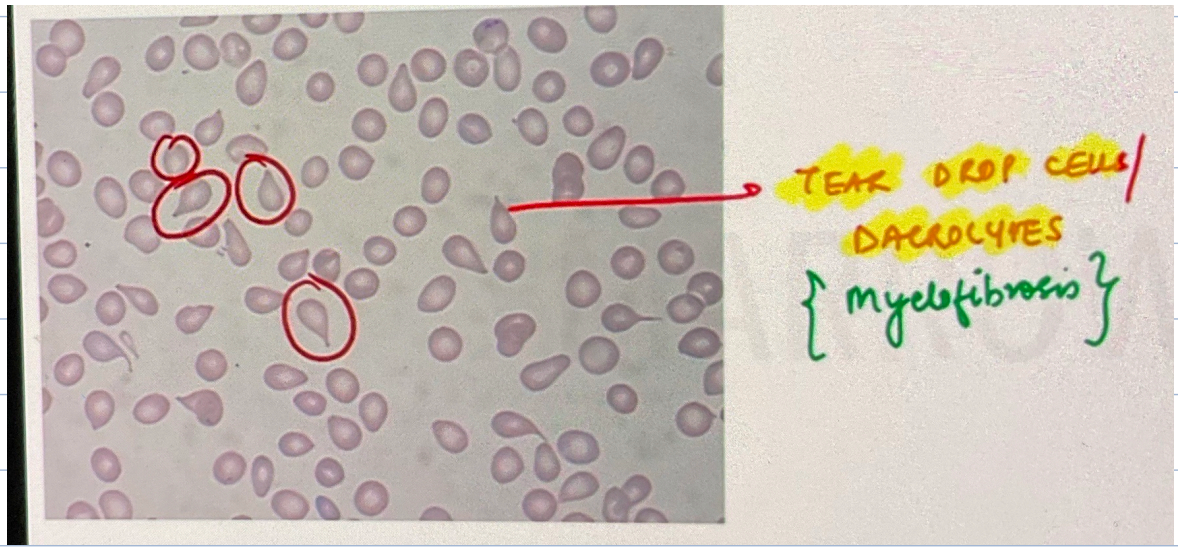
{★ ABETALIPOPROTEINEMIA}

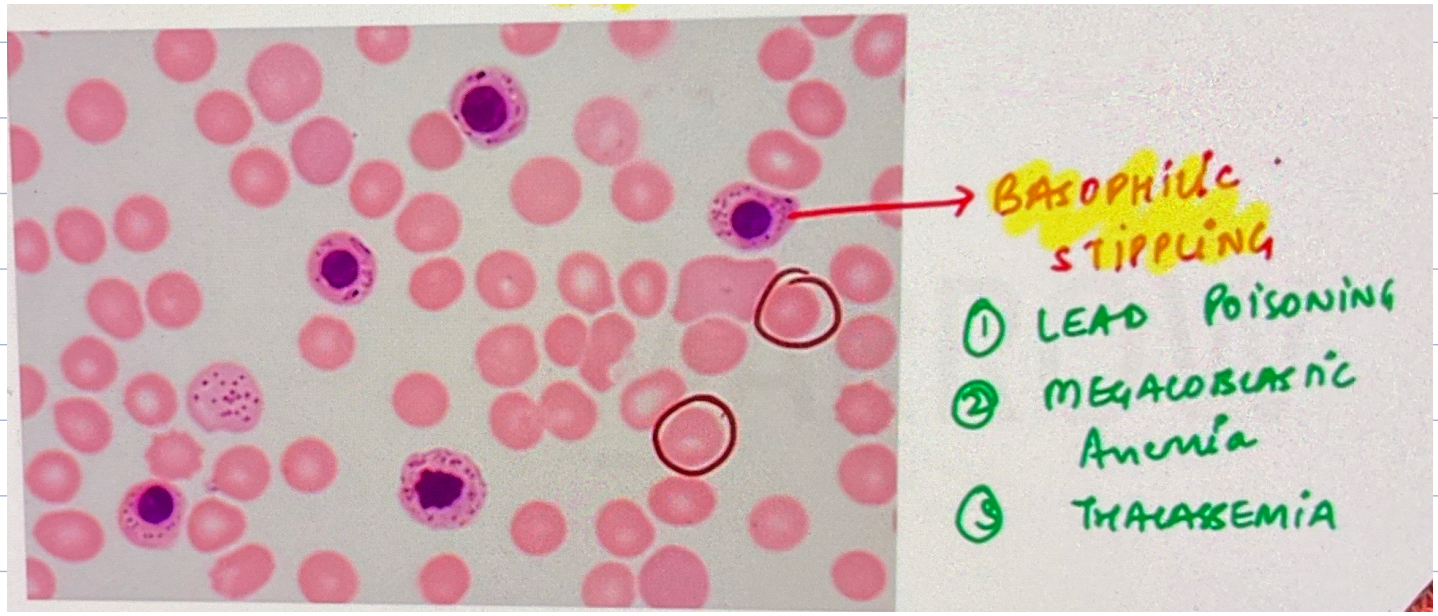


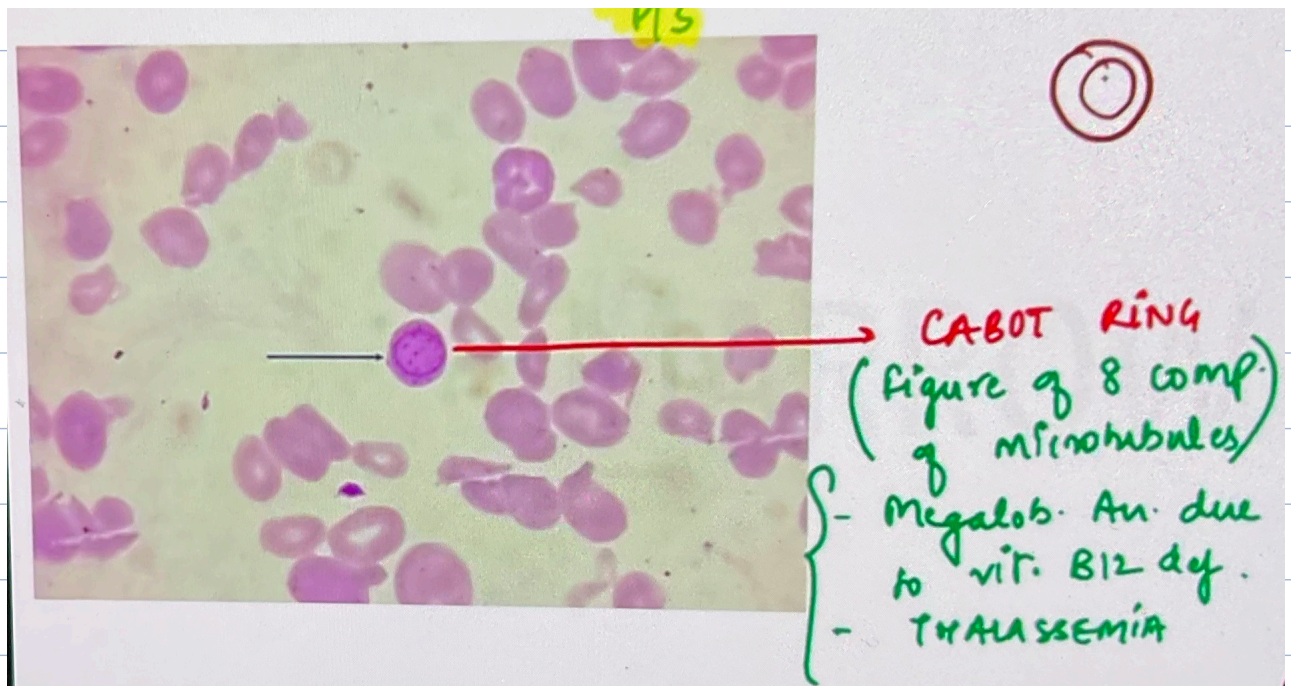
BURR CELL (CELLS WITH BLUNT PROJECTIONS)

{★ UREMIA ICAF}

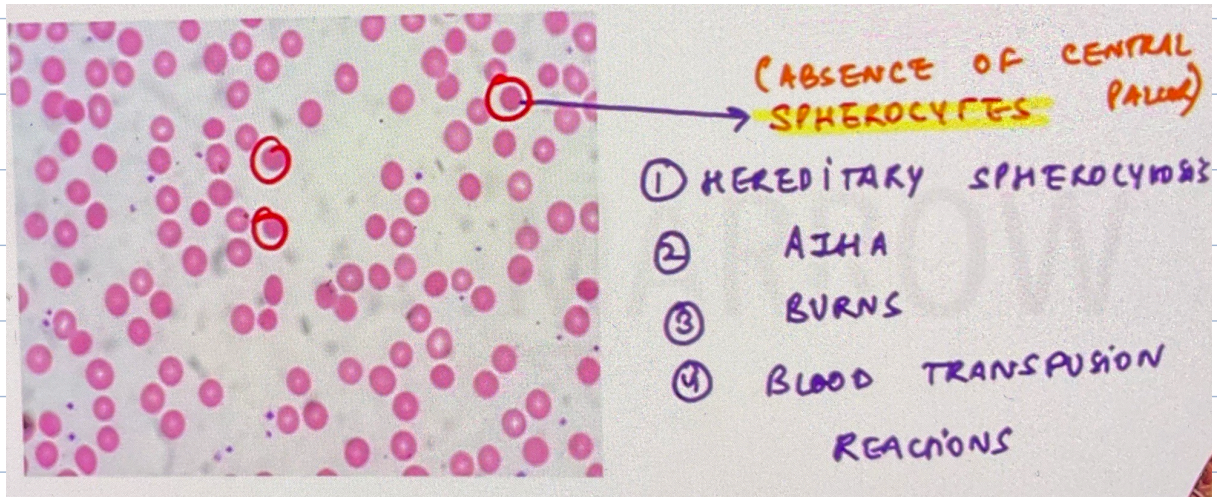
ISHITA KANODIA

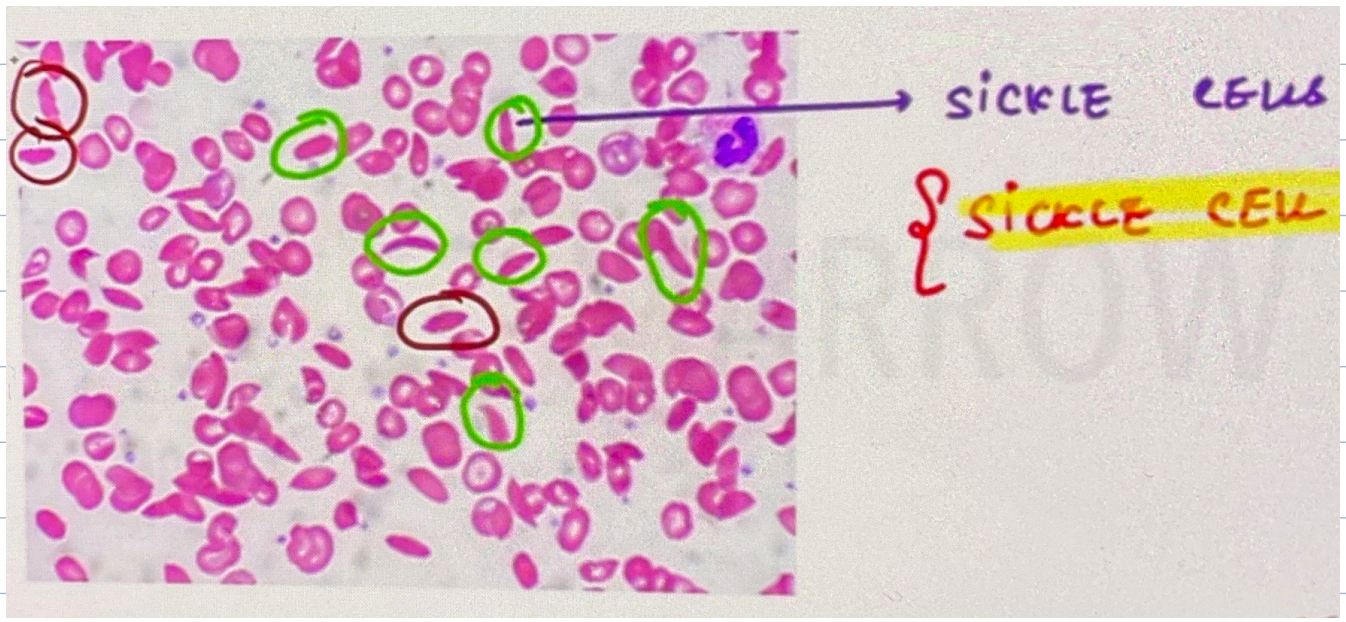


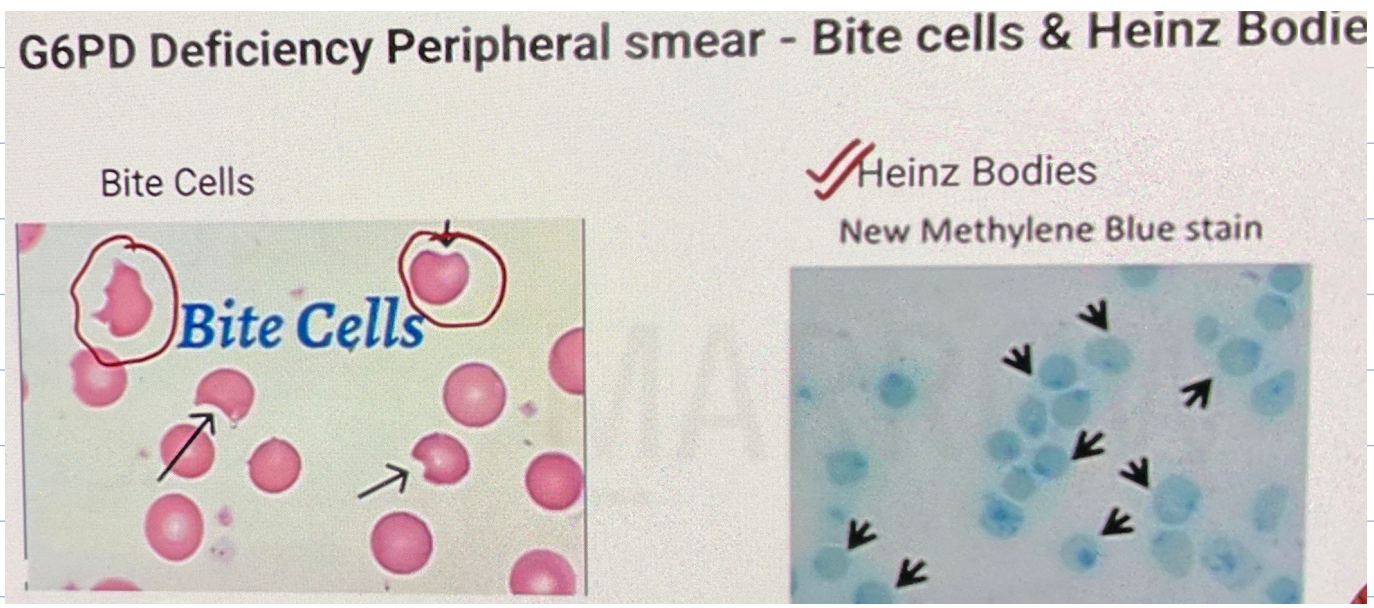



ISHITA KANODIA



→ most common cause of anemia worldwide

# Iron Deficiency Anemia (IDA): most common nutritional deficiency in the world

→ **Fe RDA:** 10 - 20 mg

→ Iron
- Haem ($Fe^{+2}$) — 80% ⇒ Hb
- Non-haem ($Fe^{+3}$)

→ Iron
- $Fe^{2+}$ (ferrous) → absorbed from intestine (most common site of absorption of Fe = Duodenum)
- $Fe^{3+}$ (ferric)

- Free iron is highly toxic
- In iron deficiency, ferritin < 12 ug/L

**Storage forms of iron:** FERRITIN, HEMOSIDERIN

Fe absorption is enhanced by vit C & is interfered with tannic acid.

**Transport form of Fe:** TRANSFERRIN.

## Causes of Iron Deficiency Anemia:

**Decreased intake**
- low socioeconomic status
- anorexia
- infants are at higher risk due to less iron in breast milk

**Increased Demand**
- pregnancy
- lactation
- puberty
- menstruation

**Impaired Absorption**
- malabsorption syndromes

**Chronic blood loss**
- GI/colon malignancies

→ Most common worm which causes IDA : Ancylostoma duodenale (Hookworm)

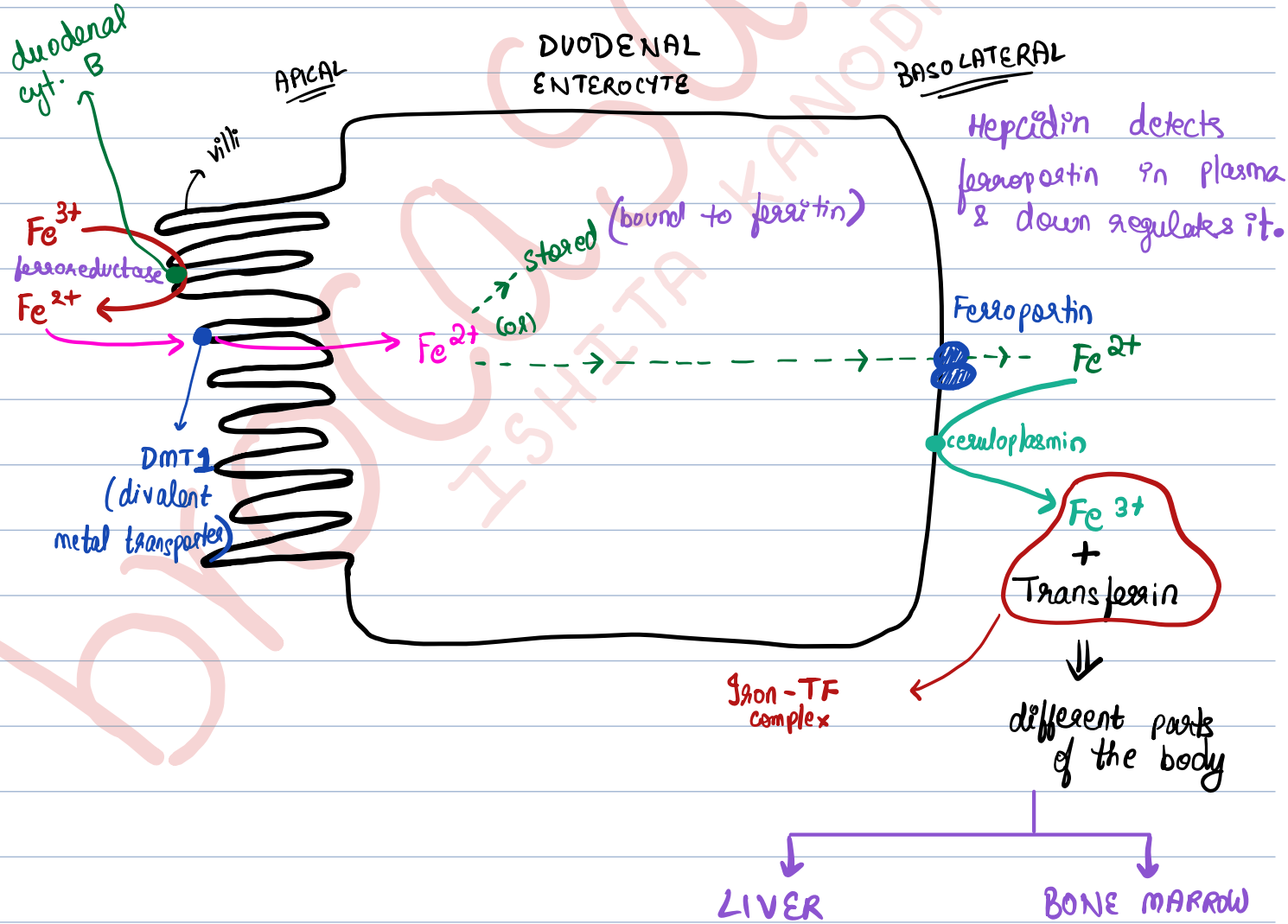
## Mechanism of Fe Absorption:

### Factors Increasing Fe Absorption

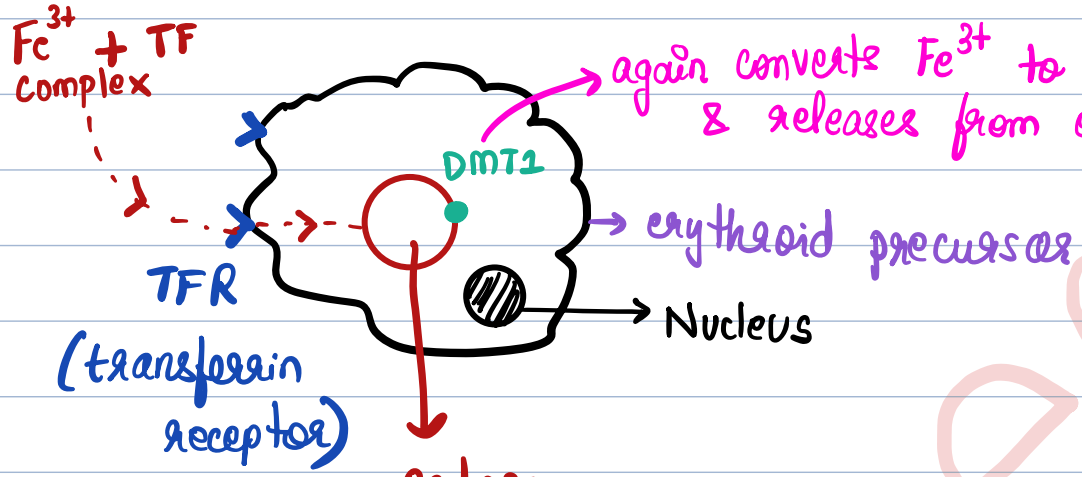
- acidic pH
- vit. C / ascorbic acid
- Amino acids
- Citric acid

### Factors decreasing Fe absorption

- Alkaline pH
- Tea
- Tannates
- Phytates



# BONE MARROW



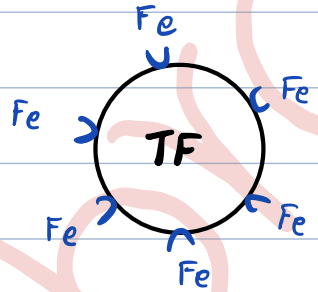

**DMT 1**: present in – duodenal enterocytes
– placenta
– macrophages
– erythroid precursors

- early erythroid precursors have more TFRs.
- late erythroid precursors start shedding TFRs.

**Transferrin (TF)**: transport form of Iron ($Fe^{3+}$)

→ one molecule of TF binds to 6 $Fe^{3+}$ IDEALLY

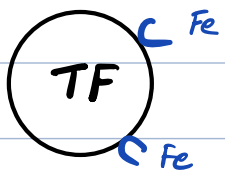
→ PRACTICALLY, 1 molecule of TF can only combine with 2 $Fe^{3+}$.




∴ TF saturation

$= \frac{2}{6} \times 100$

$= 33\%$.



## Soluble TFR Ratio: [STFRc]

→ measure of the erythropoietic activity of bone marrow.

**Hepcidin**: molecule that inhibits iron absorption
- Hep → Liver
- cidin → inhibition

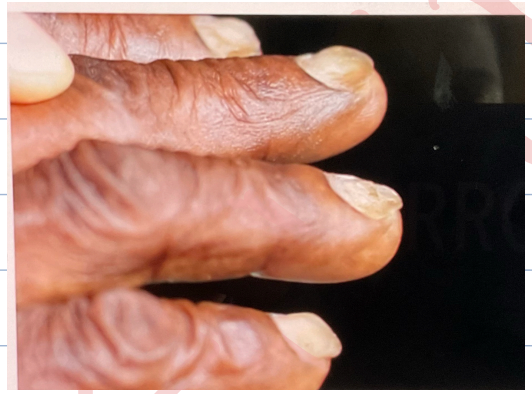
→ produced by Liver
→ master regulator of Fe metabolism
→ increased Hepcidin ⇒ decreased iron & vice versa

→ Hepcidin binds to ferroportin & degrades it
∴ decreased serum iron
⇓
microcytic hypochromic anemia.

→ also an acute phase reactant.

→ genes which regulate hepcidin
- HFE } mutation ⇒ Hemachromatosis.
- HJV }
- TMPRSS6 → mutation ⇒ IRIDA [Iron refractory Iron deficiency anemia].

## Clinical Presentation of IDA:



- → pallor
- → fatigue
- → dyspnea
- → palpitations
- → Angular stomatitis
- → Cheilitis
- → Koilonychia (spoon-shaped nails)
- → Pica (tendency to eat clay / mud / sand)
- → PLUMMER VINSON SYNDROME: aka Paterson Brown Kelly Syndrome
  - Fe deficiency anemia
  - Esophageal webs
  - Atrophic glossitis

  } usually seen in middle-aged women.

→ Chronic Fe deficiency may lead to upper GI bleeds
⇓
Malena
⇓
black & tarry stools

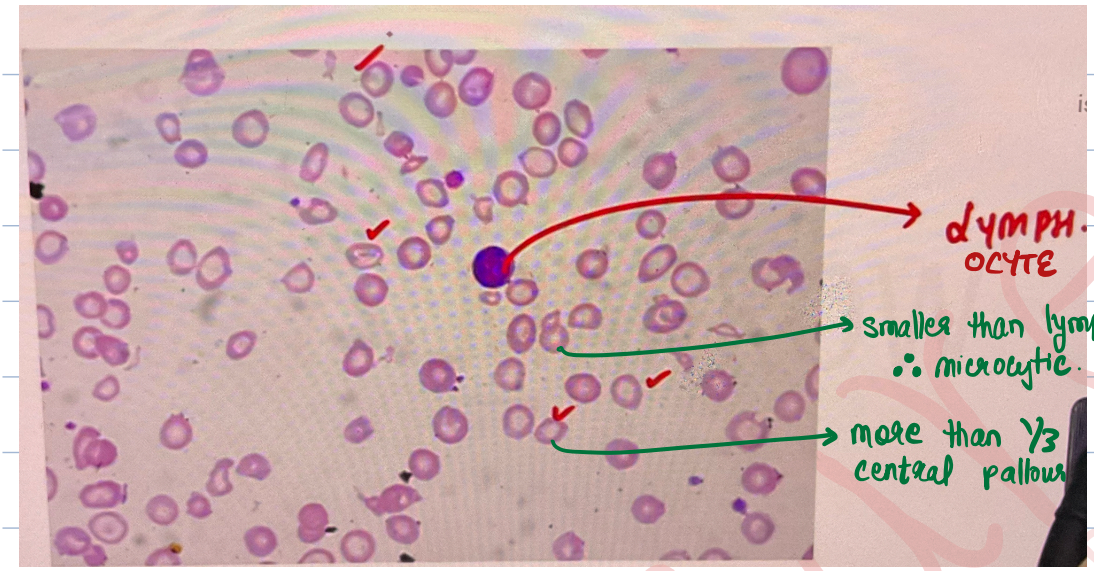
## Lab Diagnosis:

CBC
- decreased Hb
- decreased Rbc mass
- normal TLC
- normal to high platelet count [Reactive thrombocytosis]
- Reticulocyte count is normal / decreased.

MCV, MCH, MCHC } decrease.

RDW ⇒ increases. ⇒ marked.

P/s :
- Microcytic hypochromic red cells
- Pencil shaped cells, elliptocytes
- Anisopoikilocytosis




## Fe Studies :

→ Serum $Fe^{2+}$ : decreased  N = [< 15 mcg/L]
→ Serum ferritin : decrease → sensitive test
→ Serum total iron binding capacity : increases
→ transferrin saturation ratio : decreases.
→ Free erythrocyte protoporphyrin : increases

**Bone Marrow Iron** : Gold standard test for Fe deficiency anemia.
→ **Stain** : Prussian blue / Perl's stain ⇒ show's decreased iron stores.
→ In case of IDA ⇒ decrease in stainable Fe

3 DD :
- Thalassemia major
- Anemia of chronic disorders
- Sideroblastic anemia.

## Sensitive Tests for IDA:

- STFR_c Assay / Log ferritin > 1·5 ⇒ IDA  < 1·1 ⇒ AoCD
- STFR_c Assay
- Serum ferritin.

## 3 stages of IDA:

**Stage I**: stage of decreased storage
→ decreased serum ferritin.
↳ earliest indicator for diagnosis.

**Stage II**: Stage of Fe deficient erythropoiesis

**Stage III**: Stage of Fe deficient anemia
→ P/S findings observable

## Treatment:

Iron Therapy
→ Retic count starts increasing in 5-7 days of Fe therapy.

**Mentezer Index (MI):** $= \frac{MCV}{RBC\ count}$

**Significance:**

MI > 13 ⇒ IDA

MI < 13 → Thalassemia.

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# Anemia of Chronic Diseases : [AoCD]

## Causes:

- Chronic Infection
  - Bronchitis
- Chronic Inflammation
  - Rheumatoid arthritis
  - SLE
- chronic neoplasm
  - Hodgkin's lymphoma
- Idiopathic

## Pathogenesis of AoCD:

Chronic infection

↓

IL-1, IL-6 → most important

↓


↓

Increased Hepcidin synthesis

↓

Ferroportin

↓

decreased release of Fe

↓

Microcytic hypochromic anemia

CFU erythroid

↓

decreased RBCs

↓

Normocytic normochromic anemia

## Lab diagnosis:

- Hb ↓
- TLC
- Platelet count

} normal

- MCV
- MCH
- MCHC

} normal or decrease

P/S: – normocytic normochaemic usually
– sometimes microcytic hypochaemic

- ESR : increases

## Fe Profile:

→ Serum Iron: low
→ Serum ferritin: increased
→ Serum total Fe binding capacity: decreased.

$$\frac{\text{STFR}_c \text{ Assay}}{\text{Log Serum ferritin}} < 1.5 \Rightarrow \text{AOCD.}$$

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# Sideroblastic Anemia : (SA)

→ Fe overload (but this Fe cannot be utilised for Hb synthesis)

→ excess iron in the immature precursors.

## Causes:

### Genetic

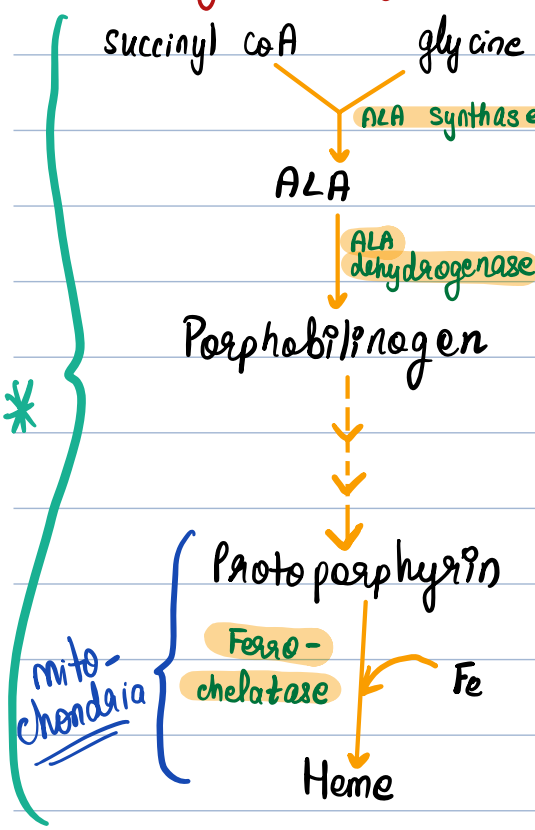
- enzyme deficiency
- X-linked SA

### Acquired

- Alcohol *
- ATD like Isoniazid
- vit. B6 deficiency

## Pathogenesis of SA:

* ∵ it is a mitochondrial poison




⇒ 3 enzymes

* vit. B6 required for this pathway

{ Iron accumulates in the mitochondria of immature precursors.

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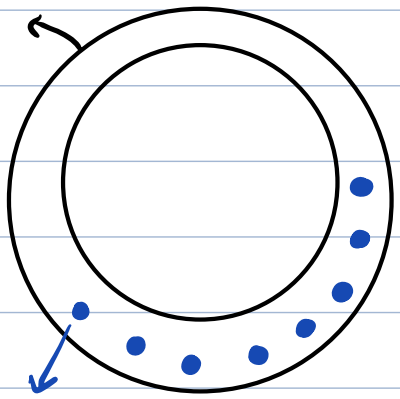



⟹ **RINGED SIDEROBLAST** ⟹ Seen in bone marrow aspirate

↓

Presence of ≥ 5 Fe granules in perinuclear location & covering ≥ 1/3 of the nucleus

P/S: Pappenheimer bodies (Fe in mature RBCs)

- Hb: ↓
- TLC
- Platelet count } normal
- MCV
- MCH
- MCHC } decrease

- **BASOPHILIC STIPPLING:** ↓ coarse

**Fe profile:**

→ Serum iron: ↑

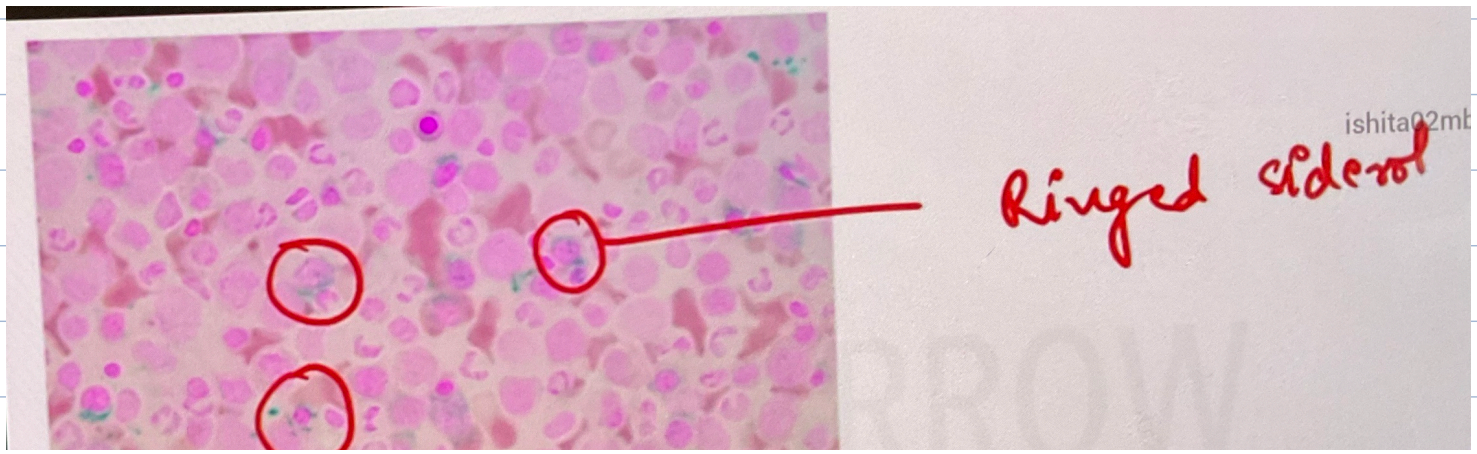
→ Serum ferritin: ↑

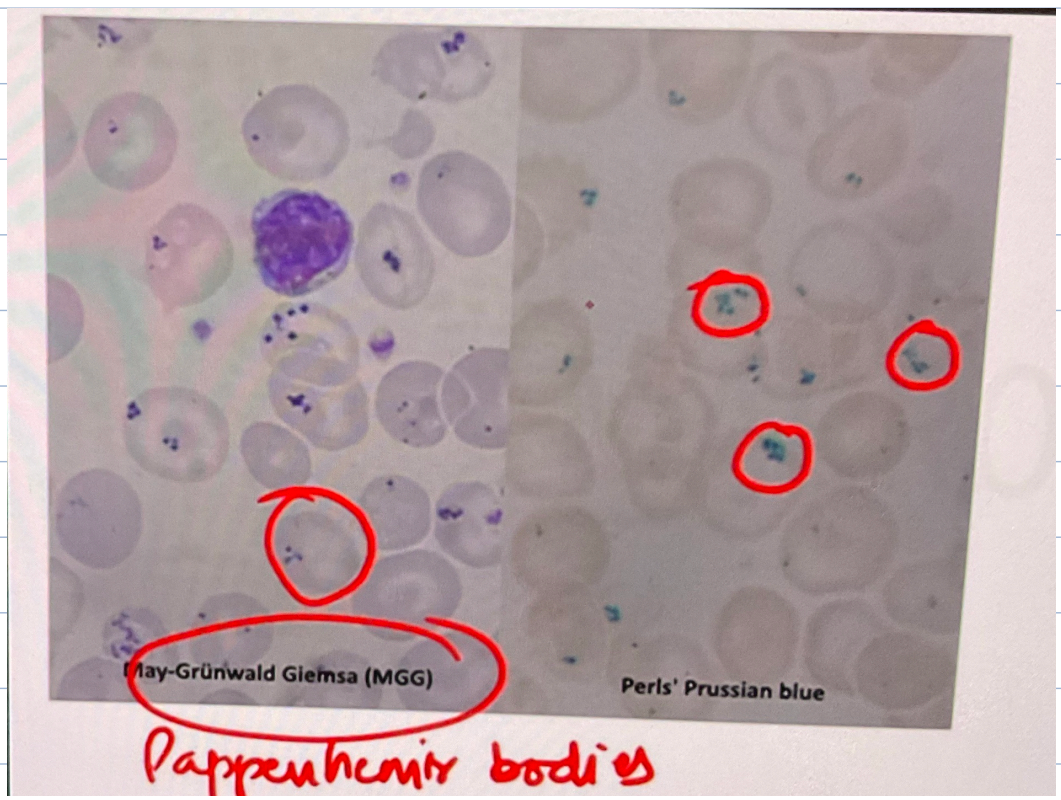
→ Total iron binding capacity: ↓

→ Transferrin saturation: ↑

## Treatment :

- Phlebotomy
- Iron chelators.



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# Megaloblastic Anemia :

- B12 deficiency
- Folate deficiency
- Pernicious anemia

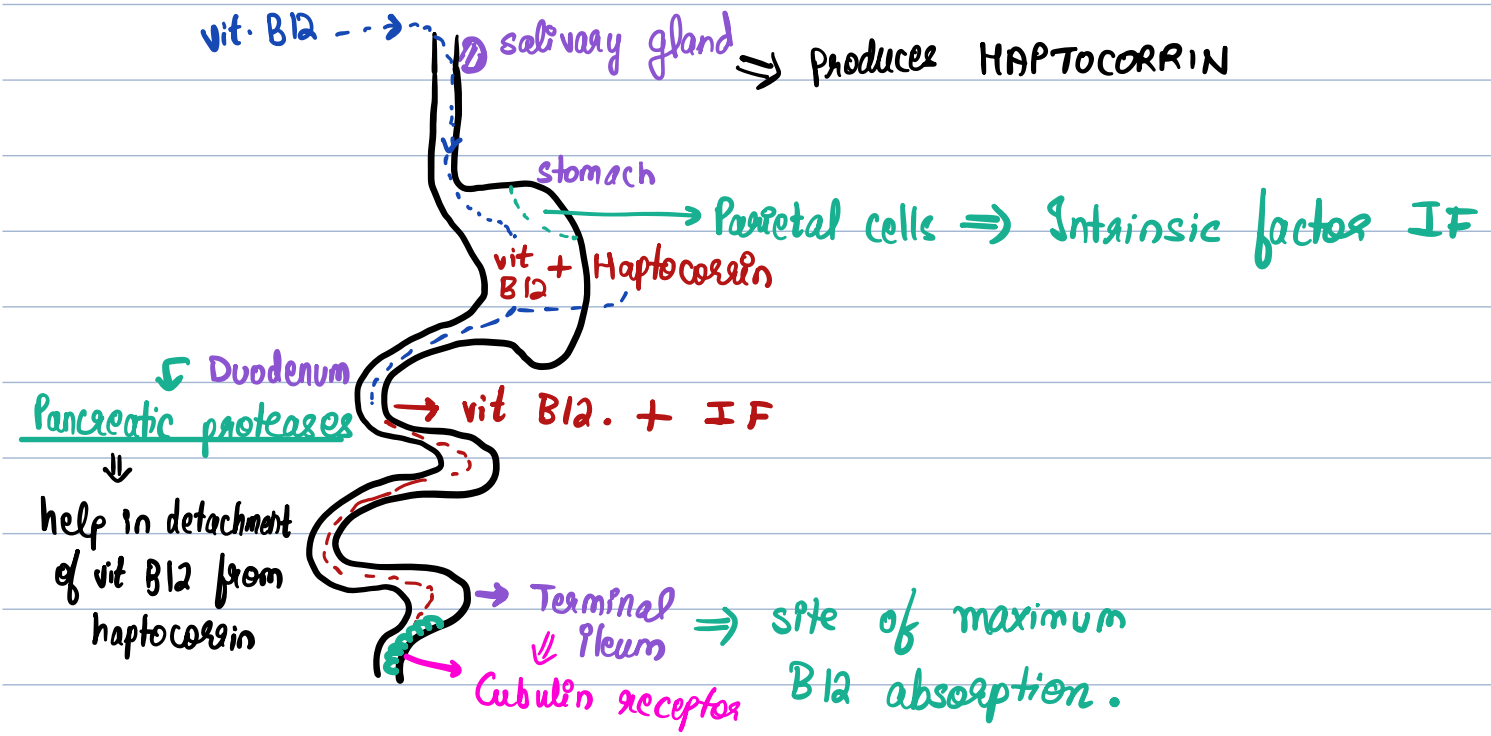
## B12 Deficiency: [Cyanocobalamin]

→ RDA = 2-3 µg

→ Sources: egg, meat, fish, milk

∴ B12 deficiency is very common in vegetarians.

## Mechanism of Absorption of vit. B12:




**Transport molecule:** Transcobalamin II .

# Causes of B 12 deficiency Anemia:

## Decreased Intake
- Low socioeconomic status
- vegetarian diet

## Increased Demand
- Pregnancy
- Lactation
- Puberty

## Impaired Absorption
- Pernicious Anemia
- Malabsorption syndromes
- Partial gastrectomy
- Pancreatic insufficiency
- Ileal resection.

# Biochemical Reactions Catalysed By Vit. B12:

(1) dUMP → (vit. B12) → dTMP

vit B12 deficiency: decreased thymine synthesis
↓
- nucleus does not mature
- cytoplasm continues to mature

↓
Nuclear-cytoplasmic asynchrony
↓
Maturation arrest
↓
Pancytopenia (RBCs, WBCs, platelets ⇒ all undergo maturation arrest)
↓
Ineffective hematopoiesis

(2) Homocysteine $\xrightarrow{\text{vit. B12}}$ Methionine

vitamin B12 deficiency ⇒ decreased methionine & increased homocysteine

↓

increased atherosclerosis & increased arterial thrombosis

(3) Methyl malonyl CoA $\xrightarrow{\text{vit. B12}}$ Succinyl CoA

vit. B12 deficiency ⇒ decreased succinyl CoA

↳ neuronal lipids, myelin sheath

↓

impaired myelinogenesis

↓

**NEUROLOGICAL COMPLICATIONS.**

# Clinical Presentation of Vit. B12 Deficiency :

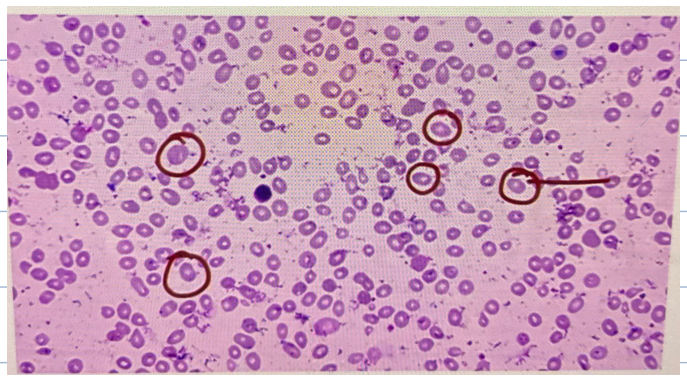
→ pallor
→ fatigue
→ spleenomegaly
→ jaundice
→ neurological complications – subacute combined degeneration of spinal cord

## Lab Diagnosis :

CBC
- Hb
- TLC
- Platelet count

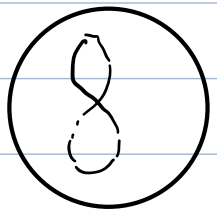
} decreased (pancytopenia)

- MCV ⇒ ↑
- MCH ⇒ ↑
- MCHC ⇒ normal

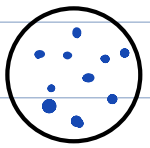


P/S :- Macroovalocytes → earliest finding
- decreased / loss of central pallor

- CABOT rings :
  - formed by microtubules



- basophilic stippling (fine)

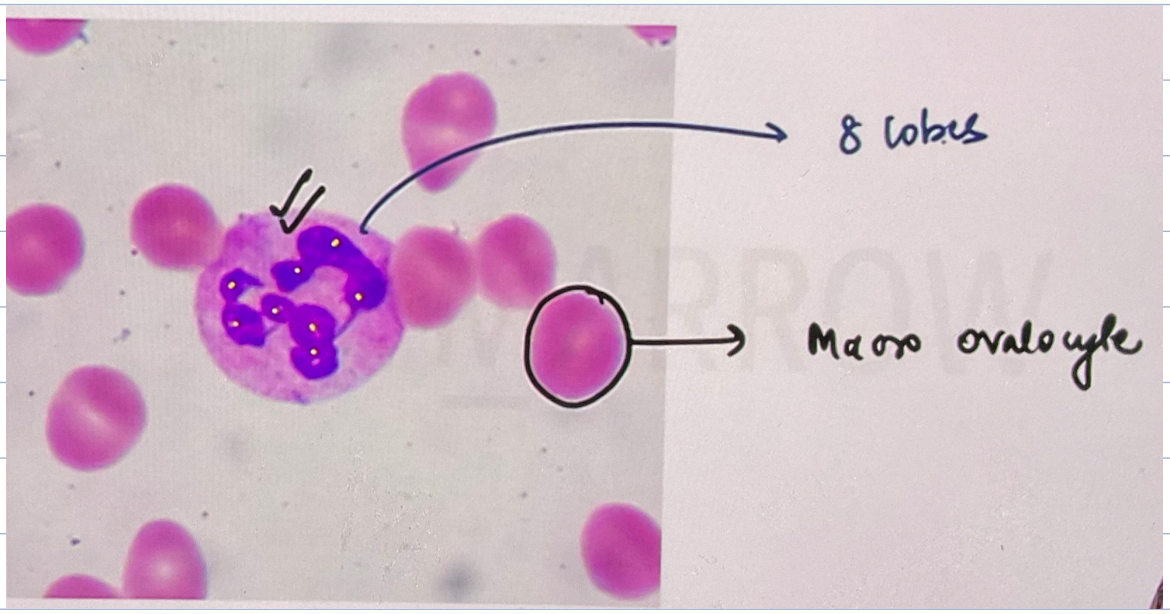


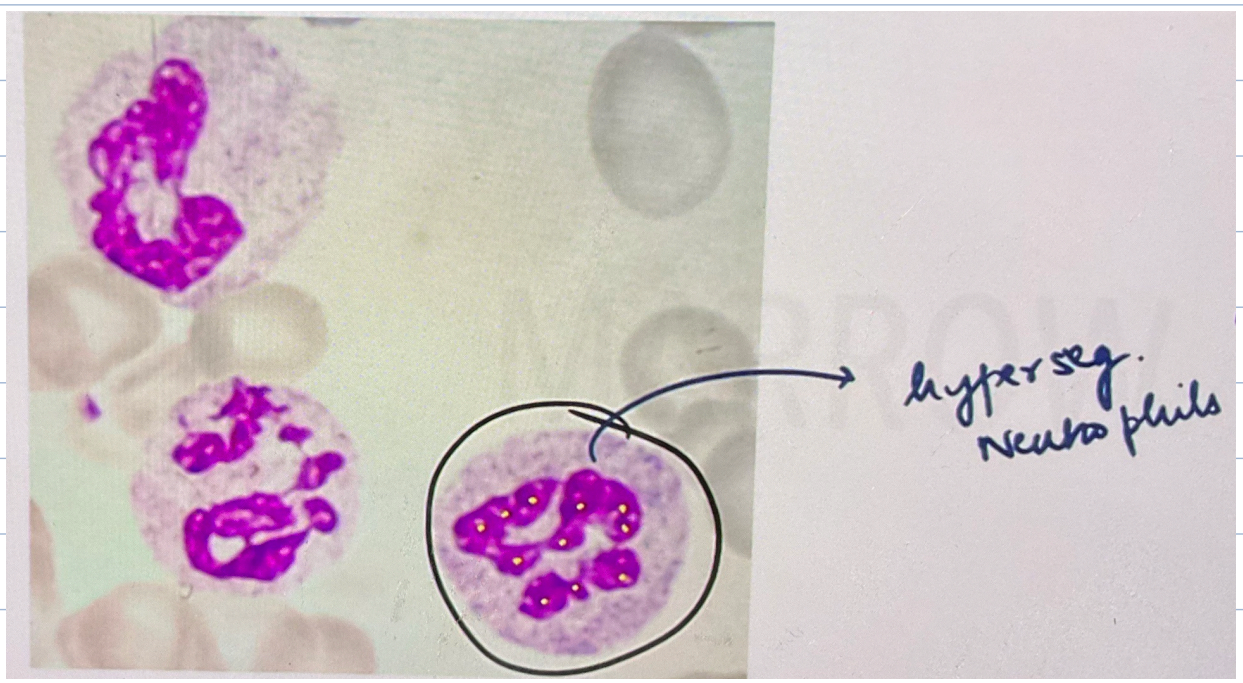
- HOWELL JOLLY BODY :→ also seen post-splenectomy
  remnant of nucleus

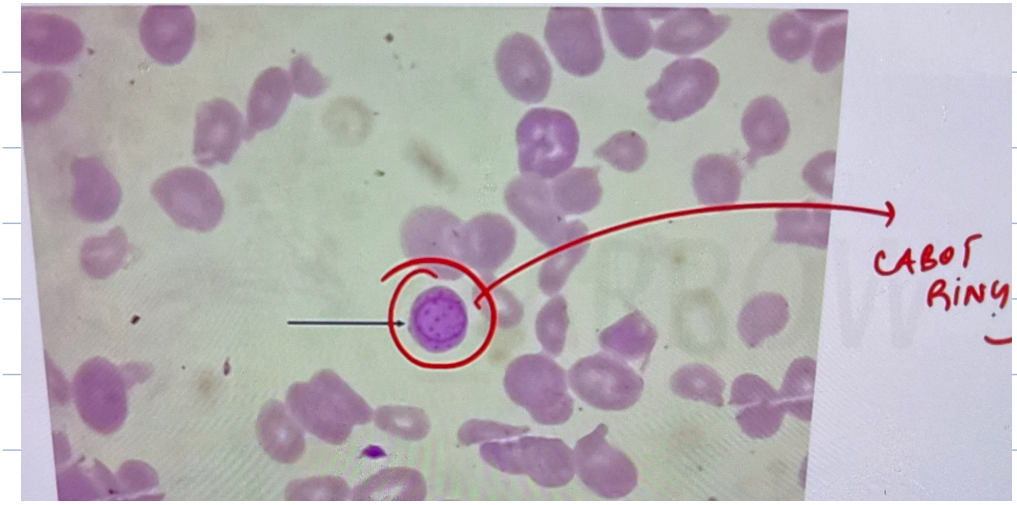
WBC: – hypersegmented neutrophil (≥5 lobes in nucleus)

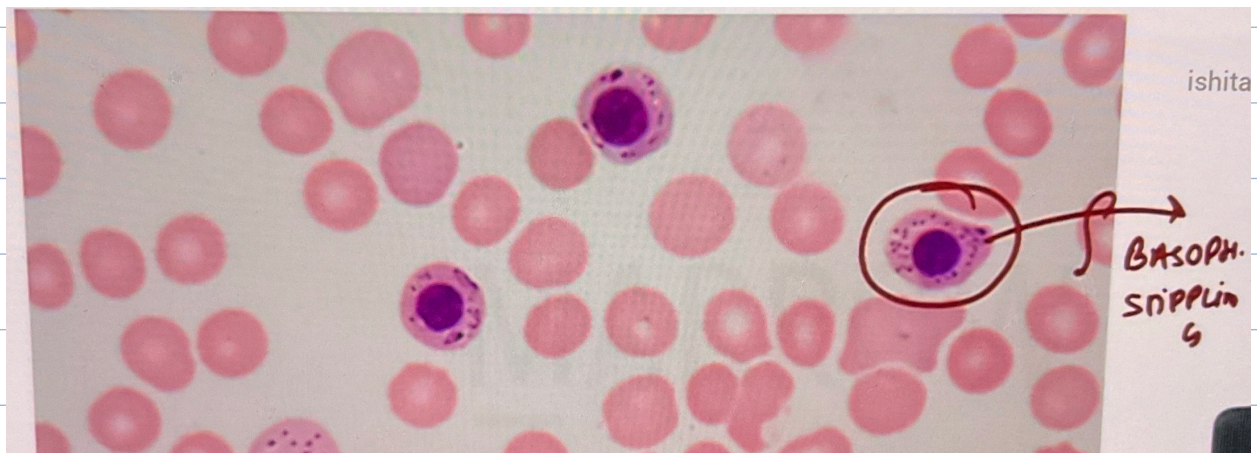
- more than 5% neutrophils with 5 or more lobes or a single neutrophil with 6 or more lobes.

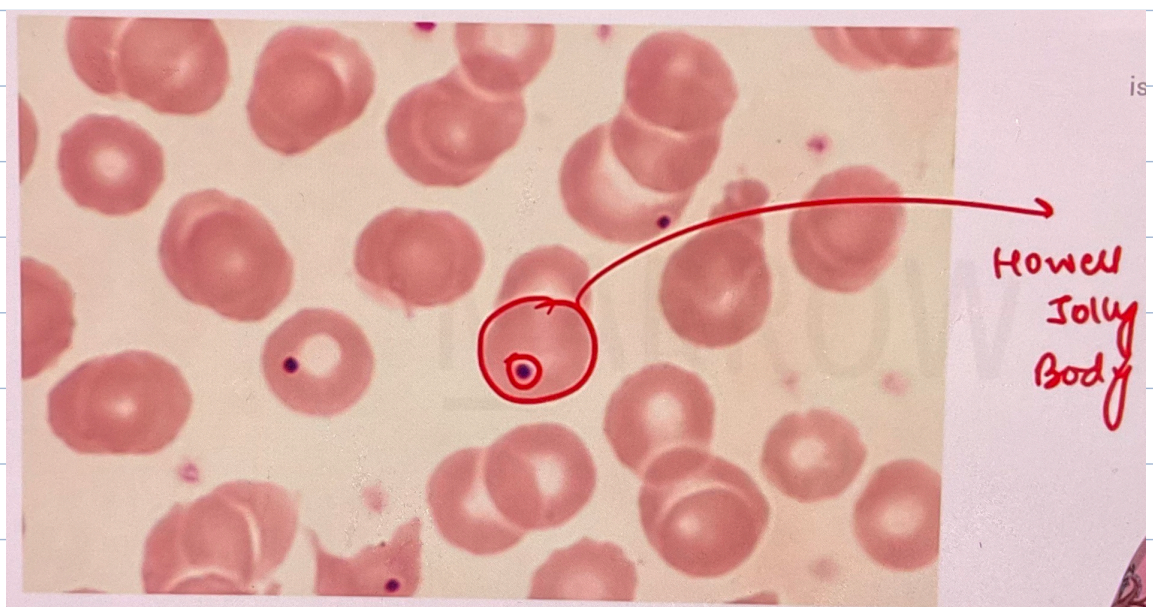
Reticulocytopenia.





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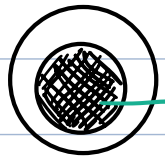
## Bone Marrow Aspirate:

- Erythroid hyperplasia
- Reversal of M:E ratio
- Large immature precursors
  - ↓
  - Megaloblast (erythroid precursor)



→ Sieve like (immature) Chromatin

- Giant metamyelocytes & band forms
- Giant megakaryocytes.

## Biochemical Investigations:

- Vit. B12 assay
- serum homocysteine
- serum methyl malonyl coA
- serum LDH

# Folate Deficiency Anemia:

→ Source: green leafy vegetables
→ Site of absorption: jejunum.
→ Alcoholics can show folate deficiency
→ No neurological complications.

# Pernicious Anemia:

→ it is a type II hypersensitive reaction (Antibody mediated)
→ autoimmune reaction

**Pathogenesis:**

- Antibody I: blocks binding of B12 to IF
- II: binding Ab as it blocks binding of B12-IF complex to ileal receptor
- III:
  - ↳ anti-parietall cell Ab.

**Clinical Presentation:**

→ pallor
→ fatigue
→ increased risk of other autoimmune diseases
→ " " " gastric adenocarcinoma
→ Beefy tongue
→ Atrophic glossitis.
→ fundic gland atrophy.

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# Schilling's Test: [obsolete]

→ purpose: to find the cause of B12 deficiency anemia

→ give radiolabelled vit. B12

- < 8% excretion in 24 hrs ⇓ B-12 deficiency
  - CAUSE:
    - malabsorption
    - Pernicious Anemia
  - ↓ Radiolabelled vit. B12 + IF
    - excretion improves ⇓ IF deficiency
    - excretion remains same ⇓ Malabsorption
- > 8% excretion in 24 hrs ⇓ NORMAL

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# Hemolytic Anemia (HA):

- SCA
- hereditary spherocytosis [HS]
- G6PD deficiency
- Paroxysmal nocturnal Hb. uria (PNH)
- Thalassemia
- AIHA

→ anemia caused due to excessive destruction of RBCs

## HEMOLYTIC ANEMIA

### Intracorpsculsr Defects

#### Hereditary

- MEMBRANE DEFECTS
  - hereditary spherocytosis
  - hereditary elliptocytosis
- ENZYME DEFICIENCY
  - G6PD deficiency
  - Pyruvate kinase deficiency
  - Hexokinase deficiency
- HEMOGLOBINOPATHIES
  - Sickle cell anemia
  - Thalassemia

#### Acquired

- PNH (only acquired intra-corpuscular defect)

### Extracorpuscular Defects

#### Immune mediated

- Autoimmune hemolytic anemia (AIHA)
- Alloimmune hemolytic anemia
  - Hemolytic transfusion reaction
  - Hemolytic disease of newborn
- Drug induced

#### Non-Immune mediated

- Infections like malaria

| Intravascular Hemolysis | Extravascular Hemolysis |
|---|---|
| → hemolysis inside blood vessels. | → hemolysis occurs outside a vessel. (eg: liver, spleen) |
| → absent usually | → splenomegaly / hepatomegaly are present usually |
| → Serum haptoglobin is reduced (haptoglobin in blood, binds to free Hb released due to RBC destruction in vessels) | → Serum haptoglobin is not usually decreased |
| → Hemoglobinuria + | → absent |
| → Hemosiderinuria + | → absent |

## Clinical Features of Hemolytic Anemia:

TRIAD:
- Pallor
- Jaundice → Increased unconjugated bilirubin (Pre-hepatic)
- Splenomegaly

→ chronic hemolysis leads to an increased risk of cholelithiasis. (pigment gall stones)

## Infections causing HA:

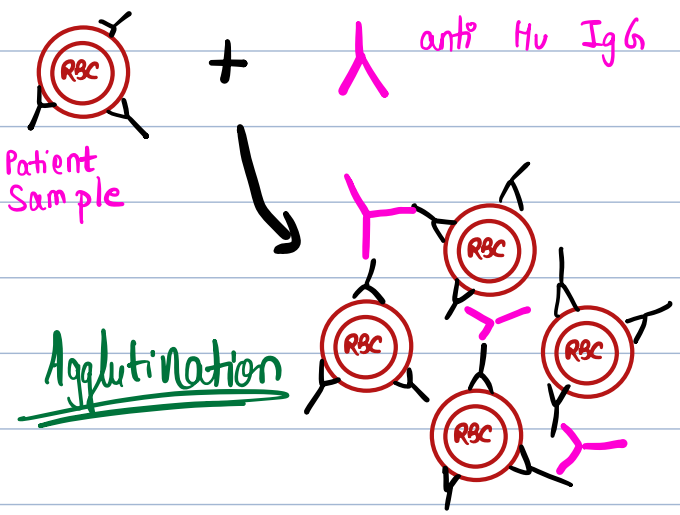
- Falciparum malaria
- Babesiosis
- Bartonella
- Meningococcal sepsis
- HIV
- other viruses
- Atypical mycobacteria
- Pneumococcal sepsis
- Snake, spider bites

# Lab Tests for Hemolytic Anemia:

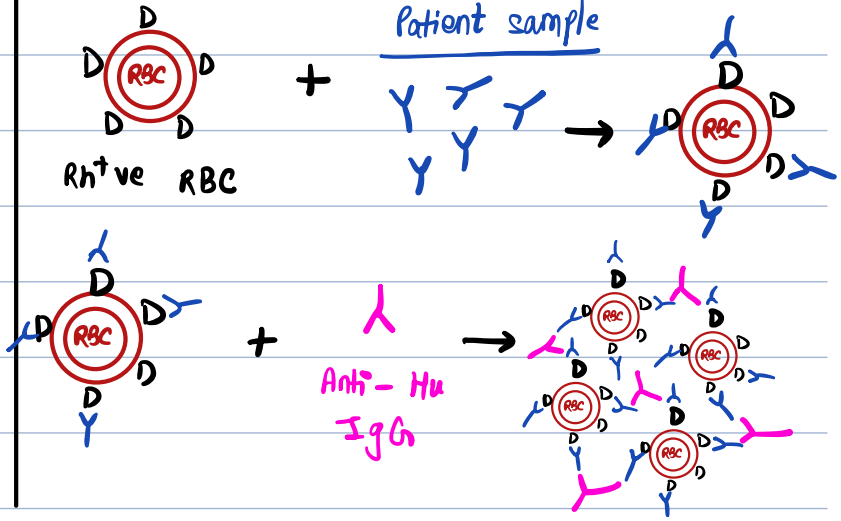
- Hb : ↓
- P/s → specific for that anemia
- LFT → deranged
  (increased unconjugated bilirubin)
- Retic count : ↑
- Serum Haptoglobin : ↓
- Hemoglobinuria
- Hemosiderinuria
- Serum LDH : ↑.

**Coomb's Serum:** serum from a rabbit or other animal previously immunised with purified human globulin to prepare antibodies directed against IgG & complement is used in the Coomb's Test
aka antihuman globulin

## Direct Coomb's Test




## Indirect Coomb's Test



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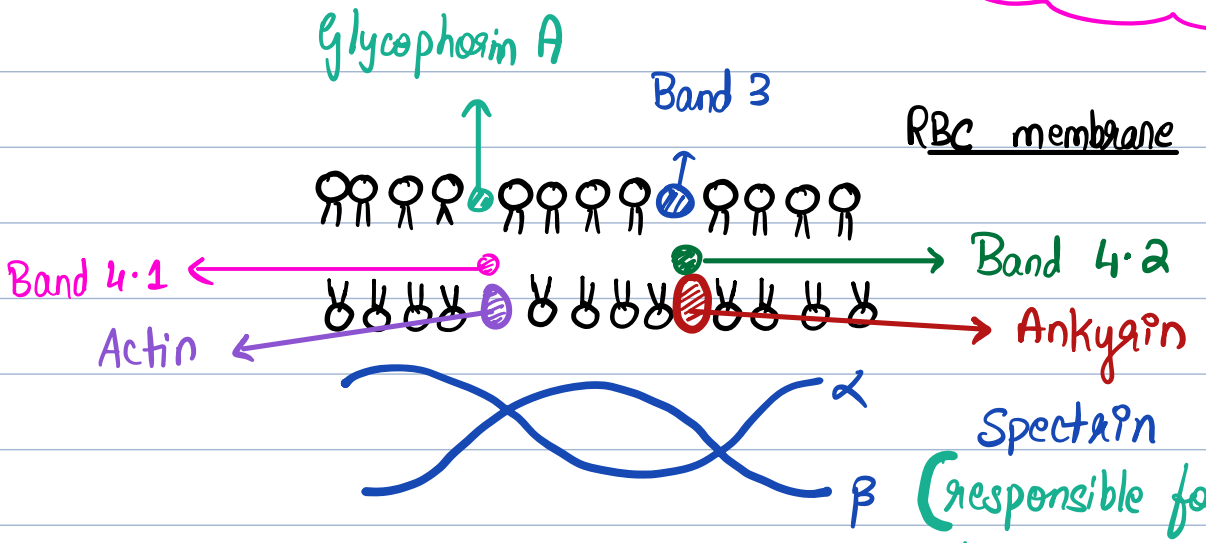
# Hereditary Spherocytosis (HS):

**Mode of Inheritance:** 75 % cases ⇒ autosomal dominant

**Pathogenesis:**

RBC life span = 10 - 20 days




Mutation of any of these proteins

↓

Unstable RBC membrane (Loss of RBC membrane)

↓

RBC tries to have minimum surface : volume ratio

↓

∴ RBC becomes spherical (with no central pallor)

↓

when it passes through the spleen, it is destroyed / trapped in splenic sinusoids

↓

∴ extravascular hemolysis

→ Increased MCHC in HS is due to loss of $K^+$ & water due to dehydration.

→ most important / common protein which is defective in HS: Ankyrin.

→ Spectrin mutations
- common in hereditary elliptocytosis
- produce most severe defects

→ protein which is not defective in HS: Glycophorin A.
↓
most abundant protein in RBC.

## Clinical Presentation:

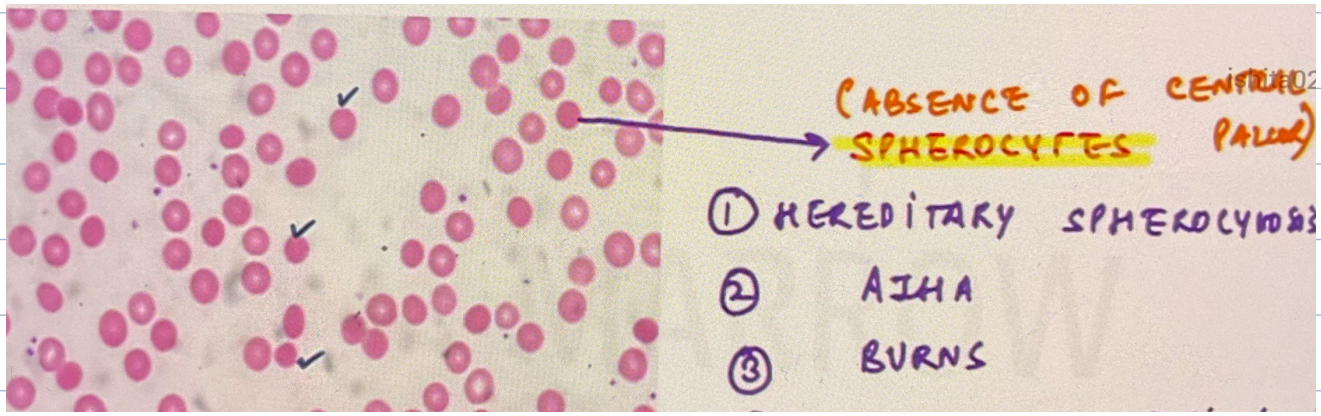
→ Pallor
→ Jaundice
→ Splenomegaly
→ Increased risk of cholelithiasis

### Aplastic Crisis
- Parvovirus B19 infection

### Hemolytic Crisis
- Epstein Barr Virus

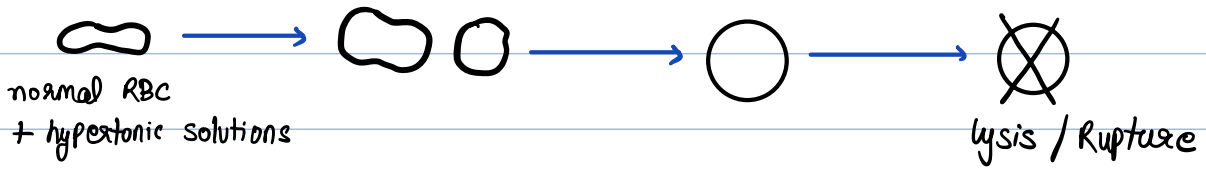
## Lab Tests:
- Hb: ↓
- MCV: ↓
- MCHC: ↑ (due to dehydration & loss of $K^+$ & water)
- RDW: ↑
- Reticulocytosis

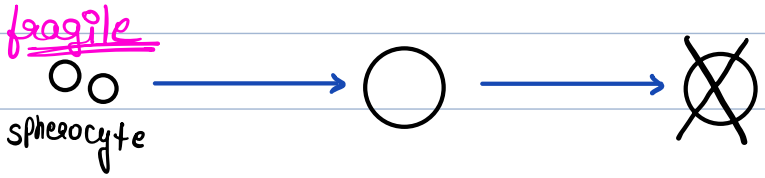
P/s:- spherocytes



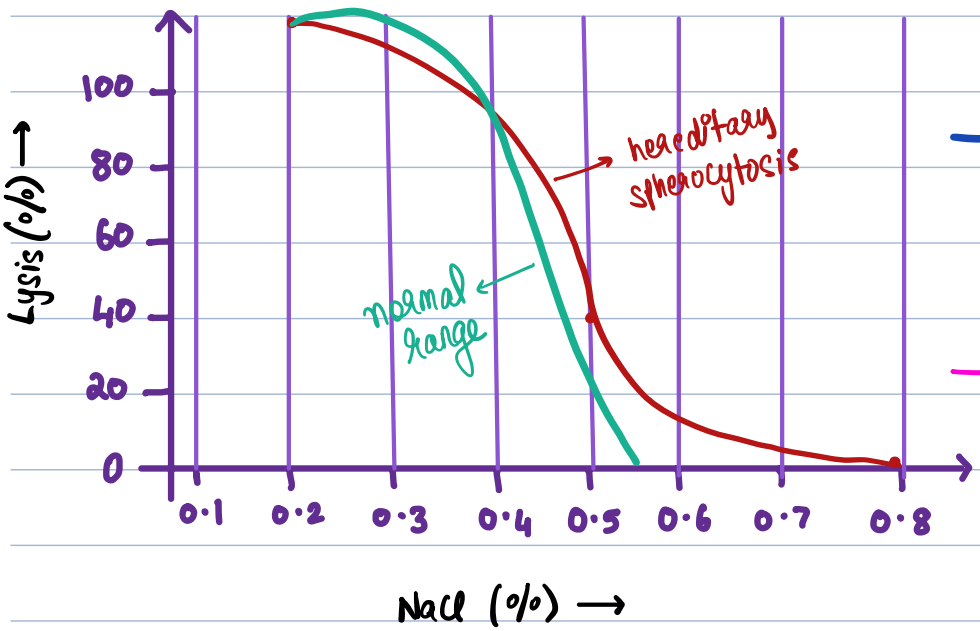

## Screening Test: Osmotic Fragility Test

PRINCIPLE: normal RBCs are isotonic with 0.9 % Nacl




at much lower Nacl concentrations




→ Normal RBC lysis starts at 0.5 % & completes by 0.2 % Nacl

→ In HS, lysis starts at 0.8 %

→ osmotic fragility curve shifts to right.

→ Osmotic fragility test curve shifts to the left in thalassemia.

Confirmatory Test: EMA binding test done by flow cytometry

Treatment: - splenectomy

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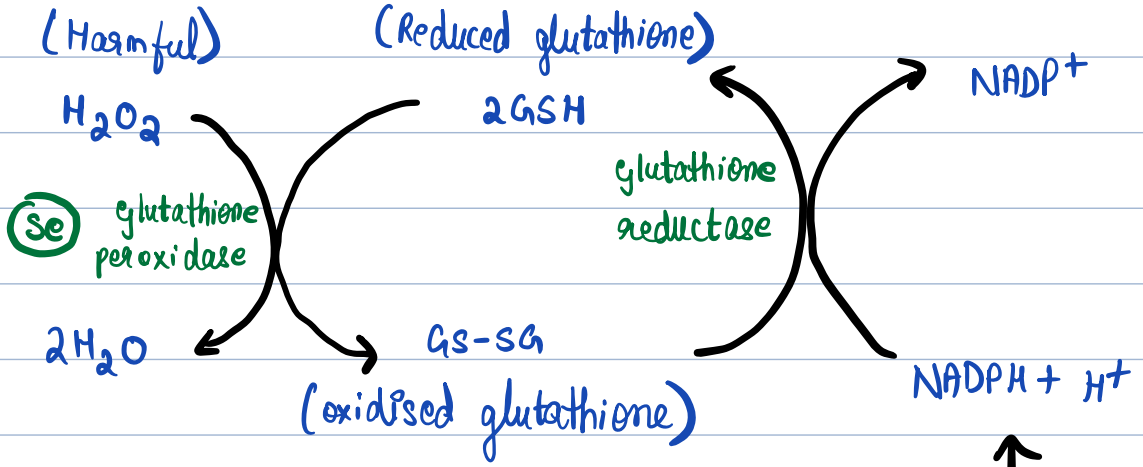
# G6PD Deficiency:

→ X - linked recessive inheritance
(male >>> female)

## Pathogenesis:

→ G6PD enzyme is used in Hexose monophosphate shunt (HMP shunt).

- Glucose 6-P —(Glucose 6-P dehydrogenase)→ 6-phospho-gluconate —(6-P-gluconate dehydrogenase)→ Ribulose 5-P




G6PD deficiency

↓

increased $H_2O_2$ in a cell

↓

oxidative stress

↓

RBC lysis when there is oxidative stress

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# Conditions Causing Hemolysis in G6PD Deficiency:

## Chronic Infections
- Pneumonia

## Drugs
- Antimalarials
- Primaquine

## Fava beans
from Viccia faba
(Favism)

→ G6PDD is more common in people of African or Mediterranean decent.

→ G6PDD provides protection against Plasmodium falciparum.

oxidative stress

- intravascular hemolysis
- cross-linking of SH groups in Hb
  - denaturation of Hb
    - HEINZ BODIES in RBCs (denatured precipitates of Hb)
      - pass through spleen
        - splenic macrophages try to pluck the heinz bodies
          - membrane loss
            - Bite cells
              - extravascular hemolysis

## Clinical Presentation:

- Pallor
- Jaundice
- Hemoglobinuria

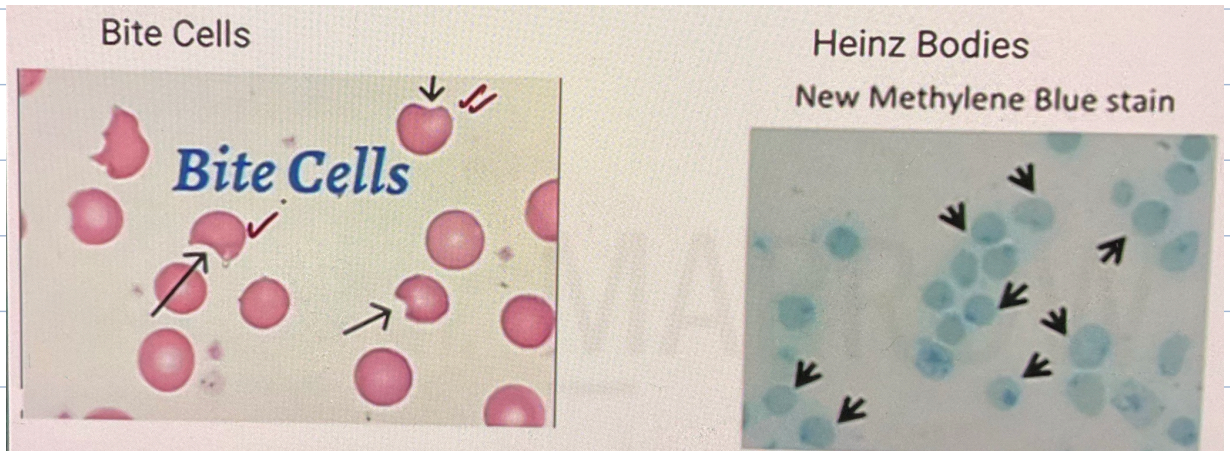
} only when there is a cause for oxidative stress ∴ episodic / intermittent

- no splenomegaly or gall stones (features of chronic hemolysis)

## Lab Tests:

- Hb : ↓
- Reticulocytosis
- Unconjugated bilirubin : ↑

P/S: – Bite cells / Degmacytes
– Heinz bodies (not seen on Romanowsky stain)
↳ seen on supravital staining



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- Methemoglobin Reduction Test
- G6PD enzyme assay

[hemolysis occurs more in the older RBCs than the newer ones]

Treatment: avoid oxidative stress.

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# PNH:

→ only acquired intracorpuscular defect

→ defect at the level of stem cells.

## Pathogenesis:

Normal

Phosphatidy inositol glycan A → PIGA gene → GPI anchor

most common/important protein defective in PNH: CD 59 (MIRL)

↓

synthesis of GPI anchored proteins

complement regulatory proteins:
- CD 55 (DAF - decay accelerating factor)
- CD 59 (MIRL - membrane inhibitor of reactive lysis)
- C8 binding proteins

↓

decrease activity of complement

PNH

↓

mutation of PIGA

↓

no GPI anchors

↓

no synthesis of GPI anchored proteins

↓

* ← increased complement activity → complement mediated hemolysis → intravascular hemolysis

* activates endothelium → Thrombosis

## Clinical Presentation:

- Pancytopenia
- Nocturnal hemoglobinuria [seen only in 25 % cases]

sleep
↓
↓ pH
↓
↑ complement activity ⟶ ↑ hemolysis

- Thrombosis (most common cause of disease related death in PNH)

## Complications:

- AML
- MDS
- Aplastic anemia

## Lab Tests:

- Hb
- TLC
- Platelet count

↓

P/S:- normocytic normochromic anemia
- pancytopenia

- Reticulocytosis
- Increased unconjugated bilirubin (sometimes)

- Ham's Test [Acidified Serum Lysis Test]
- Sucrose lysis Test

BEST TEST : flow cytometaric evaluation of CD55 and/or CD59.

Treatment: – stem cell transplantation
– Eculizumab
↳ this drug is a complement inhibitor

Sickle Cell Anemia
Thalassemia
} HEMOGLOBINOPATHIES.

→ Normal Adults
- HbA : $\alpha_2\beta_2$ → 95-97%
- HbF : $\alpha_2\gamma_2$ → < 1%
- $HbA_2$ : $\alpha_2\delta_2$ → 2-3.5%

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# Sickle Cell Anemia : [SCA]

→ autosomal recessive
[male = female]

→ protects against malarial parasite Plasmodium falciparum

→ more common in African or Mediterranean people.

**Pathogenesis:** caused by missense point mutation in which glutamic acid is replaced by valine at the 6th position of β chain of hemoglobin.

glutamic acid → -vely charged

valine

- HbA is replaced by HbS

## SCA

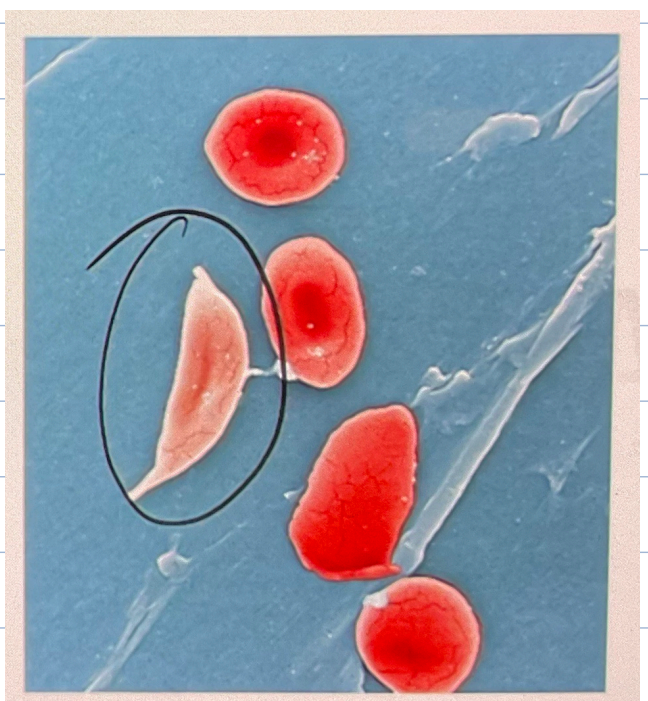
### Sickle cell Trait

- HbAS
- HbA - 60%
  HbS - 40%

### Sickle cell disease

- HbSS
- > 90% HbS

Sickle cells [3 S]
- Stiff
- Sticky
- Solubility decreased



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Normal RBCs

↓

Hypoxia

↓

Sickle RBCs

↓

Restoration of oxygen

↓

Desickling

↓

Repeated cycles of sickling, desickling & resickling.

↓

Formation of Sickle cell polymer

↓

Polymers pass through spleen

↓

trapped in splenic sinusoids

↓

extravascular hemolysis

[Splenomegaly]

Enter a blood vessel

↓

Gets attached to maggins of lumen of vessels

↓

Microvascular occlusions

(most common complication of SCA)

Osmotic Fragility Test

↓

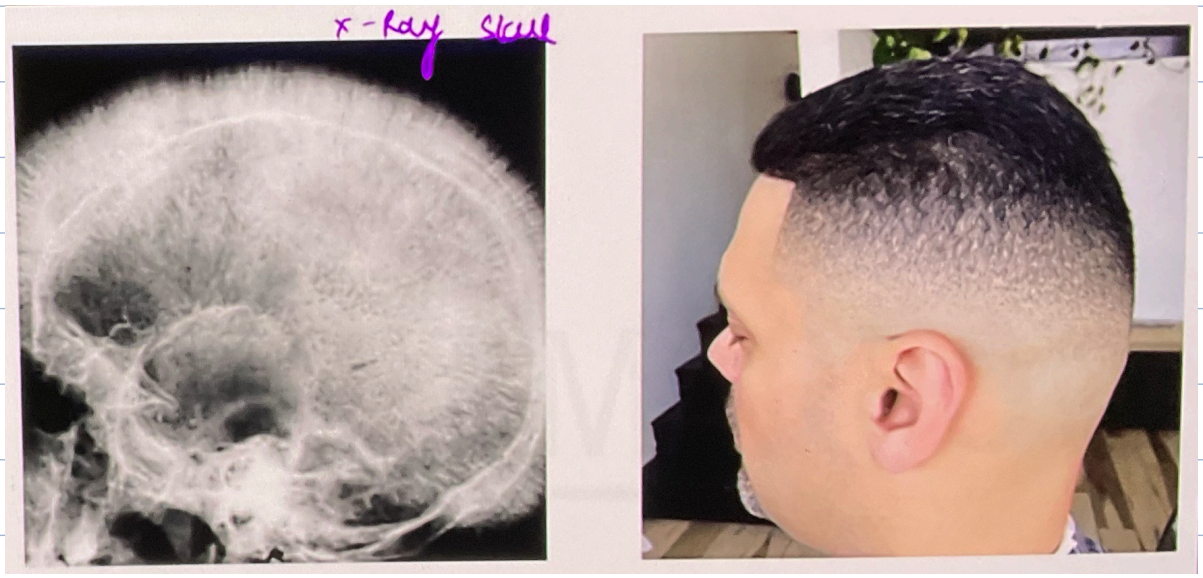
curve shifts to the left

(also in thalassemia)

| Factors which increase Sickling | Factors which decrease sickling |
| --- | --- |
| • Hypoxia<br>• Dehydration<br>• Increased MCHC<br>• Decreased pH / acidosis | • HbF<br>• clinical manifestation of SCA do not manifest until 6 months of age<br>• treatment of SCA is also hydroxyurea which increases the level of HbF |

## Clinical Presentation:

- Pallor
- Jaundice
- Splenomegaly —(later)→ autosplenectomy (once a period of time, all sickle cell polymers get deposited in the spleen which produces many infarcts causing shrinkage)
- X-ray Skull: Crew-cut / Hair-on-end appearance (due to extramedullary hematopoiesis occurring in skull bone)
  - also seen in thalassemia



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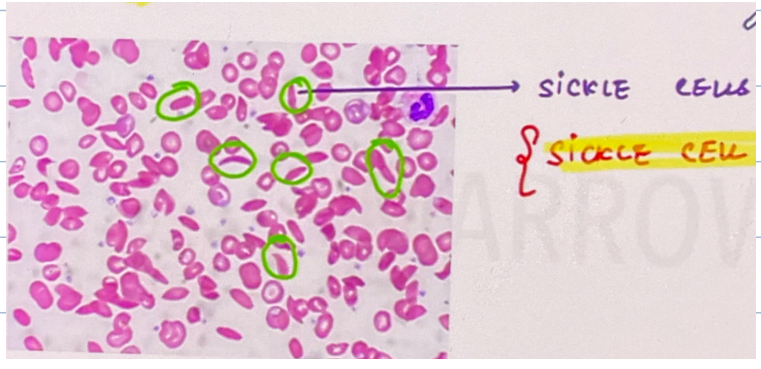
## Complications:

- **Vaso occlusive Crisis**: due to microvascular occlusion (most common crisis in SCA)
  - → Brain: Stroke / TIA (transient ischemic attacks)
  - → Bones: dactylitis, fish mouth vertebrae
  - → Lung: Acute chest syndrome
  - → Priapism
- **Aplastic Crisis**: due to Parvovirus B19.
- **Sequestration Crisis**: spleen sequestered with blood
- **Hemolytic Crisis**: Epstein Barr Virus.

### Lab Tests:

- Hb: ↓
- Reticulocytosis
- Unconjugated bilirubin: ↑
- ESR: ↓




- P/S: — sickle cells

- **Sickling Test**:

0 % %

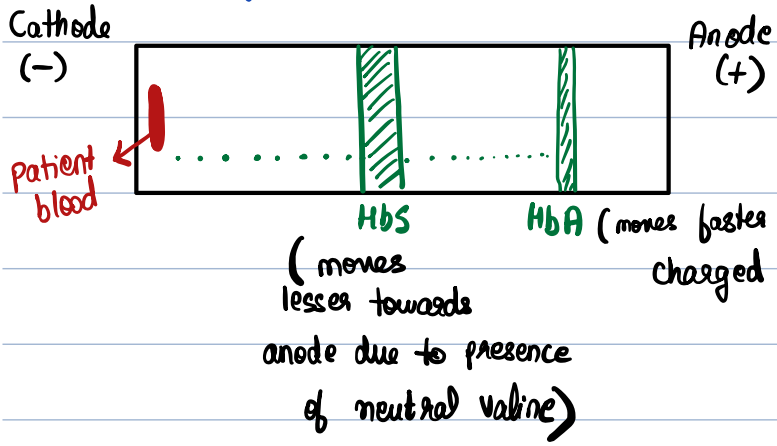
glass slide ↓ *

1 drop of patient's blood
+
1 drop of 2% Na metabisulphite / Na dithionate → oxygen-consuming agents ⇓ creates hypoxia

* ↓ 2hrs

Increase in sickle cells.

- Solubility Test
- Hemoglobin electrophoresis :

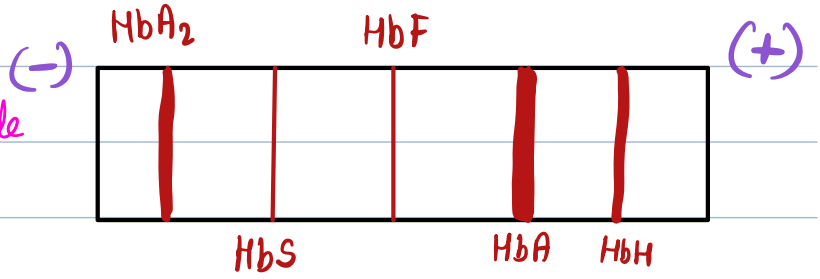


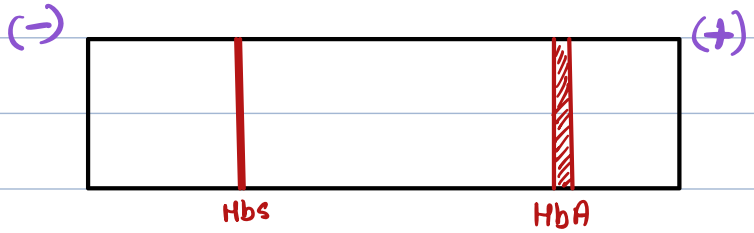

SCA

(moves lesser towards anode due to presence of neutral valine)

(moves faster towards anode due to negatively charged glutamic acid)

HAFSA ⇒ from anode to cathode




Sickle cell Trait

- electrophoresis cannot quantify the amounts of different Hb.
- at one site, multiple hemoglobins can occur
  - Hb $A_2$, C, E, O
  - Hb S, D, G, Lepore

disadvantages

- **HPLC**: Gold standard test for hemoglobinopathies
[high pressure/performance liquid chromatography]

→ % of various hemoglobins is accurately obtained directly.

**Treatment**: – stem cell transplant
– Hydroxyurea

# Thalassemia: [Thalassa = sea] → autosomal recessive

→ more common in regions around Mediterranean Sea

→ In India, common in Punjabis or Sindhis.

→ provides protection against Plasmodium falciparum

## β Thalassemia

- reduced synthesis of β chain
- more common
- gene for β chain synthesis on chromose 11
- most cases are due to mutation

## α Thalassemia

- reduced synthesis of α chain
- less common
- gene for α chain synthesis on chromosome 16
- most cases are due to gene deletion

# β- Thalassemia:

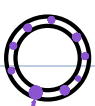
→ Splicing mutations ⇒ most common cause of β+ thalassemia
→ Chain terminator mutations ⇒ β° thalassemia (most common cause)
→ frame shift mutation
→ transcription mutation
→ 619 bp deletion

- **β thalassemia MAJOR** — $\beta^0/\beta^0$
- **β- thalassemia MINOR** $\beta^+/\beta$ — β-thalassemia Trait
- **β-thalassemia Intermedia** — $\beta^+/\beta^+$

- β ⇒ normal chain
- $\beta^+$ ⇒ partial deficiency of β chain
- $\beta^0$ ⇒ complete deficiency of β chain

## Pathogenesis:

reduced synthesis of β chain

- reduced $\alpha_2\beta_2$ → reduced HbA → microcytic hypochromic anemia
- increased $\alpha_2\gamma_2$ & $\alpha_2\delta_2$ → increased HbF, $HbA_2$
- α chain precipitates in the erythroid precursors
  - 
  - $\alpha_4$ tetramers
  - → destruction of erythroid precursors in bone marrow
  - → ineffective erythropoiesis

→ * Some erythroid precursors escape

↓

trapped in splenic sinusoids

↓

extravascular hemolysis

* → extramedullary hematopoiesis in skull

↓

Crew-cut / Hair-on-end appearance

* → erythroid hyperplasia

↓

increased Fe absorption

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## Clinical Presentation:

| Features | β Thal Major | β thal intermedia | β thal minor |
|---|---|---|---|
| | • markedly reduced synthesis of β chain | • moderately reduced synthesis | • synthesis reduced minorly |
| Clinically | - severe pallor<br>- Jaundice<br>- Hepatosplenomegaly<br>- h/o repeated blood transfusion<br>- chipmunk facies | - Pallor<br>- jaundice<br>- hepatosplenomegaly | - mild pallor<br>- asymptomatic<br>- no response to iron therapy |
| Hb | 3-5 gm% | 5-8 gm% | > 8gm % |
| P/s | - many target cells<br>- basophilic stippling<br>- cabot ring | - few target cells | - No target cells |
| RBC indices | -MCV<br>-MCH<br>-MCHC<br>↓<br>- RDW - (N) | -MCV<br>-MCH<br>-MCHC<br>↓<br>- RDW - (N) | Not much change |
| Iron profile | Iron increased | Normal | Normal |
| Hb electrophoresis | more increased HbF<br>Raised HbF | Both increased<br>Both | $HbA_2$: 3-5-9%<br>Raised $HbA_2$ |

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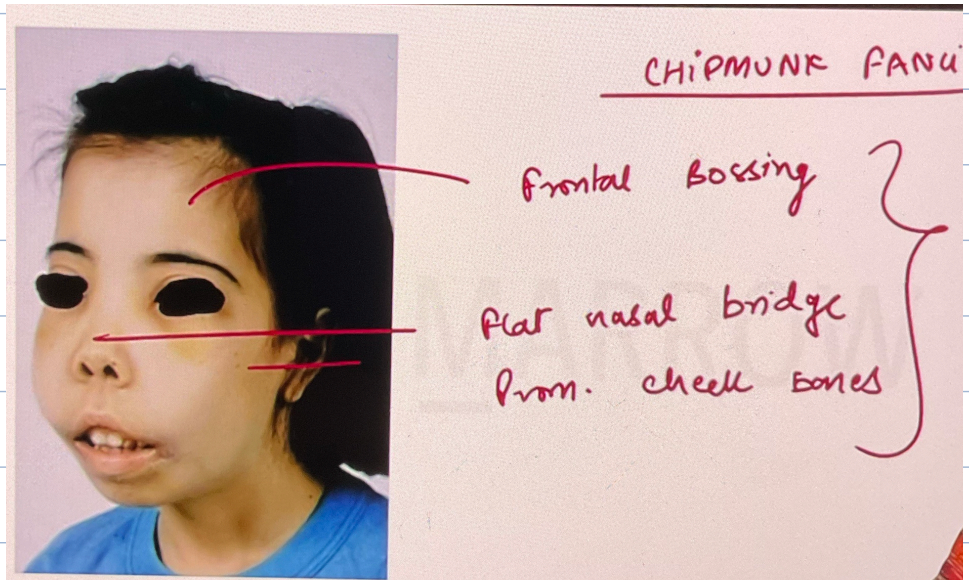



## Lab Tests :

**β- Thalassemia Major:**

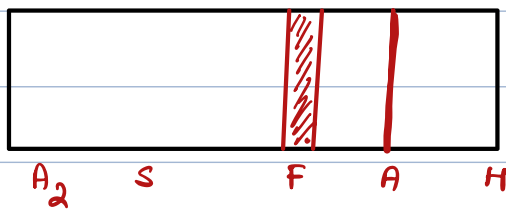
- Hb : ↓ (3-5 g%)
- MCV
- MCHC
- MCH

  } ↓

- RDW : normal

**P/S:**
- microcytic hypochromic red cells
- target cells / codocytes → rigid
- basophilic stippling
- HOWELL JOLLY BODIES

**Hb electrophoresis:**
- ↑ Hb F → rises most
- ↑ Hb $A_2$ → variable



**HPLC** (gold standard): ↑ HbF

→ Most common cause of death in β-thalassemia major: Iron overload → CHF

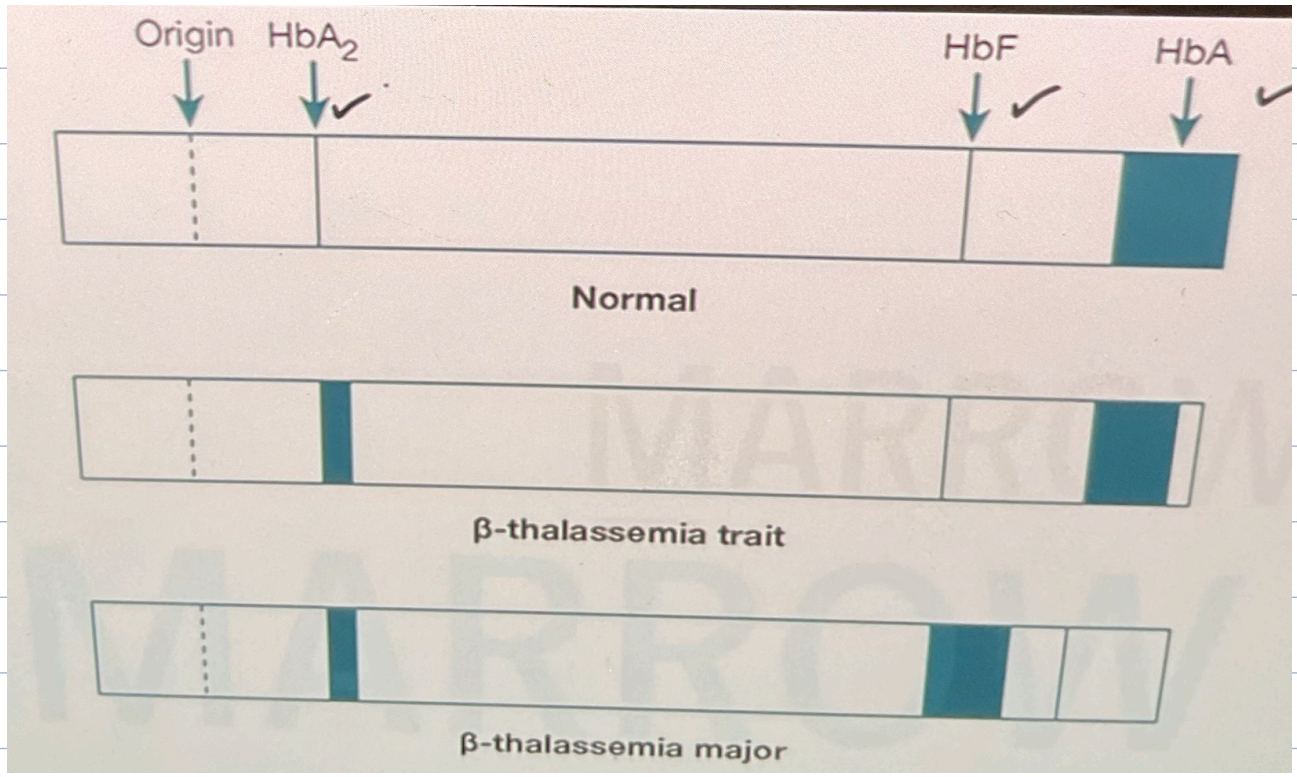
**β-Thalassemia Minor:**
- Hb ↓ (8-10 g%)
- MCV, MCH, MCHC } usually normal
- RDW: normal

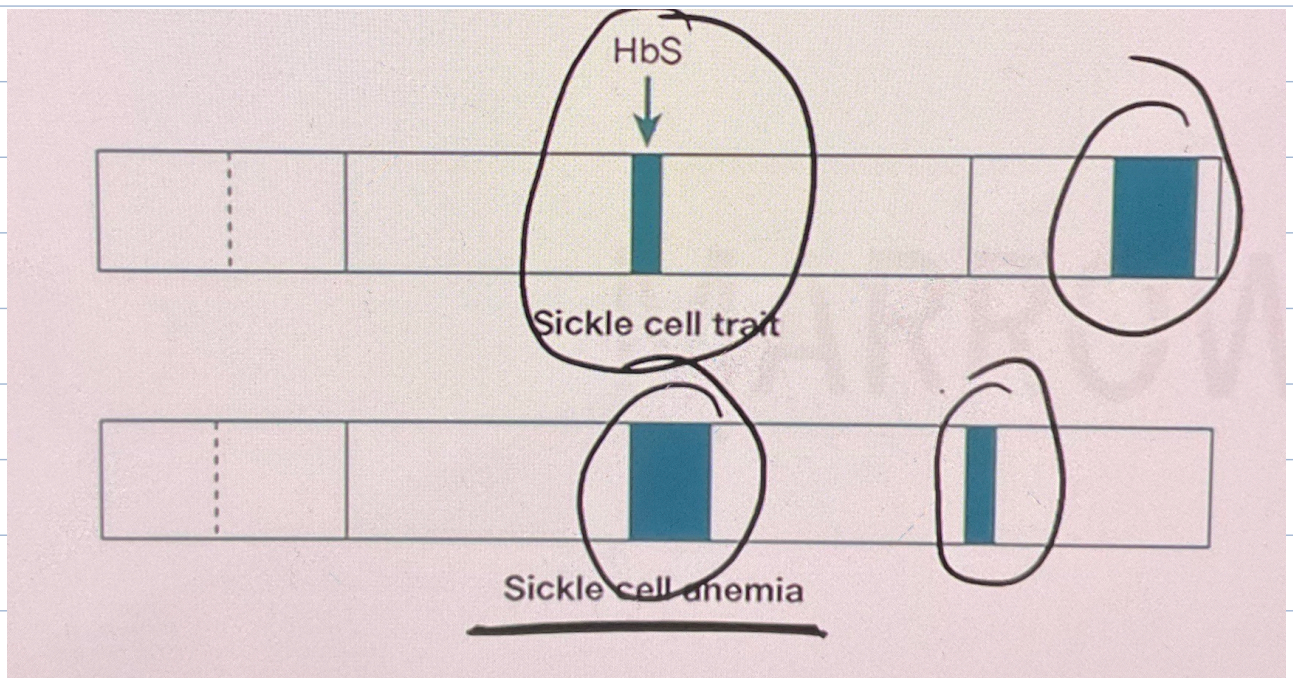
**P/S:**
- microcytic hypochromic red cells
- normocytic red cells
- few/no target cells

**Fe profile:** - normal

**Hb Electrophoresis:** $HbA_2$ ↑ (4-9 %) (→ normal = 2 to 3.5 %)
[• $HbA_2$ > 9% ⇒ HbE disease]

**HPLC:** ↑ $HbA_2$



## Screening Test for β-Thalassemia: NESTROF test

(Naked eye single tube red cell osmotic fragility test)

– curve shifts to the left

| Iron Deficiency Anemia | β Thalassemia Minor |
|---|---|
| • RDW: increases | • RDW: Normal |
| • Mentzer index > 13 | • Mentzer index < 13 |
| • HbA: normal | • $HbA_2$: 4–9% |
| • Iron profile: Abnormal | • Iron profile: Normal |

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# α Thalassemia :

## 1 α gene deletion

[αα/α-]

- silent carrier
- no manifestation

## 2 α gene deletions

(αα/--)
(α-/α-)

↓

- α thalassemia trait

## 3 α gene deletions

(α-/--)

↓

- HbH disease

↓

formation of β4 tetramers

↓

Golf ball inclusions

## 4 α gene deletions

(--/--)

↓

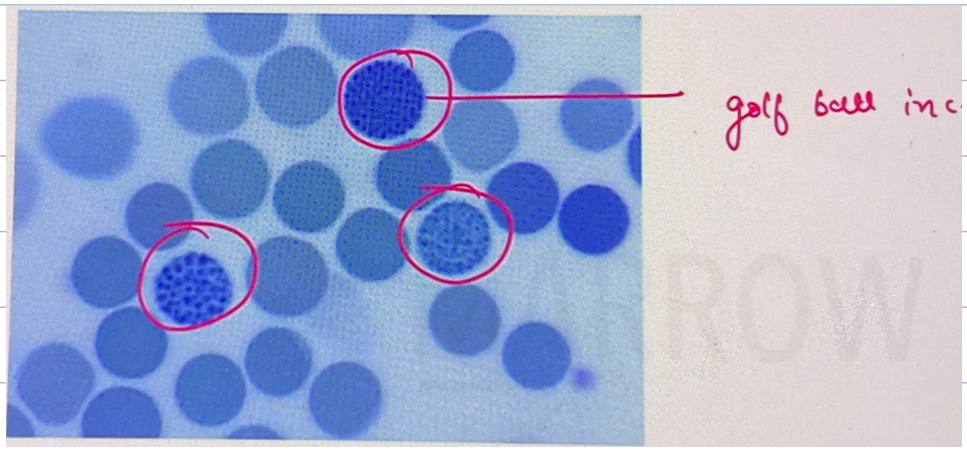
- Hb Bart's / hydrops fetalis

↓

formation of γ4 tetramers

↓

death



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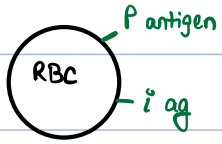
**AIHA**: Type II hypersensitivity reaction → +ve Coomb's Test
→ Ab against RBC membrane proteins

## Warm Ab AIHA

- more common
- IgG
- Ab is active at 37°c

**Causes:**
- idiopathic (most common)
- autoimmune disorders like SLE, RA
- CLL




IgG ab produced against P-ag of RBC
↓
Fc fragment of IgG is an opsonin
↓
RBC is destroyed
↓
Extravascular hemolysis

- Splenomegaly
- Hepatomegaly

## Cold Ab AIHA

- less common
- IgM
- Ab is active at 0 - 4°c

**Causes:**
- infections like mycoplasma
- EBV

### Cold agglutinin type

IgM
↓
activation of complement
↓
complement-mediated lysis

### Cold hemolysin type

Paroxysmal Cold Hb. uria [PCH]

D-L Ab (Donath Landsteiner Ab)
(biphasic Ab)
↓
both warm & cold type
↓
activation of complement
↓
lysis of RBC's

Intravascular hemolysis ∴ no organomegaly

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**P/S:** – spherocytes [AIHA is most common cause of spherocytes]
→ microspherocyte ⇒ loss of central pallor
– polychromasia

**Lab findings:**
– reticulocytosis
– jaundice
– direct Coomb's test is +ve
– blood film shows red cell autoagglutination

**Coomb's Test:** used to differentiate AIHA from HS.

## Alloimmune HA:

### Rh Incompatibility

Rh +ve father → Rh -ve mother carrying 1st Rh +ve fetus

Rh antigens from developing fetus can enter mother's blood during delivery

In response to fetal Rh antigens, mother will produce anti-Rh antibodies

If woman becomes pregnant with another Rh +ve fetus → mother's anti-Rh antibodies cross the placenta & cause agglutination & lysis of fetal RBCs

| Intravascular Hemolysis | Extravascular Hemolysis |
|---|---|
| • G6PD deficiency | • G6PD deficiency |
| • MAHA (microangiopathic hemolytic anemias) | • HS |
| • Prosthetic cardiac values | • SCA |
| • Mechanical disruption of RBCs | • Thalassemia |
| • Plasmodium falciparum malaria | • AIHA |
| • AIHA | • Drug induced HA |
| • Snake bites | • Liver disease |
| • Infections | • Infections |
| | • Toxins |

## Microangiopathic Hemolytic Anemia (MAHA):

- Hemolytic uremic syndrome (HUS)
- Thrombotic thrombocytopenic purpura (TTP)
- Disseminated intravascular coagulation (DIC).

## RED BLOOD CELL MORPHOLOGY

| Size variation | Hemoglobin distribution | Shape variation | | Inclusions | Red cell distribution |
|---|---|---|---|---|---|
| Normal | Hypochromia | Target cell | Acanthocyte | Pappenheimer bodies (siderotic granules) | Agglutination |
| Microcyte | 1+ | Spherocyte | Helmet cell (fragmented cell) | Cabot's ring | |
| Macrocyte | 2+ | Ovalocyte | Schistocyte (fragmented cell) | Basophilic stippling (coarse) | Rouleaux |
| Oval macrocyte | 3+ | Stomatocyte | Tear drop | Howell-Jolly | |
| Hypochromic macrocyte | 4+ | Sickle cell | Burr cell | Crystal formation: HbSC, HbC | |
| | Polychromasia | | | | |