Anaemia

Stem cells

↓

CFU-E

↓

Pro-erythroblast (proerythroblast)

(Proerythroblast)

↓

Normoblast

↓

Early Normoblast - Basophilic

↓

Intermediate - Polychromatophilic

↓

Late - Orthochromatic

↓

Reticulocytes - (non nucleated) — BM

(1-2 days) maturation ↓ PB

↓

RBC

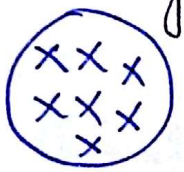
Time - 48 Hrs.

Hb Ist appears - EM, Special Stains

Hb detected - Routine stains

Nucleus Removed

1. Reticulocytes



- Reticulum like structures

Ribosomal RNA proteins

→ can be stained by Romanowsky stain (Routine stain)

1) Giemsa
2) Leishmann
3) Jenner stain
4) Wright stain

→ Supravital stain - Specific - staining live RBC

Best stain - New methylene Blue > Brilliant Cresyl Blue.

Use of Romanowsky stain -
- Reticulocytes
- Howell - Jolly Bodies
- Basophilic stippling
- Cabot Ring

Heinz Bodies - never stained by Romanowsky stain.
- methyl violet / Crystal violet stain.

Reticulocyte < Peripheral Blood
Bonemarrow.

(N) R - 0.5 to 1.5% ↑R - Hemolytic anaemia.
↓R - aplastic anaemia.
Nutritional anaemia (Fe, Vit B_{12})

In megaloblastic anaemia } mild Hemolysis, can also - Jaundice, splenomegaly, Nu-RBC

B.M: Hypercellular
M/E - Ratio Reversal (normal M/E - 3/1)
Erythroblastic Hyperplasia.

↓ B12 ⇕ folic acid → No DNA Synthesis → nonuclear maturation.
↓
Apoptosis (nucleated RBC)

Bone biopsy.

Infants - Anterior Tibia lateral part (Post. Ant Tibia medial site)

child/adults - Posterior iliac Crest

↓

Obesity - Anterior iliac Crest.

Hemolytic Anaemia

Hereditary Spherocytosis - AD > AR.
(1/15)

→ RBC membrane protein defect

a) Spectrin - m/c abundant; m/c mutation elliptocytosis

b) Ankyrin - m/c mutant in Spherocytosis

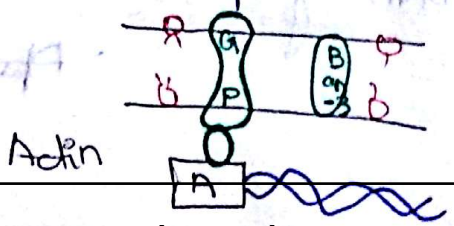
c) Band-3 (Anion Transport Protein)

d) Protein 4.2 - Palladin.

e) Glycophorin (not associated with HS)

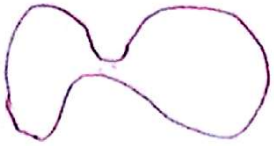
α-Spectrin mutation - Correlate Severity of HS.

RBC - Biconcave, → Spectrin - actin interaction



Integral protein → glycophorin > Band 3.

Glycophorin A > B > C > D.



RBC - 7.5 μm dia.

- Central pallor (N) - 1/3rd of diameter of RBC.

Hypochromic - CP - > 1/3 of dia of RBC.

Function - Provide flexibility/deformability to RBC.

↓

mutation

↓

No flexibility

↓

damaged membrane

↓

↓ Surface area

↓

micro Spherocytes (Smaller, no central pallor)

↓

Spleen (extravascular Hemolysis)

R - Splenectomy, But even after Splenectomy, Spherocytes persists

Hemolytic Anaemia:	HS	SCA
	mild to moderate	Severe.
→ Jaundice	+	+
→ pigmented gallstones.		

↓ Reticulocytes
Aplastic crisis } 5-7 days. (Transient)

Screening:- Pink Osmotic fragility Test.

(N) RBC $\xrightarrow{\text{Isotonic}}$ 0.9% NaCl.

Hemolysis start at → 0.5% (0.48%)

Hemolysis Completed → 0.3%

Hereditary Spherocytosis

Hemolysis → 0.5% (↑ osmotic fragility)

G6PD deficiency - XLR.

m/c a/w - Bacterial Infections

m/c Drug - primaquine > Dapsone.

→ Fava beans (favism)

malaria protective - P. falciparum

enzyme def
- G6PD deficiency
- Pyruvate kinase deficiency

Hb-pathies
- Thalassemia
- HbC
- Sickle cell (carrier)

Duffy negative
- DR + P. vivax
- NO DR

- Protective against P. vivax.

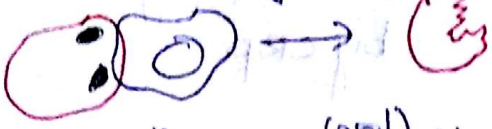
NADP ← G6PD → NADPH

↓

Reduced → glutathione.

No Reducing agent

Denatured globin chains → Heinz cells.

 → Bite cells.

3) Paroxysmal (225) Nocturnal (215) Hemoglobinuria.

Urine Sample - must be collected in → morning / 1st void / mid stream

→ 5-10% - Congenital.

→ Somatic mutation in stem cell - Phosphatidyl Inositol Glycosyl - A gene mutation coded by PIGA gene.

Stem cell → GPI+ Anchor protein.

↓

Complement Regulatory Protein

1) CD-59 - Membrane Inhibitor of Reactive lysis

2) CD-55 - Decay accelerating factor

3) - C8 Binding protein

M/Imutation: GPI-AP.

→ Amongst, complementary Regulatory protein - CD59.

PB - Pancytopenia + Hemolytic An + Thrombosis. - Triad

M/C Cause of death ~~Hepatic Vein thrombosis~~

M/c - Budd chiari Syndrome → PCV

Pathogenesis - Acidic pH
↓
Activate complement
↓
C5-9 (MAC) → generally RBC protected against MAC by CRP
↓
Damage to RBC
↓
Intravascular Hemolysis

Screening - HAMS test - m/I
Sucrose lysis Test.

Confirm → Flow Cytometry

PNH → Aplastic anaemia
↓
MDS → Acute Leukaemia

∴ Preleukaemic condition - PNH, MDS, aplastic anemia

Autoimmune Hemolytic Anaemia - AIHA

Warm
m/c Type
IgG1
m/c c/w CLL
AI disorders - SLE

Cold
Hemagglutinin
IgM
Syphilis, mycoplasma viral infections.

Hemolysin
IgG1
Donath Landsteiner Ab (against P-Ag of RBC)
PCH

m-type

Drugs- Penicillin
α-methyl Dopa

m/c - Spherocytosis *
↓
AIHA.

Cold type

IgM - Hemolysin - Paroxysmal cold Hemoglobinuria.
m/c associated with viral infection

IgG → P-Ag
RBC
↓
activate complement.
↓
M.A.C.
↓
Hemolysis

Coomb's Test

Direct

Ab in Surface of RBC

→ AIHA.

Drug induced.
- ITP (IgG → platelets)
4) Hemolytic disease of newborn.

Indirect

Abs in Serum.

Nonself
Non-Immunogenic
NTAHA.

- HDN in maternal Sample.
→ Cross matching
→ Unexpected Ab. (In Rare Blood groups)

Coomb's Test -ve: Aplastic Anemia.

→ Mismatch Blood Transfusion.
in Recipient.

Hemoglobinopathies

Embryonic Hb (8 weeks IUL)

1] Gower I → Zeta-2 Epsilon-2.

2) Gower II → α_2 ε_2

3) Postlant I → Zeta$_2$ ε_2 γ_2

4) Postbird II → Zeta$_2$ ε_2 γ_2.

8 weeks ↓

HbF → $\alpha_2\gamma_2$

↓

HbA → $\alpha_2\beta_2$. [HbA2 → $\alpha_2\delta_2$] - minor.

Hb pathies

Qualitative

Sickle cell anaemia.

β chain.

6th position

glutamic acid → Valine

[polar]-ve → neutral

Insoluble.

- Solubility is altered.

- Stability - normal.

Quantitative

α, β (quantity reduced)

α-↓-α thallassemia.

β-chain ↓ β thallassemia.

Sickle cell anaemia.

Point mutation.
missense
Partially accepted missense point mutation.

β-thallasemia

β- Point mutation
nonsense

α-thallasemia - Deletion
- loss of α chain
αα/αα.

α-thallasemia-
1α gene deletion: αα/α- : Carrier
2α gene deletion: α-/α- ; αα/-- : Trait
3α gene deletion: α-/-- - HbZ
4α gene deletion: --/-- : Hb Bartz - Hydrops fetalis.

β-thallasemia.
Q. [21/F: Hb-5g%. 1 unit of BT; Hepatosplenomegaly]
T.major, T.minor, T.trait, (AIHA)

1) T.major: Cooley A/mediterranean Anaemia.
Hb-<3g%
multiple units of BT → Iron overload.
↓
CHF → m/c COD

Usual-10 years
Rarely reach 2nd decade.

Confirm: *HPLC chromatography > Hb. electrophoresis
HbF ↑ To confirm

N HbA - 96%
HbF - <2%
~~HbA2 - 3.5%~~

HbF - 30-90%

2) Thalassemia intermedia:

Pediatric:

Hb - >7g/l

upto 4 occasional Blood Transfusion

Pediatric.

Adult → Transfusion dependent anemias.

Δ: HPLC - HbF ↑. (10-30%).

3) Thalassemia minor: Pediatric/adult

9-11g/l

No H/o blood transfusion

Δ: electrophoresis - Both HbF ↑ HbA_2 ↑.

↑HbF - (1-4%) - nodiagnostic.

HbA2 - 4-8% - diagnostic.

P.B - Microcytic Hypochromic anaemia

T. minor. IDA Q.

(N) RDW - 11.5-14.5 (N)Q ↑ RDW.

anisocytosis.

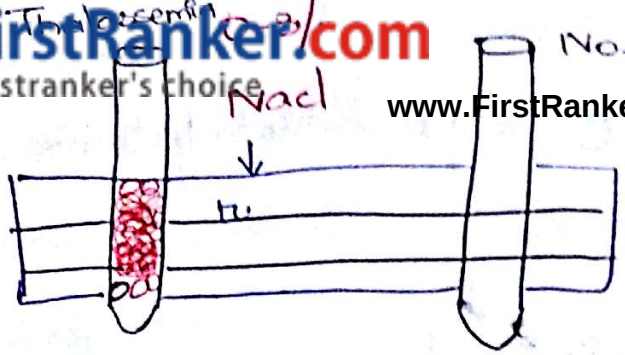
Mentzer index MCV <13 ≥13

RBC Count.

NESTROF - Best Screening method for Thalassemia

Thalassemia

Normal



Th. - Hemolysis not complete } ↓ osmotic fragility

0.3% normally Hemolysis Complete

Nolinesseen

Hemolysis - not complete

RBC membrane Stable.

Thallassemia

Able to view lines

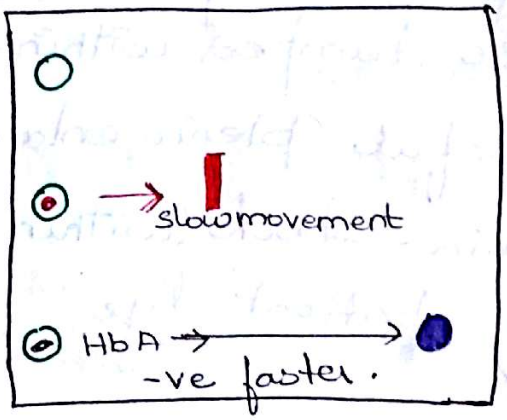
0.3% - Hemolysis complete

RBC - dissolved.

Normal

Sickle cell Anaemia

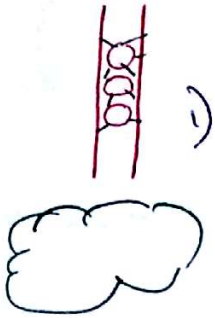
HbE



2 Bands → Carrier - Hbs + HbA

only 1 Band → Disease.

under Hypoxia, Sickle cells express adhesion molecule.



1) Vasoocclusive - m/c clinical picture

Crisis a) hand foot Syndrome

b) acute chest syndrome (no radiation) - Not resemble MI

3) fish mouth vertebra

m/c Cause of ORT in Sickle Cell Salmonella infections

4) Autosplenectomy

↓

Dyserythropoesis.

↓

PB-  nucleai Remnants - Howell Jolly Bodies

i) splenectomy

ii) megaloblastic anaemia

(ii) Aplastic Crisis - Parvovirus

(iii) Sequestration Crisis - most dangerous, life threatening crisis

→ Large volume of RBC dumped within Spleen.

i) acute painful Splenic enlargement

ii) Hypovolemia - Shock (within 4hrs)

patient - dye.

→ medical emagency.

Hemosiderin + Ca^{2+} + Fibrotic Tissue

↓

Gamma Gandy Bodies

1) SCA

2) CMTL

3) Liver cirrhosis

Sickling

i) most important factor → HbS concentration

Incauses - HbA polymerization prevents Sickling

ii) Total Hb - ↓ Sickling

Reach to adult level (<2%) - 6 months.

4 year old boy, episodic - HA/Jaundice since birth

- Least likely - Sickle cell anaemia.

iii) → HbC Anaemia | HbSC disease

↑ Sickling, HbC removes water & Sodium from cell. ↑ HbS concentration - ↑ Sickling

Features of HbSC - 1) proliferative retinopathy

2) Bone infarction.

Screening - 1) Sodium metabisulphite.

2) Sodium dithionate.

Δ: HPLC > electrophoresis.

Megaloblastic disease

i) Vit-B-12 ↓

ii) Folic - A ↓.

iii) Ab against intrinsic factor of castle (Parietal cells) → Fundus.

Pernicious anaemia

Adenocarcinoma in pernicious - Fundus

Most specific finding of Macrocytosis is most characteristic

Macrocytosis

Macrocytosis - MCV >100fL.

diameter of RBC - >9μm.

Microcytic MCV <80fL

dia of RBC - <6μm

most specific - Hypersegmented neutrophils

1 Neutrophil - >6 lobes of neutrophil <<

>5 lobes - >5% neutrophils.

dyserythropoiesis

i) Howell-Jolly Bodies

ii) Fine Basophilic stippling

iii) Cabot Ring - figure of 8

Target Cells

Sickle cell Anaemia

Thallasemia (most characteristic)

Iron deficiency Anaemia

Megaloblastic Anaemia.

MCV > 100fL.

MCHC - 33-37 gm/dl

→ H.S ↑, megaloblastic - normal. (not characteristic

Iron deficiency Anaemia

m/c presentation → fatiguability, irritability

Latent Iron deficiency Anaemia - ↓ Iron Stores without Anemia

Hb within normal limits

Fe-absorbed - Fe^{2+}

↓

S.I → proximal duodenum

Storage - RES - BM, liver, spleen.

Fe^{3+} B.M ⟹ Peripheral Blood.

↓

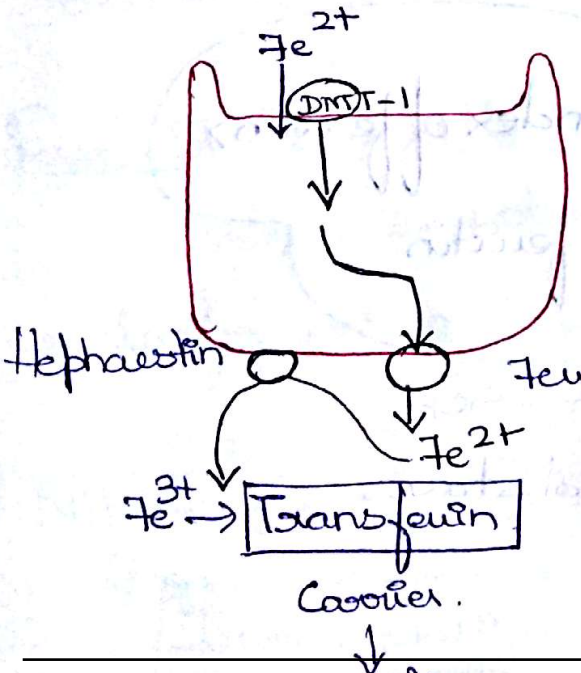
Serum ferritin/Storage Iron.

*

Hepcidin - master Regulator of iron metabolism

↓ 1) Iron absorption

2) ↓ Iron mobilisation from storage sites.



DMT - non specific Transporter, all divalent metal ions

Lab findings

i) Serum ferritin - ↓ earliest / most Sensitive

ii) ↑ Transferrin

iii) ↑ TIBC - normal 300-400 µg/dl
< 300 - Anemia of chronic disease.

iv) ↓ % Saturation of Transferrin (N: 22-50%
Q IDA - <16%)

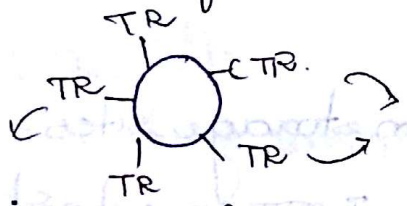
v) Hepcidin - ↓

IDA with inflammation / infection

S. ferritin ↑ (Acute phase Reactants)

i) gold standard Bone marrow Biopsy - with special stain (Perls prussian blue) for iron stores.

ii) S. Transferrin Receptors - ↑



Q.

S. Transferrin Receptor Ratio with log index of ferritin>

S. Transferrin Receptor > S. ferritin.

Best investigation to diff - IDA & ACA.

1) Bone marrow with Special Stains.

2) S. Transferrin Receptor.