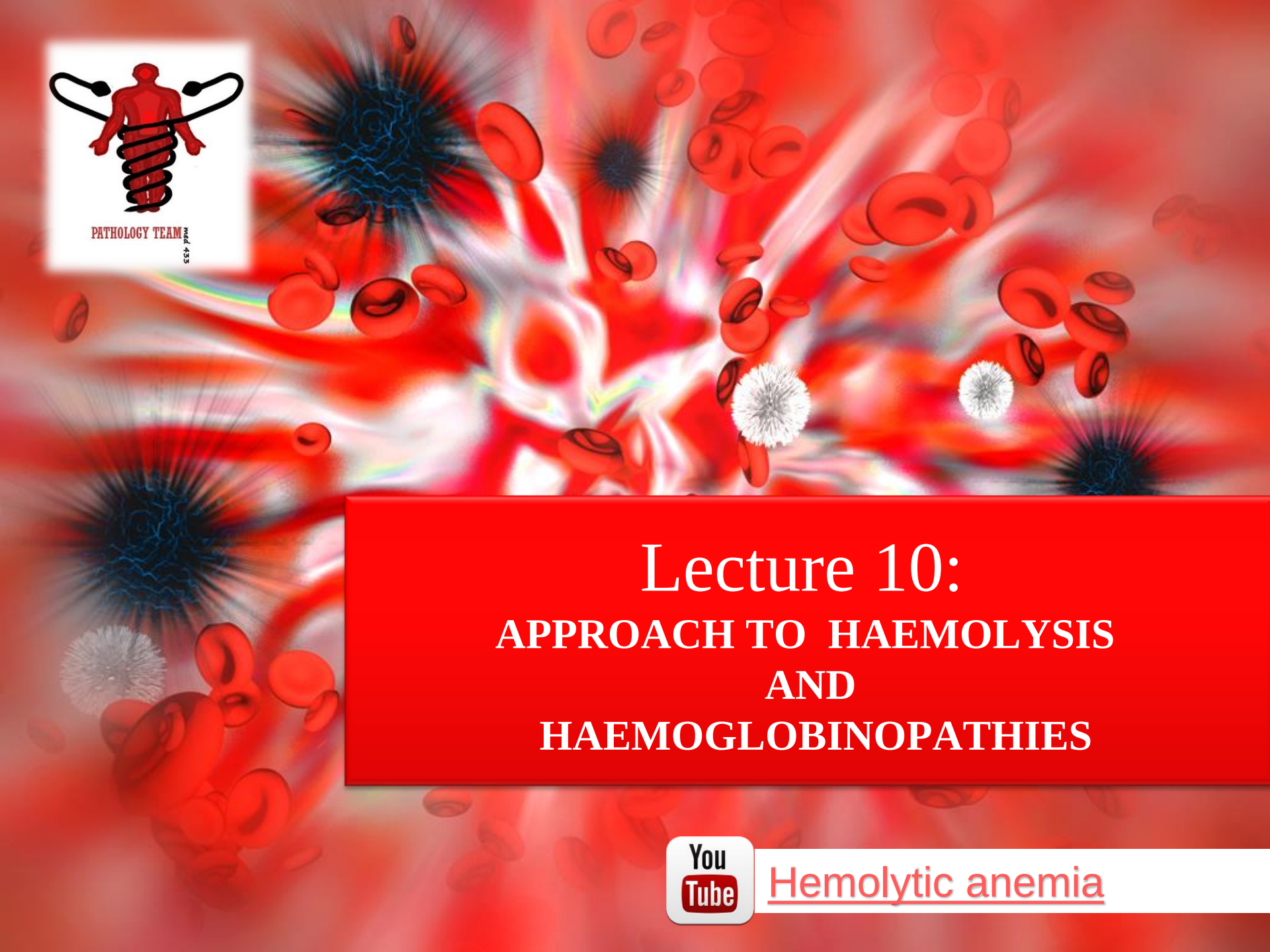
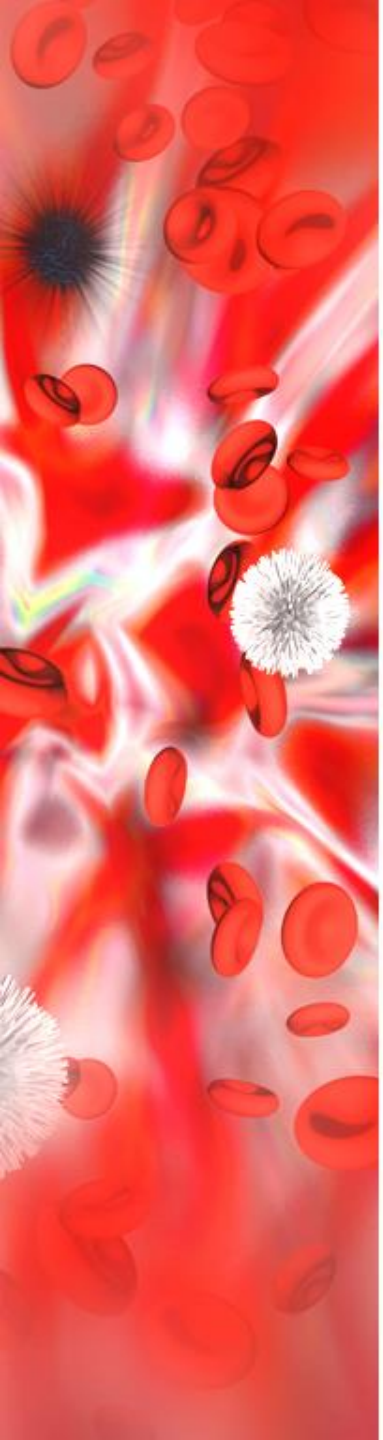


Lecture 10:

APPROACH TO HAEMOLYSIS AND HAEMOGLOBINOPATHIES

You Tube Hemolytic anemia



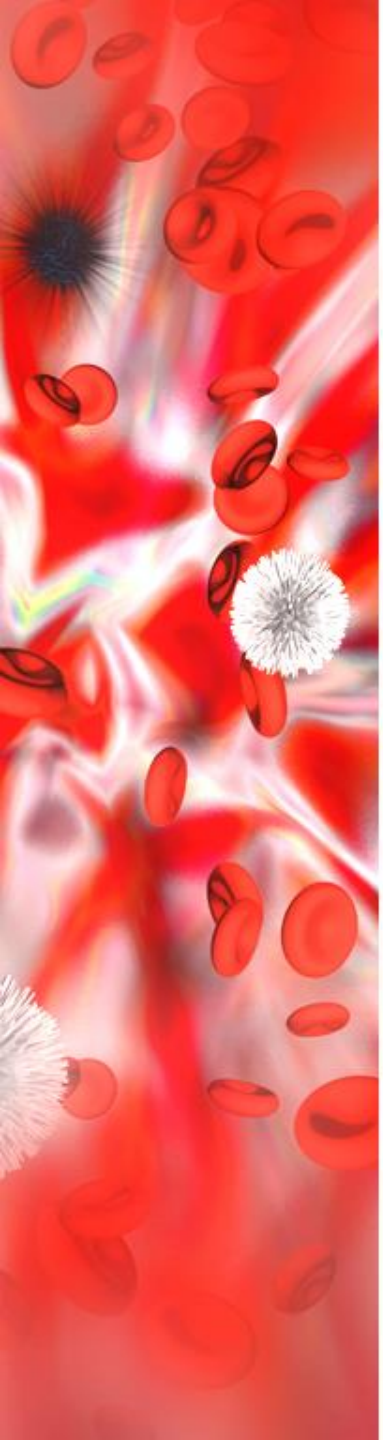
Hemolysis:

- Premature destruction of RBCs.
- According to the site of destruction ,it could be intravascular or extravascular .
- Hemolysis could be due to:

  a. Defect in the RBCs (intra-corpuscular) as in congenital hemolytic Anemia.

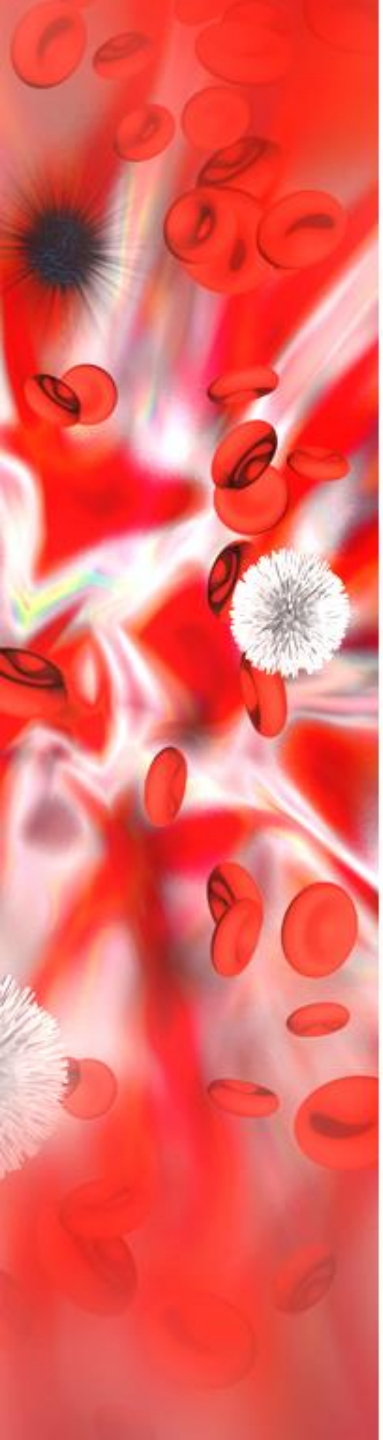
  b. Defect in the surrounding environment (extracorpuscular) as in acquired Anaemia.

REMEMBER: normally after 120 days , old RBC s are destroyed by Reticuloendothelial system 'RES', (Extravascular) in bone marrow, liver and spleen.



Clinical feature of Hemolysis

- Pallor, lethargy
- Jaundice
- Splenomegaly
- Gall stones (pigment – bilirubin)
- Dark urine (urobilinogen)
- Bone deformity (in some types of
- Haemolytic anaemia
- Leg ulcers (in some types of haemolytic anaemia).

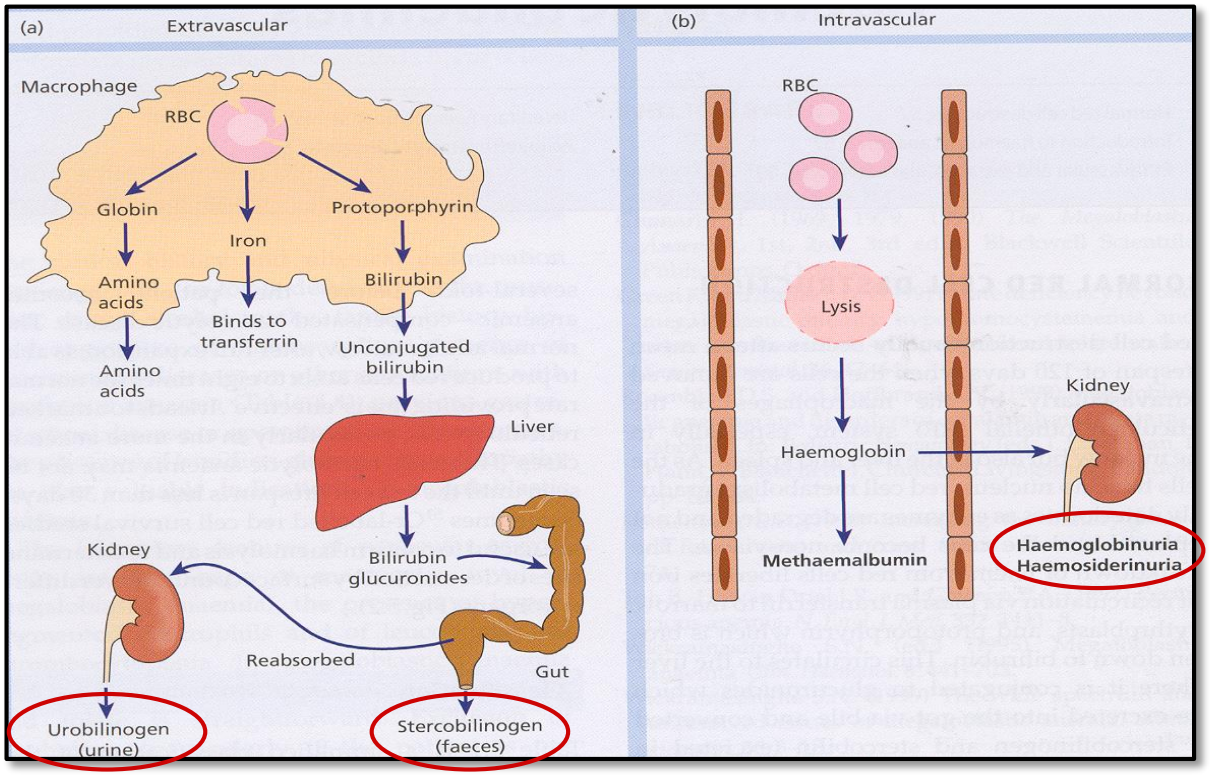


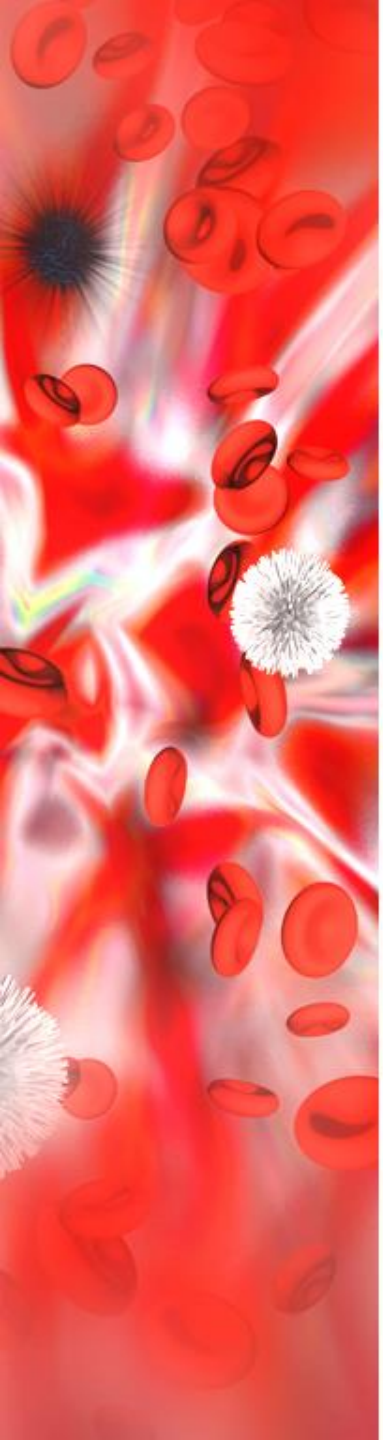
Laboratory Feature of Hemolysis

Features of increased red cell breakdown.	Features of increased red cells production.	Damaged red cells.
↑ serum bilirubin is raised (unconjugated and bound to albumin).	Bone marrow erythroid hyperplasia.	Morphology (e.g. microspherocytes, elliptocytes, red cells fragmentation).
↑ urine urobilinogen.	Reticulocytosis* *stage before mature erythrocyte	Increased osmotic fragility, autohaemolysis ,etc).
↑ faecal stercobilinogen.		Shortened red cell survival (This can be shown by **51Cr** labeling with study of the sites of destruction.
Absent serum haptoglobins*	NOTE: *It is abscent because it bacome saturated with Hemoglobin and the complex removed by RES). **Lactate dehydrogenase is an enzyme found in body tissues It's released when there's a tissue damage and it is abundant in red blood cells and can function as a marker for hemolysis	
↑ lactate dehydrogenase (LDH)**.		

Intravascular and extravascular haemolysis.

Intravascular haemolysis	Extravascular haemolysis
the process of breakdown of red cells directly in the circulation. (direct)	excessive removal of red cells by cells of RE system in the spleen and liver.





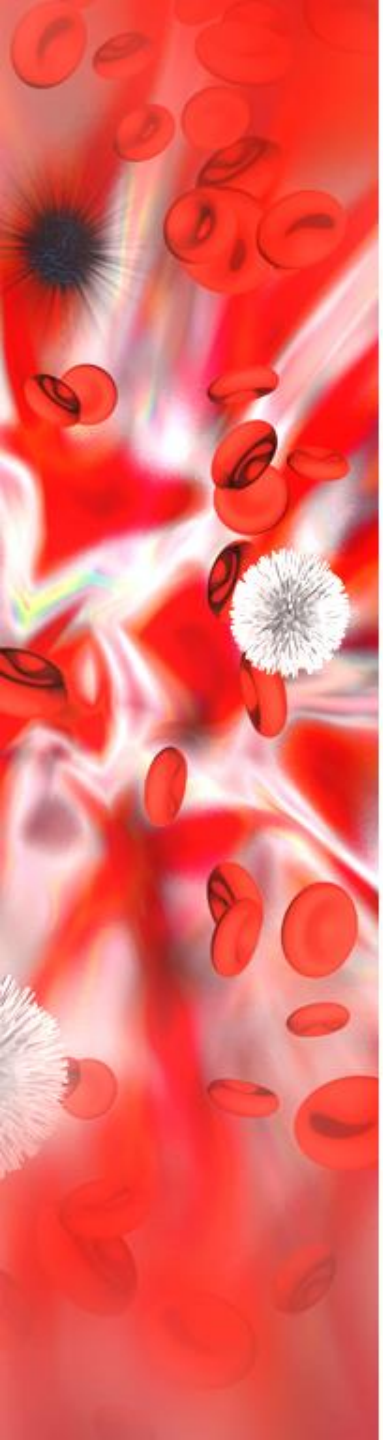
The main laboratory features of intravascular haemolysis are as follows:

1-Haemoglobinaemia and haemoglobinuria.

2- Haemosiderinuria* (Iron storage protein in the spun deposit of urine).

3-Methaemoglobinemia.

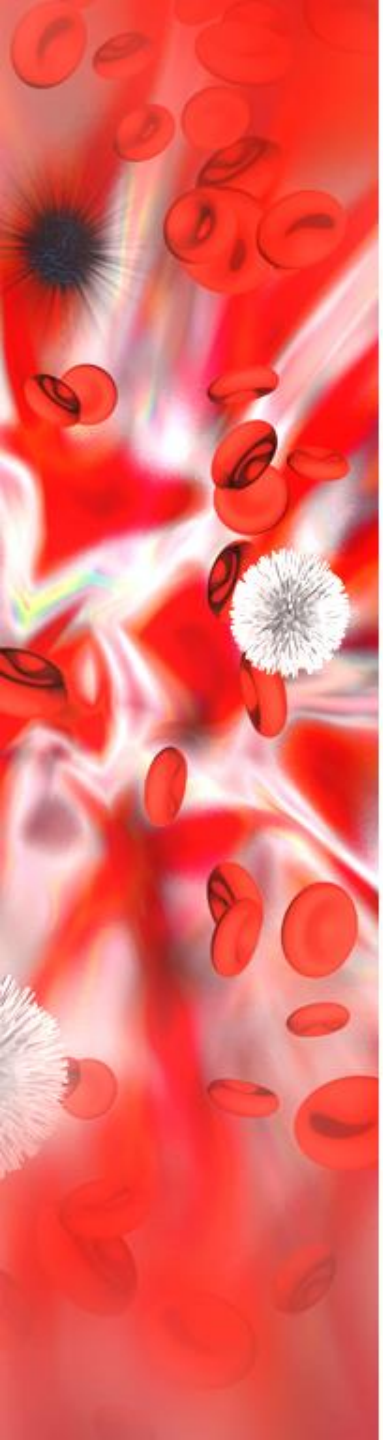
*Haemosiderinuria occurs in chronic intravascular hemolysis . Destruction of RBCs lead to release of Hb , Hb will bind to Haptoglobin and removed by RES but excess HB will be filtrated by Glomerulus and then reabsorbed by proximal tubules . Excess amount of Hb will be excreted with urine. Haemosiderin will be released from Hb and appear in urine (Brownish Urine).



Causes of intravascular haemolysis

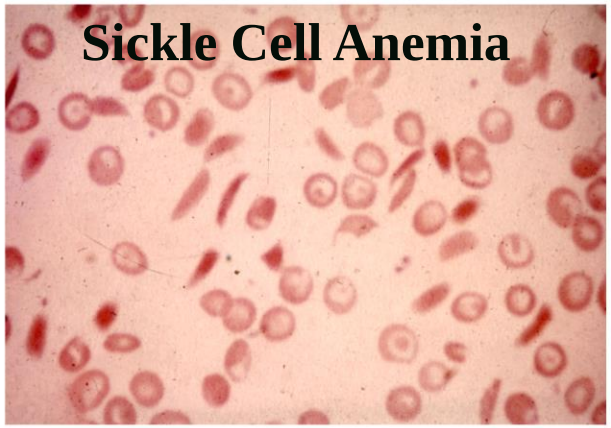
- Mismatched blood transfusion (usually ABO)
- G6PD deficiency with oxidant stress
- Red cell fragmentation syndromes (in case valve replacement)
- Some autoimmune haemolytic anaemias (specially in SLE)
- Some drug-and infection-induced haemolytic anaemias
- Paroxysmal nocturnal haemoglobinuria*
- March haemoglobinuria *
- Unstable haemoglobin

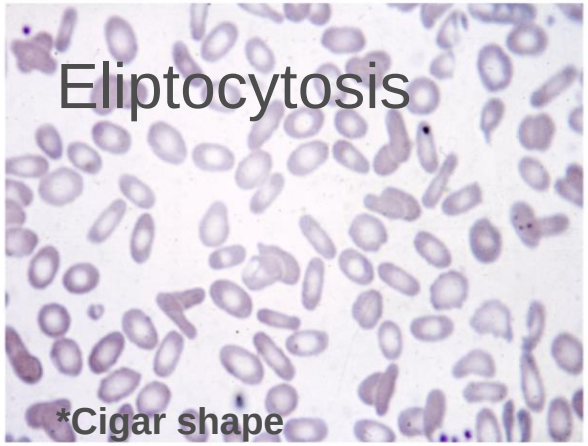
*↑ acidosis during night lead to activation of complement lead to intravascular hemolysis .
* is form of mechanical hemolysis (Damage of RBCs of feet due to prolonged running)



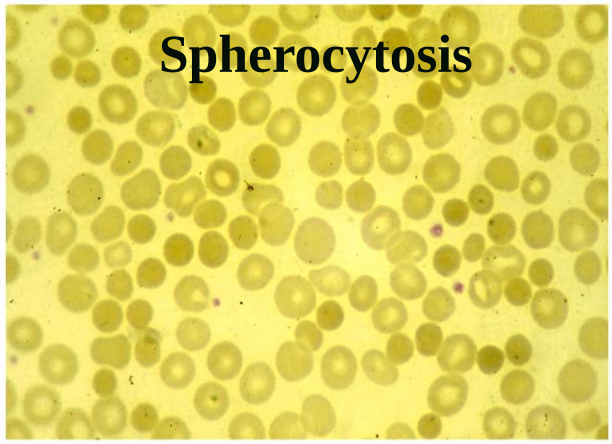
Classification of Hemolysis

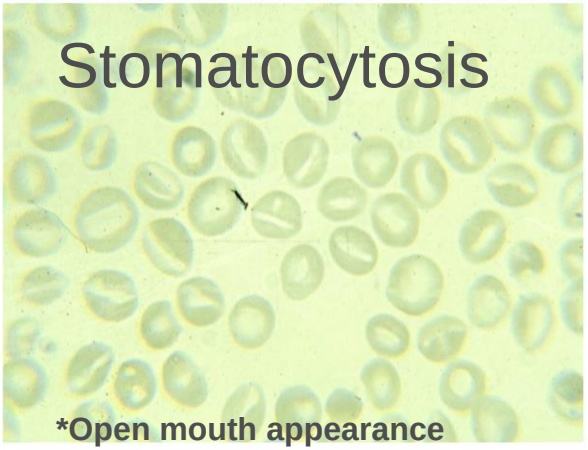
Congenital	Acquired
• Sickle cell disease & other Haemoglobin disorders (Hb genetic abnormalities: HbS,HbC,unstable). • Thalassaemias. • Enzymopathies • E.g. G6PD deficiency, PK deficiency. • Membranopathies • E.g. Hereditary spherocytosis, Elliptocytosis, Acanthocytosis.	Allografts, especially marrow transplantation. o Drug associated. o Red cell fragmentation syndrome. o Arterial grafts, cardiac valves. o Microangiopathic. o Thrombotic Thrombocytopenic Purpura, Haemolytic Uraemic syndrome. o Meningococcal sepsis o Pre-eclampsia o Disseminated intravascular coagulation o March haemoglobinuria - Infections: o Malaria, clostridia. - Chemical and physical agents: o Especially drugs, inductrial/domestic substances, burns. - Secondary: o Liver and renal disease. o Paroxysmal nocturnal Haemoglobinuria. o **Autoimmune Haemolytic Anaemias.**

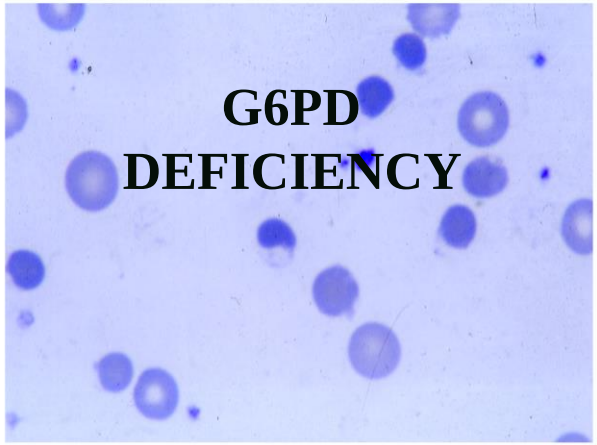

Sickle Cell Anemia

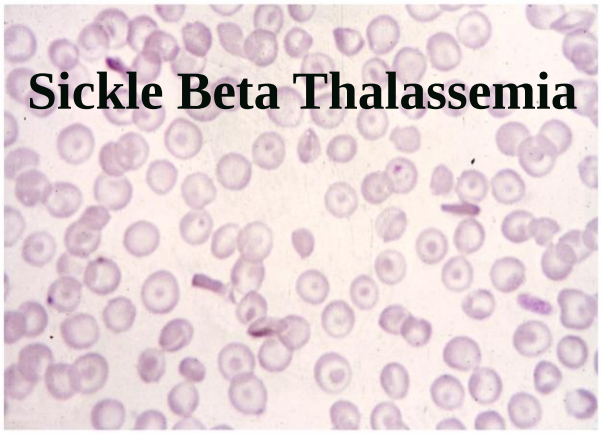

Eliptocytosis
*Cigar shape

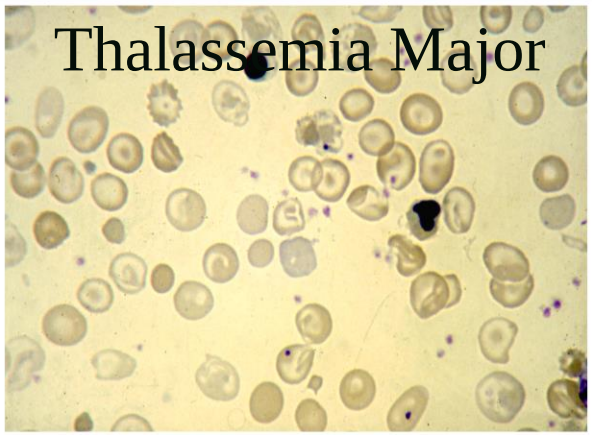

PK DEFICIENCY

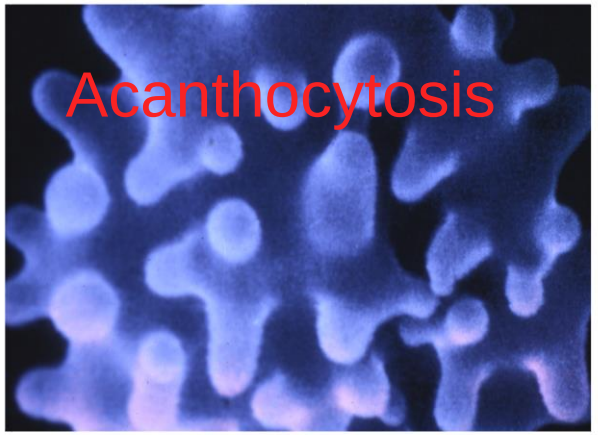

Spherocytosis


Stomatocytosis
*Open mouth appearance

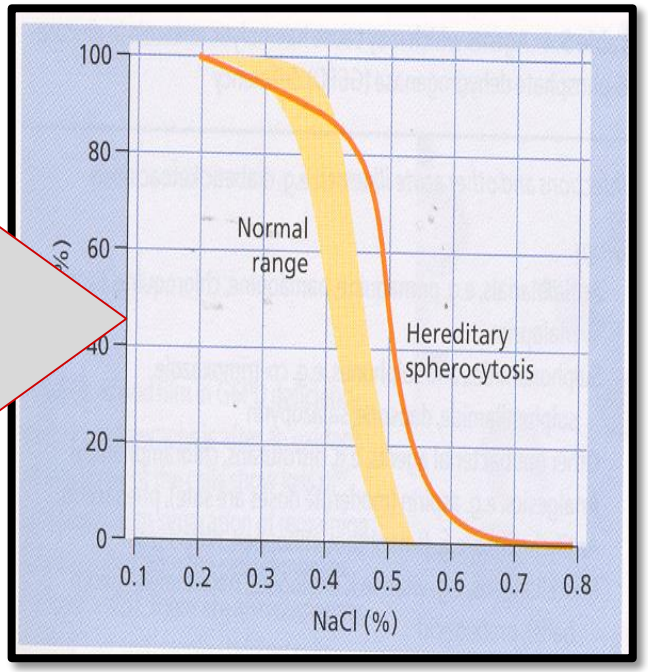

G6PD
DEFICIENCY


Sickle Beta Thalassemia

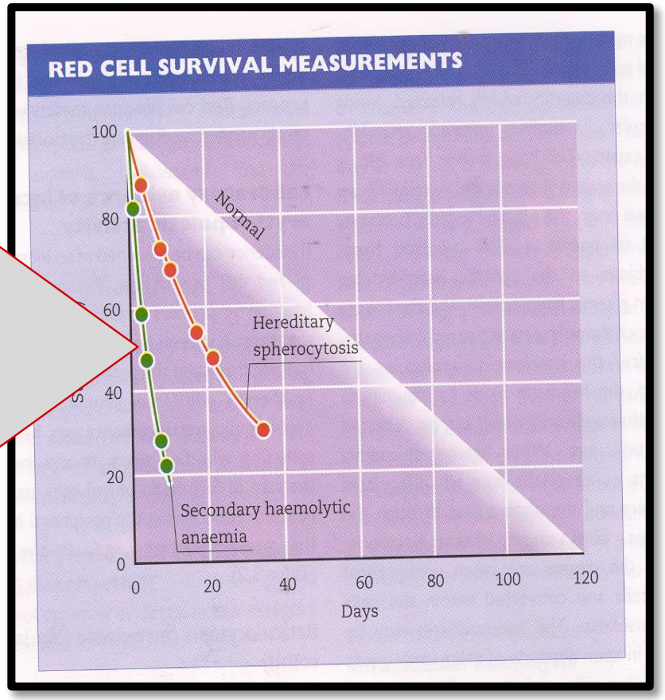

Thalassemia Major


Acanthocytosis

Osmotic fragility test measures red blood cell (RBC) resistance to hemolysis when exposed to a series of increasingly dilute saline solutions. The sooner hemolysis occurs, the greater the osmotic fragility of the cells. here it shows that in Hereditary spherocytosis it's highly increased



Red Cell Survival Measurements: to confirm hemolysis. Anything before the white line indicates hemolysis and in Hereditary spherocytosis (lifespan of RBC 30d) and in 2ndry hemolytic anaemia (15d)



Abnormal haemoglobins (Haemoglobinopathies)

- Some Known Haemoglobin Mutants

NAME	SUBSTITUTION
Hb. S	α2 β2 6 GLU → VAL
Hb. C	α2 β2 6 GLU → LYS
Hb. E	α2 β2 26 GLU → LYS
Hb. O ARAB	α2 β2 121 GLU → LYS
Hb. D PUNJAB	α2 β2 121 GLU →GLN

Molecular changes in Genetic Hb disorders:

1) HbA, HbS, Hb,C

Amino acid sequences of the peptides 4 in haemoglobins A, S and C.

	1	2	3	4	5	6	7	8	9	10
HbA	Val	His	Leu	Thr	Pro	*Glu*	Glu	Lys	Ser	Ala - etc
HbS	Val	His	Leu	Thr	Pro	*Val*	Glu	Lys	Ser	Ala - etc
HbC	Val	His	Leu	Thr	Pro	*Lys*	Glu	Lys	Ser	Ala - etc

Sickle Cell Anemia

DNA Coding for the Amino-Acid in the sixth position in the β-chain

Normal

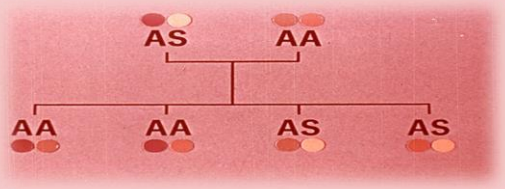
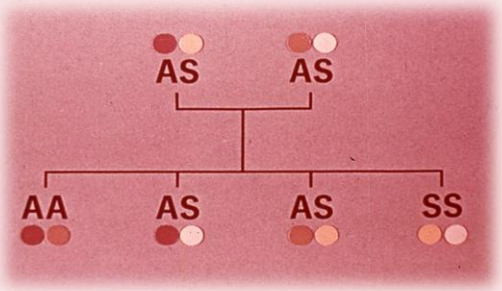
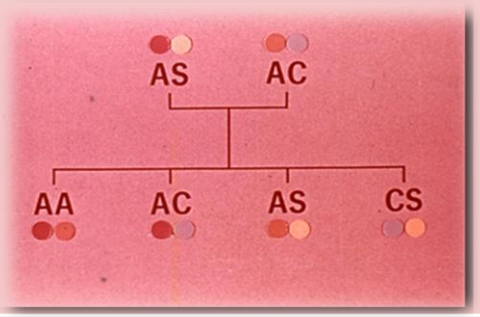
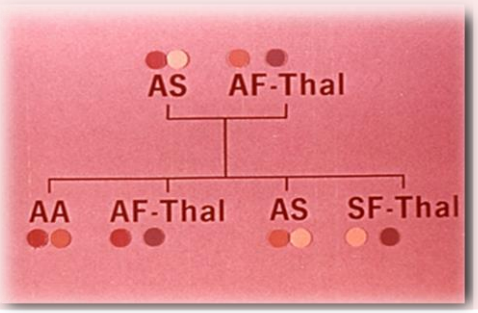
	5	6	7
Amino Acid	pro	glu	glu
DNA Base Composition	CCT	G A G	G A G

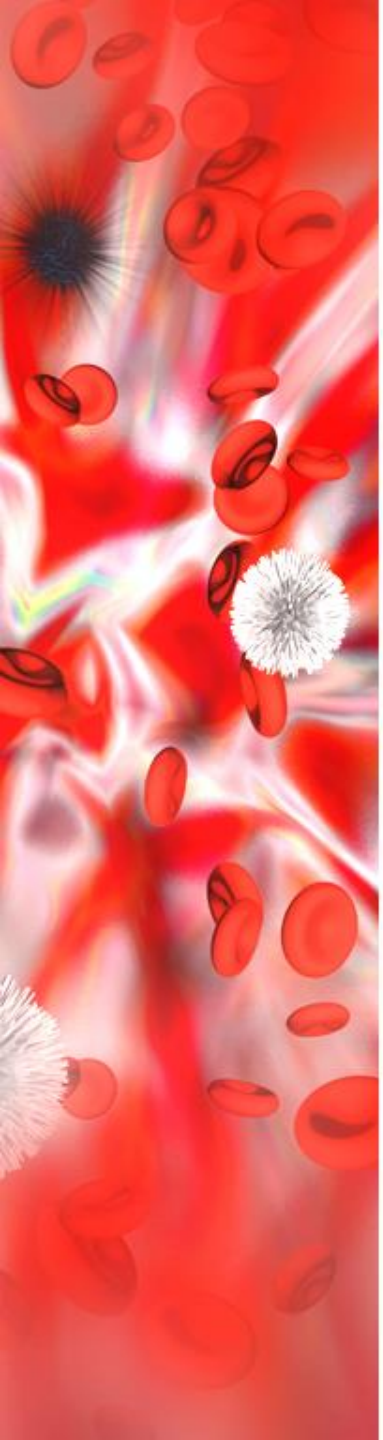
Sickle

DNA Base composition	CCT	G T G	G A G
Amino Acid	pro	val	glu
	5	6	7

Molecular change in sickle cell anaemia:

There is a single point mutation in the DNA coding for the amino acid in the sixth position in the β-globin chain (adenine is replaced by thymine). This leads to an amino acid change from glutamic acid to Valine .

Case 1 If one parent was a carrier (trait) of an abnormal allele (of HbS) 50% of their children will be carriers (trait)	
Case 2 If both parents were carriers (traits) of an abnormal allele of the same type (HbS) 25% of their children will be severe sickle cell patients (diseased) Termed as ***homozygous sickle cell disease (sickle cell anemia) (SS)***	
Case 3 If both parents were carriers of an abnormal allele of different types (one HbS the other HbC) 25% of their children will be severe sickle cell patients or diseased. Termed as ***double heterozygous sickle cell disease (sickle cell anemia) (CS)***	
Case 4 If both parents were carriers of an abnormal allele of different types (one HbS the other Hb F- thalassemia) 25% of their children will be severe sickle cell patients or diseased. Termed as ***double heterozygous sickle cell disease (sickle cell anemia) (SF-thal)***	



Properties of HbS:

- Decrease Solubility .
- Conformational changes:"tactoid formation"

→ Sickled cells

→ Irreversible sickled cells

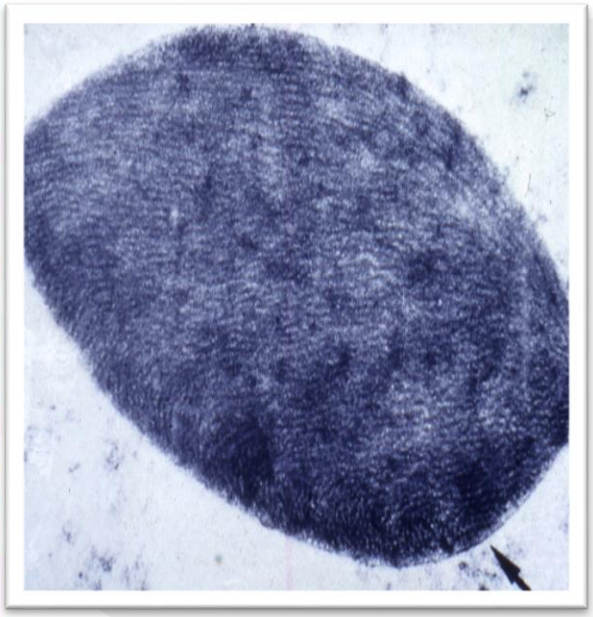
- Increase Mechanical fragility lead to hemolysis.
- Increase Viscosity lead to organ infarction.

Factors affecting sickling:

1. Oxygen tension (50-60mmHg for SS - 20-60mmHg for AS)
2. pH – inhibited at alkaline pH and exacerbated by acidification
3. Increase Concentration of HbS
4. Presence of other hemoglobin:
5. Polymerization: S > D > C > J = A > F

Factors precipitating crises:

1. Infection (especially malaria) 2. Pyrexia 3. Exposure to cold
4. Dehydration 5. Pregnancy



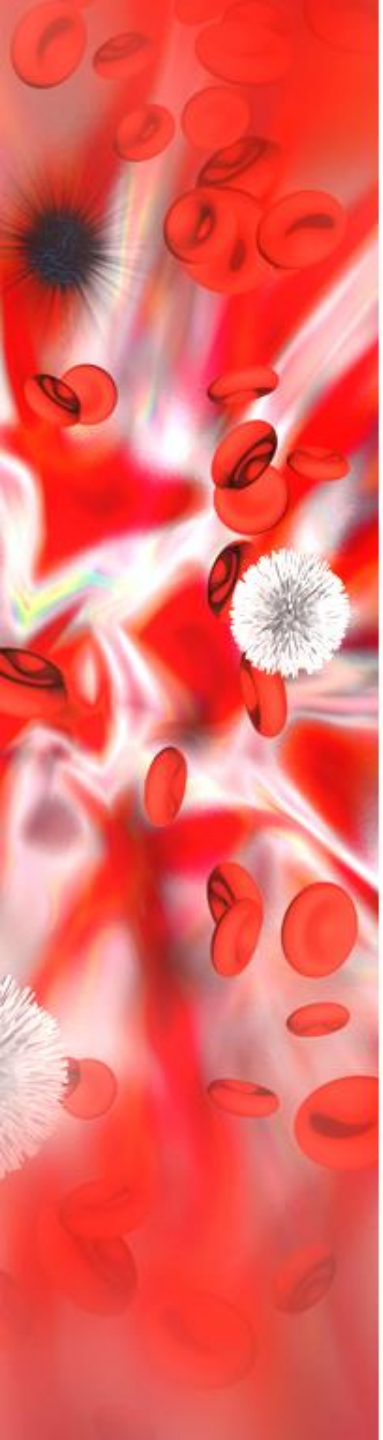
Precipitation of HB molecules inside of RBCs (Tactoid formation)

Crises in sickle cell disease:

1. Hyperhaemolytic
2. Aregenerative or aplastic
3. Small vessel occlusion

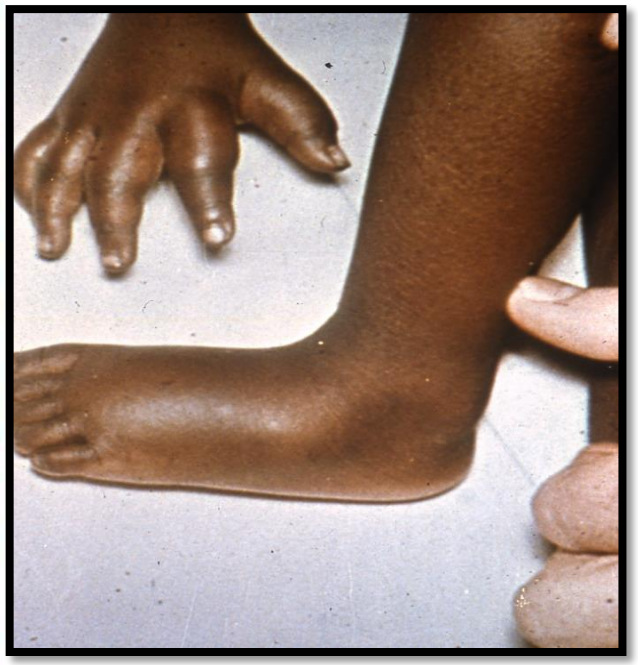
Clinical manifestations of Sickle cell disease:

1. Hemolytic anemia
2. Tissue infarction

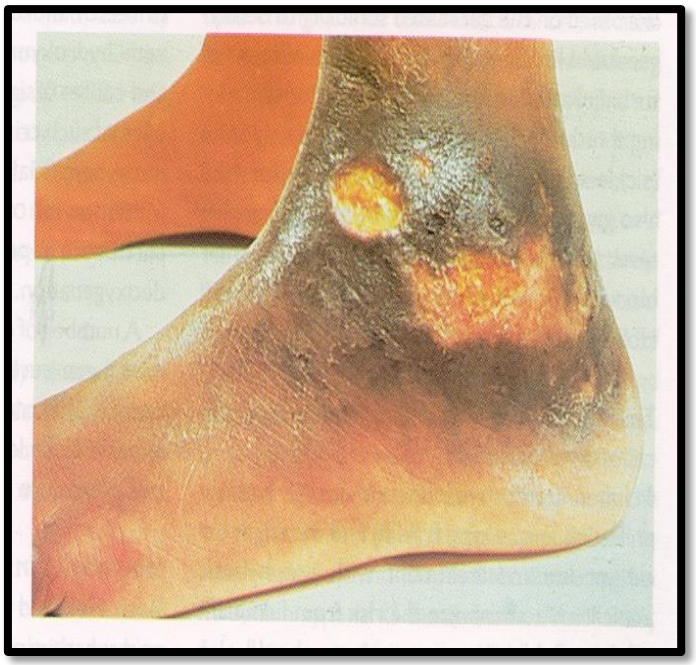


Clinical Manifestations in Sickle Anaemia:

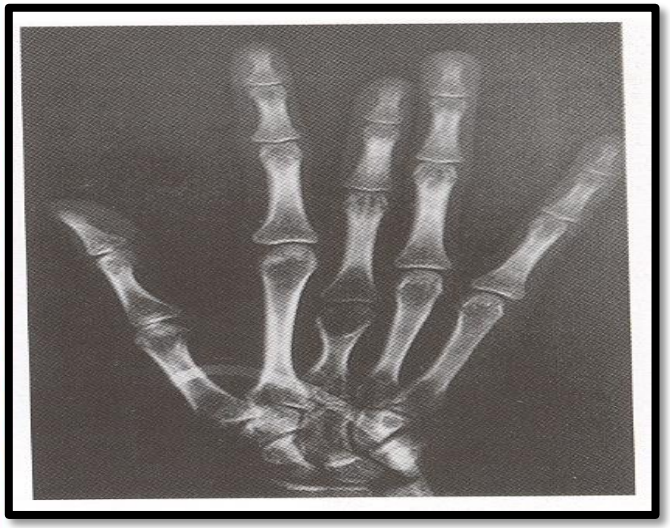
- Pallor (Anaemia)
- Jaundice & Dark Urine
- Apathy & Anorexia
- Hand-Foot Syndrome (Young Children).
- Splenic sequestration (Young Children), Hepatic Sequestration.
- Bones, Joints Pain, Abdominal Pain
- Recurrent Infections & Chest Symptoms (Acute Chest Syndrome).
- Hepato-Splenomegaly :
  - (Early Childhood)
  - (Association with Thalassaemias)
- CNS Presentations (from the infarctions).
- Leg Ulceration (very common).
- Skeletal Deformity.



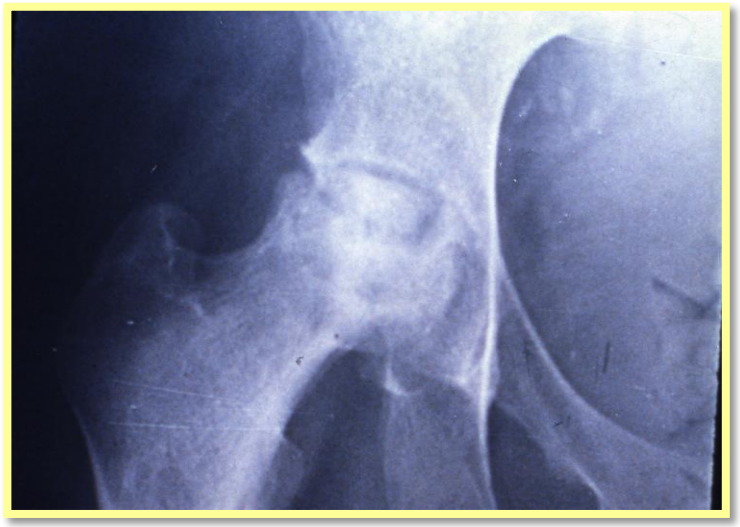
Foot and Hand syndrome is painful and swollen hand and foot due to Dactylitis (infarction of small bones of hand and foot)



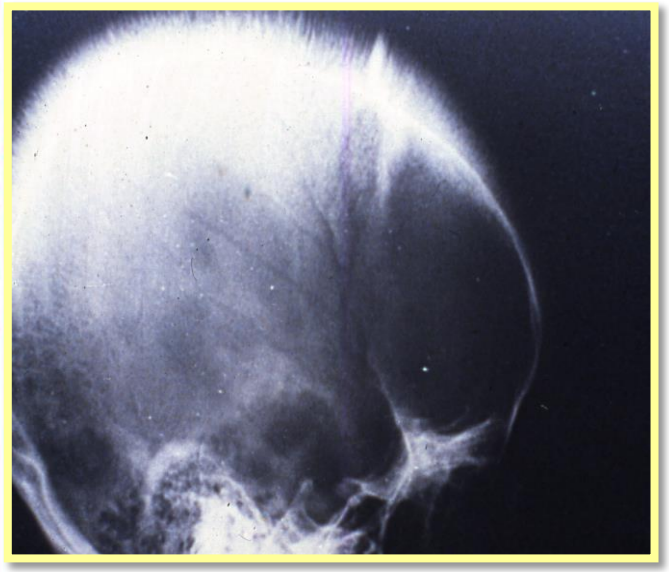
Leg ulcer very common in sickle cell anemia due to infarction



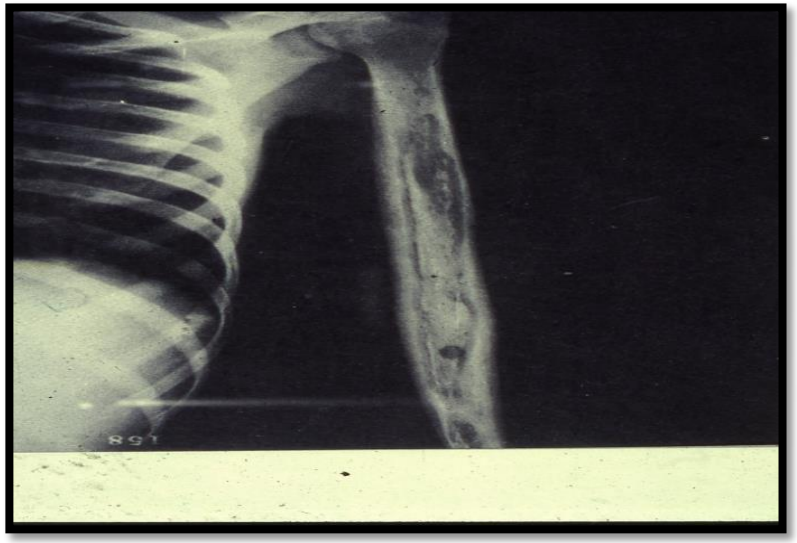
Short middle finger



Infarction of head of femur lead to damage of hip joint .



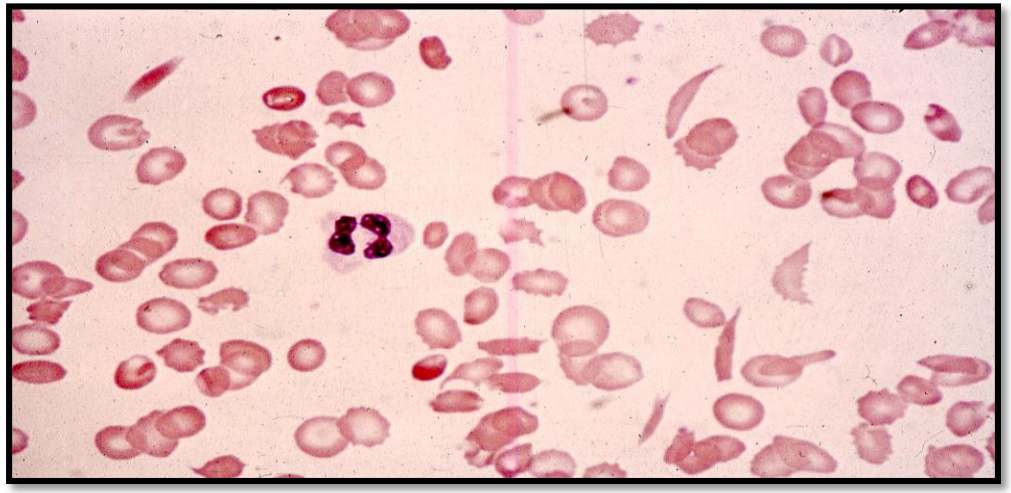
Hair on end due to marrow expansion



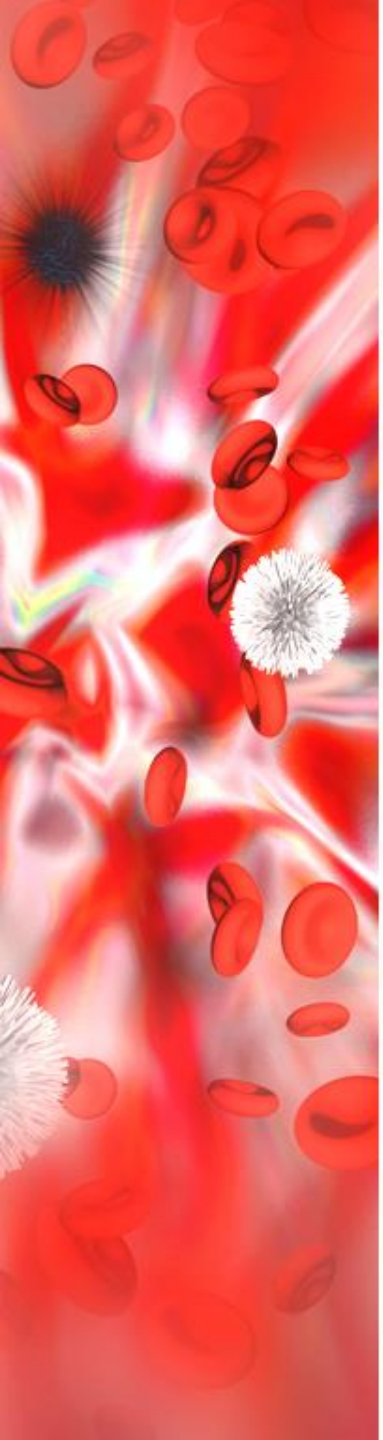
Infarction of humorous and salmonella(most common) osteomyelitis

Laboratory Diagnosis :

- CBC.
- Blood Film.(shows irreversible sickle cells).
- Sickle Solubility Test.(usually positive)
- Hb Electrophoresis* (most accurate).
- Genetic Study.



***if HbS <45% this person is trait**
if >45% this person has sickle disease.

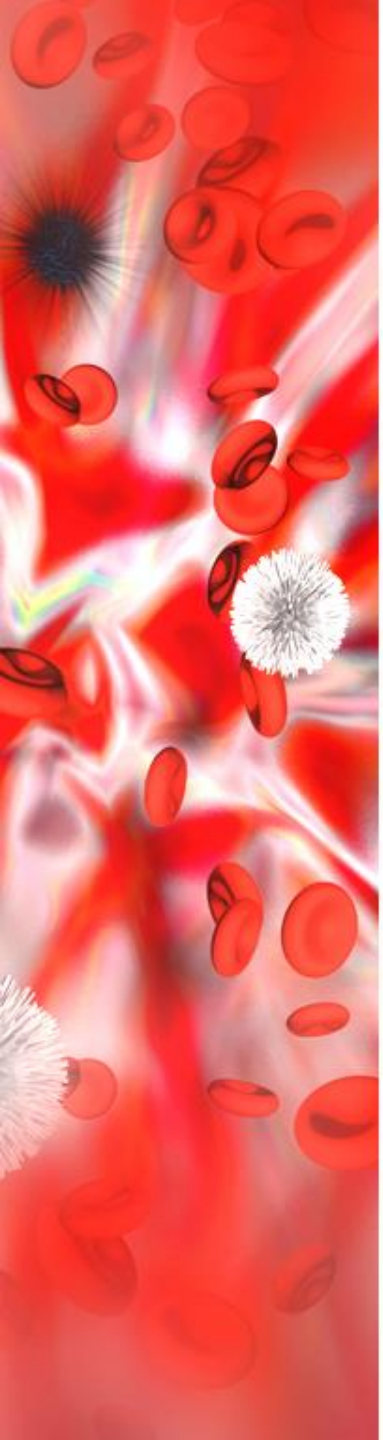


Indications for Blood Transfusion in Sickle Cell Anaemia:

- Splenic sequestration
- Hepatic sequestration
- Aplastic crisis
- Overwhelming infections
- Elective or emergency surgical operation
- Severe painful crisis associated with severe hemolysis
- Pregnancy

Indications for exchange transfusion:

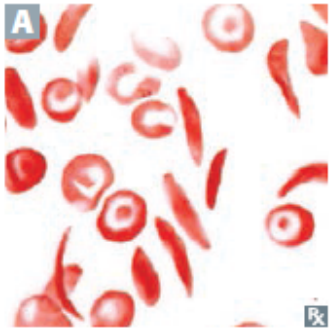
- Strokes
- Pulmonary infarcts with infection
- Pregnancy (Severe persistent painful crisis)
- Priapism
- Preparation for major surgery



Summary from Essential Haematology for Hemolysis anemia

- Haemolytic anaemia is caused by shortening of the red cell life. The red cells may break down in the reticuloendothelial system (extravascular) or in the circulation (intravascular).
- Haemolytic anaemia may be caused by inherited red cell defects, which are usually intrinsic to the red cell, or to acquired causes, which are usually caused by an abnormality of the red cell environment.
- Features of extravascular haemolysis include jaundice, gallstones and splenomegaly with raised reticulocytes, unconjugated bilirubin and absent haptoglobins. In intravascular haemolysis (e.g. caused by ABO mismatched blood transfusion), there is haemoglobinaemia, methaemalbuminaemia, haemoglobinuria and haemosiderinuria.
- Genetic defects include those of the red cell membrane (e.g. hereditary spherocytosis), enzyme deficiencies (e.g. glucose-6-phosphate dehydrogenase or pyruvate kinase deficiency) or haemoglobin defects (e.g. sickle cell anaemia).
- Acquired causes of haemolytic anaemia include warm or cold, auto- or allo-antibodies to red cells, red cell fragmentation syndromes, infections, toxins and paroxysmal nocturnal haemoglobinuria.

Summary from First Aid for Sickle cell anemia

	DESCRIPTION	FINDINGS
Sickle cell anemia (E) 	HbS point mutation causes a single amino acid replacement in β chain (substitution of glutamic acid with valine) at position 6. Pathogenesis: low O_2, dehydration, or acidosis precipitates sickling (deoxygenated HbS polymerizes), which results in anemia and vaso-occlusive disease. Newborns are initially asymptomatic because of ↑ HbF and ↓ HbS. Heterozygotes (sickle cell trait) have resistance to malaria. 8% of African Americans carry the HbS trait.	Sickled cells are crescent-shaped RBCs A. "Crew cut" on skull x-ray due to marrow expansion from ↑ erythropoiesis (also in thalassemias). Complications in sickle cell disease (SS): ▪ Aplastic crisis (due to parvovirus B19). ▪ Autosplenectomy (Howell-Jolly bodies) → ↑ risk of infection with encapsulated organisms; early splenic dysfunction occurs in childhood. ▪ Splenic sequestration crisis. ▪ *Salmonella* osteomyelitis. ▪ Painful crisis (vaso-occlusive): dactylitis. (painful hand swelling), acute chest syndrome (most common cause of death in adults), avascular necrosis, stroke. ▪ Renal papillary necrosis (due to low O_2 in papilla; also seen in heterozygotes) and microhematuria (medullary infarcts). Diagnosis: hemoglobin electrophoresis. Treatment: hydroxyurea (↑ HbF) and bone marrow transplantation.

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Good luck …