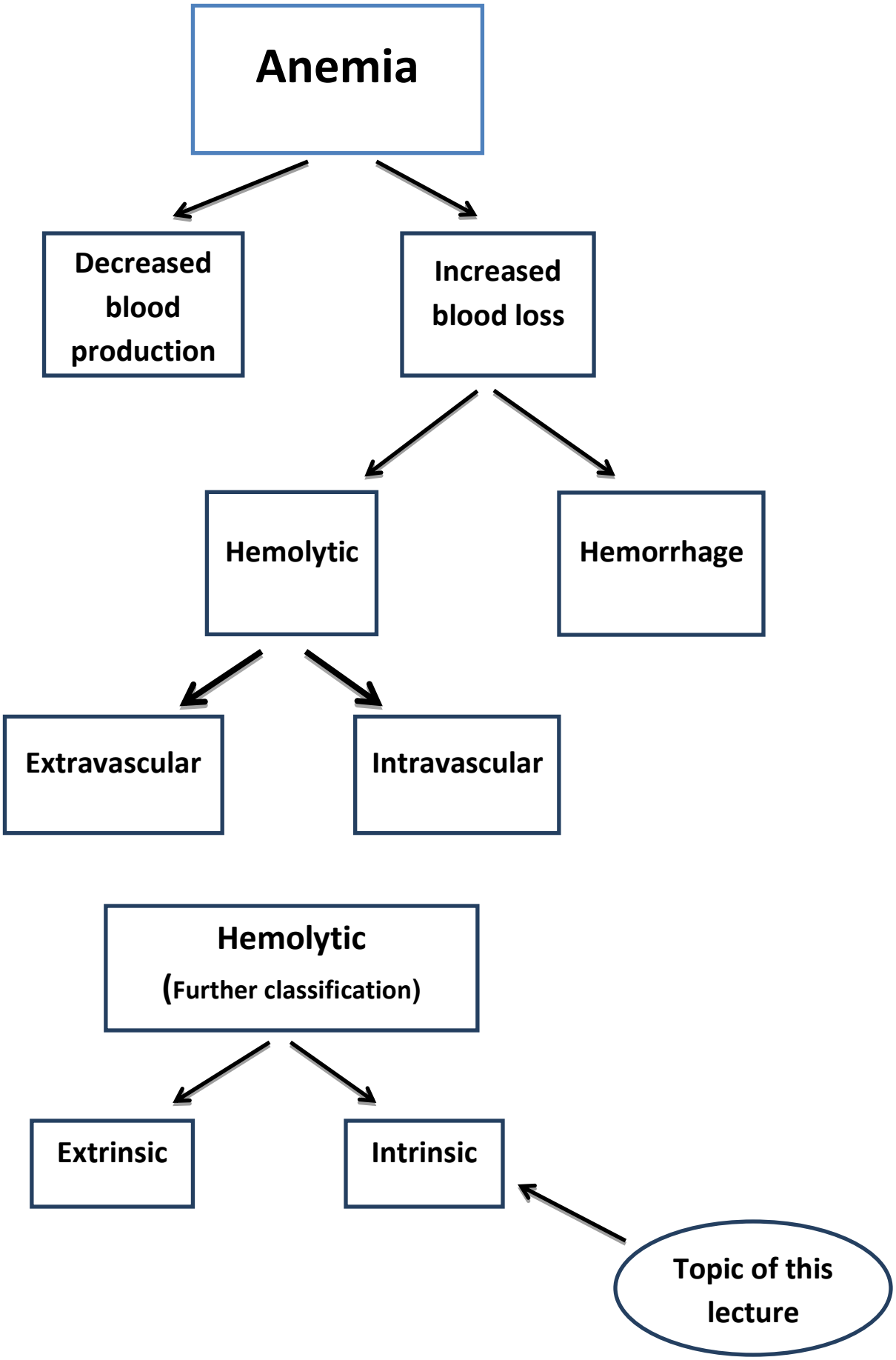
HEMATOLOGY
&LYMPH SYSTEM

Pathology sheet

Number	4
Done BY	Jumana Jihad
Correction	Dr. Ahmad Mansour
Doctor	Dr. Ahmad Mansour


Anemia
Decreased blood production
Increased blood loss
Hemolytic
Hemorrhage
Extravascular
Intravascular
Hemolytic
(Further classification)
Extrinsic
Intrinsic
Topic of this lecture

Here's an exception:
Anemia of chronic blood loss → (is actually) → anemia of decreased production (iron deficiency)→ with chronic blood loss, iron stores are gradually depleted. Iron is essential for hemoglobin synthesis and erythropoiesis, and its deficiency leads to chronic anemia of underproduction. Iron deficiency anemia can occur in other settings as well.

Quick review:

Anemia results from decreased RBC mass reflected in a decrease in Hb and hematocrit resulting in asymptomatic anemia or in general symptoms of anemia, for example:

- Fast heart rate (tachycardia)
- High respiration rate (O2 intake)
- Pale skin (blood will be shifted from skin to more vital organs)
- Muscle weakness
- Mental confusion

Topics of this sheet:

<u>Hemolytic anemia of intrinsic cases</u>

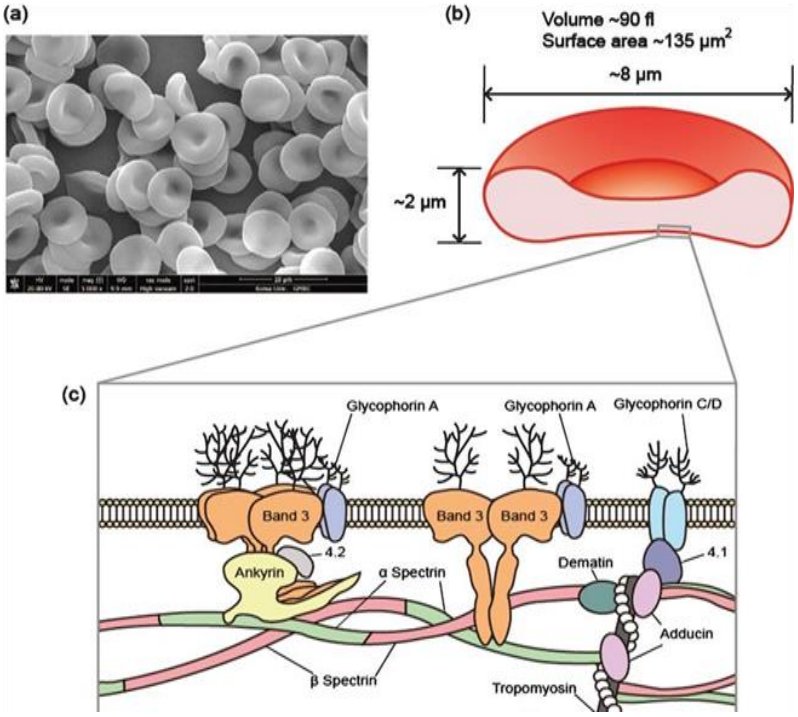
1. Hereditary:
   - Membranopathies - spherocytosis
   - Hemoglobinopathies – thalassemia, sickle cell disease
   - Enzymopathies - G6PD deficiency

2. Acquired:
   - Paroxysmal nocturnal hemoglobinuria.

REMEMBER

Hemoglobin is composed of:

1. Iron
2. Heme ring
3. 4 polypeptide chains of 2 types (Alpha and Beta).



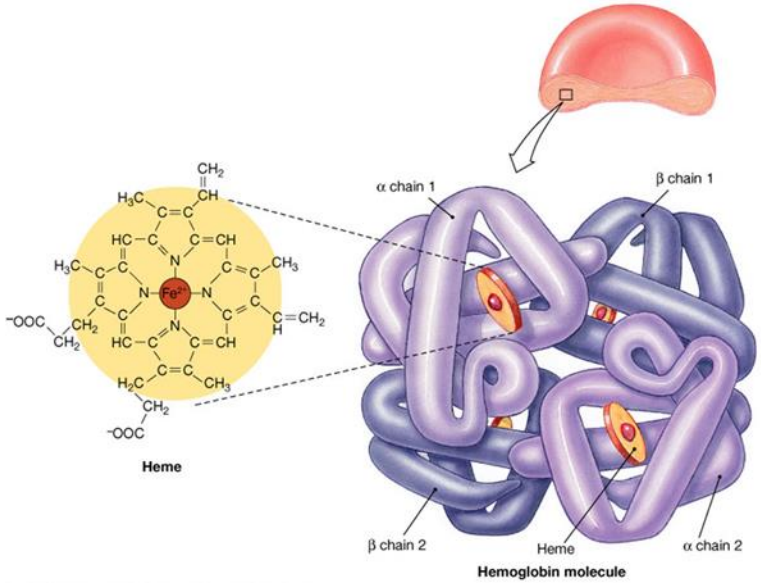
- When the problem is in the <u>beta</u> chain it could be either **thalassemia** or **SCA**
- If the problem in the <u>alpha</u> chain → **alpha thalassemia**
- If there is problem with the <u>heme ring</u> →**sideroblastic anemia**
- If the problem is in <u>iron</u> → **IDA**

1- <u>Hereditary spherocytosis:</u>

We've mentioned previously the structure of RBCs and its membrane which is very important in forming the biconcave shape of RBCs.

- **What is the importance of RBCs biconcavity?**

1- It helps increase the surface area allowing more gas exchange.
2- This shape gives the flexibility needed for RBCs to pass through small capillaries.



RULE OF PHYSICS: A spherical shape is the most economical shape for a certain volume.

- **What does maintain this biconcave shape of RBCs?**

As we learned in histology, a set of proteins (ankyrin, spectrin, and actin) support the membrane and stabilize this biconcave shape.

Hereditary spherocytosis → Mostly AUOSOMAL DOMINANT

Usually this disease occurs due to a mutation in one of the following proteins (ankyrin, band 3, or spectrin). This will result in the abnormal spherical shape of RBCs because the membrane is no more stable or well supported.

Here's how it gets nasty:

RBCs will keep losing parts of their membrane while moving through capillaries to fit. While this loss occurs, the Hb content of cells will not change; therefore, MCHC will increase.

Hereditary spherocytosis is the only type of anemia where MCHC is high

These spherocytes spend abnormally longer time in the spleen because they can't pass easily like regular RBCs. This is called (**splenic trapping**).

- **Why is spending more time in the spleen a problem?**

Because it will cause a decrement in glucose and pH → RBCs will be more vulnerable for hemolysis. Macrophages in the spleen will eventually lyse these RBCs.

- **Which type of hemolytic anemia do you think spherocytosis is?**

EXTRAVASCULAR (in the spleen)

- **What is the most common casus of SPHEROCYTES?**

Auto-hemolytic anemia

Hereditary spherocytosis it's a very rare disease

Notes taken from slides:

- Moderate clinical course, mostly.
- Can be complicated by aplastic crisis (parvo B19). This virus affects only the nucleated erythroid progenitors in the bone marrow and kills them.
- Anemia, jaundice, gallbladder stones, (splenomegaly)→ one of the largest (huge spleen).
- MCHC is high.
- Diagnosis involves osmotic fragility test.
- No definitive treatment
  - Symptomatic treatment with splenectomy for patients above 5 years of age.

- **What is the osmotic fragility test?**

The osmolality of the plasma and interior of RBCs is **0.9** .

- **What would happen if we put RBCs in pure water?**

Water will enter the cells. Normal RBCs (which are larger in volume than spherocytes) can tolerate this to a certain extent. The biconcave shape helps in this toleration, but eventually RBCs will rupture. Normal RBCs differ from the spherocytes in that they are more flexible and therefore can tolerate more water entering before finally rupturing. Sherocytes, on the other hand, have less volume and as a result will allow less amount of water to enter before rupturing. In other words,

they have more restriction on the amount of water they can tolerate and will rupture in a higher concentration than normal RBCs.

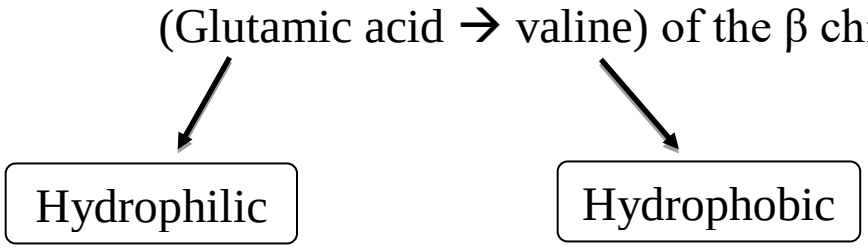
In spherocytosis the rupture will appear in higher concentration

In Normal RBCs the lysis will begin at a concentration of 0.5 and in spherocytosis it will start at 0.85. The hemolysis will appear as a pink color in the tube.

2- Hemoglobinopathies - Thalassemia and Sickle Cell Disease

I. Sickle Cell anemia:

As it's been made well known for us, SCA results from a mutation in the β chain.



- **Where will you see these patients?**
  In the ER because of the chronic pain they'll present with (a pain crisis is described as one of the worst presentations). Many of them will be addict to analgesics.

- **Why do they have this pain?**
  When this mutation occurs, an abnormal hemoglobin forms (HbS). HbS in the deoxy state will polymerize forming fiber like structure inside RBCs and this will damage the membrane.

So there will be:

Deformities in the deoxygenized environment

↓

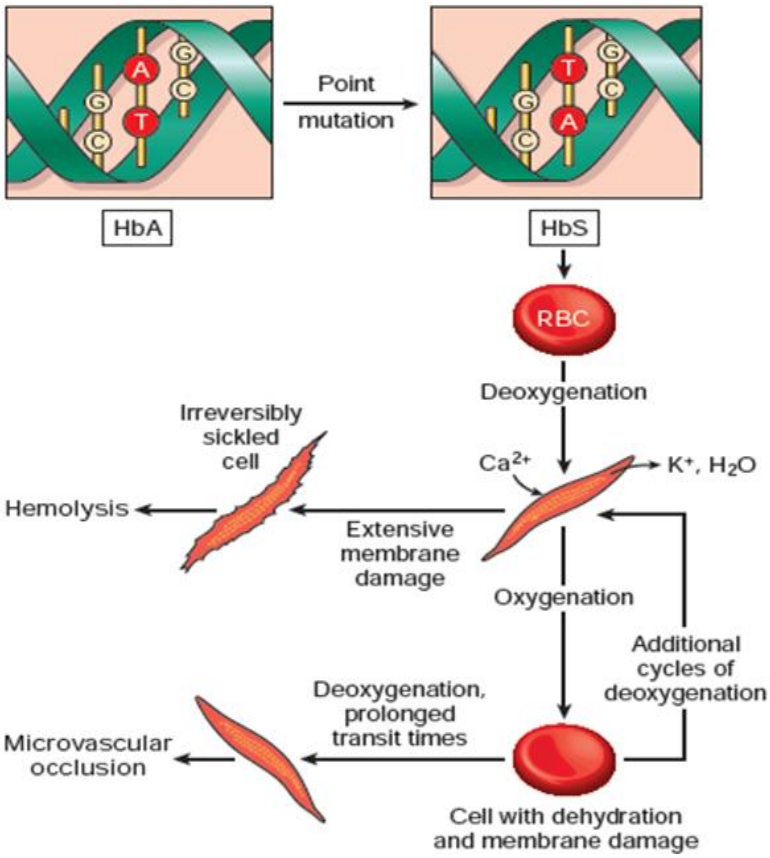
RBCs can't enter to throughcapillaries

↓

Ischemia

↓

Chronic pain



- It is an AUTOSOMAL RECESSIVE disease.
- When the patient is a **heterozygotes** (have mutation in only one gene) ½ of its hemoglobin is normal → so s/he will have mild anemia → does not cause any problem.
- But if the patient **homozygotes** most of her/his hemoglobin will be HbS.
- **8% in black Americans** (they have a high gene frequency because their origin is from West Africa where malaria is endemic).

➢ **Why sickling?**

There are three important factors that influence sickling in the body:

a) Presence of hemoglobin types other than HbS

If we are talking about a carrier:
S/he will have **HbS** and **HbA**→ Sickling will be very weak; HbA will dominate.

In newborns, **HbF** will also overcome the effect of **HbS**.

- **HbA** ($\alpha 2\beta 2$) = weak
- **HbF** ($\alpha 2\gamma 2$) = weak
- **HbC** = strong

****HbC** will increase sickling******

If we have **HbC** + **HbS** → It will be like having complete **HbS**

b) Intracellular concentration of hemoglobin

More Hb → more sickling
Less Hb → less sickling

Dehydration is one of the precipitating factors in a sickling crisis.
In α thalassemia, we have a lower concentration of Hb, so it will be less severe.

c) Transit time for RBCs within the vasculature

If RBCs are moving fast in the vessels → no sickling.
If RBCs happen to move slowly (like in small capillaries) → sickling, and small capillaries will be obstructed.

Your patient will present with:

- **Chronic hemolytic anemia.**
- **Fatty change in the heart, liver and renal tubules.**

➢ **Do these patients develop splenomegaly?**

In the beginning YES they will, but after that **autosplenectomy** will take place because of the recurrent attacks of ischemia.

- **Reticulocytosis and erythroid hyperplasia in bone marrow.**
- **Bone changes**, prominent cheekbones and crew-cut skull this is because of the activation of hematopoiesis in these bones which are normally inactive.
- **Extramedullary hematopoiesis in liver and spleen** but eventually we will have **autosplenectomy**.
- **Increase risk of infections, salmonella osteomyelitis.**
- **Vessel occlusion, bone pain, acute chest syndrome, stroke**→it's what kills the patients with SCA.
- ***Aplastic crisis*** if they affected by Parvo19 virus**.**

(Temporary cessation of red cell production)

- **Diagnosis:**

1. Hemoglobin electrophoresis to demonstrate **HbS**.
2. Fetal DNA via amniocentesis or chorionic villi biopsy.

Sickle cell trait is mostly ASYMPTOMATIC

- **Treatment:**

1- HYDROXYUREA:
- Increase HbF.
- Anti-inflammatory due to decreases WBC production.

- Increase MCV (Hb concentration will decrease → less sickling).
- Production of NO which causes vascular dilatation.

2- BONE MARROW TRANSPLANT.

II. -Thalassemia:

We have two type of thalassemia:

α and **β**

- **What is the difference between thalassemia and SCA?**

In SCA we have a mutation is the β-chain.
In thalassemia → there is no structural deformities but there is a decrease in the quantity of hemoglobin chains.

In other words:
SCA is qualitative defect, while thalassemia is quantitative defect.

The thalassemia syndromes are a heterogeneous group of disorders caused by inherited mutations that decrease the synthesis of either the **α-globin** or **β-globin** chains that compose adult hemoglobin, HbA (α2β2), leading to anemia, tissue hypoxia, and red cell hemolysis

- **Why?**

Because α-chains decreased → β-chains will increase and will precipitate causing hemolysis (imbalance in globin chain synthesis).

Thalassemia involve diminished production and hemolysis

- **In what regions is thalassemia found?**

Africa, Mediterranean, and Asian regions were malaria is endemic.

- **Premarital test (فحص ما قبل الزواج):**

It measures MCV.
Thalassemia carriers have very low MCV.

- **4 alpha genes, on chromosome 16**
- **2 beta genes, on chromosome 11**

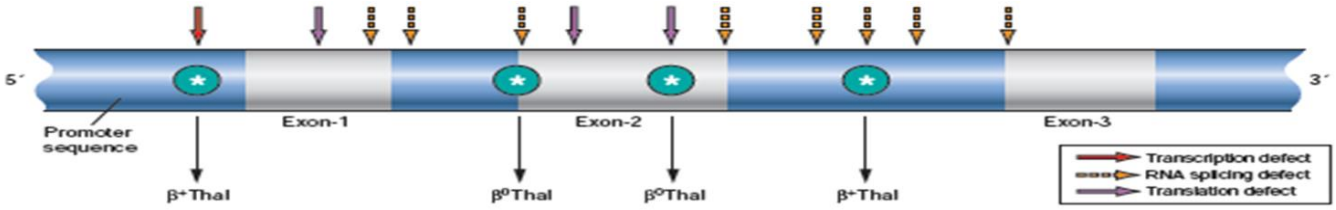
- β thalassemia:

We have two category of mutations

1) β0→ no protein is produced

2) β+→decreased protein production

****Unlike sickle cell disease, the amino acid sequence is INTACT!****



- **Types of mutations:**

1. Promoter region mutations.
   (It is the region before the gene which controls how much gene is expressed).
2. Splicing mutations.
3. Chain termination mutations.
   (The presence of a stop codon instead of an amino acid codon).

In β thalassemia → point mutations
In α thalassemia → larger deletions

Two mechanisms contribute to the anemia in **β** thalassemia:

1- Decreases production and 2- hemolysis.

Hemolysis occur because there is an imbalance in **β-globin** and **α-globin** chain synthesis → α chines will precipitate → go to the spleen → phagocytosis →RBC life cycle will decrease to become less than 120 days.

Anemia → hypoxia → bone marrow expansion trying to compensate → skeletal deformities →**α globulin** aggregation in RBCs forming **Heinz bodies**.

-***B thalassemia major*** → 2 genes are affected / could result from 2 genes affected with severe mutations **(β0/β0**).
-***Beta thalassemia minor*** → 1gene is affected.
-***B thalassemia intermedia*** **(Variable)** → either 1 gene or 2 genes are affected, but there is no complete absence of production → only reduction one is affected and the other is normal **(β0/β)** or the 2 gene is affected with modest mutation **(β+/β+).**

- Common in the Mediterranean areas and the Middle East-Anemia manifests 6-9 months of life, **WHY?** → As hemoglobin synthesis switches from **HbF (α2γ2)** to **HbA (α2β2).**
- Low hemoglobin 3-6g/dL
- Low MCV
- Elevated **HbF** its thalassemia and **HbA2 (α2δ2)**
- **HbA2** is very sensitive for **beta** thalassemia.

• Morphology:

- No specific morphology
- Presence of target cells, but they are present in other diseases as well (not specific for diagnosis).
- Heinz bodies → they are **α** precipitation when they go to the spleen→ phagocytised → and we call them "bite cells" or "blister cells".

- X-ray of the skull shows perpendicular radiations resembling a crew-cut).

- **Manifestations:**

- Hepatosplenomegaly.
- Cardiac disease.
- Transfusion dependent (role of chelation therapy to decrease iron overload).
- Really bad prognosis rarely lives beyond their twenties.
- Stem cell transplantation is the only hope for cure.

Beta thalassemia minor:

- Usually asymptomatic
- Increased erythropoietin
- Bone MARROW EP (ERYTHROPOIESIS PROGENITOR) hyperplasia
- Elevated **HbA2**

It's important to differentiate between **beta minor** and **IDA**:

Minor
-Increased RBCs count
-Normal Hb
-Low MCV
-RDW is normal

IDA
-High RDW
-low MCV
-Decreased RBC count

- # α Thalassemia:

✓ 4 genes are normal→ **Healthy**
✓ 1 gene is affected→ **Silent Carrier** (little decrease in MCV)
✓ 2 genes →**Alpha thalassemia trait** (microcytosis and mild to no anemia).

- ✓ 3 genes → **HbH** (moderately severe anemia similar to B-thalassemia intermedia)
- ✓ 4genes are affected → **Hydrops fetalis** (lethal in utero)

1- What is the mode of inheritance in the vast majority of spherocytosis cases?

A. **Autosomal dominant**
B. **Autosomal recessive**
C. **X-linked dominant**
D. **X linked recessive**

2- The amino acid present at the sixth position of the normal alpha-globin chain is replaced by which one of the following amino acids in sickle cell disease?

A. **LysineB.ValineC.SerineD.AlanineE.None of the above**

3- In thalassemia disorders, when only one alpha gene is affected, what do we call that?

A. **Normal**
B. **Silent carrier**
C. **Thalassemia trait-*cis***
D. **Thalassemia trait-*trans***
E. **HbH disease**

4- Gallbladder stones are a frequent complication of G6PD deficiency?

TRUE **FALSE**

5- Paroxysmal nocturnal hemoglobinuria results from an acquired mutation in which of the following genes:

A. **Alpha hemoglobin**
B. **Beta hemoglobin**
C. **Erythropoietin**
D. **PIGA**
E. **G6PD**