Hemolytic Anemia
Hemoglobinopathies

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Hemolytic anemia
Outlines

- Definition
- Causes and Features
- Hereditary Spherocytosis
- G6PD deficiency
- Immune mediated hemolytic anemia
- PNH
- Hemolytic Anemia Due to Mechanical Trauma to RBC

Hemolytic anemia

- Hemolytic anemias are a diverse group of disorders that have as a common feature **accelerated red cell destruction (hemolysis)**.
- There are several ways to organize hemolytic anemias.
- One approach groups them according to whether the pathogenic red cell defect is intrinsic (intracorpuscular) or extrinsic (extracorpuscular) .
- A second more clinically useful approach groups hemolytic anemias according to whether hemolysis is primarily extravascular or intravascular.
- Extravascular hemolysis is caused by defects that increase the destruction of red cells by phagocytes, particularly in the spleen

Intrinsic (Intracorpuscular) abnormalities:

A.Hereditary defects

1.Defects in RBC membrane: Spherocytosis

2.Enzyme defects: G6PD Deficiency

3. Disorder of Hemoglobin synthesis:

- Structurally abnormal hemoglobin synthesis (Hemoglobinopathies): Sickle cell disease
- Deficient globin synthesis: Thalassemia syndromes

B. Acquired Defects

1. Membrane defect: PNH (paroxysmal nocturnal hemoglobinuria)

Hemolytic anemia

Destruction can occur :

1. Intravascular

2. Extravascular (Within tissue macrophage, mainly liver and spleen)

Clues?

Intravascular Hemolysis:

- Hb-uria, Hb-emia, and Hemosiderinuria
- Unconjugated hyperbilirubinemia and Jaundice (Due to conversion of heme to bilirubin)
- Marked decrease in Haptoglobin (circulating protein that binds and clears free hemoglobin)

- Incresed LDH (Lactate dehydrogenase)

Hemolytic Anemia

- **Extravascular Hemolysis:**
  - More common
  - No Hb-uria, Hb-emia, and Hemosiderinuria
  - Jaundice
  - Mild decrease in Haptoglobin
  - Splenomegaly

Extrinsic (Extracorpuscular) abnormalities:

- **Antibody- mediated**

- **Mechanical trauma to red cells**

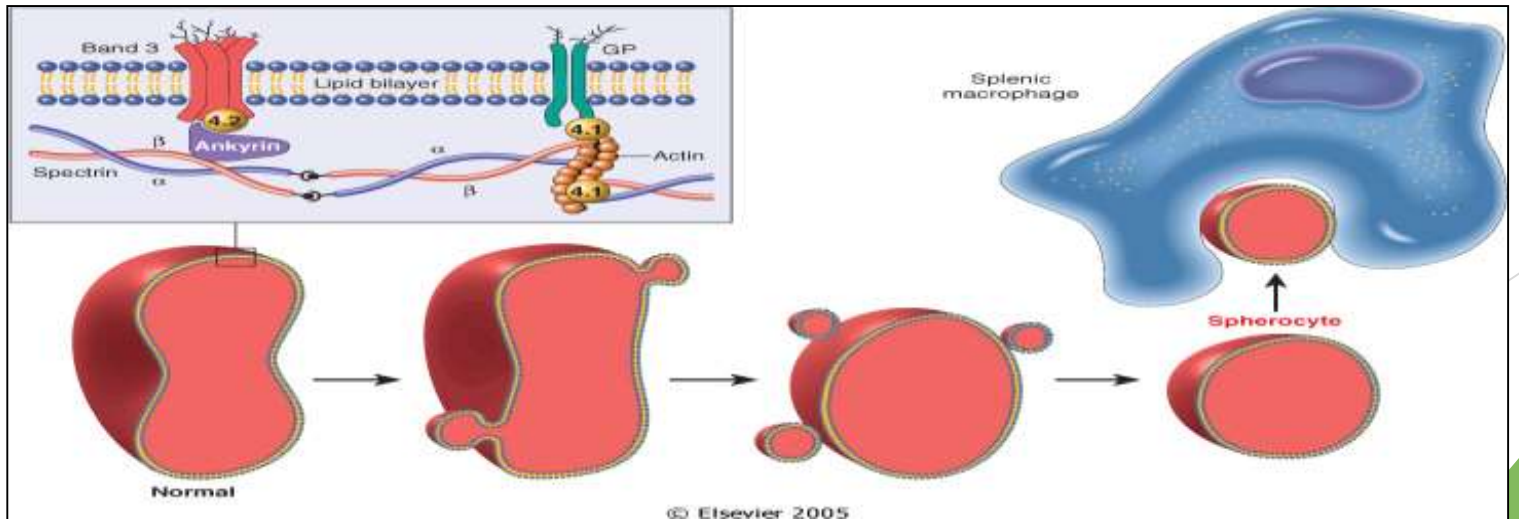
- **Infection: Malaria**

Features of Hemolytic Anemia

- **Shortened RBCs survival**
- **Elevated erythropoietin level leading to increased erythrpoiesis (erthyroid hyperplasia) and early release of RBCs from marrow**
- **Reticulocytosis in peripheral blood**
- **Retention of the products of degraded red cells (including iron) by the body.**
- **Jaundice and Increase LDH**
- **In severe hemolytic anemia: Extramedullary hematopoiesis in the liver, spleen, and lymph nodes.**

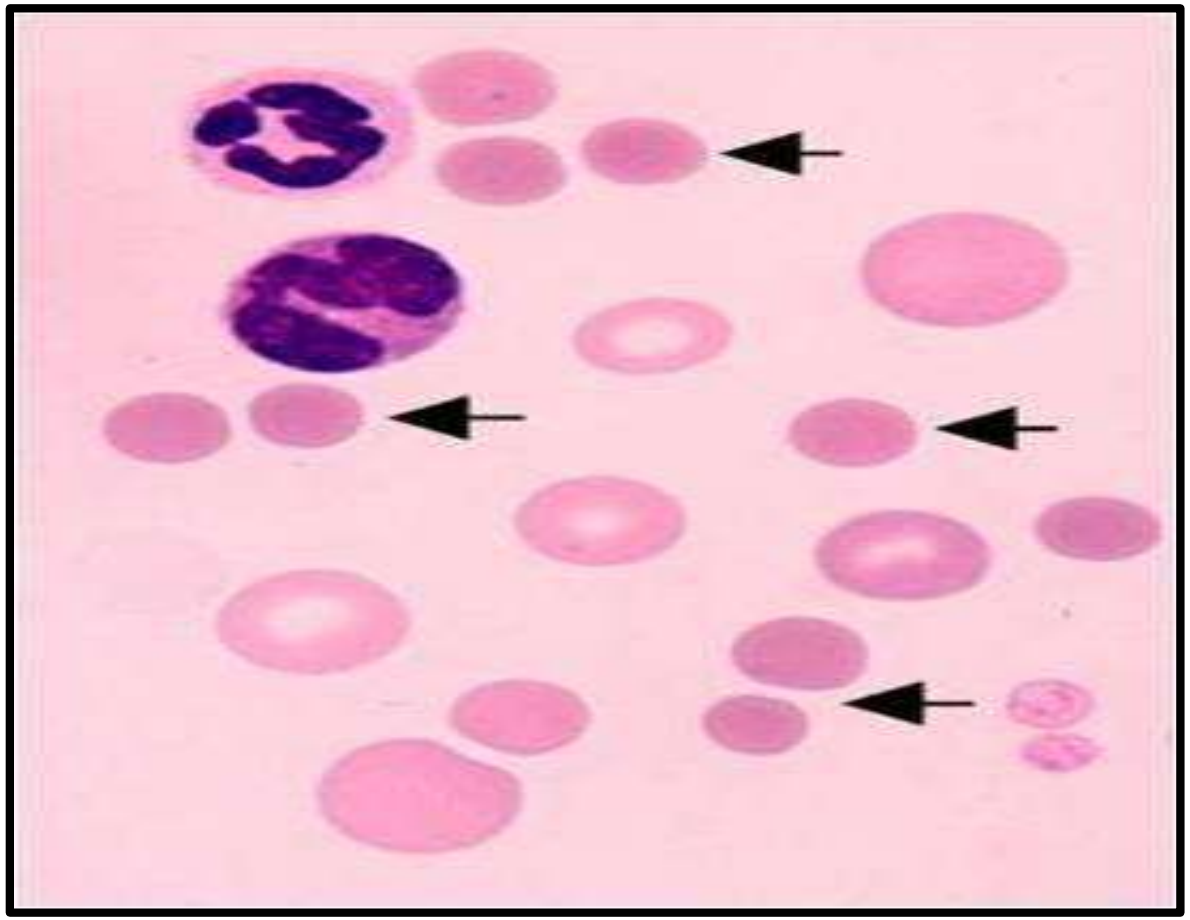
HEREDITARY SPHEROCYTOSIS

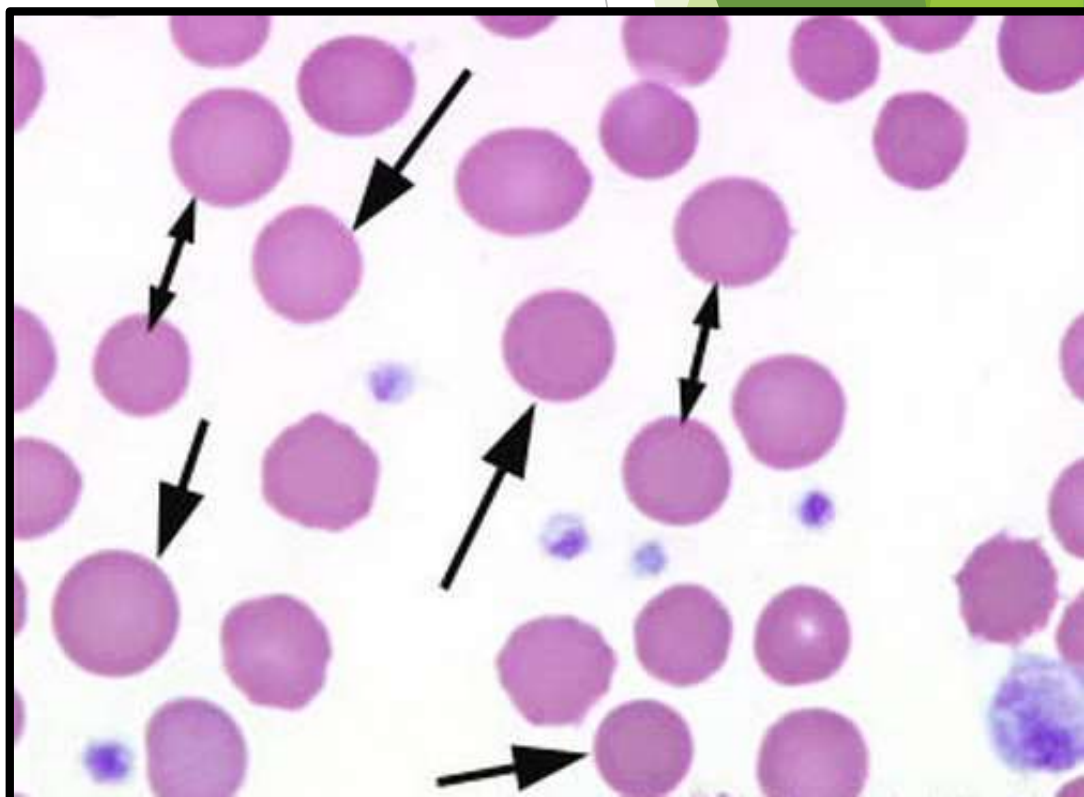
- **Inherited** defect in red cell **membrane**
- Fromation of **spherocytes**, nondeformable cells: highly vulnerable to sequestration and destruction in the spleen



HEREDITARY SPHEROCYTOSIS

- Etiology: mutations in **Spectrin, Ankyrin, and band 3**
- General features:
  - **Autosomal dominant** trait
  - Spherocytes in peripheral blood: Dark red, lack central pallor, Increase MCHC
  - High reticulocyte count
  - Increased osmotic fragility especially after incubation for 24 hours at 37^{o}C (in hypotonic)
  - Destruction in the spleen
  - Splenomegaly
  - Splenectomy helps in correcting anemia, but red cell defect persist





CLINICAL FEATURES

- Anemia, splenomegaly and jaundice
- Severity of anemia correlates with spectrin deficiency.

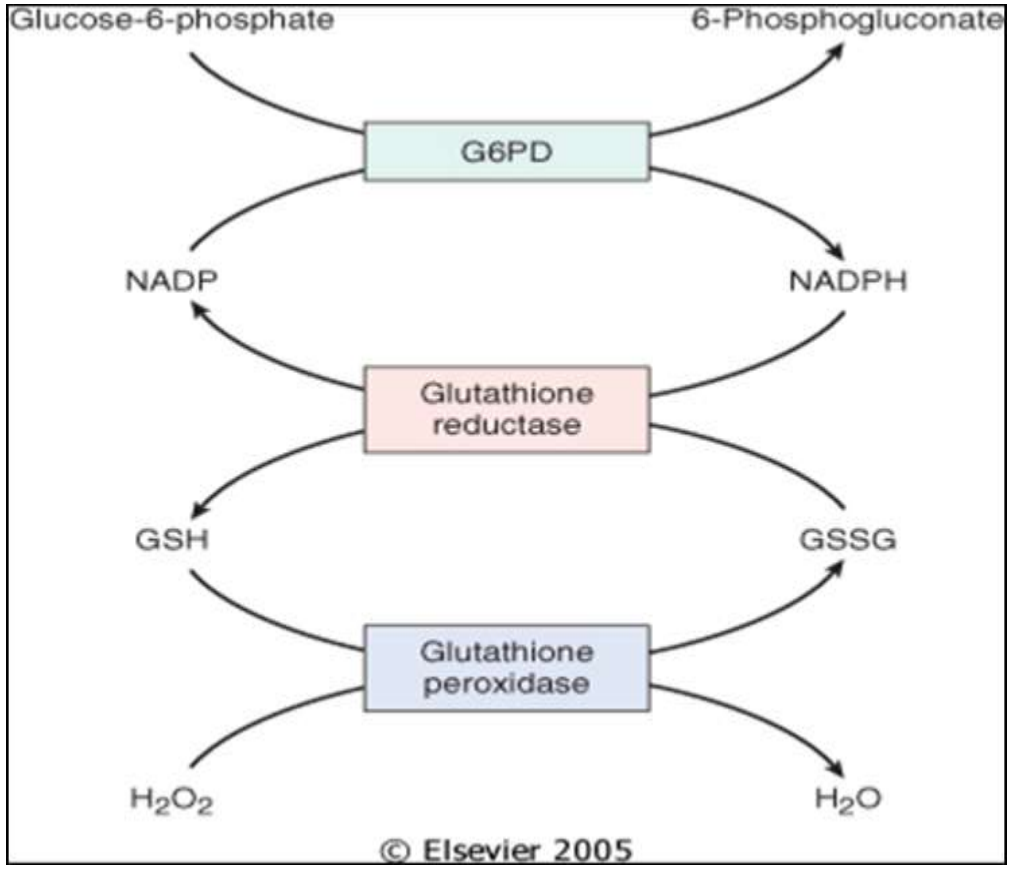
Complications:

- Gallbladder stones
- Aplastic crisis: Due to infection by Parvovirus B19

G6PD Deficiency

Role of glucose-6-phosphate dehydrogenase (G6PD) in defense against oxidant injury

- **The disposal of H2O2, a potential oxidant, is dependent on the adequacy of reduced glutathione (GSH), which is generated by the action of NADPH. The synthesis of NADPH is dependent on the activity of G6PD**



G6PD

- G6PD gene located on chromosome X.

- Decrease in GSH causes hemolysis in cells exposed to oxidant agent.

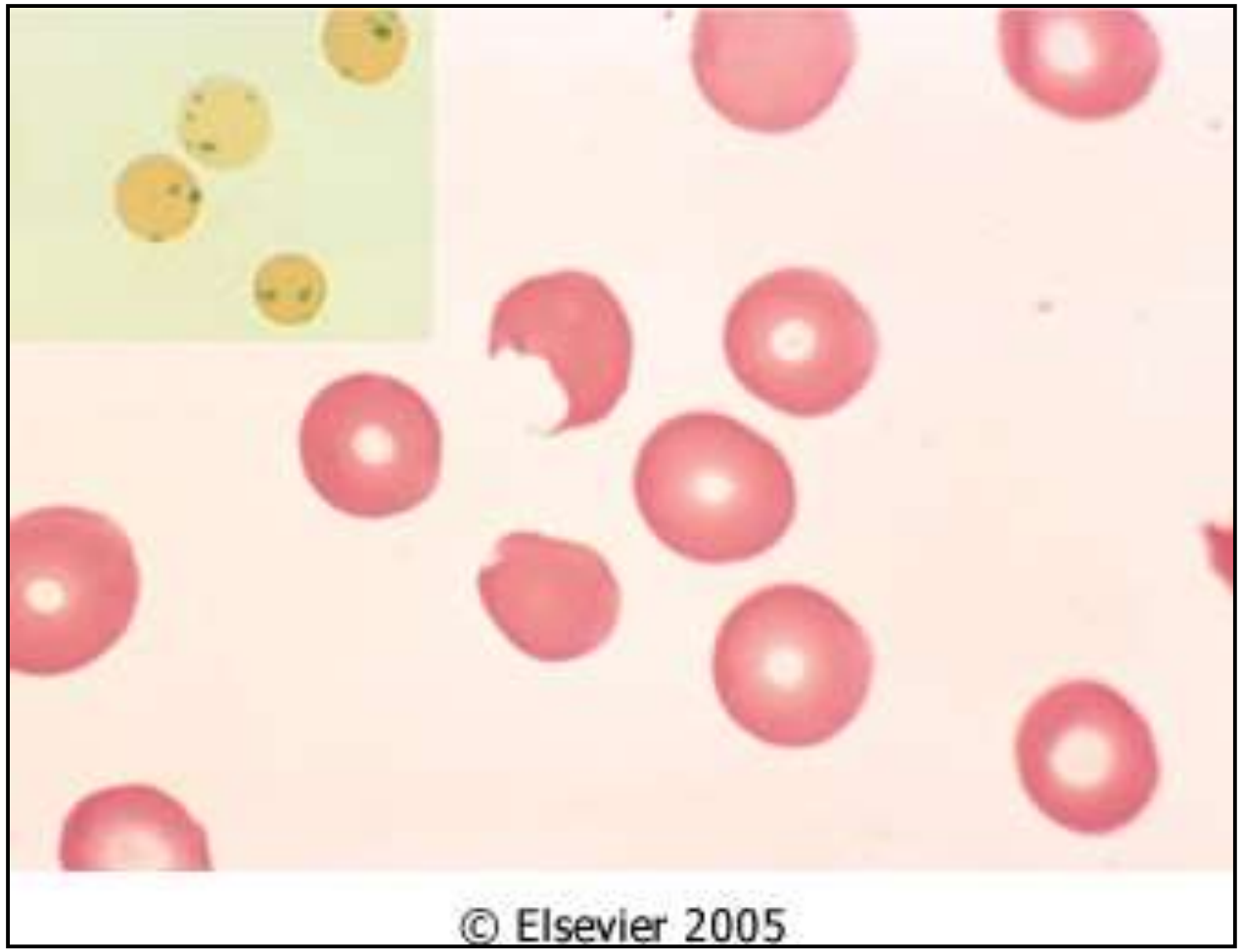
- In the A- (African) type: Normal enzyme but decrease half life
- Only aging RBCs have decreased G6PD

- (Med) Mediterranean type: more severe, Enzyme deficiency

G6PD deficiency

- Patients are **asymptomatic** until exposed to environmental factors that produce oxidants and so hemolysis:
  - Drugs: eg. Antimalarial, sulfonamides, furantoins,…etc.
  - Favism
  - Products of free radicals in infections.

- Features of Extra/Intravascular hemolysis
- Oxidation leads to **denaturation** of globin chains, and precipitation at membranes forming **Heinz bodies**
- RBCs: **Bite cells and Heinz bodies**
- Males more vulnerable than female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IMMUNE HEMOLYTIC ANEMIA

- Due to **antibodies**
- Antibodies may arise spontaneously or be induced by exogenous agent such as chemicals or drugs.
- **Uncommon** and classified according to:

(1) The nature of the antibody

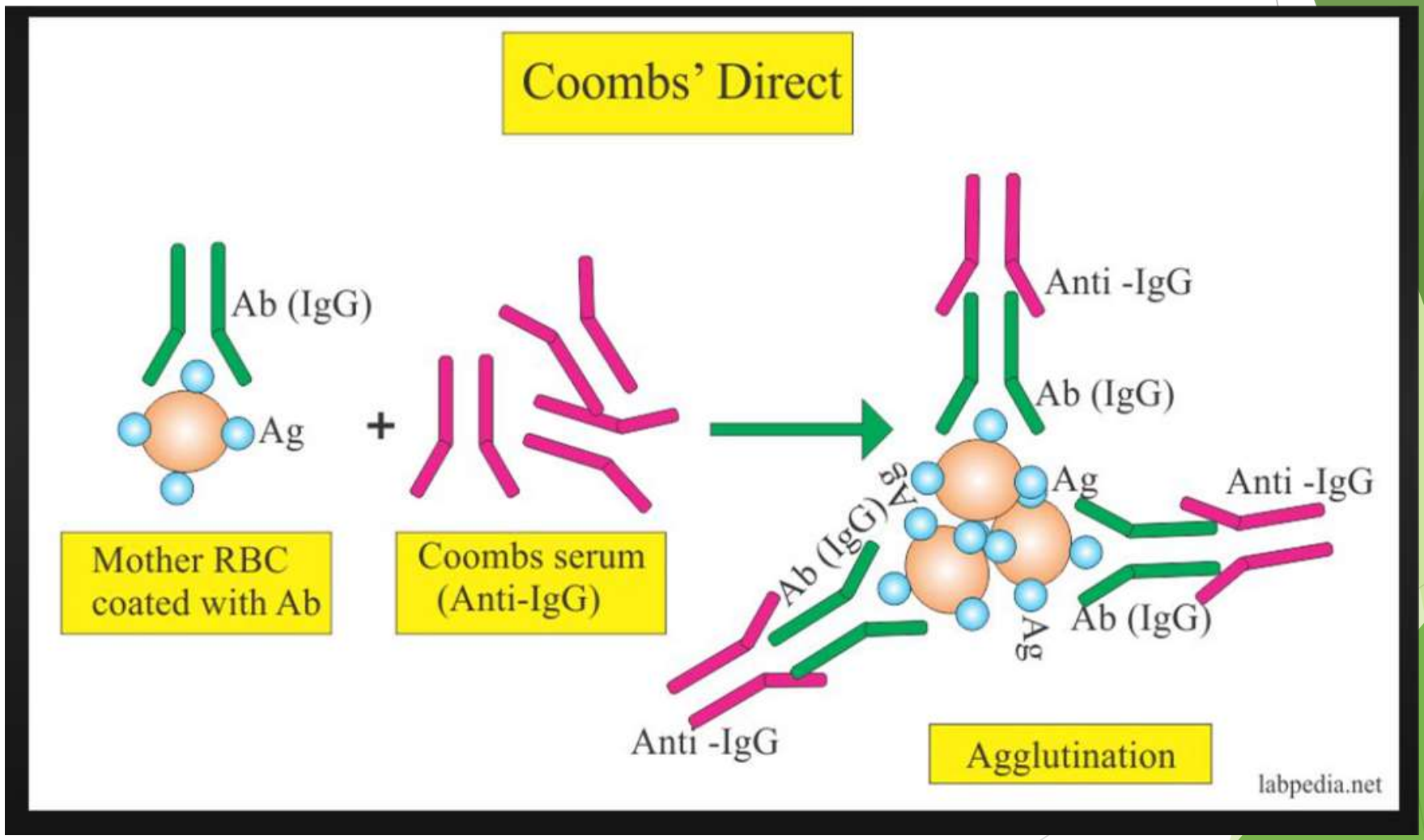
(2) The presence of predisposing conditions.

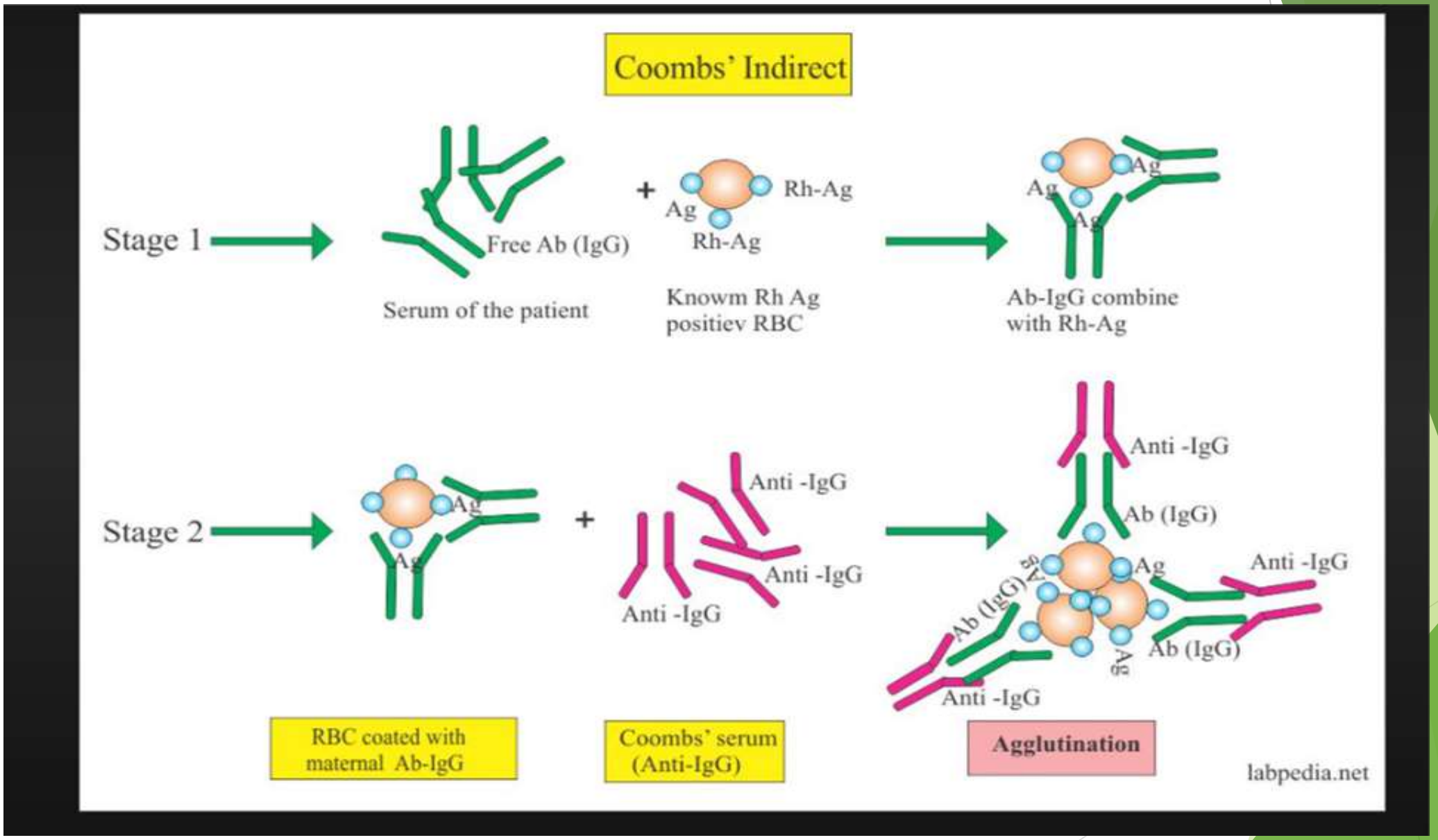
Diagnosis

- Detection of antibodies and/or complement on red cells:

- Direct Coombs antiglobulin test
- Indirect Coombs test

COOMB's Test


Coombs' Direct
Ab (IgG)
Ag
+
Mother RBC coated with Ab
Coombs serum (Anti-IgG)
Anti -IgG
Ab (IgG)
Ag
Anti -IgG
Ab (IgG)
Ag
Ag
Ab (IgG)
Anti -IgG
Agglutination
labpedia.net


Coombs' Indirect
Stage 1
Free Ab (IgG)
Serum of the patient
+
Ag
Rh-Ag
Rh-Ag
Knowm Rh Ag
positiev RBC
Ag
Ag
Ag
Ab-IgG combine
with Rh-Ag
Stage 2
Ag
Ag
+
Anti -IgG
Anti -IgG
Anti -IgG
Anti -IgG
Ab (IgG)
Ag
Ag
Ab (IgG)
Ab (IgG)
Anti -IgG
Anti -IgG
RBC coated with
maternal Ab-IgG
Coombs' serum
(Anti-IgG)
Agglutination
labpedia.net

IMMUNE HEMOLYTIC ANEMIA

Warm Antibody: IgG type active at 37 °C

- **80% of immune hemolytic anemia**
  - **Primary (70%)**
  - **Secondary:**
    - **B cell lymphoid neoplasm (CLL)**
    - **SLE**
    - **Drugs**

Cold Antibody: IgM type active below 30°C

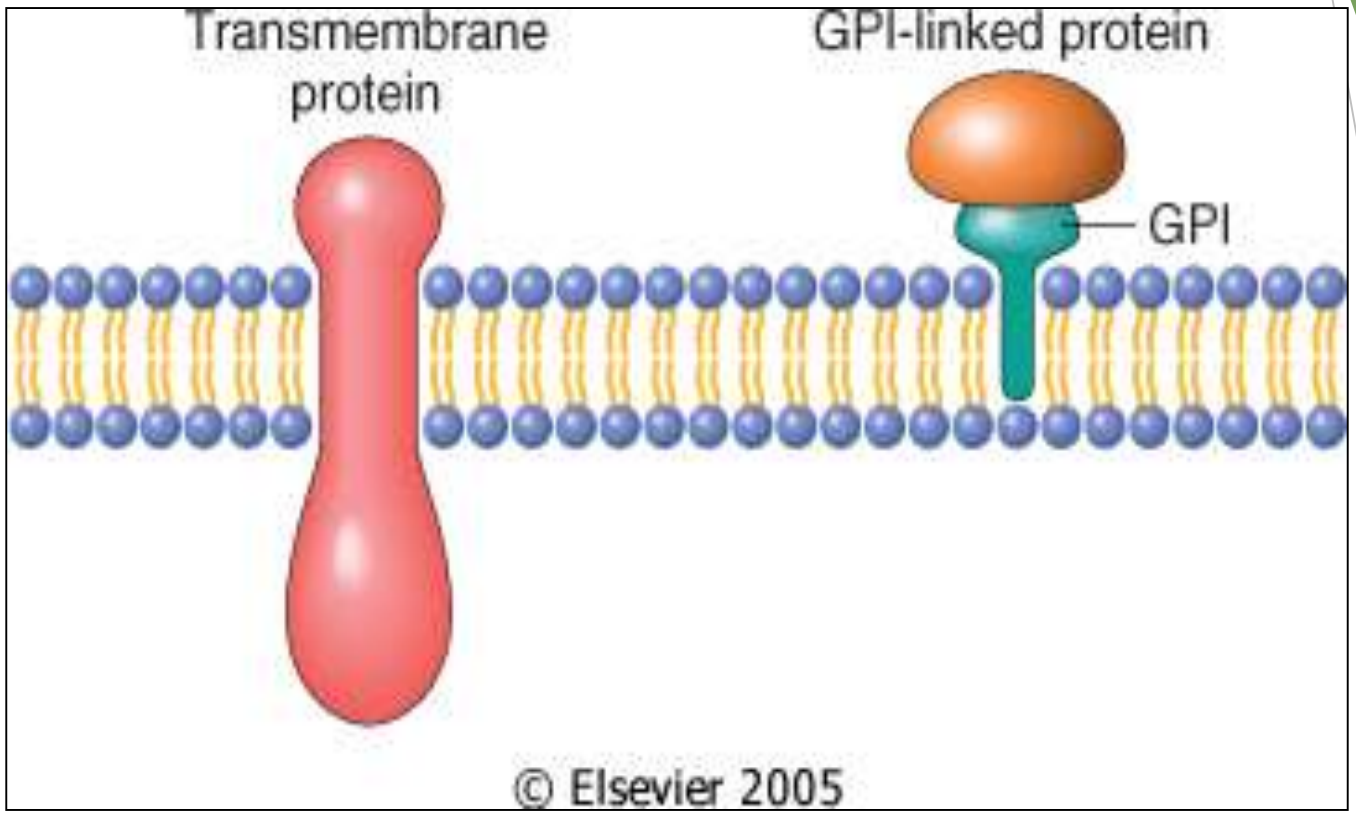
- **Acute**
  - **Infectious mono**
  - **Mycoplasma infection**
- **Chronic:**
  - **Idiopathic**
  - **B cell Lymphoid neoplasm (Lymphoplasmacytic lymphoma)**

Paroxysmal Nocturnal Hemoglobinuria

- Acquired mutations in gene PIGA (required for the synthesis of phosphatidylinositol glycan (PIG), a membrane anchor that is a component of many proteins).

- The affected and thus deficient (GPI-linked proteins) include several proteins that limit the activation of complement: **CD59**, a potent inhibitor of C3 convertase.

- PIGA-deficient precursors give rise to red cells that are sensitive to complement-mediated lysis



Two kinds of membrane proteins: trans membrane and glycosyl phosphatidyl inositol (GPI)-linked
The latter are anchored to cell membranes through a covalent attachment to a glycosyl phospatidyl inositol moiety.
In PNH, GPI cannot be synthesized, leading to a global deficiency of GPI-linked membrane proteins

PNH

- Young adults
- Chronic hemolysis
- Normochromic normocytic anemia
- Reticulocytosis
- **Venous thrombosis**

- Diagnosis:

- Positive sucrose hemolysis test
- Positive acidified serum test (**Ham test):** low sensitivity and specificity
- Flow cytometry for expression of CD59, CD55

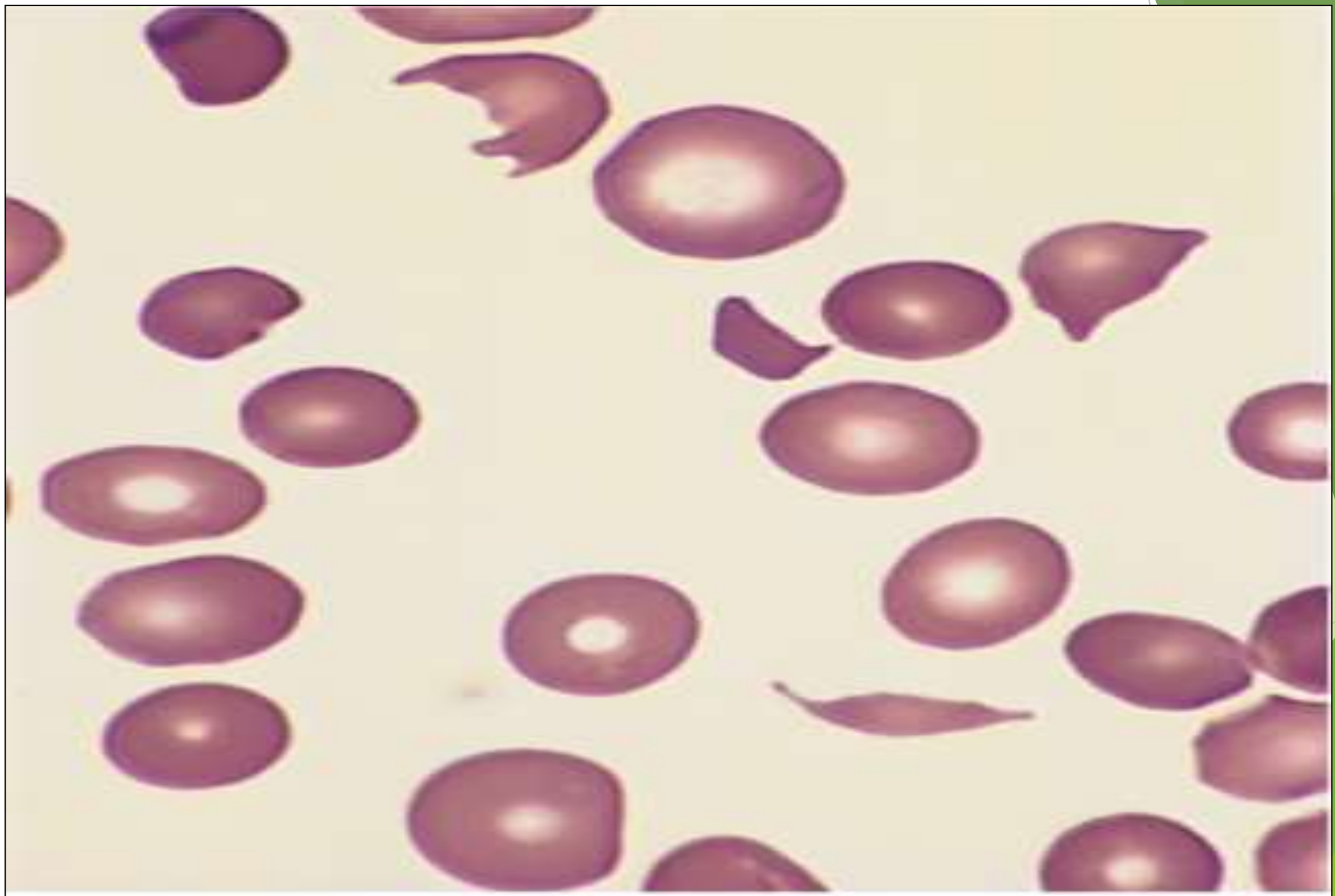
Hemolytic Anemia Due to Mechanical Trauma to RBC

Etiology

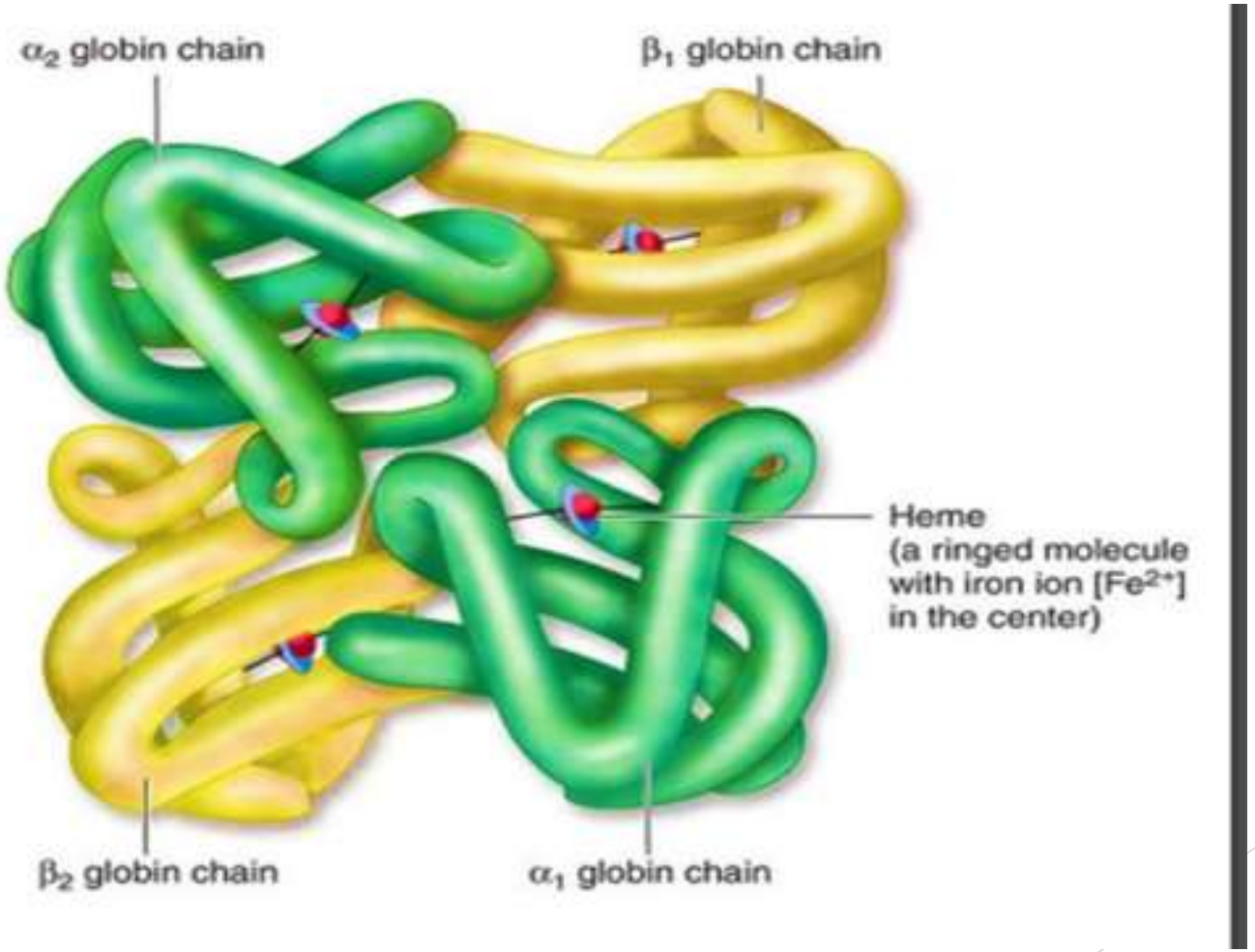
- Artificial valves
- Microangiopathic hemolytic anemia
  - DIC
  - Malignant hypertension
  - TTP
  - Hemolytic uremic syndrome

Morphology

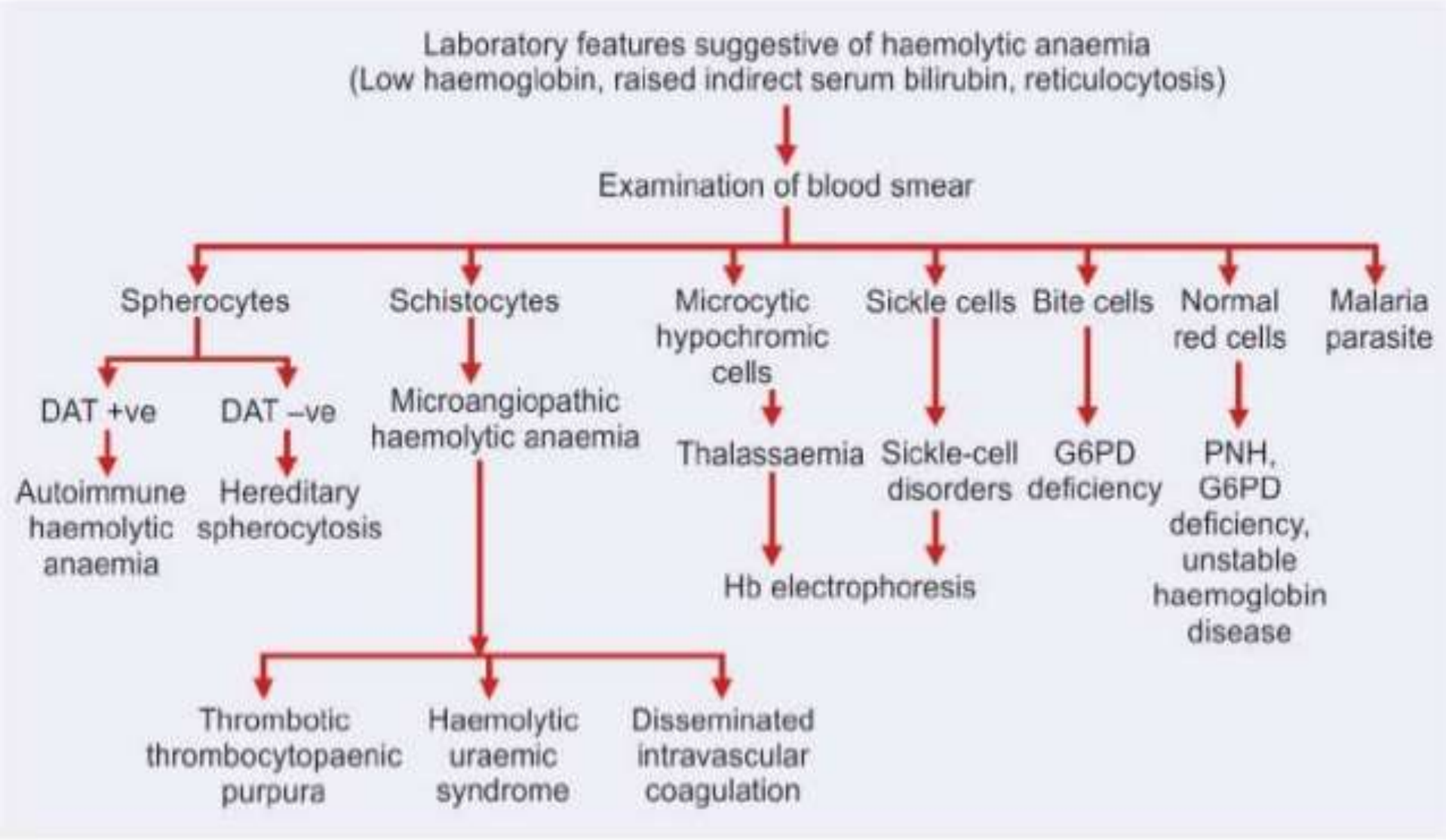
- Significant poikilocytosis with helmet cells/ schistocytes , burr cells, and triangle cells



HEMOGLOBINOPATHIES



A simplified approach to diagnosis of haemolytic anaemias



Hemoglobin types

- Normal types of hemoglobin include:

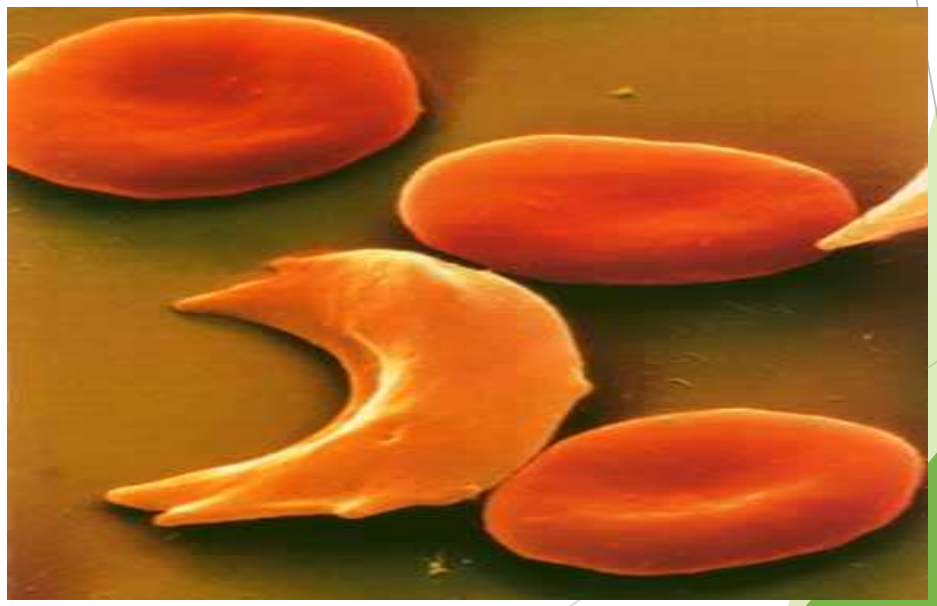
- **Hemoglobin (Hgb) A**, the most common type of hemoglobin in healthy adults
- **Hemoglobin (Hgb) F**, fetal hemoglobin. This type of hemoglobin is found in unborn babies and newborns. HgbF is replaced by HgbA shortly after birth.
- If levels of HgbA or HgbF are too high or too low, it can indicate certain types of anemia.

NORMAL HUMAN HEMOGLOBINS

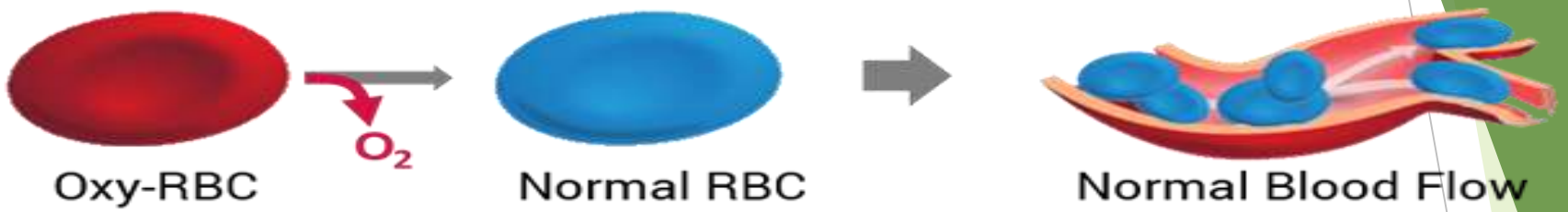
- Hemoglobin A ($\alpha_2\beta_2$): 95% of adult hemoglobin
- Hemoglobin A2 ($\alpha_2\delta_2$): 3% of adult hemoglobin
- Hemoglobin F ($\alpha_2\gamma_2$) :
  - 75% at birth
  - < 5% at 6 months
  - < 1% in adults

SICKLE CELL DISEASE

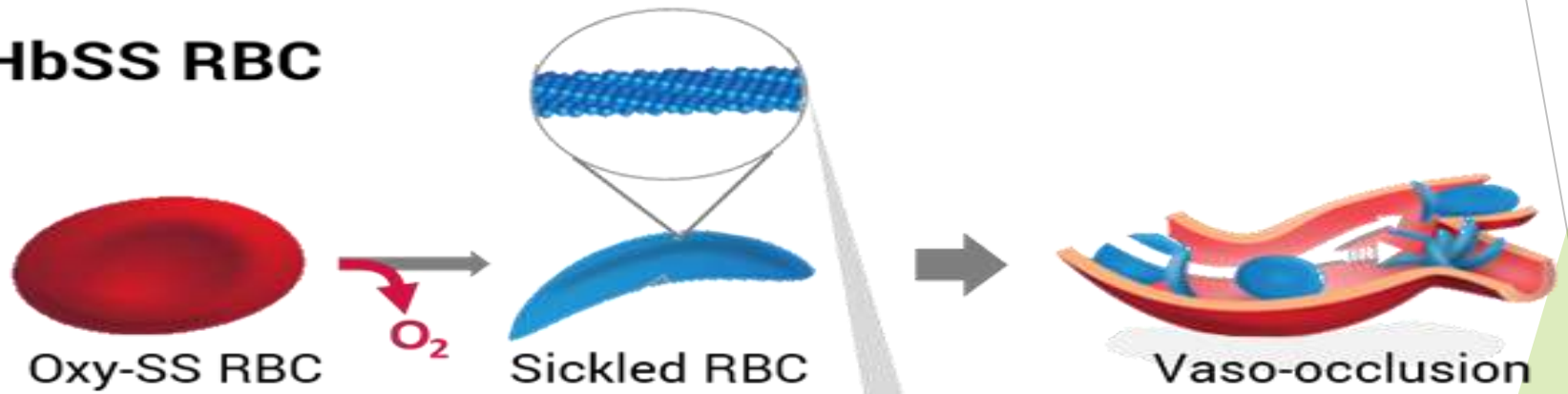
- Most common familial hemolytic anemia in the world
- Recessive inheritance
- Point mutation resulting in the substitution of valine for the glutamic acid at the 6th position of the β chain of Hb

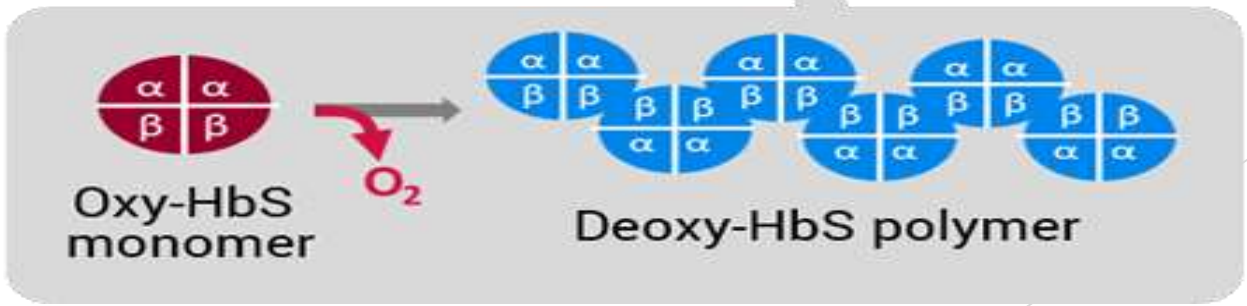


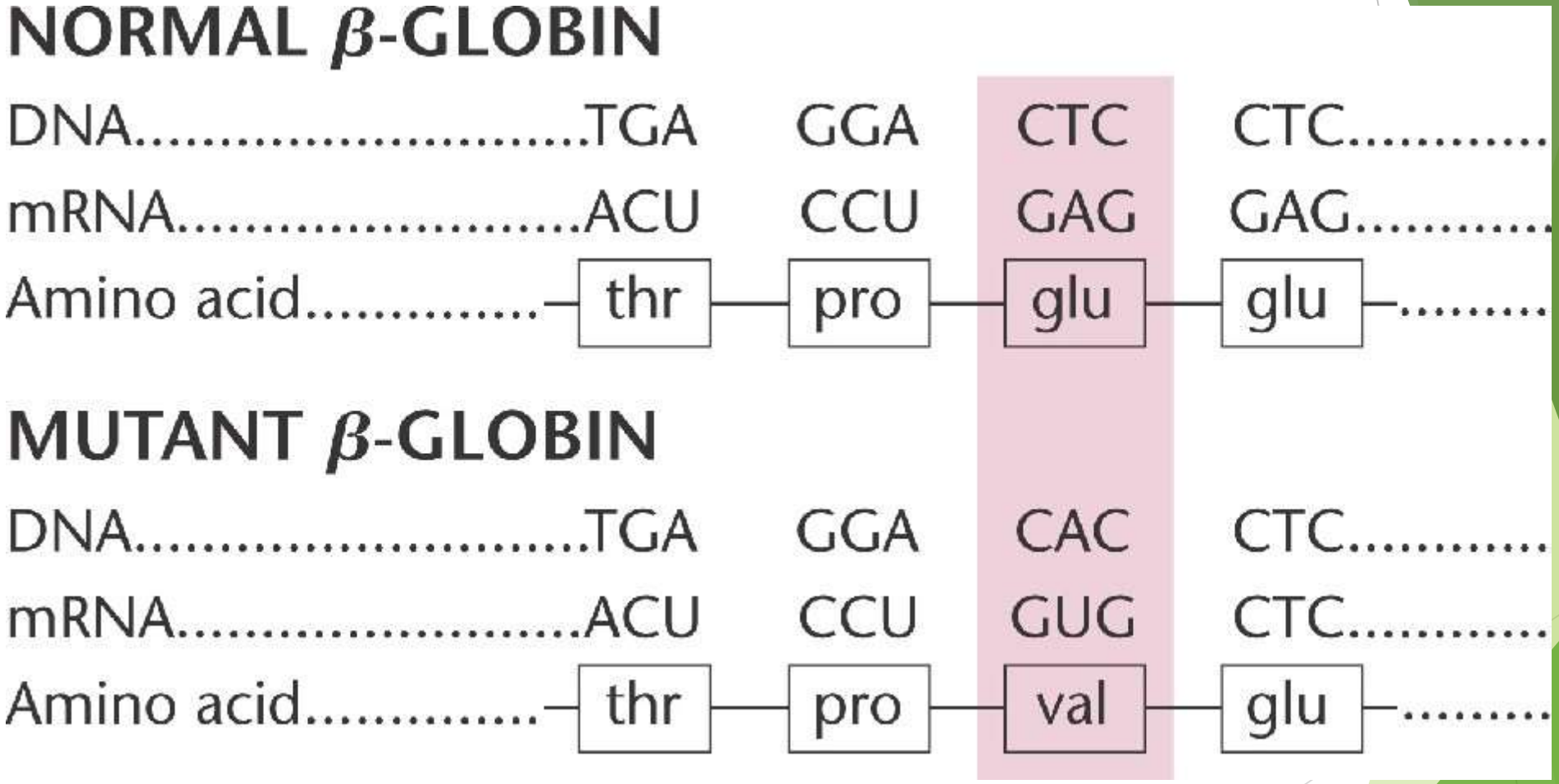
Normal RBC

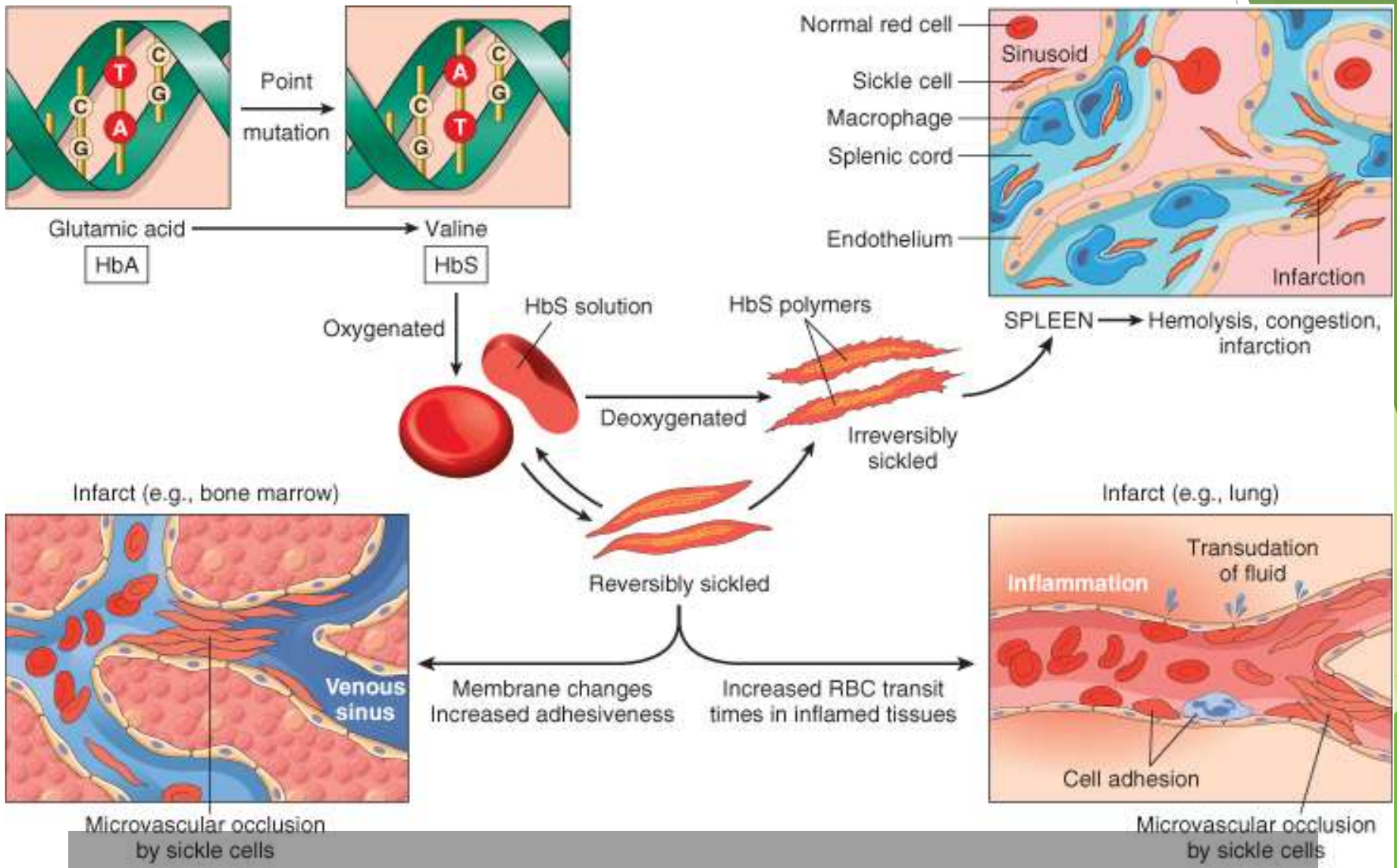


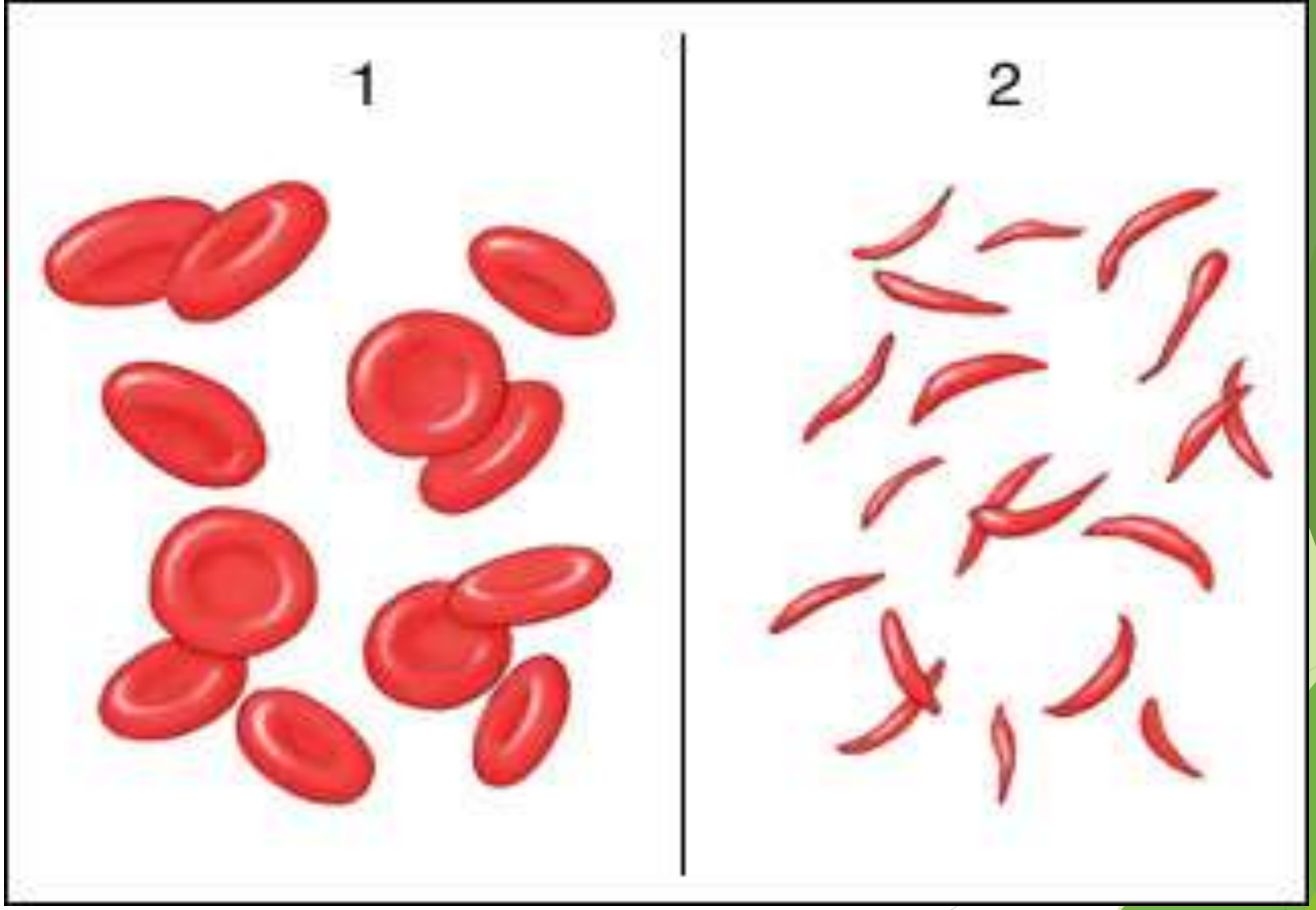
HbSS RBC





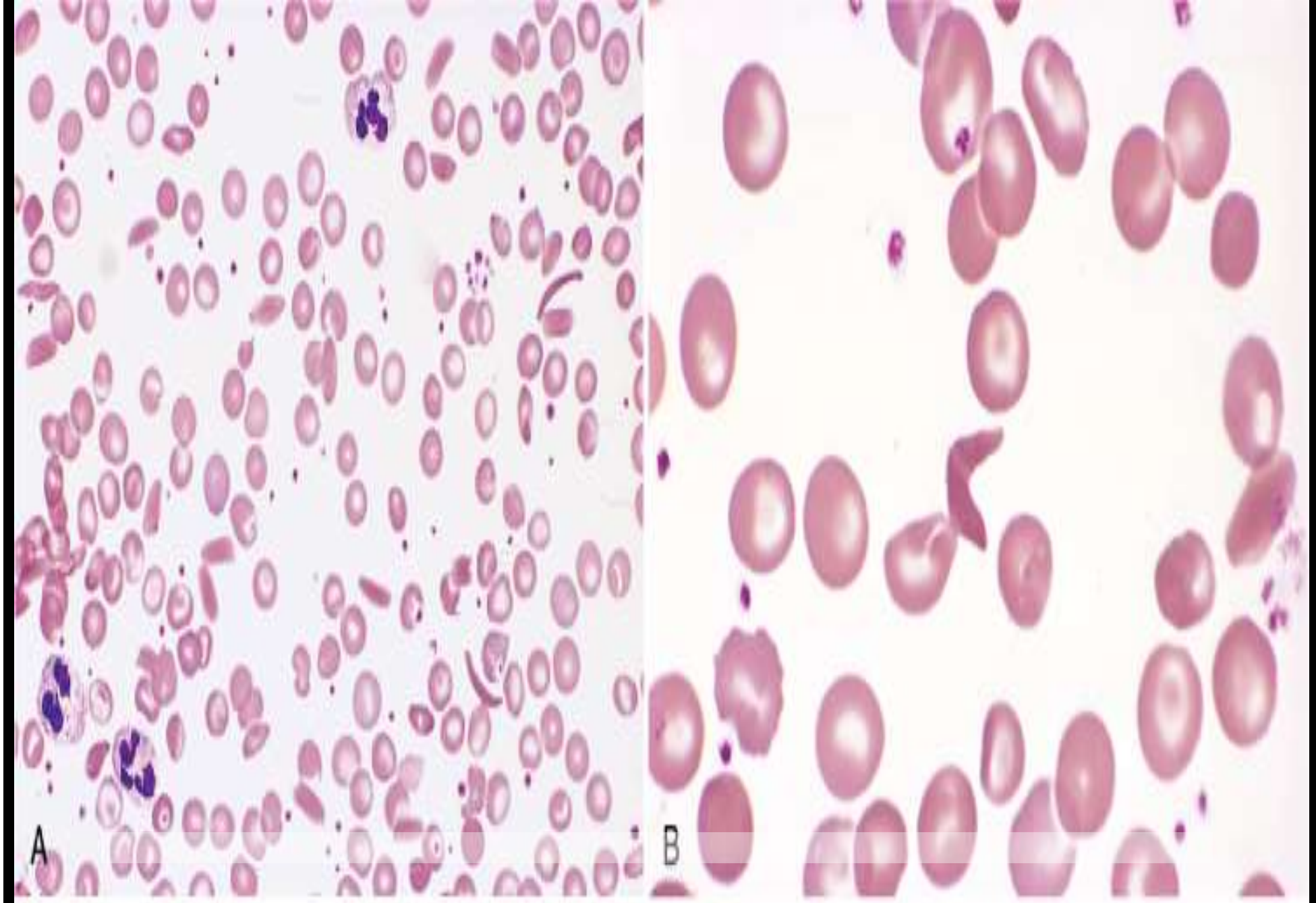

NORMAL β-GLOBIN
DNA.....TGA GGA CTC CTC.....
mRNA.....ACU CCU GAG GAG.....
Amino acid.....thr pro glu glu.....
MUTANT β-GLOBIN
DNA.....TGA GGA CAC CTC.....
mRNA.....ACU CCU GUG CTC.....
Amino acid.....thr pro val glu.....





Normal RBCs

Sickled RBCs



FACTORS AFFECTING THE DEGREE OF SICKLING

(1) **Presence of Hb other than HbS**:

- Heterozygous HbA: HbS 60:40: - Sickle cell trait
- HbC: Mutant β-globin, lysine residue instead of the normal glutamic acid residue at position 6: aggregate with HbS
- HbSC is a milder form the disease
- HbF: Newborns with sickle cell anemia do not manifest the disease until HbF falls to adult levels, generally around the age of 6 months.

) **Intracellular Concentration of HbS:**

- Red cell Dehydration: Increase MCHC, Facilitates sickling

(3) **Transit time for red cells through microvasculature**

- Sickling in microvascular beds is confined to areas of the body in which blood flow is sluggish: Spleen and BM
- Inflammation: slows the flow by increasing the adhesion of leukocytes and RBC’s

CLINICAL CONSEQUENCES OF SICKLE CELL DISEASE

) **Chronic hemolytic anemia** (life span decreased from a normal 120 days to 20 days) to 10-20 days.

(2) **Microvasculature obstruction**: Ischemic tissue damage: Pain Crises

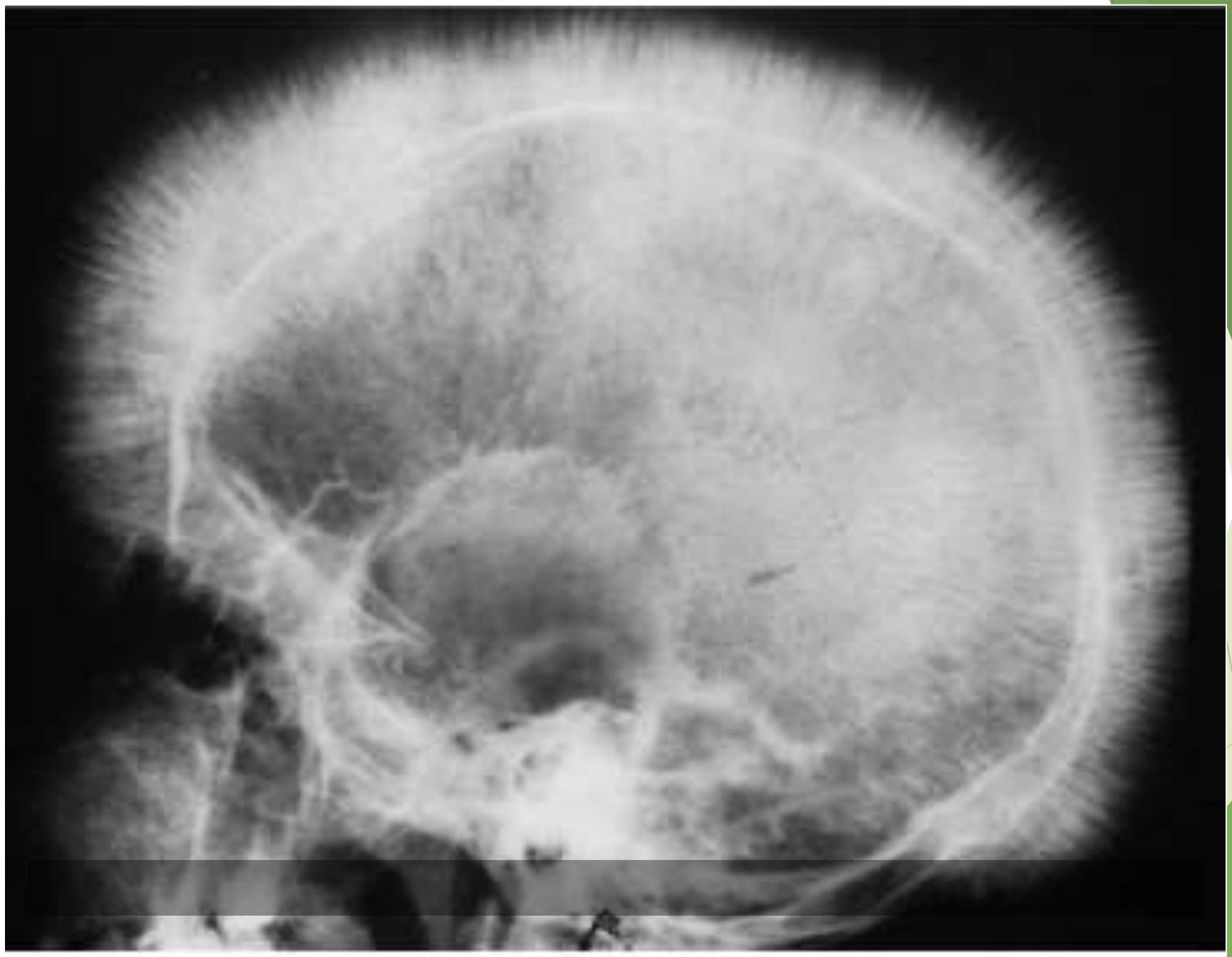
- bones, liver, kidneys, skin, retina,…etc.

(3) Crises:

1. Vaso-occlusive or painful crises
2. Aplastic crises: Due to infection by Parvovirus B19
3. Hemolytic crises

Extramedullary hematopoiesis

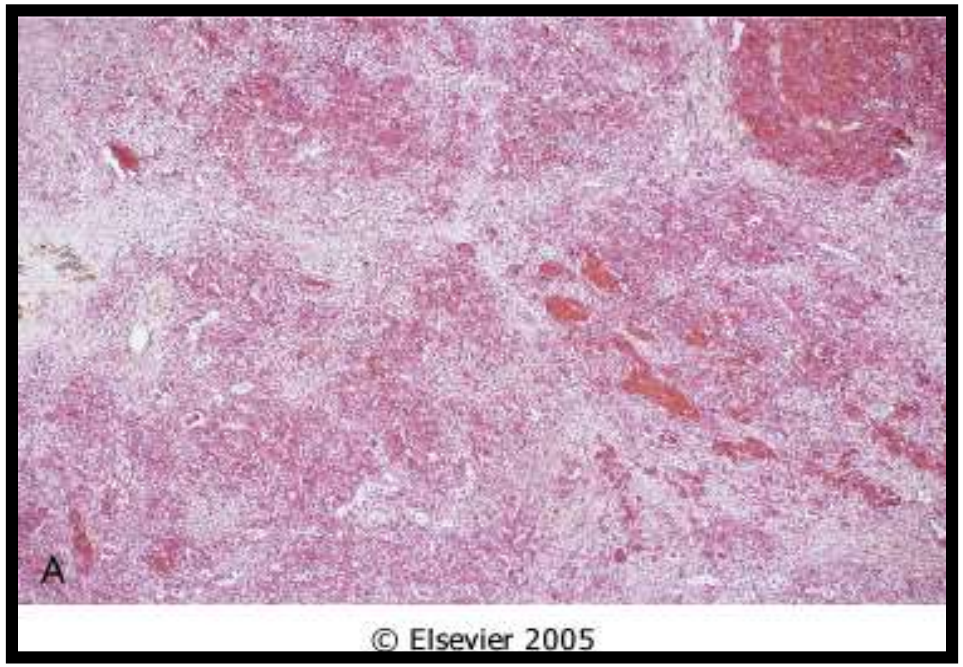
- There is a compensatory hyperplasia of erythroid progenitors in the marrow:
- Causes bone resorption → secondary new bone formation → prominent cheekbones and changes in the skull resembling a “crewcut” in radiographs.
- Extramedullary hematopoiesis may appear in the liver and spleen.



Splenomegaly- auto splenectomy

- In children: moderate splenomegaly due to red pulp congestion caused by entrapment of sickled red cells.
- chronic splenic erythrostasis → hypoxic damage and infarcts, which over time reduce the spleen to a useless fibrous tissue (autosplenectomy) → Increased risk of infections
- Confers protection against Plasmodium falciparum malaria

Spleen in Sickle Cell Anemia



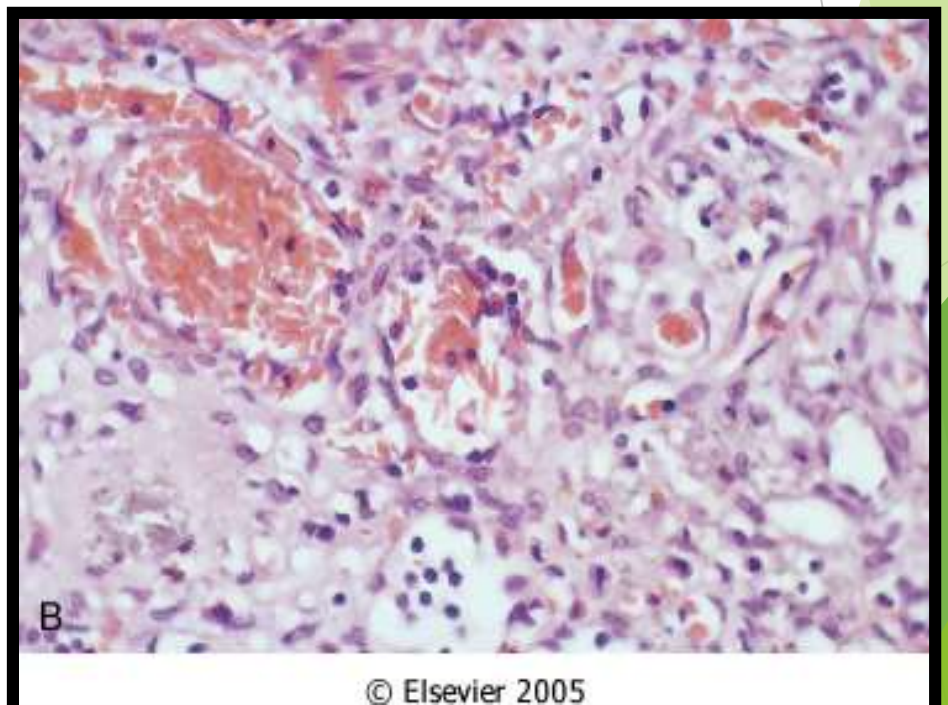


Figure 13-12: Splenic remnant in sickle cell anemia. (Courtesy of Drs. Dennis Burns and Darren Wirthwein, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)

Complications

- Acute chest syndrome
- Stroke

Two leading causes of ischemia-related death

Laboratory diagnosis of sickle cell anemia

- Clinical History:

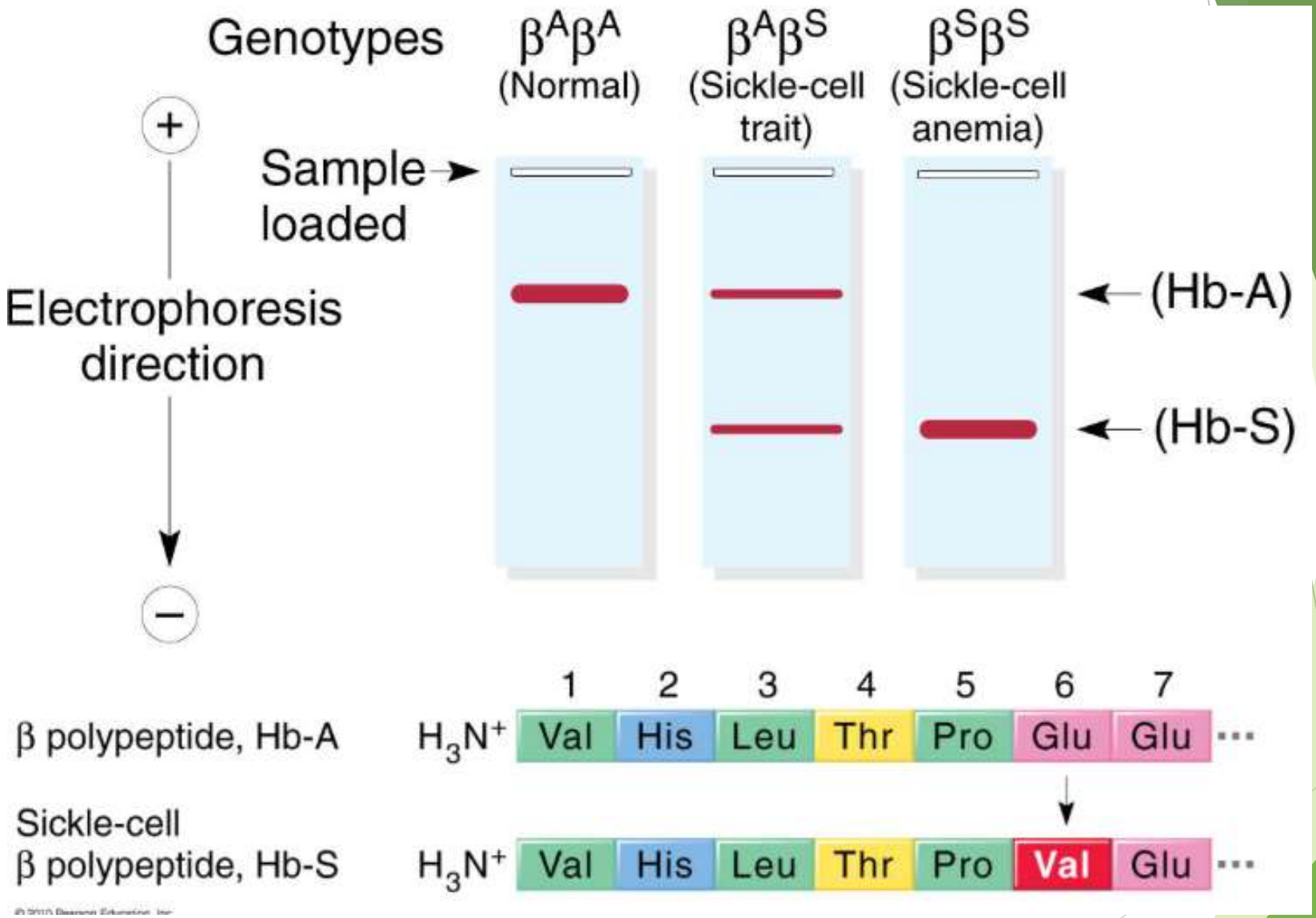
- Asymptomatic till 6 months of age.

- Moderate to severe anemia (6-8 g/dl).

- Unremitting course punctuated by sudden crises

- CBC and blood smear

- Hemoglobin electrophoresis


Genotypes
$\beta^A\beta^A$ (Normal)
$\beta^A\beta^S$ (Sickle-cell trait)
$\beta^S\beta^S$ (Sickle-cell anemia)
+
Sample loaded
Electrophoresis direction
−
(Hb-A)
(Hb-S)
1 2 3 4 5 6 7
β polypeptide, Hb-A
H_3N^+ Val His Leu Thr Pro Glu Glu ...
Sickle-cell β polypeptide, Hb-S
H_3N^+ Val His Leu Thr Pro Val Glu ...

Treatment

1- Adequate hydration
2- Pain relief
3- Antibiotic therapy
4- Hydroxyruea
5- In severe cases, exchange transfusion to reduce the Hgb S.

Thalassemia

FEATURES OF THALASSEMIAS

- Inherited
- Mutations that decrease globin chain synthesis (Alpha or Beta) leading to hypochromic microcytic anemia.
- Quantitative defect → Imbalance of globin chains →

  Reduced Hb synthesis and anaemia
- Precipitation of abnormal Hb → haemolysis and ineffective erythropoiesis

§ Alpha

§ Beta

§ Delta-beta

§ Gamma-delta-beta

β-Thalassemia

Mutations associated with β-thalassemia :

(1) β0: No β-globin chains are produced

(2) β+: Reduced (but detectable) β-globin synthesis.

Mutations leading to aberrant RNA splicing are the most common cause of β-thalassemia

β −Thalassemia

- β-thalassemia minor (β-thalassemia trait):
  - Persons inheriting one abnormal allele
  - Asymptomatic or mildly symptomatic.

- β-Thalassemia major:

- Persons inheriting any two β0 and β+ alleles

Mechanisms of Anemia

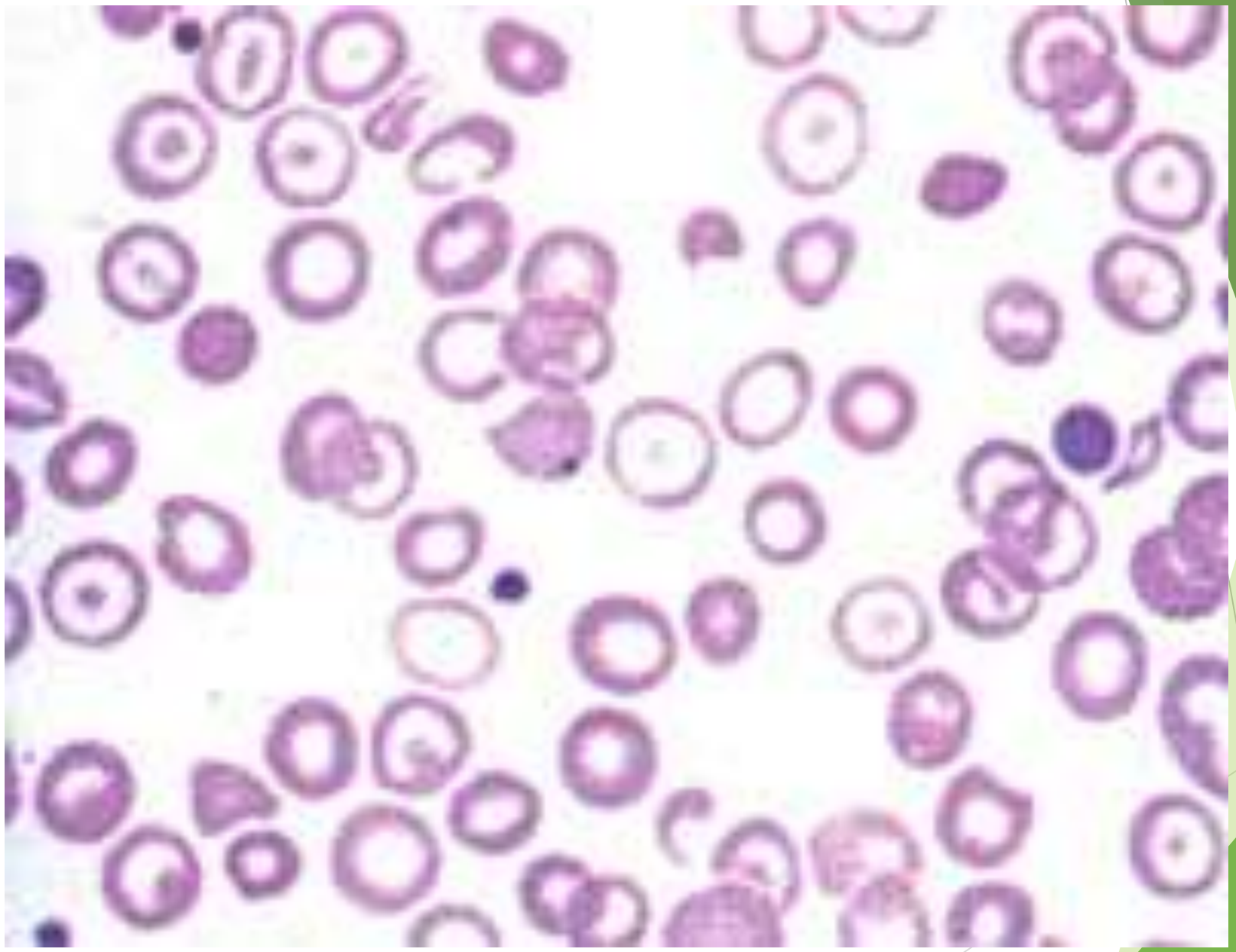
- Reduced synthesis of β-globin → Inadequate HbA formation → hypochromic microcytic anemia
- Imbalance in β- and α-globin chain synthesis → excess unpaired α chains → aggregate into insoluble precipitates → bind and severely damage the membranes of both red cells and erythroid precursors → ineffective erythropoiesis
- Few red cells that are produced have a shortened life span due to extravascular hemolysis

β−THALASSEMIA MAJOR

- Genotype: β^0/β^0, β^+/β^+, β^0/β^+
- Age of manifestations: 6-9 months
- Hb. Level: 3-6 gm/dl (if un-transfused)
- Transfusion dependent

β−THALASSEMIA MAJOR

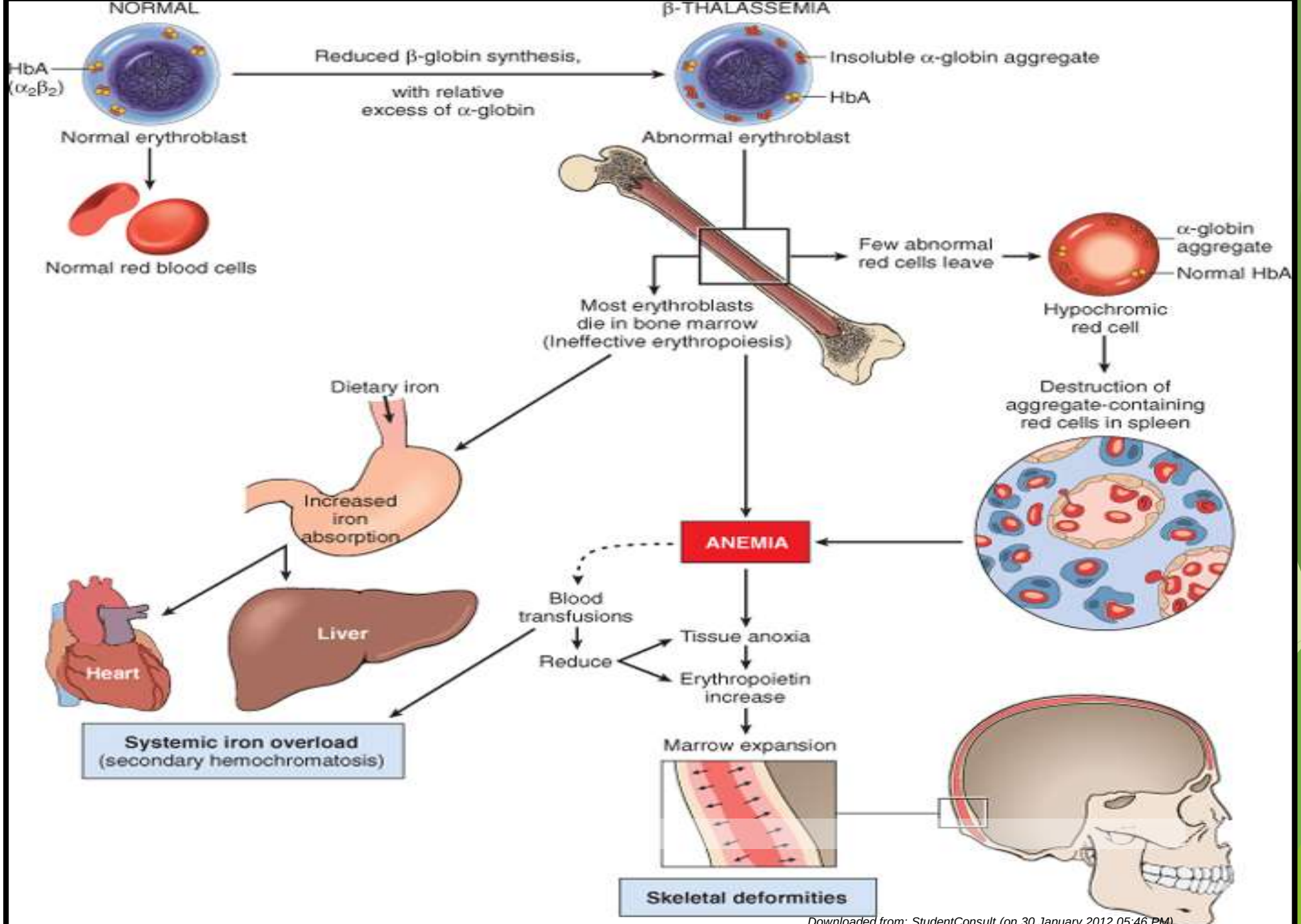
- Very high HbF, absent or decreased HbA, HbA2 Normal or increased
- Expansion of bone marrow, impair bone growth: Skeletal deformities
- Extramedullary hematopoiesis and hepatosplenomegaly
- Stunted growth and cachexia
- Secondary hemochromatosis: May lead to cardiac dysfunction
- Bone marrow transplantation at an early age is the treatment of choice



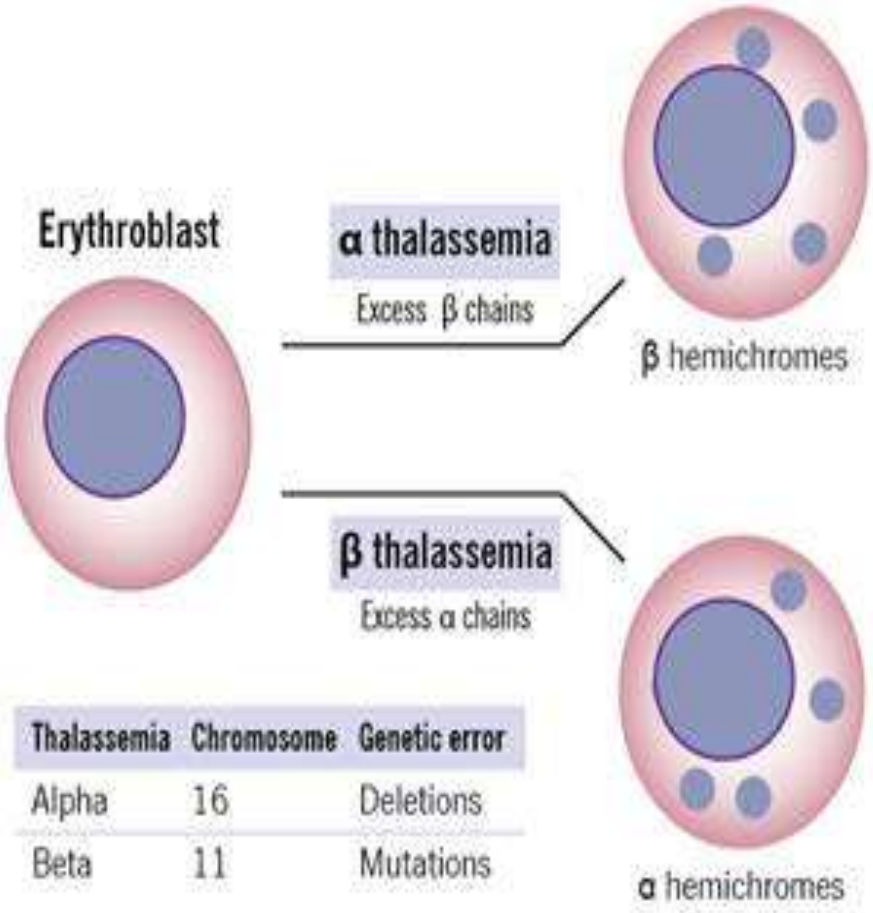
β −THALASSEMIA MINOR

- Heterozygous for β^0 oρ β^+ gene
- increased HbA2 (> 3.5%) and/or HbF (1- 5%)
- Mild microcytic anemia (Hb 9-11 g/dL)
- Differential Dx: Iron deficiency anemia

	Genotype	Hb A	Hb A2	Hb F
Homozygous	β^0/β^0	0%	Variable	>95%
	β^+/β^+	5-35%	Variable	20-80%
	β^0/β^+	2-10%	Variable	>85%
Heterozygous	β^0 thal	Slight	3.5-7%	up to 5%
	β^+ thal	Slight	3.5-7%	up to 5%



Alpha and beta thalassemias



Thalassemia	Chromosome	Genetic error
Alpha	16	Deletions
Beta	11	Mutations

Inclusion body
In HbH disease (a type of α thalassemia), excess β chains precipitate as hemoglobin H (β4) inclusion bodies in the cell. In β thalassemia major, excess α chains can also precipitate as inclusion bodies.

Heinz body
A type of inclusion body containing denatured hemoglobin. Classically associated with G6DP deficiency, these can be found in the thalassemias as well. Heinz bodies are typically larger than the inclusion bodies mentioned above. When a functional spleen is present, Heinz bodies lead to bite cells.

Howell-Jolly body
A type of inclusion body containing DNA. Like Heinz bodies, they are usually removed by splenic macrophages. Howell-Jolly bodies can be seen when red cells fail to fully mature or when a functional spleen is absent.

Because α chains dissociate into monomers more readily than β chains, the β chains form hemichromes at a faster rate; therefore making β thalassemia clinically more severe.

α- Thalassemia

α-Thalassemia

- Caused mainly by deletions involving one or more of the a-globin genes.
- Severity of the disease is proportional to the number of a-globin genes that are missing

α-Thalassemia

- -α/αα: silent carrier state, asymptomatic
- --/αα, -α/-α : α thal minor, asymptomatic
- --/-α : Excess beta: **Beta 4:** HbH disease
- --/-- : Excess Gamma : Hb Barts

  Death in utero
  (Hydrops fetalis)

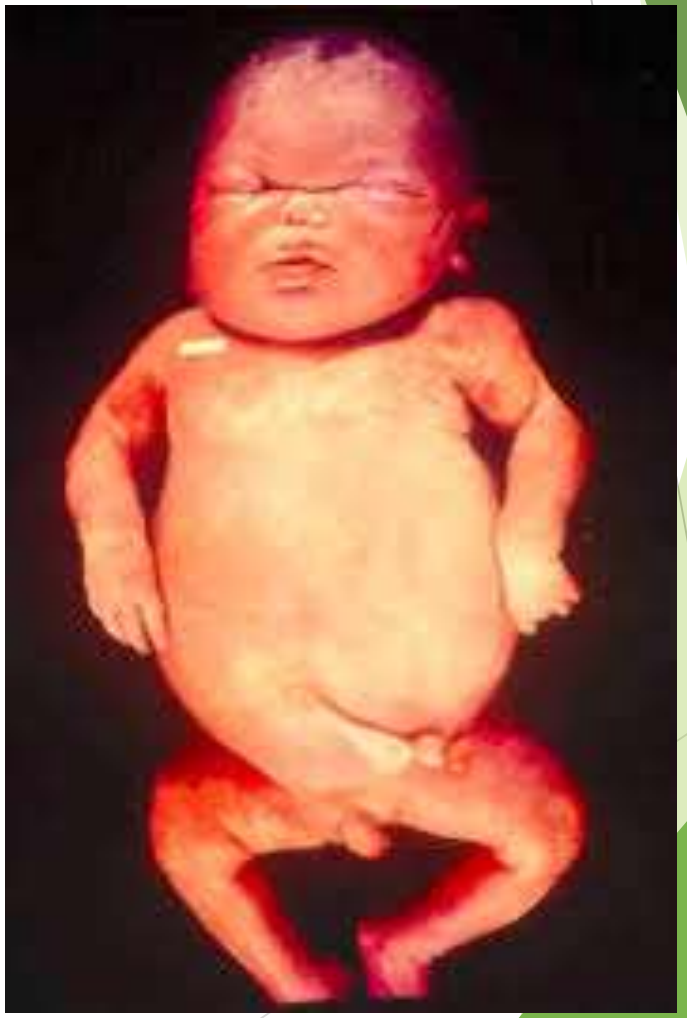


Table 1 Clinical classification of α-thalassemia

Condition	Clinical characteristics
Silent carrier	Clinically and hematologically normal
Thalassemia trait	Microcytosis, hypochromia, and mild anemia
HbH disease	Moderate to severe microcytic, hypochromic, hemolytic anemia, mild jaundice, moderate hepatosplenomegaly
Hb Bart hydrops fetalis syndrome	Severe anemia, generalized edema, ascites, marked hepatosplenomegaly, skeletal and cardiovascular malformations, usually death in utero

Questions

GOOD LUCK