Hematology

3 /11

Haemolysis & Haemoglobinopathies

432 ***Hematology*** *Team*

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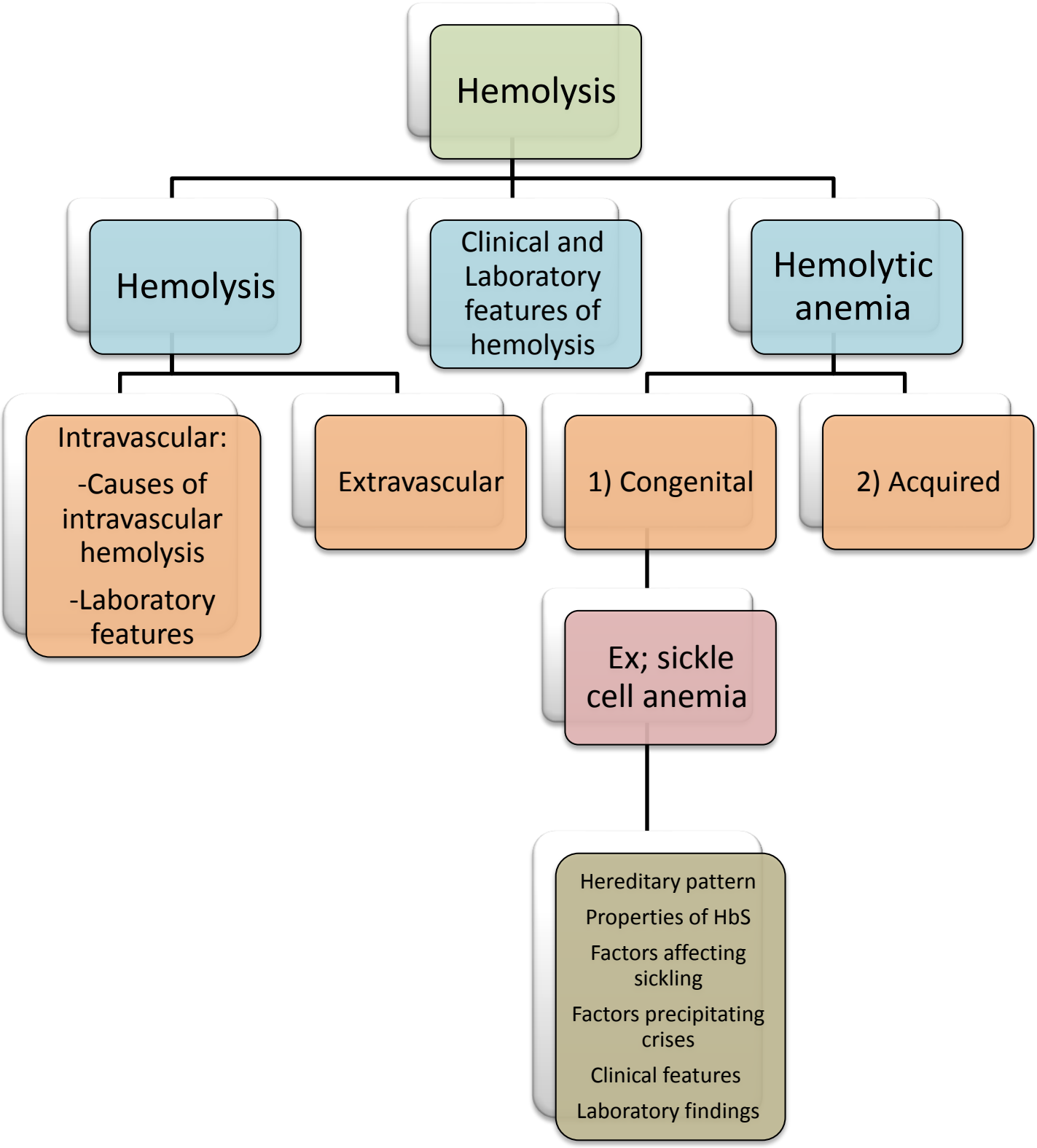
Color Index: Female notes are in Green. Male notes are in Blue. Red is important. Orange is explanation.

Haemolysis & Haemoglobinopathies

Mind Map:



Hemolysis

Hemolysis:

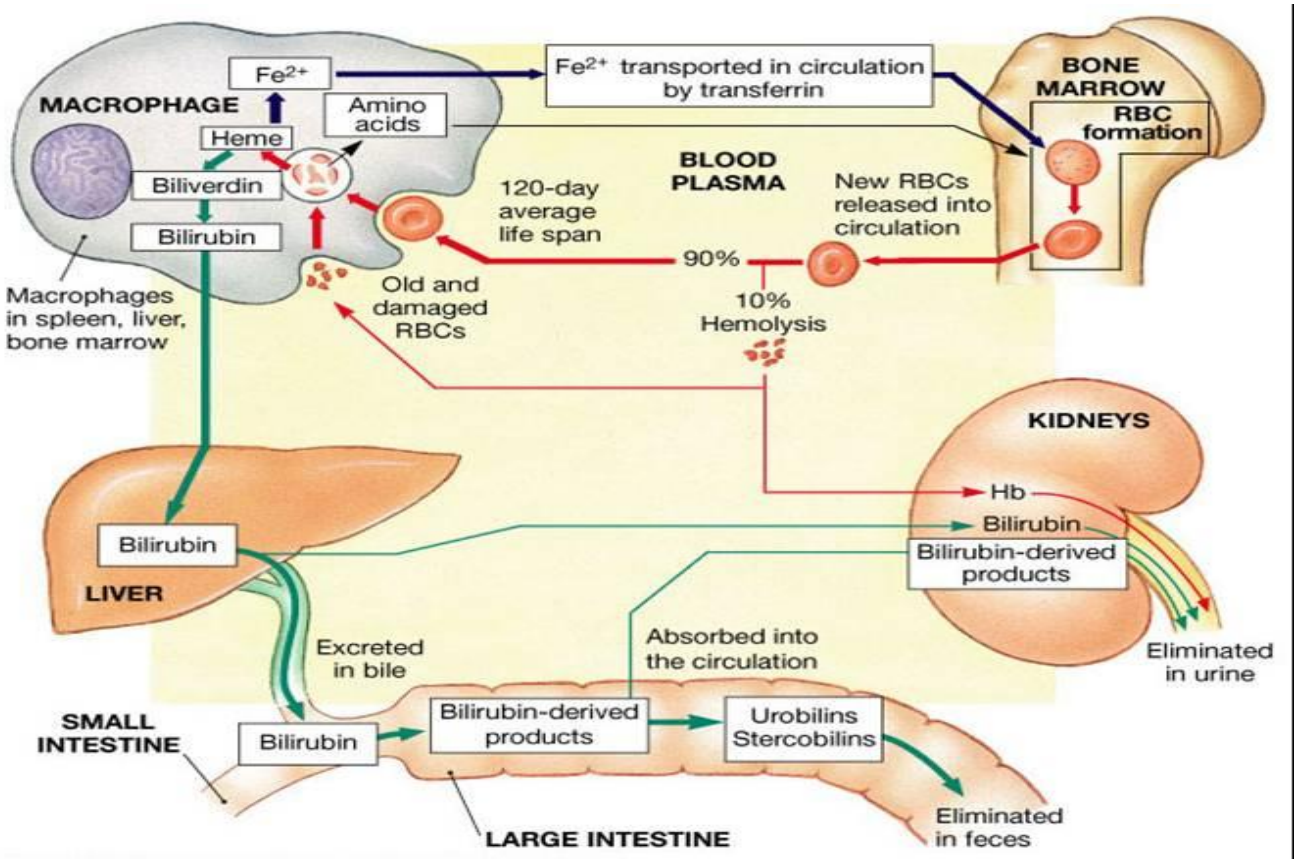
Is premature destruction of RBCs. (Destruction can be extravascular and Intravascular).

- ***Hemolysis could be due to:***
  - Defect in the RBCs (intra-corpuscular) as in congenital hemolytic Anaemia.
  - Defect in the surrounding environment (extra-corpuscular) as in acquired Anemia. *(**Anemia** also spelled **Anaemia**)*

REMEMBER:

Normal RBC lifespan is 120 days then the cells are removed extravascularly by macrophages of *Reticuloendothelial system*, especially in the bone marrow but also in the liver and spleen. The intravascular hemolysis (breakdown of RBC inside the vessels) plays little or no part in normal RBC destruction.

The picture below just a reminder of the normal RBC destruction and its contents' fate (Extra information).



Clinical Features 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 Features of Hemolysis:

1. Features of increased red cell breakdown:

a) ↑ Serum bilirubin is raised (unconjugated and bound to albumin).
b) ↑ Urine urobilinogen.
c) ↑ Faecal stercobilinogen.
d) Absent serum Haptoglobins. (Haptoglobin: are proteins present in normal plasma and are capable of binding to hemoglobin).
e) ↑ Lactate Dehydrogenase (LDH).

2. Features of increased red cells production:

1) Reticulocytosis
2) Bone marrow Erythroid Hyperplasia.

3. Damaged red cells: (criteria)

- Morphology (e.g. Microspherocytes, Elliptocytes, red cells fragmentation). (**Elliptocytes** are abnormally shaped red blood cells that appear oval or elongated).
- Increased osmotic fragility, Autohaemolysis, etc.
- Shortened red cell survival (This can be shown by 51Cr labeling with study of the sites of destruction).

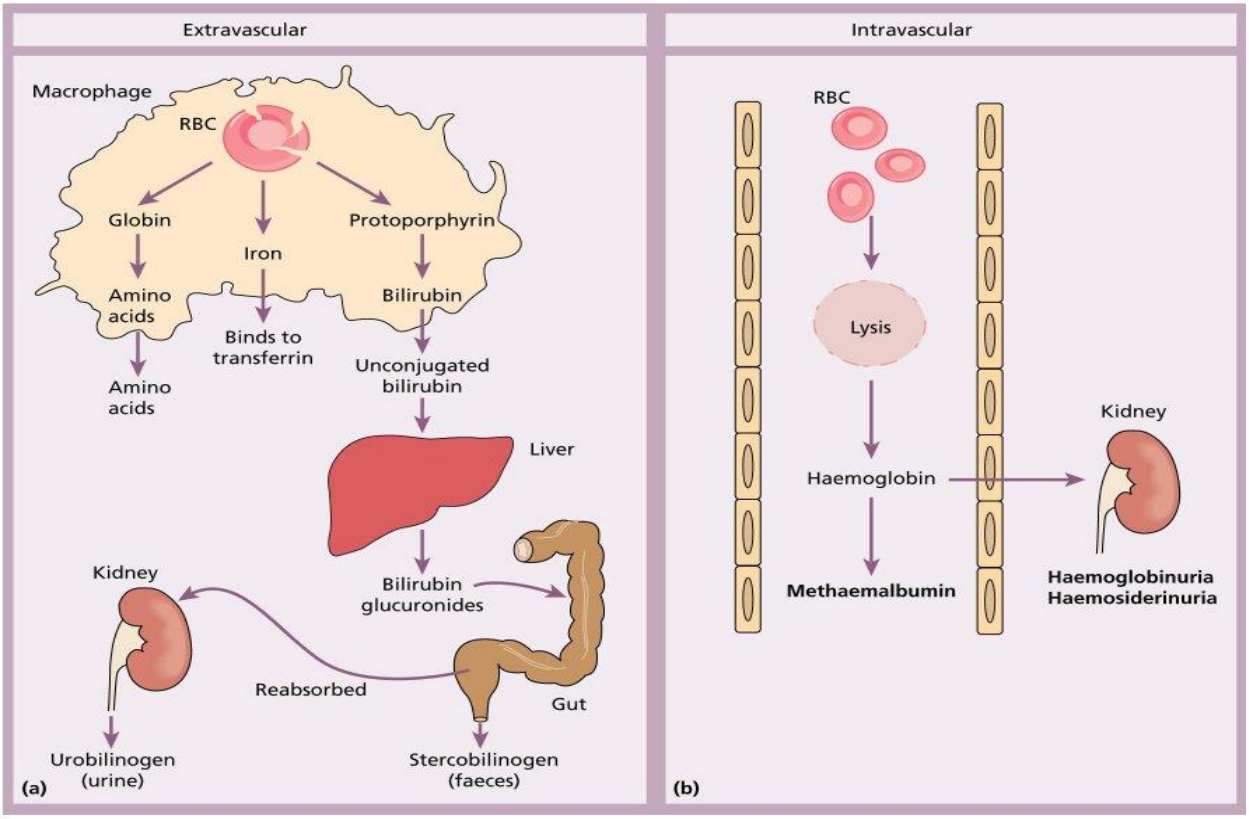
Explanation: -Lactate dehydrogenase is an enzyme found extensively in body tissues, such as blood cells and heart muscle. It's released when there's a tissue damage which makes it a significant marker of common injuries and disease.
- LDH is abundant in red blood cells and can function as a marker for hemolysis.
- The osmotic fragility test (OFT) is used to measure erythrocyte resistance to hemolysis while being exposed to varying levels of dilution of a saline solution.

Intravascular and extravascular haemolysis:

1) Intravascular haemolysis, the process of breakdown of red cells directly in the circulation.
2) Extravascular haemolysis, 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) "Brown urine".



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Explanation -**hemosiderinuria** occurs with chronic intravascular hemolysis, in which hemoglobin is released from RBCs into the bloodstream in excess of the binding capacity of Haptoglobin. The excess hemoglobin is filtered by the kidney and reabsorbed in the proximal convoluted tubule, where the iron portion is removed and stored in ferritin or hemosiderin. The tubule cells of the proximal tubule slough off with the hemosiderin and are excreted into the urine, producing a "brownish" color. It is usually seen 3-4 days after the onset of hemolytic conditions (good marker for hemolysis stays for several weeks).

-Hemoglobinuria disappears more quickly than hemosiderin.

Causes of intravascular haemolysis:

- ✓ Mismatched blood transfusion (usually ABO).
- ✓ G6PD deficiency with oxidant stress.
- ✓ Red cell fragmentation syndromes >> The red cell fragmentation syndrome can occur due to abnormalities of the heart or the blood vessels or vascular malformations.
- ✓ Some autoimmune Haemolytic Anaemia.
- ✓ Some drug-and infection-induced Haemolytic Anaemia.
- ✓ Paroxysmal Nocturnal Haemoglobinuria >> complement-induced intravascular hemolytic anemia (activating of complements during night)
- ✓ March Haemoglobinuria >> damage of RBCs between the small bones of the feet due to prolonged marching or running
- ✓ Unstable Haemoglobin >> Type of hemoglobin disorder

Haemolytic Anaemia:

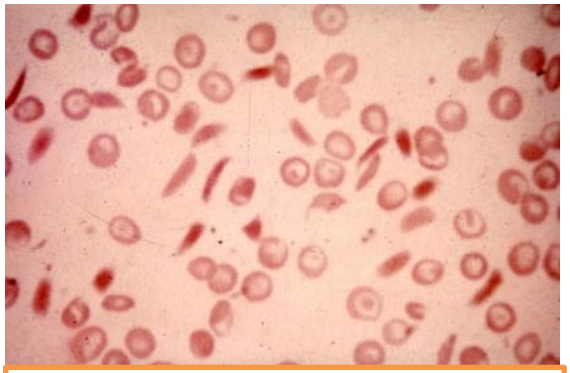
I. Congenital
   - Sickle cell disease & other Haemoglobin disorders (Hb genetic abnormalities: HbS,HbC,unstable).
   - Thalassaemias.
   - Enzymopathies >> Eg. G6PD deficiency, PK deficiency.
   - Membranopathies >> Deficiencies in the proteins\ structural abnormalities of the RBCs membranes Eg. Hereditary spherocytosis, Elliptocytosis, Acanthocytosis.

II. B. Acquired (from the table) Imp in red only, like the causes of the intravascular hemolysis.
   - Allografts, especially marrow transplantation.
   - Drug associated.
   - Red cell fragmentation syndrome.
   - Arterial grafts, cardiac valves.
   - Microangiopathic.
   - Thrombotic Thrombocytopenic Purpura, Haemolytic Uraemic syndrome.
   - Meningococcal sepsis
   - Pre-eclampsia
   - Disseminated intravascular coagulation
   - March haemoglobinuria

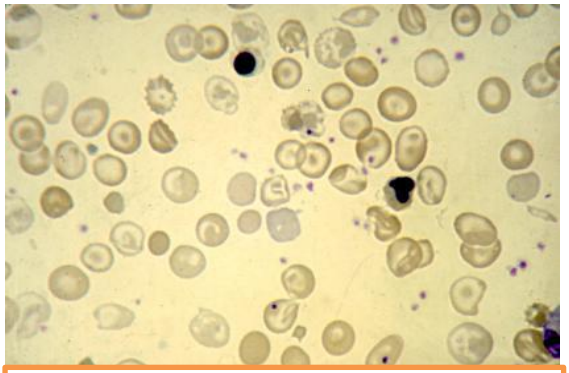
-Infections:
- Malaria, clostridia.

-Chemical and physical agents:
- Especially drugs, inductrial/domestic substances, burns.

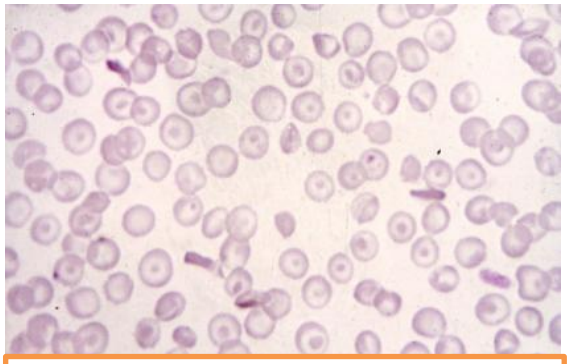
-Secondary:
- Liver and renal disease.
- Paroxysmal nocturnal Haemoglobinuria.
- **Autoimmune Haemolytic Anaemias.**



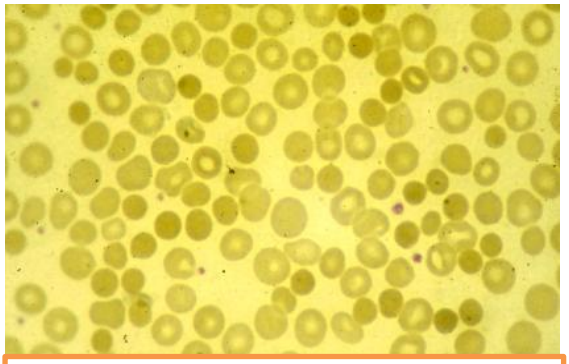
Sickle Cell Anemia



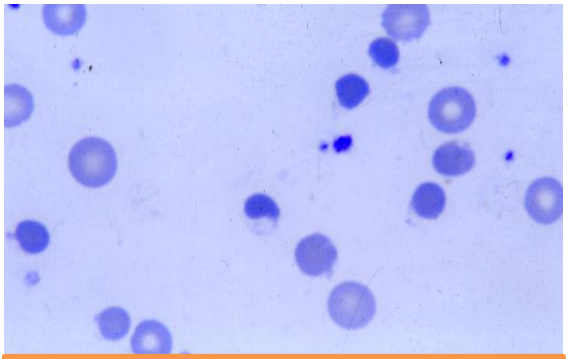
Thalassemia Major



Sickle Beta Thalassemia



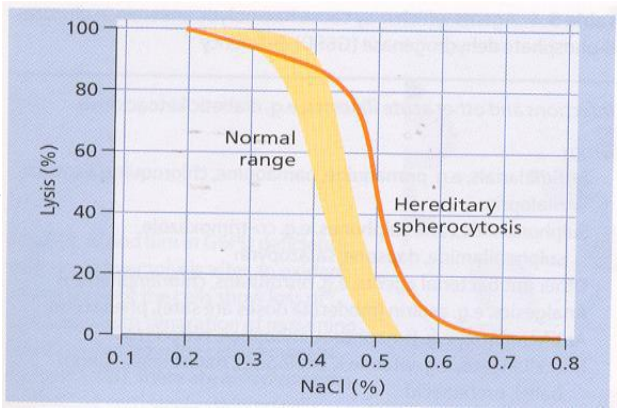
Spherocytosis



G6PD

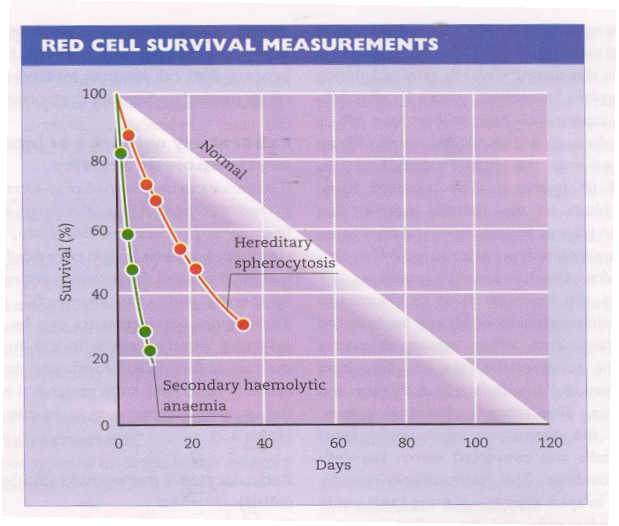
Other hereditary hemolytic anaemias (doctor said not important but just in case) Spherocytosis in new born, Eliptocytosis (Cigar shape) Stomatocytosis (Open mouth shape), Acanthocytosis (fingers like projections) all are due to membrane abnormality, Pyruvate kinase deficiency anaemia.

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
Some known Hemoglobin mutants:	
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**

Only these five are important with knowing the substituted amino acid.

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

You should only know the changes that happened to the 6th amino acid of each and the abnormal generated Hb.

2) Sickle cell disease

Amino acid	pro	glu	glu
Normal β–chain Base composition	CCT	GAG	GAG
Base composition	CCT	GTG	GAG
Sickle β–chain Amino acid	Pro	val	glu

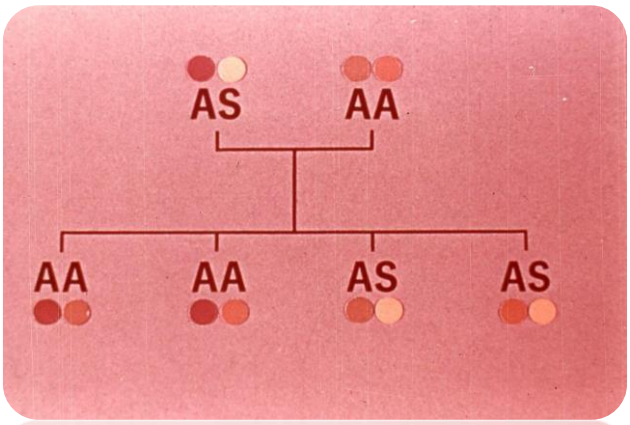
- A, adenine
- C, cytosine
- G, guanine
- glu, glutamic acid
- pro, proline
- T, thymine
- val, valine

Molecular pathology of sickle cell anaemia:

There is a single base change 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. (You must know the mutation in a sickler pt in which base and the newly formed amino acid).

Defect in the glutamic acid in β globin causes the sickle cell anemia.

Hereditary pattern in sickle cell disease:

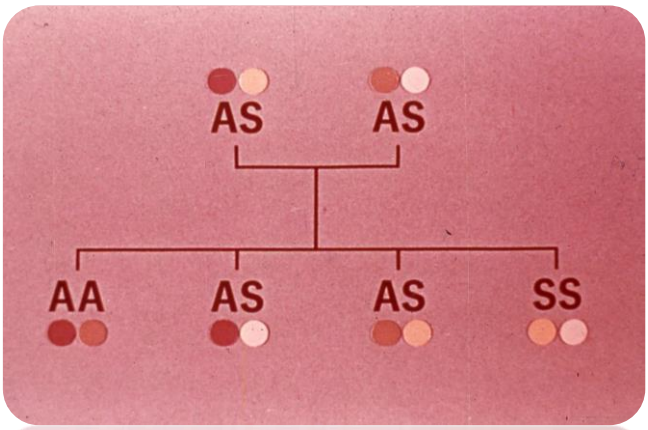


Case 1

If one parent was a carrier (trait) of an abnormal allele (of HbS)

50% of their children will be carriers (trait)

These will be living normally. However , if certain factors aggravate them they will manifest the disease (high altitude (low O_2) , surgery ..)

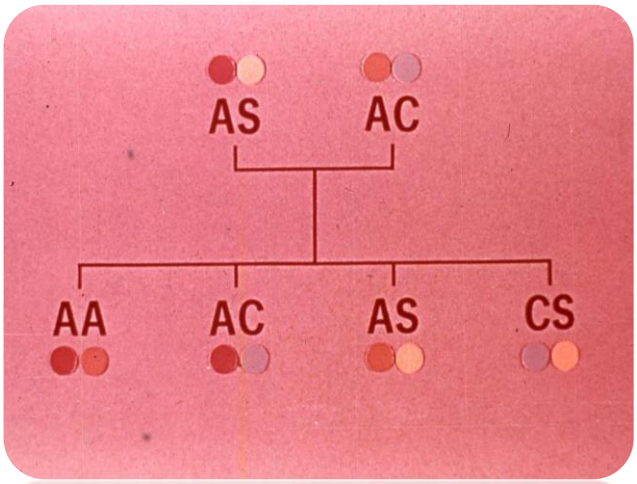


Case 2

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)***

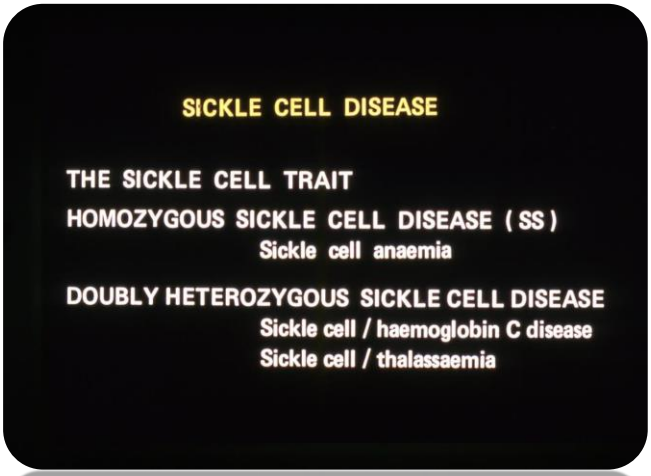


Case 3

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) and in this case sickle cell/HbC disease .because,in other cases instead of HbC it can be seen with thalassemia***



This summaries cases 2 and 3 and explains how the25% would manifest the disease if both parents were traits

Very important:

How to know whether this person was a trait or a sickled patient?

You do *Hb electrophoresis*→if *HbS <45%* this person is *trait* and if *>45%* this person has sickle *disease*

When we say heterogeneous → having non-similar alleles at corresponding chromosomal loci (here its seen as the case of a trait person having one allele as normal and the other Hbs mutation (AS) or in case of sickle cell disease you can say double heterogeneous meaning the person has the disease but from two different allele as in the case of (SC or S-thal)

When you say homozygous →having, similar alleles such as ones seen in normal people (AA) or sickled patients with the same type of allele anomaly (SS)

Properties of HbS:

- Solubility ↓
- Conformational changes:
  "tactoid formation"
  → Sickled cells
  → Irreversible sickled cells

Erythrocytes containing mutant HbS have abnormal properties, and under conditions of low oxygen tension, interaction between the abnormal valine residue and complementary regions on adjacent molecules results in the formation of intracellular, rod-shaped polymers. These abnormal hemoglobin polymers aggregate to disrupt the cytoskeleton and distort the shape of the erythrocytes, making them brittle and poorly deformable. Thus, unlike normal erythrocytes, the sickle-shaped cells cannot squeeze through the microcirculatory vessels, blocking blood flow and resulting in local hypoxia.

- ↑ Mechanical fragility ⟶ hemolysis
- ↑ Viscosity ⟶ organ infarction

Factors affecting sickling:

- Oxygen tension (50-60mmHg for SS - 20-60mmHg for AS)

In case of O_2 tension : the trait can tolerate the drop on O_2 to a certain limit 20-30 mm Hg if it drops further → signs of the disease will occur. However, in the case sickle cell patients they can't deal with very high drops and only can accommodate a range of 50-60 mm Hg.

- pH – inhibited at alkaline pH and exacerbated by acidification Higher acidity worsens the disease
- Concentration of HbS
- Presence of other hemoglobin:

  Polymerization: S > D > C > J = A > F

- The presence of high concentrations of HbS or other forms associated with it worsens the disease further.

Factors precipitating crises:

1. Infection (especially malaria)
2. Pyrexia
3. Exposure to cold
4. Dehydration
5. Pregnancy She is at high risk of mutli-organ infarctions

Crises in sickle cell disease:

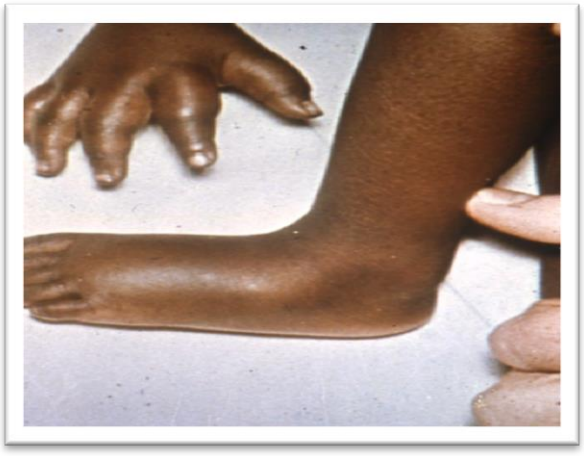
1. Hyperhaemolytic
2. aregenerative or aplastic (= shutting down of the bone marrow)
3. Small vessel occlusion

Clinical manifestations of Sickle cell disease:

- Hemolytic anemia
- Tissue infarction

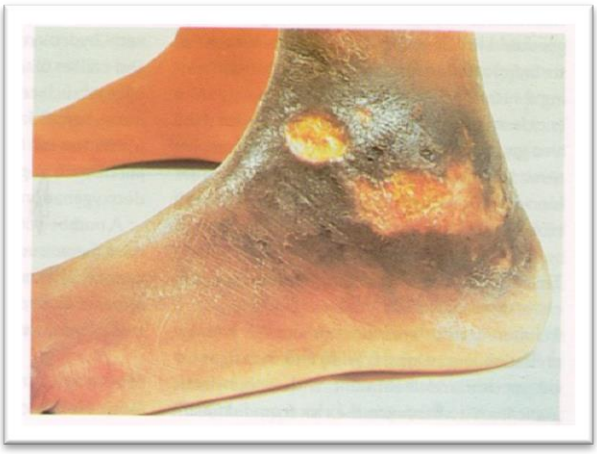
Clinical Manifestations in Sickle Anaemia:

- **Pallor (Anaemia)**
- **Jaundice & Dark Urine**
- **Apathy & Anorexia**
- **Hand-Foot Syndrome (Young Children)**
- **Splenic sequestration (Young Children)Hepatic Sequestration (they are taking up a lot of blood leading to their enlargement)**
- **Bones, Joints Pain >> very severe**
- **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**



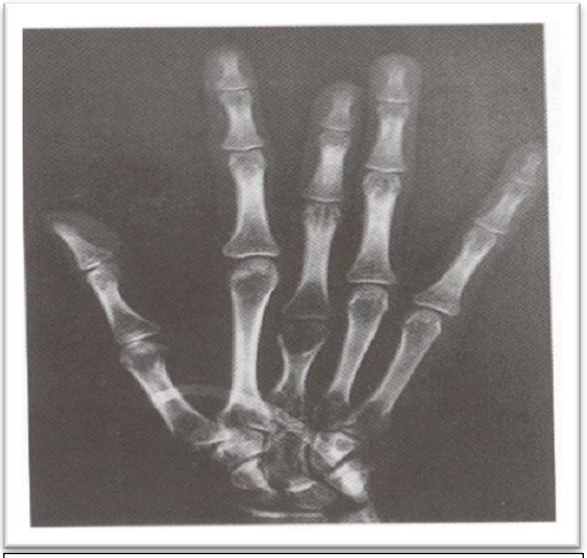
Foot and hand syndrome >> foot and hand swollen and severe pain

This effect is due to dactylitis which is secondary to (multiple infarctions, and salmonella infection)

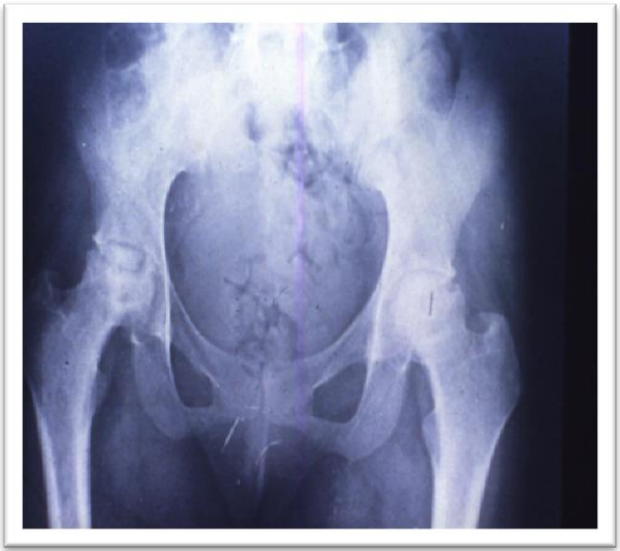


Leg ulcer very common in sickle cell anemia secondary to infarction

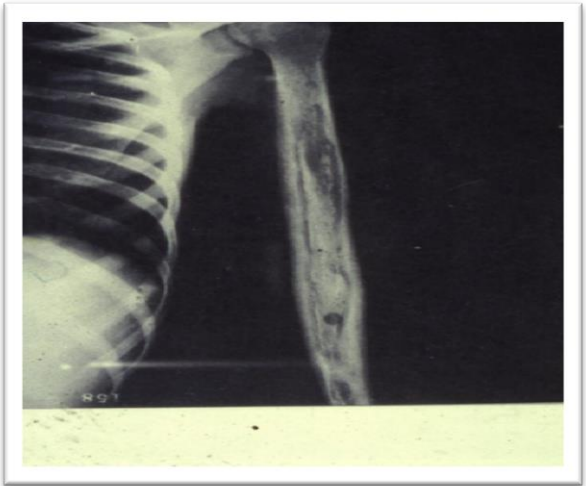
On X-RAY:



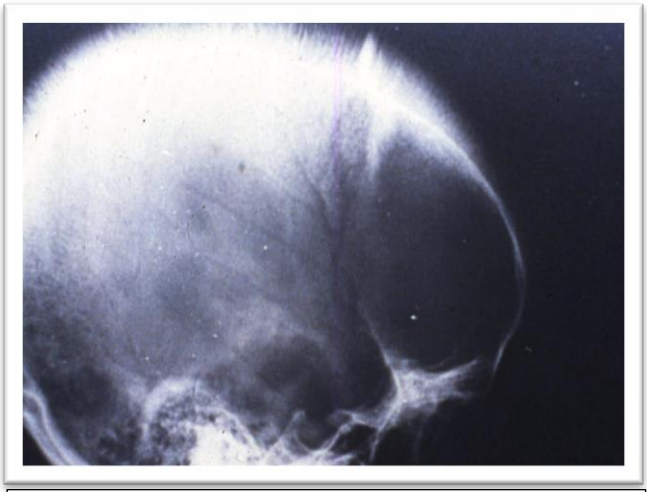
Short middle finger (specific feature)



Avascular necrosis of the hip joint



Dactylitis

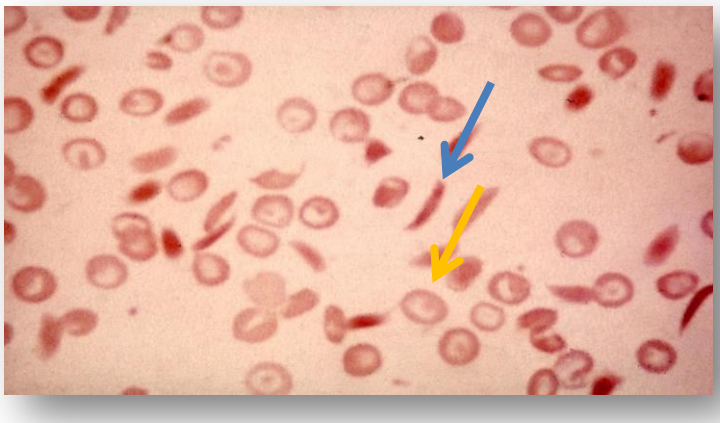


Hair on end (due to ineffective erythropoiesis and bone marrow expansion)

Papillary necrosis

Laboratory Diagnosis:

- CBC >> Low Hb and hypochromic microcytic anemia
- Blood Film >>irreversible sickle cells (blue arrow) and target cells (orange arrow)
- Sickle Solubility Test (usually positive)
- Hb Electrophoresis >> It is the most important and confirmative test for the sickle cell anemia
- Genetic Study



Indications for Blood Transfusion in Sickle Cell Anaemia:

- Splenic sequestration
- Hepatic sequestration
- Aplastic crisis
- Overwhelming infections
- Elective or emergency surgical operation >> e.g. for the removal of gallstones
- Severe painful crisis associated with severe haemolysis
- Pregnancy if they are at labor to prevent major crisis

Indications for exchange transfusion:

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

*Please note what's mentioned above goes according to what the doctor has mentioned in the slides there for, please refer to the lecture for some picture (that weren't explained on by the doctor)

Summary (from Essential Hematology book)

- **Haemolytic anaemia is caused by shortening of the red-cell life. The red cells may breakdown 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 acquire causes, which are usually caused by an abnormality of the red cell environment.**
- **Features of the extravascular haemolysis include jaundice, gallstones and splenomegaly with raised reticulocyte, unconjugated bilirubin and absent haptoglobins. In intravascular haemolysis example caused by ABO (or mismatched blood transfusion) there is haemoglobinemia methaemoglobinaemia, haemoglobinuria and haemosiderinuria.**
- **Genetic defects include those of the red cell membrane example, hereditary spherocytosis, enzyme deficiencies example, glucose-6-phosphate dehydrogenase or pyruvate kinase deficiency or haemoglobin defect, example sickle-cell anaemia.**
- **Acquired causes of haemolytic anaemia include: warm or cold, auto or allo antibodies to red cells (acquired autoimmune hemolytic anaemias), red cell fragmentation syndromes, infections, toxins and paroxysmal nocturnal haemoglobinuria.**
- **The most frequent structural defect of Hb is the sickle mutation in the Beta globin chain causing, in the homozygous form, a severe haemolytic anaemia, associated with vaso occlusive crisis.** These may be painful affecting bone or soft tissues eg: chest, spleen, CNS) crisis may also be haemolytic or aplastic**.**

Questions

1/ Which ONE of the following is a feature of a extravascular haemolytic anaemia?

(A) Raised serum conjugated bilirubin
(B) Gall stones
(C) Low reticulocytes count
(D) Hypocellular Bone marrow

2/ Which one of the following is a cause of congenital hemolytic anemia?

(A) Pyruvate kinase deficiency
(B) ABO incompatibility
(C) Malaria
(D) Red cell fragmentation syndrome

3/ A 10-year-old black girl is brought to the emergency room. She complains of severe pain in her chest, abdomen, and bones. Physical examination reveals jaundice and anemia. Her parents state that she has been anemic since birth. A CBC shows normocytic anemia with marked poikilocytosis. Hemoglobin electrophoresis demonstrates hemoglobin S. This child's chest and bone pain is most likely caused by which of the following mechanisms?

(A) Amyloidosis
(B) Coagulopathy
(C) Infection
(D) Ischemia

Answers:

- 1- B
- 2- A
- 3- D

اللهم إني استودعك ما قرأت و ما حفظت و ما تعلمت فرده عليَ عند حاجتي إليه انك على كل شيء قدير

If there is any mistake or feedback please contact us on: 432PathologyTeam@gmail.com