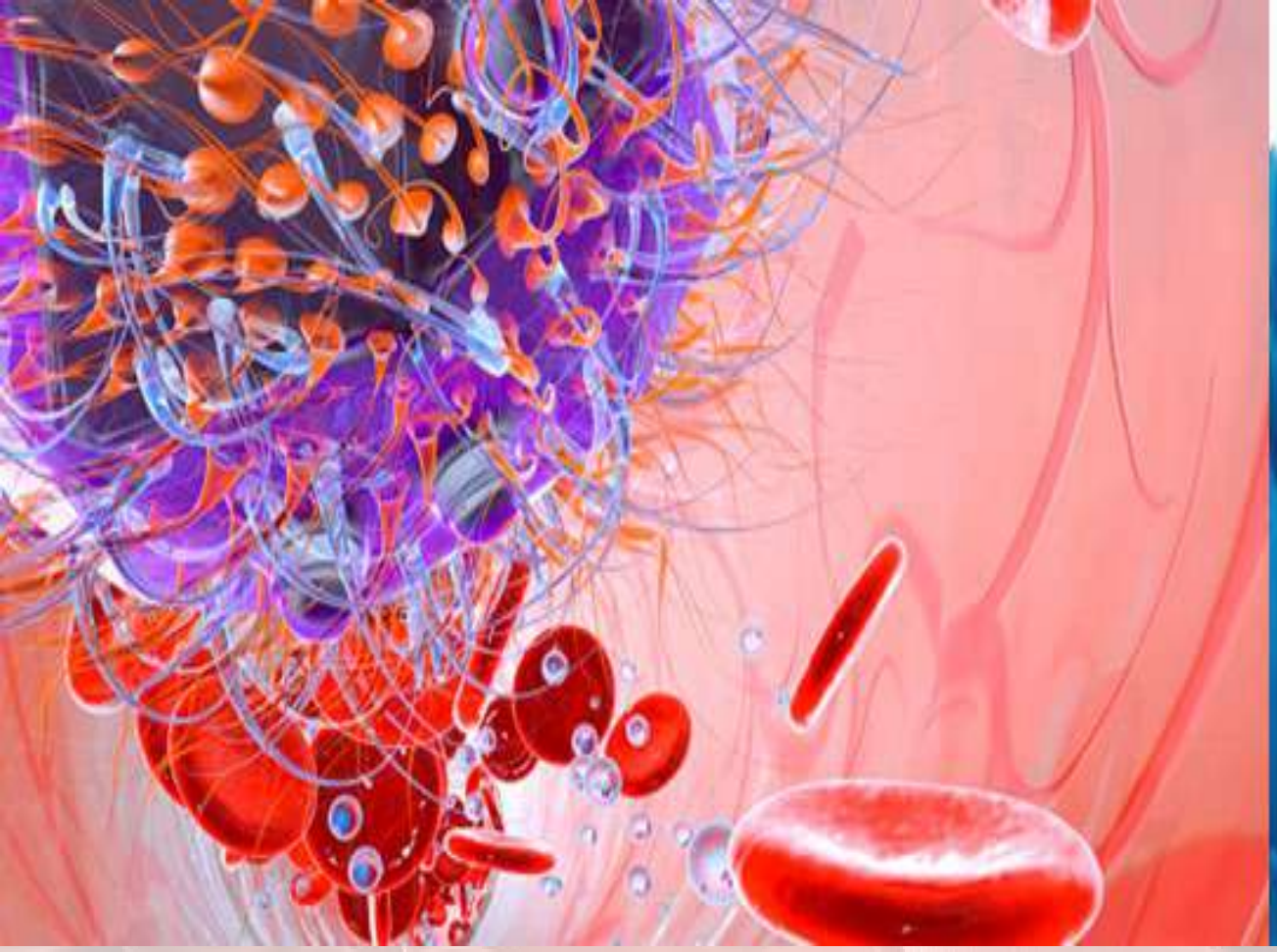

Haematology

Hematology

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4th Year – Under Graduate
College of Medicine
University of Anbar

Objectives

1. Hemopoiesis.
2. **Anemia, Types and Related Disorders.**
3. Granulopoiesis and White Blood Cell Disorders.
4. Hematological Malignancies.
5. Hemostasis.
6. Transfusion Medicine.

3. Genetic Disorders of Hemoglobin (Hemoglobinopathies)

One of the most difficult topics in hematology.

Most common genetic defects all over the world.

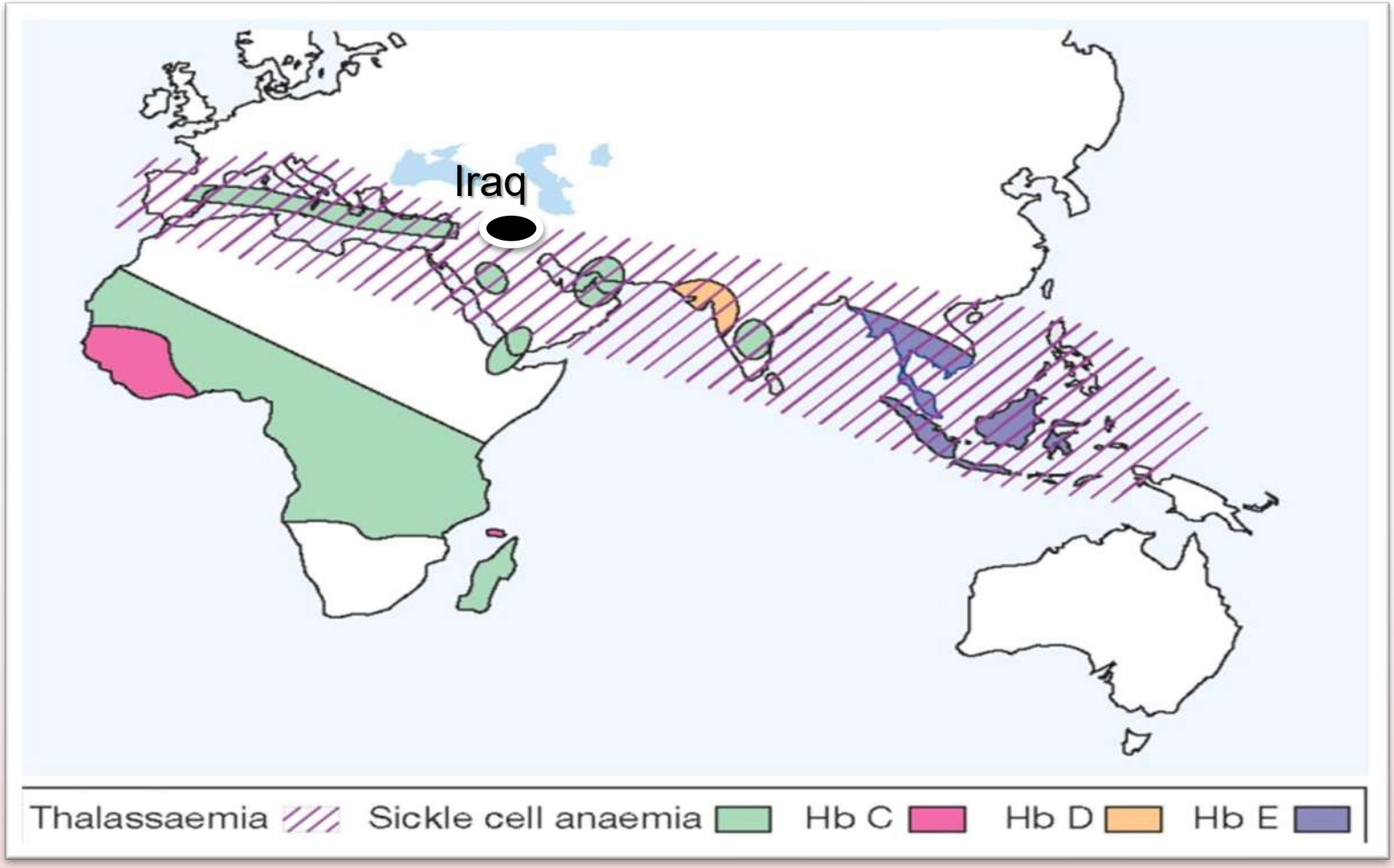
HB ABNORMALITIES (hemoglobinopathies):

Inherited abnormalities of globin genes leading to synthesis of a variant hemoglobin , including:

1- Thalassemias, usually but not always; Hb is structurally normal, but reduced Hb synthesis (i.e; **Quantitative** defect)

2- Sickle cell anemia; produced hemoglobin is abnormal (i.e; **Qualitative** defect); Hb S (Sickle cell), Hb C, D, E ..etc.

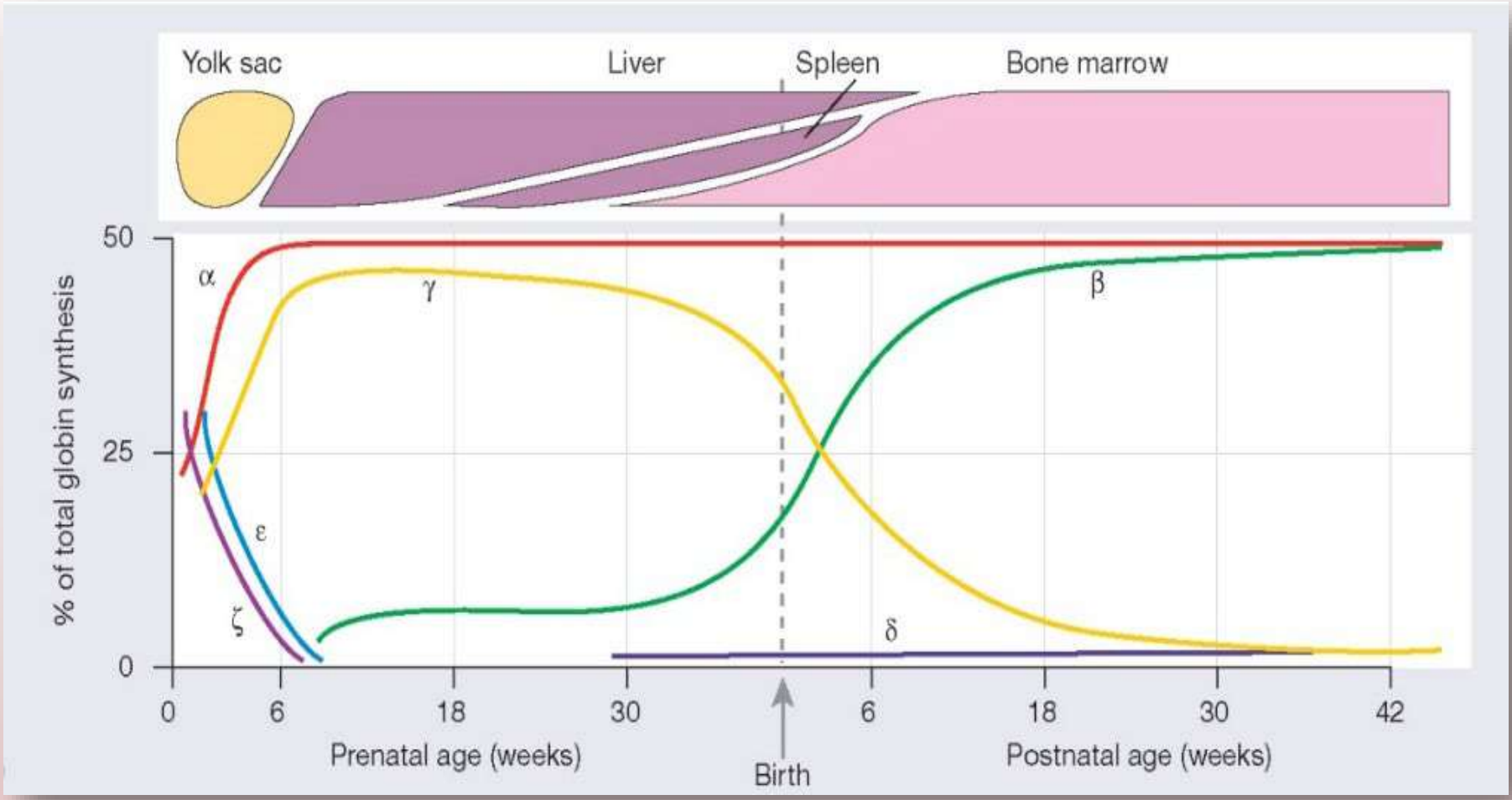
3- Other; **unstable** hemoglobin.



- Most common genetic defects all over the world, mostly in tropical and subtropical area.
- Carriers are affords some protection against malaria

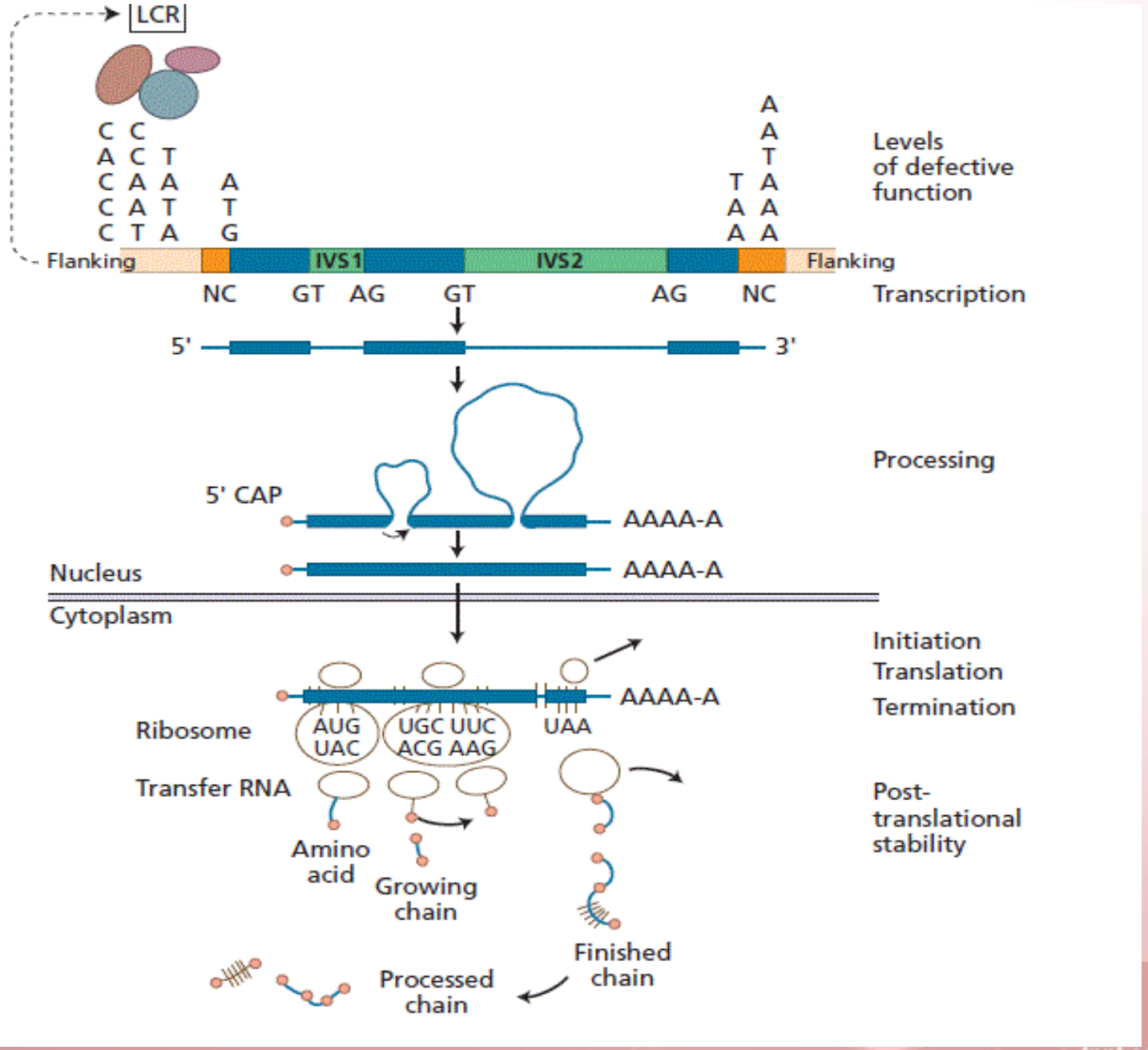
- Major adult Hb component is Hb A ($\alpha_2 \beta_2$) (96–98%)
- Hb A2 ($\alpha_2\delta_2$) and Hb F ($\alpha_2\gamma_2$) constituting 1.5–3.2% and 0.5–0.8% respectively
- Genes for globin chains located in 2 cluster on chromosomes; 11 for β, γ, δ, and ε while chromosome 16 for α and ζ
- Globin chains synthesized on ribosomes.
- Hbs; A, A2, and F are of the clinical importance.

α; alpha, β; Beta, δ; delta, γ; gamma, ζ; zeta



Synthesis of individual globin chains in prenatal and postnatal life.

Globin gene and the genetic control of globin chain synthesis

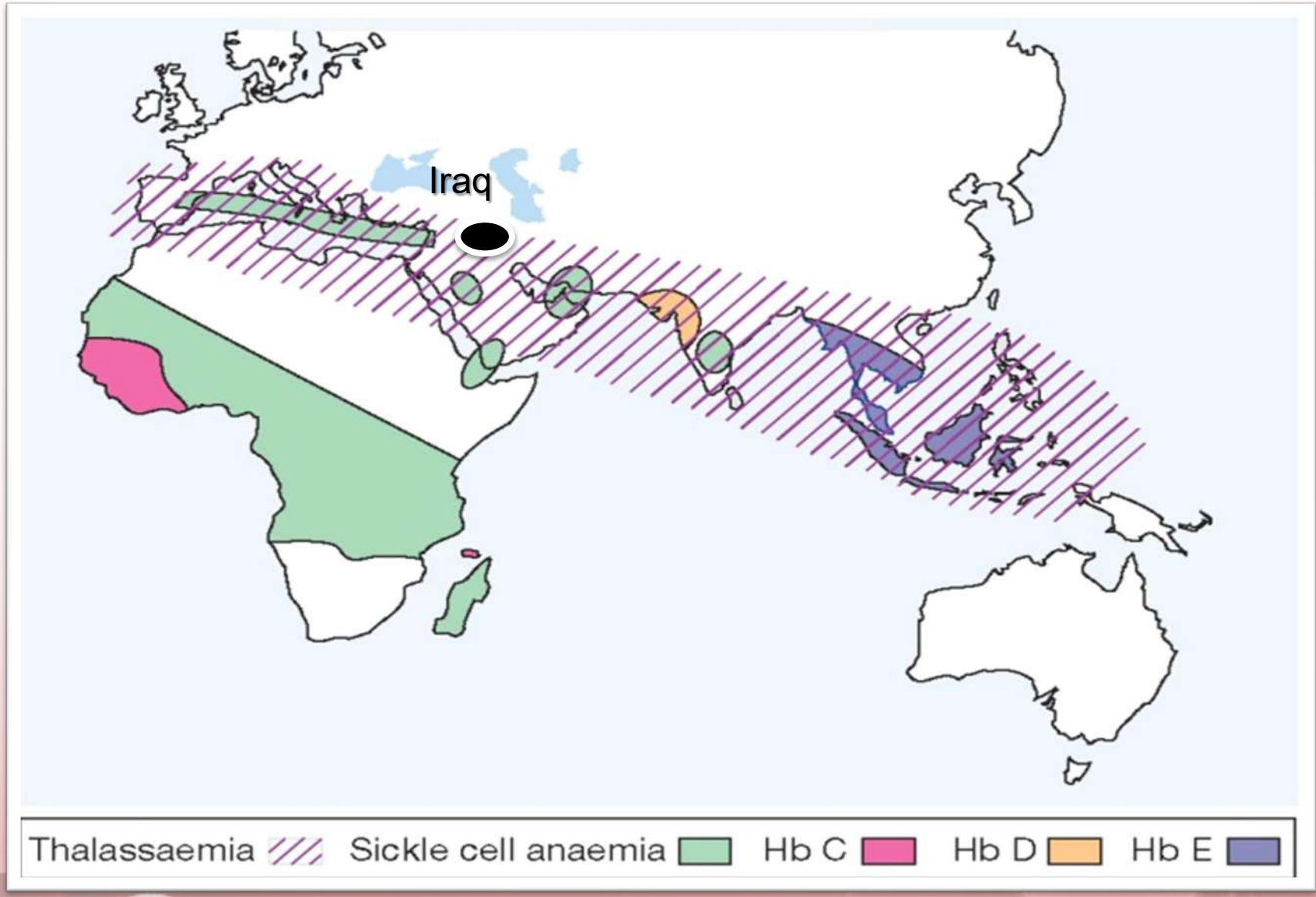


THALASSEMIAS

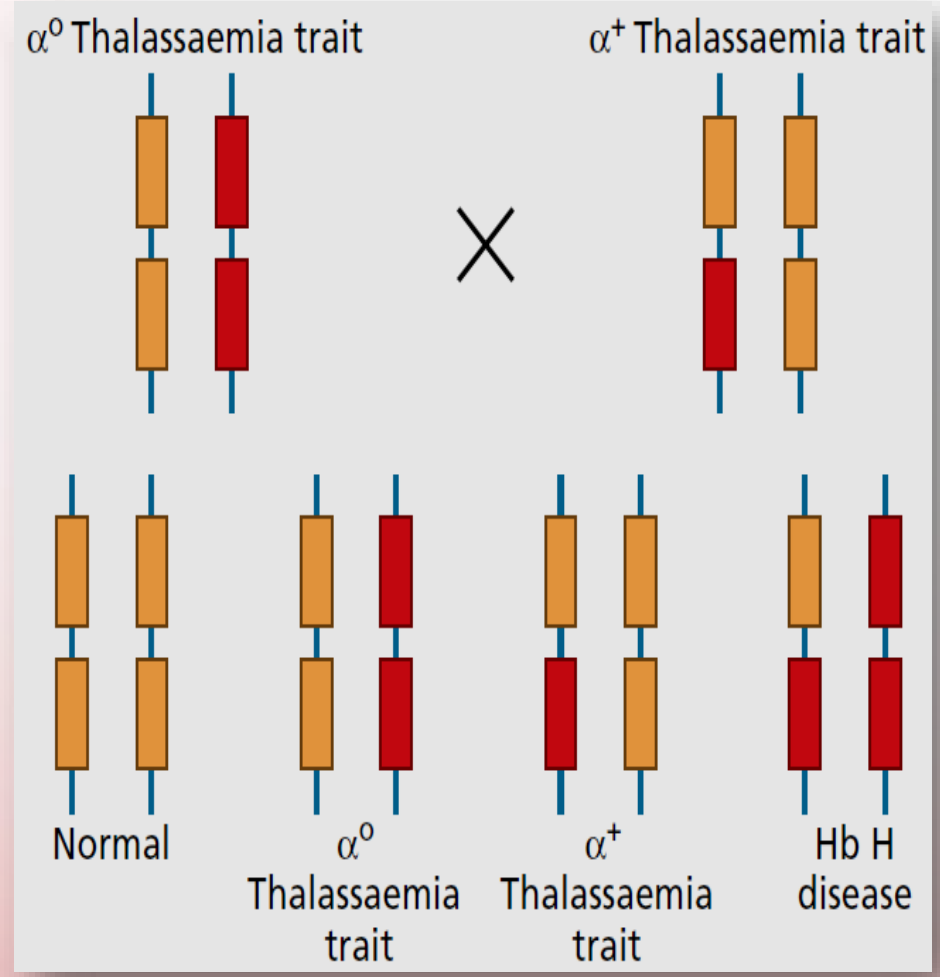
- Genetic disorder due to reduced rate of globin chains synthesis (mostly α and β).
- Functionally, some thalassemia mutations cause a complete absence of globin chain synthesis, and these are called α^0- or β^0-thalassaemias; in others, the globin chain is produced at a reduced rate and these are designated α^+- or β^+-thalassemias
- Most common single-gene disorder all over the world.
- Most common health problem all over the world.
- β-thalassemias mostly in Mediterranean while α-thalassemias in Far East, but no area spared from it all over the world, with a variable prevalence (2-30%).
- In Iraq its prevalence about 4-5%.

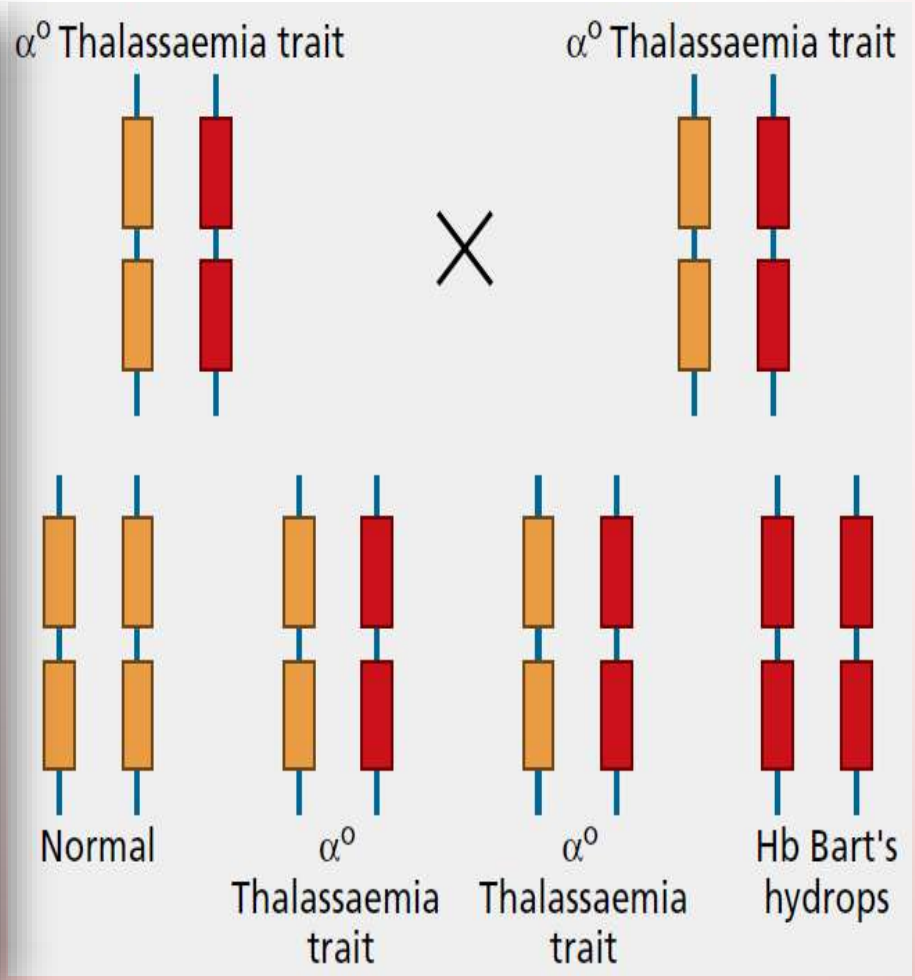
α-thalassemia:

- Mostly of gene deletions of one or more of the 4 alpha (α) genes.
- Results from absence or decreased production of α globin chains.
- Each erythrocyte precursor cell has two α globin genes on each chromosome16 that determine alpha globin production. Thus, there are a total of four alpha globin genes.
- The α-thalassaemias and β-thalassaemias follow similar distribution, extending throughout sub-Saharan Africa, the Mediterranean region, the Middle East, the Indian subcontinent and Southeast Asia.



❖Inheritance of α-thalassemia





❖ Clinical Forms;

α-thalassemia can in one of the following forms;

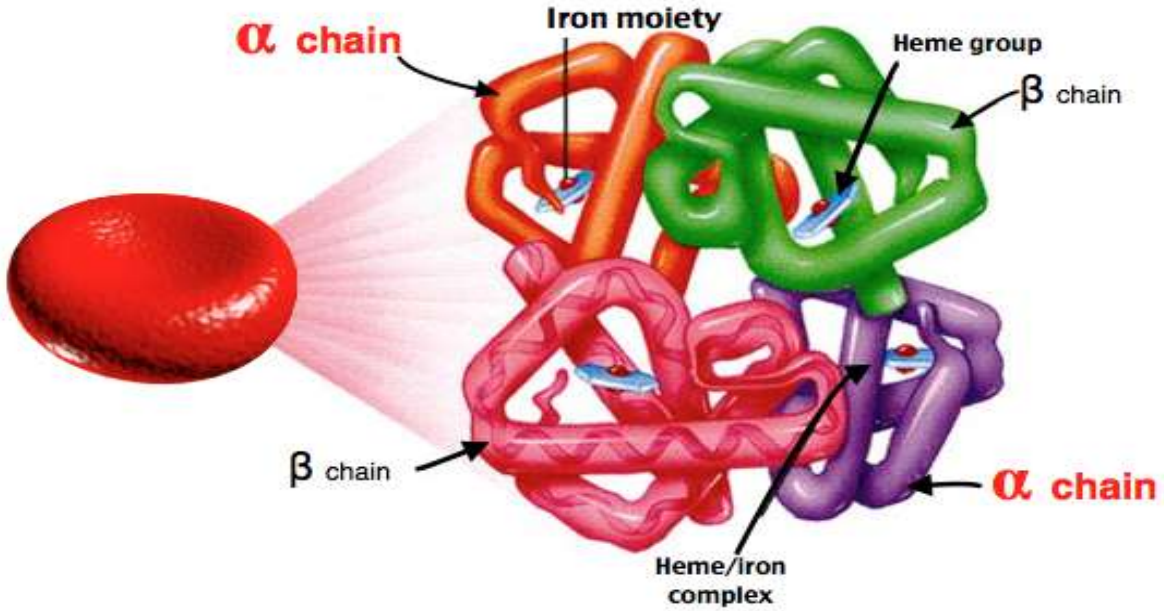
1- α-thalassemia trait; absence of 1 or 2 α-genes leading mild changes in red blood indices (MCV and MCH) and blood film shows mild degree of hypochromia.

2- Hb H disease; absence of 3 α-genes forming β_4 tetramers (HbH) leading to moderate to severe hypochromic microcytic anemia with splenomegaly.

3- Hydrops fetalis (severe edema); complete absence of the α-genes which form γ_4 (because it's the part of fetal Hb) tetramers (Hb Bart's) leading to failure of Hb synthesis in fetal life.

Alpha Thalassemia

Occurs when **one or more** of the **four** **alpha globin chain** genes fails function



Normal	α α / α α	**Normal:** No deletions
Carrier	α α / α α	**No abnormalities:** 1 deletion
Minor	α α / α α or α α / α α	**Alpha thalassemia minor: 2 deletions** • Usually asymptomatic • Mild microcytic anemia
Hb H Disease	α α / α α	**Hemoglobin H Disease: 3 deletions** • Splenomegaly, hemolytic & microcytic anemia • Avoid oxidative drugs
Hydrops fetalis	α α / α α	**Hydrops fetalis: 4 deletions** • Fetal demise • Total body edema

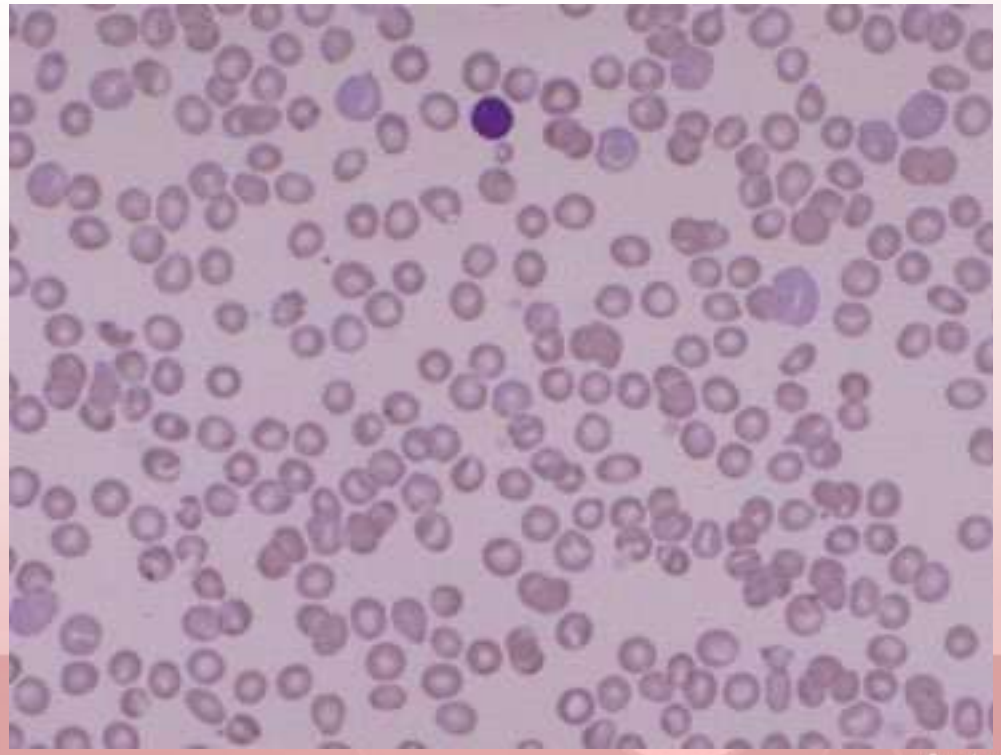
❖ Classification of α-thalassemia

CLINICAL CLASSIFICATION	GENOTYPE	NO. OF GENES PRESENT
Silent carrier	aa/- a	3 genes
a thalassemia trait	- a/- a or aa/- -	2 genes
Hemoglobin H disease	-a/- -	1 gene
Hb Barts / Hydrops fetalis	- -/- -	0 genes

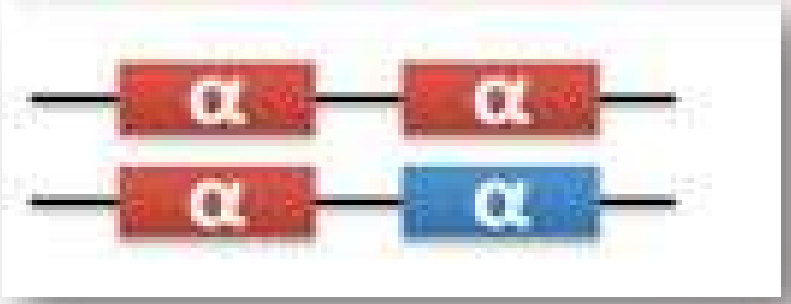
α -Thalassemia trait:

- The diagnosis of α thalassemia trait is difficult and requires DNA analysis. Usually it is a presumptive diagnosis only, when no other explanation can be found for microcytic red cells.

This film, from an individual with α-thalassemia trait attributable to loss of two α genes, shows hypochromia and microcytosis (mild).

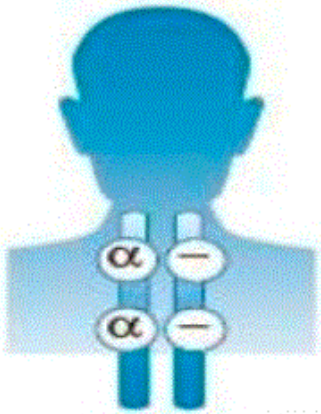


Hemoglobin H (HbH) Disease

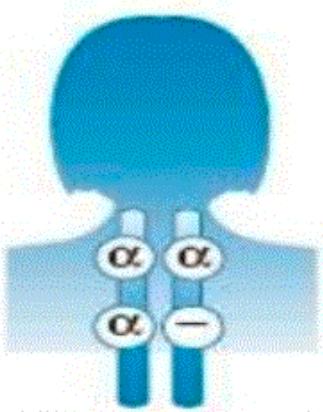


- Seen primarily in Asian populations
- Three α-genes deletion.
- HbH; its of tetramer of β chains
- Has high affinity to oxygen (O2 dissociation curve similar to myoglobin)
- It is unstable, and leads to shortened red cell life span.
- Variable degree of chronic hemolytic anemia and splenomegaly
- Infection or exposure to toxic drugs may exacerbate the condition compromising the already anemic patient.

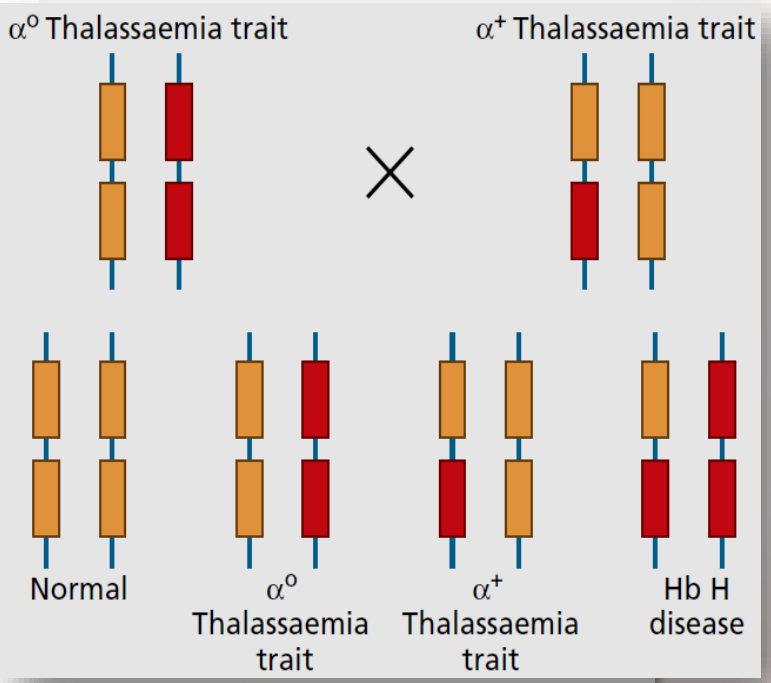
❖Inheritance of HbH Disease α-thalassemia

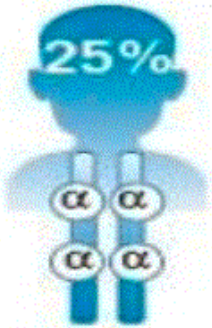


one parent has alpha thalassemia trait with two abnormal alpha globin genes on the same chromosome

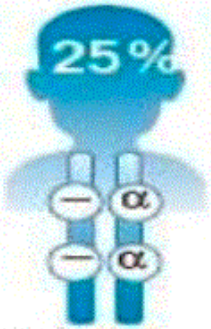


and the other parent has the silent carrier state with a single abnormal alpha globin gene on one chromosome

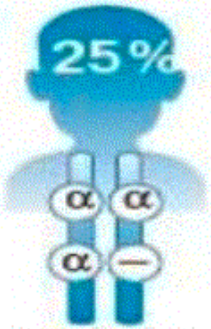




normal hemoglobin

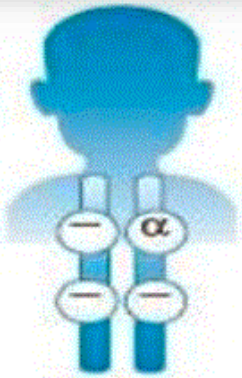


alpha thal trait



alpha thal silent carrier

25% hemoglobin H disease



❖Lab. Findings:

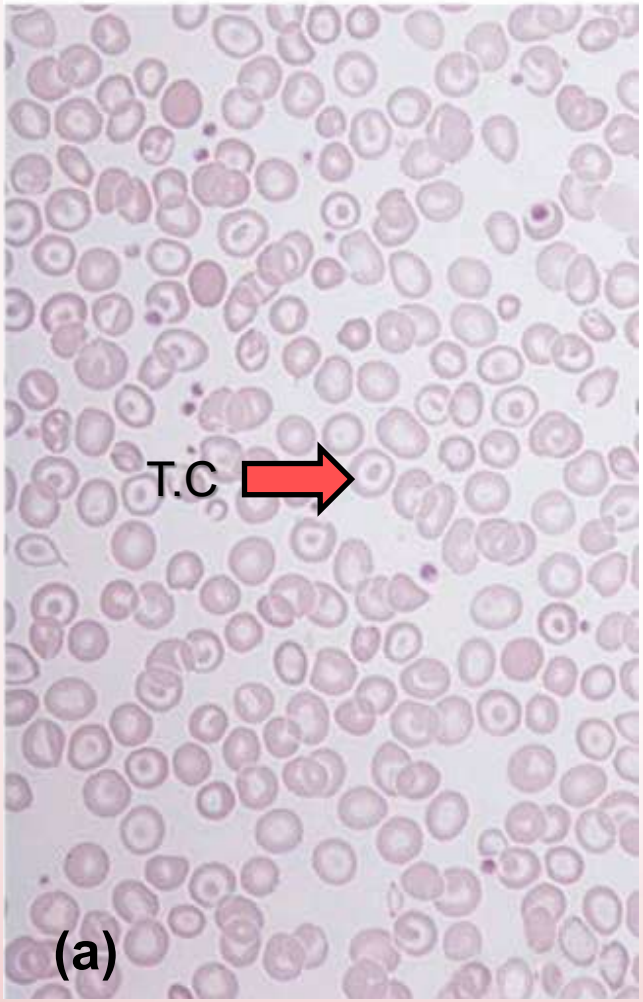
- Variable degree of anemia (Hb; 7-10 g/dL)
- **Blood film shows;**

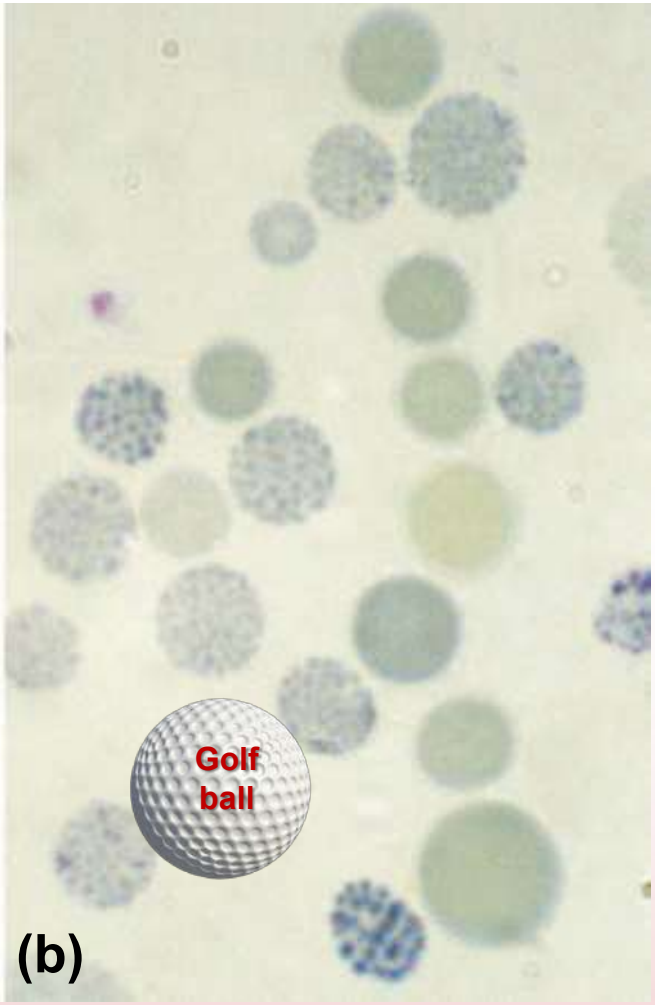
✓ Marked hypochromia, anisocytosis, poikilocytosis and target C.

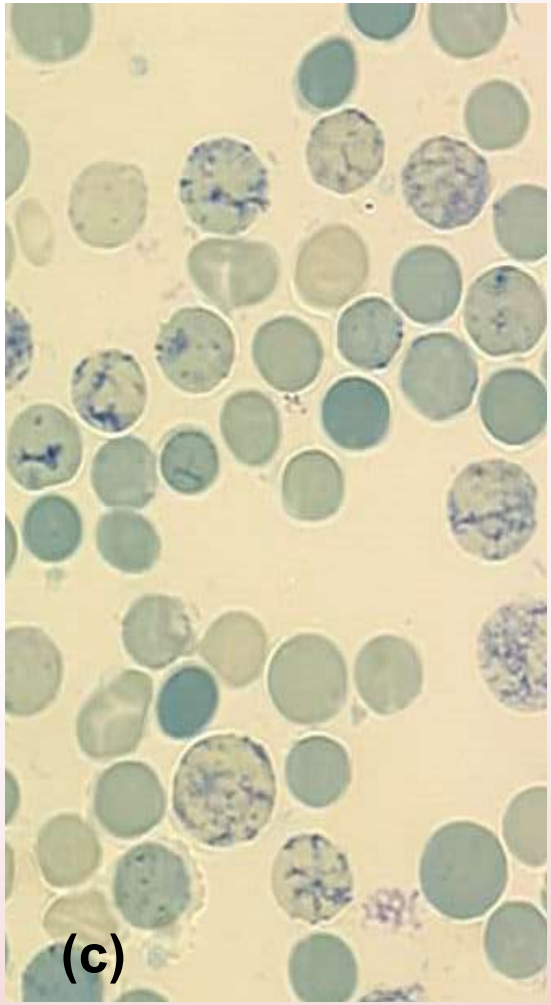
✓ The reticulocyte count is increased.

✓ Incubation of the red cells with brilliant cresyl blue, numerous inclusion bodies are generated by precipitation of HbH under the redox action of the dye. These small pale blue inclusions distributed evenly through a red cell, giving an appearance called; **'golf ball'** cells.

✓ The diagnosis of hemoglobin H disease is confirmed by demonstration of hemoglobin H on hemoglobin electrophoresis and by genetic analysis.



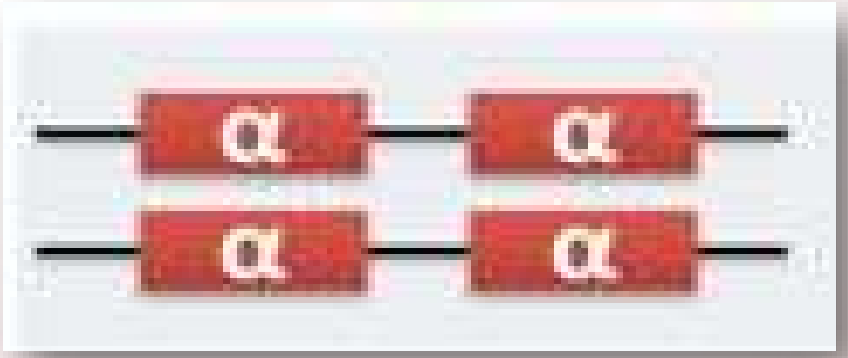




(a); The blood film shows marked hypochromic microcytic cells with target cells (T.C) and poikilocytosis. **(b);** Supravital staining with brilliant cresyl blue reveals multiple fine, deeply stained deposits (' golf ball ' cells) caused by precipitation of aggregates of β-chains. **(c);** shows reticulocytes

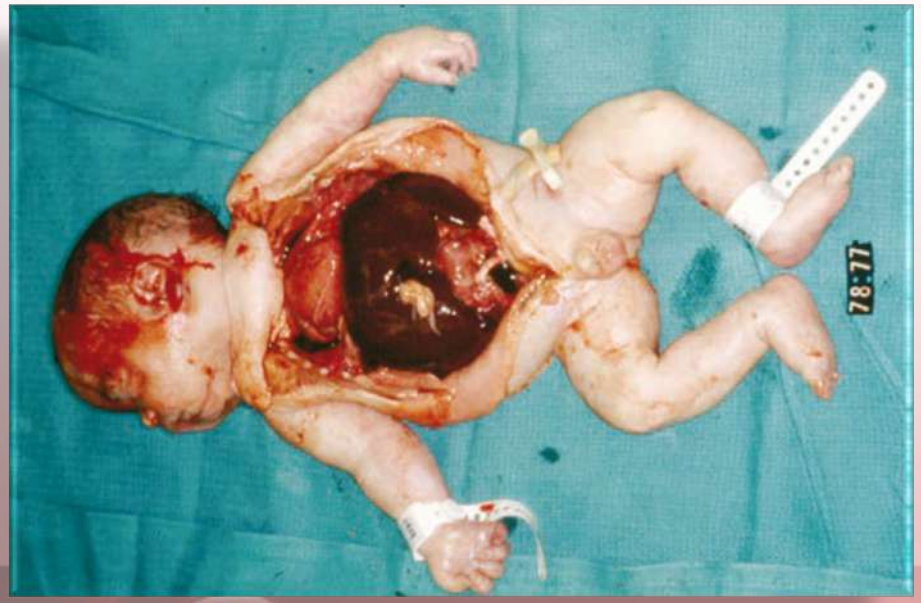
α-thalassemia Major
(Hb Bart's Hydrops Fetalis)

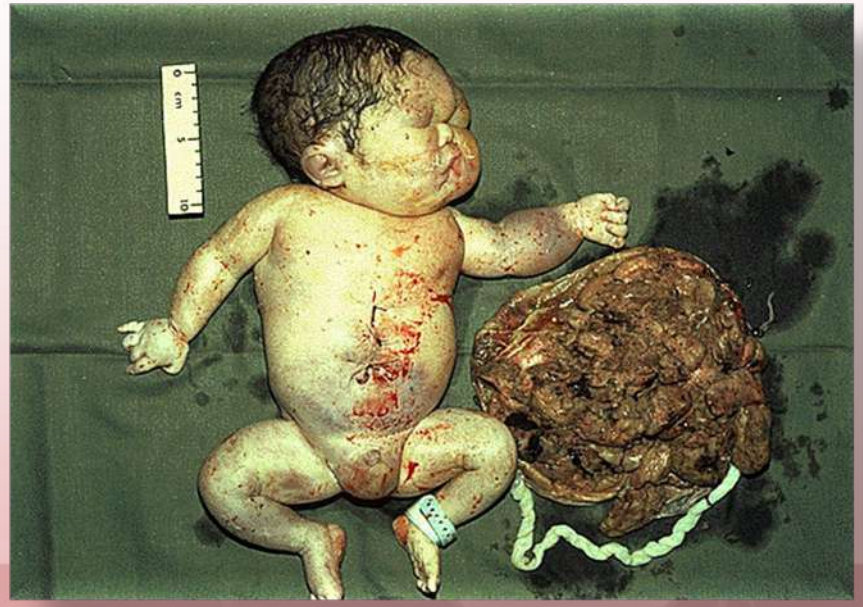
- A severe α thalassemia syndrome caused by deletion of all four α-genes. Since there can be no α-chain synthesis, no Hb A is produced.
- The major hemoglobin 80% Hb Bart's (γ4) and 20% Hb Portland ($\zeta_2\gamma_2$). There may also be some hemoglobin H, which has β_4 chains.



- This condition is generally incompatible with life although a few fetuses have survived after transfusion either in utero or after premature delivery. Some of these infants have had brain damage, probably consequent on fetal hypoxia.

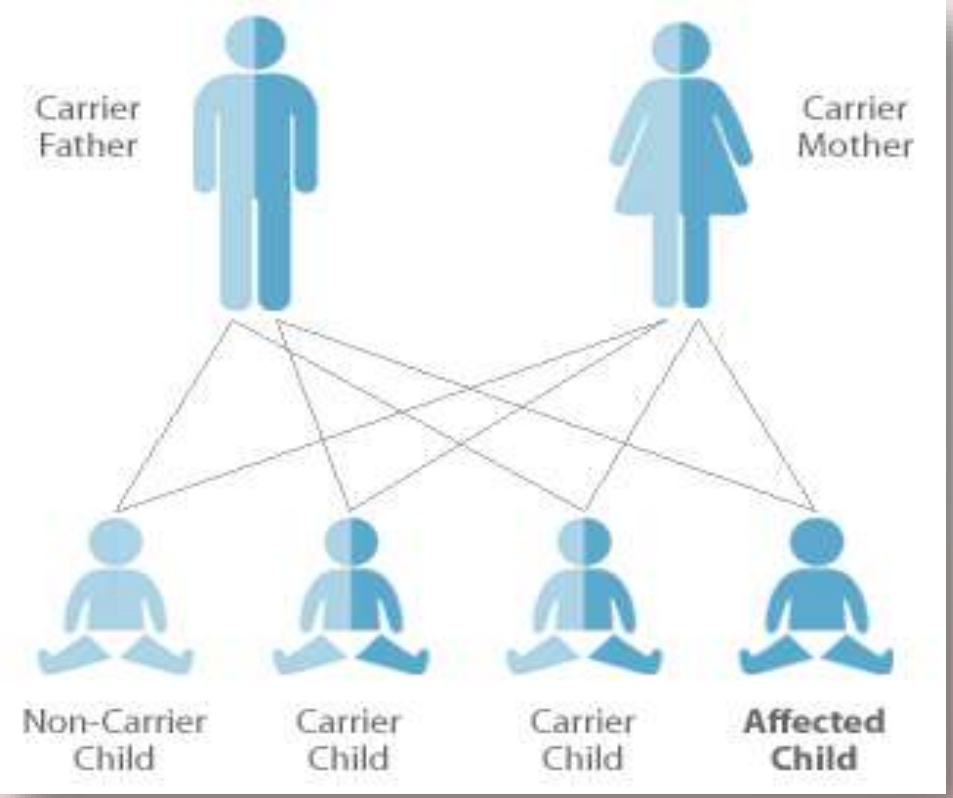
- Hemoglobin Bart's hydrops fetalis causes severe anemia, hepatomegaly, splenomegaly and gross edema of the fetus and the placenta.
- The features are consequent on hypoalbuminemia and on the failure of hemoglobin Bart's to transport oxygen (hypoxia).





β-thalassemia Syndromes

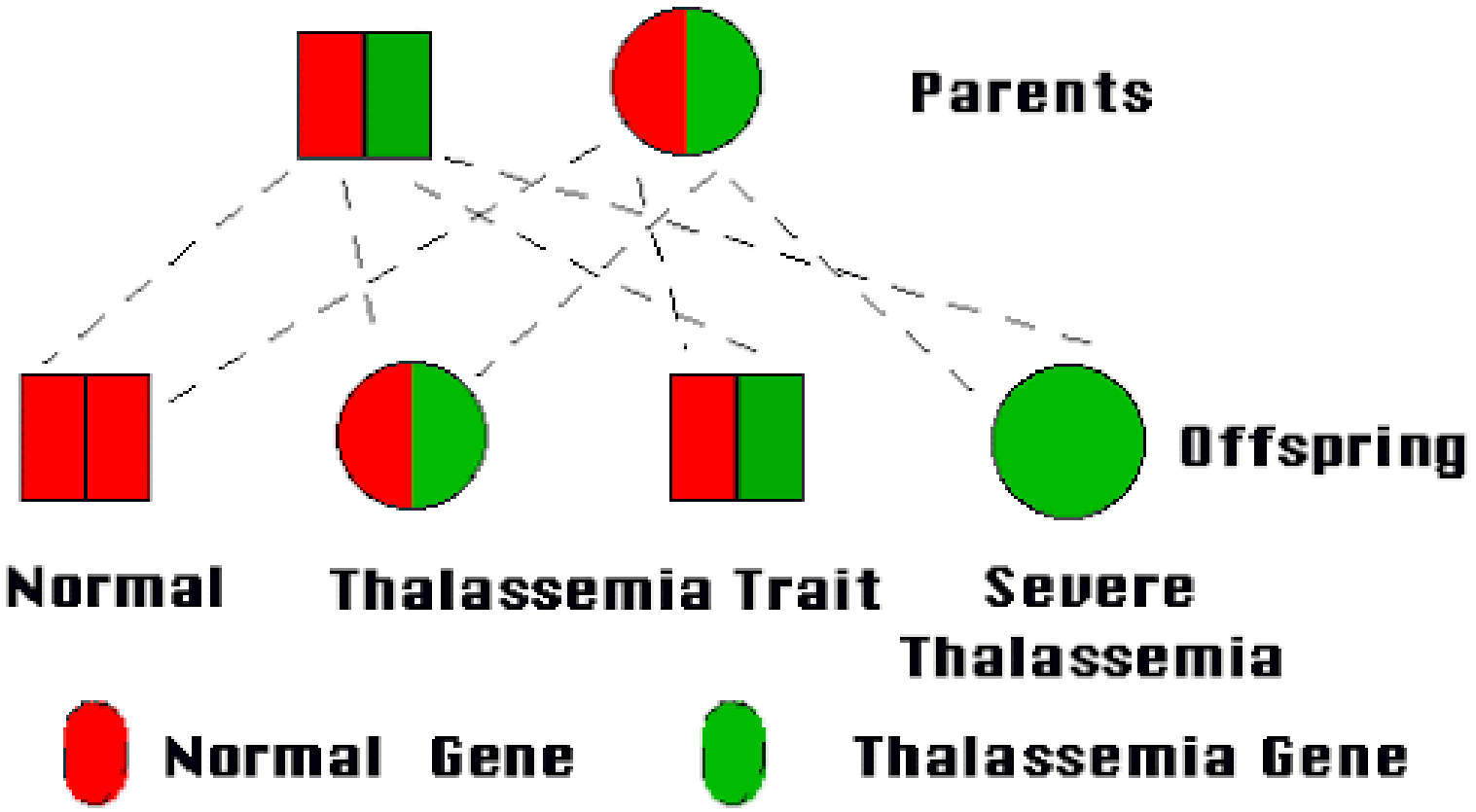
Autosomal recessive; inheritance of one abnormal β gene can lead to a carrier state and the inheritance of two abnormal β-globin genes is required to produce a clinically detectable phenotype.

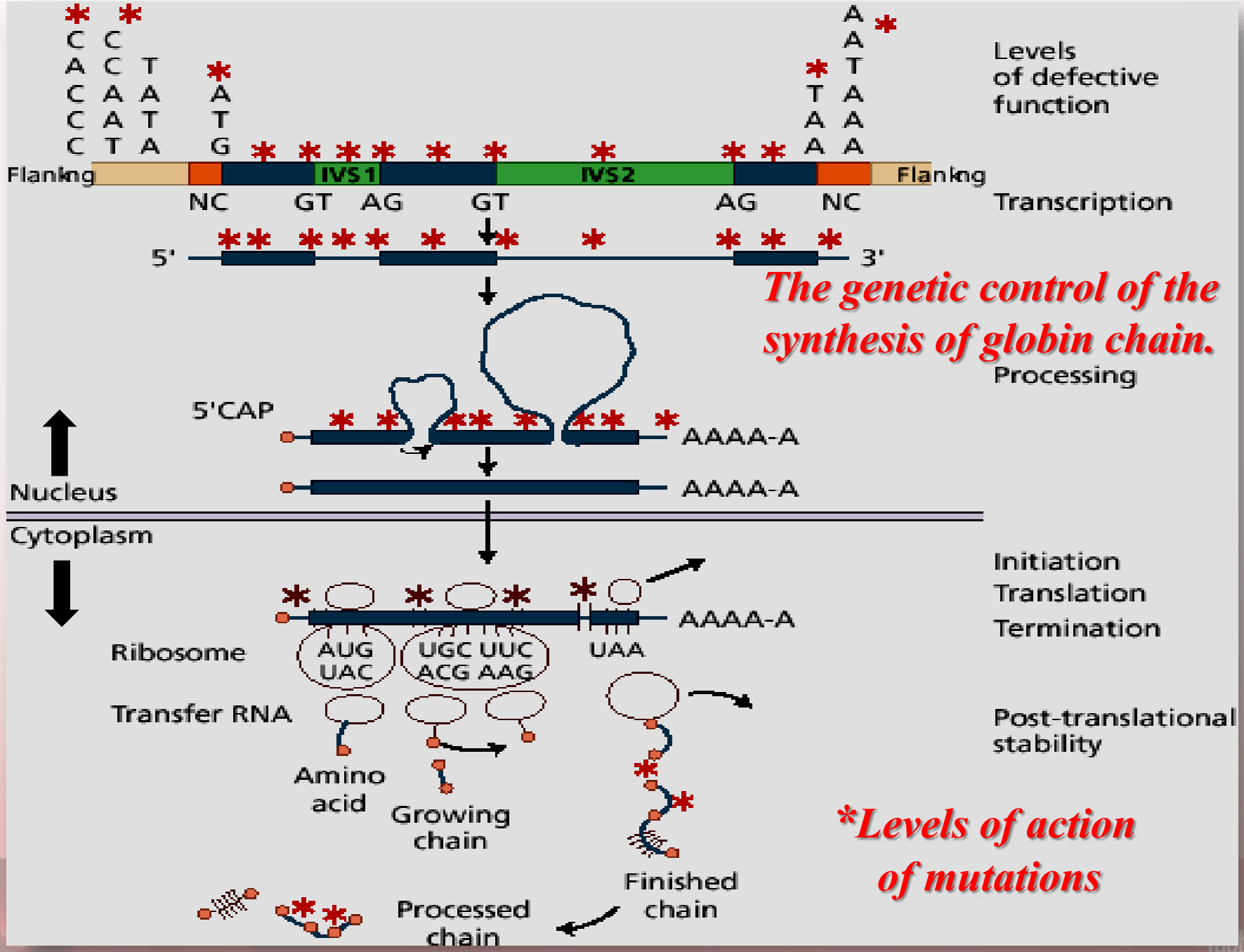


- Mostly point mutation.
- Can be one of following forms

1. Thalassemia Major.
2. Thalassemia Intermedia.
3. Thalassemia Minor (trait).
4. Association with other Hb defects; δβ, βγ (Hb Lepore) or HbS.

Thalassemia Inheritance
Two Carriers




Levels of defective function
Flanking
IVS 1
IVS 2
Flanking
NC
GT
AG
GT
AG
NC
Transcription
5'
3'
The genetic control of the synthesis of globin chain.
Processing
5'CAP
AAAA-A
AAAA-A
Nucleus
Cytoplasm
Initiation
Translation
Termination
AAAA-A
Ribosome
AUG
UAC
UGC UUC
ACG AAG
UAA
Transfer RNA
Post-translational stability
Amino acid
Growing chain
Finished chain
Processed chain
*Levels of action of mutations

β-thalassemia Syndromes

Clinical Syndromes	Genotype	Clinical Features	Molecular changes
1- β-Thalassemia Major	Homozygous β-thalassemia (β^0/β^0, β^+/β^+, β^0/β^+)	Severe; requires blood transfusions	Mainly *point* ***mutations*** (i.e. single-base substitutions) and small ***insertions*** or ***deletions*** of one to two bases may involve any step in globin chain production: transcription, translation or post-translational stability of the globin gene product
2- β-Thalassemia Intermedia	Variable (β^0/β^+, β^+/β^+, β^0/β, β^+/β)	Severe but does not require regular blood transfusions	
3- β-Thalassemia Minor	Heterozygous β-thalassemia (β^0/β, β^+/β)	Asymptomatic with mild or absence of anemia; red cell abnormalities seen	

β-THALASSEMIA MAJOR:

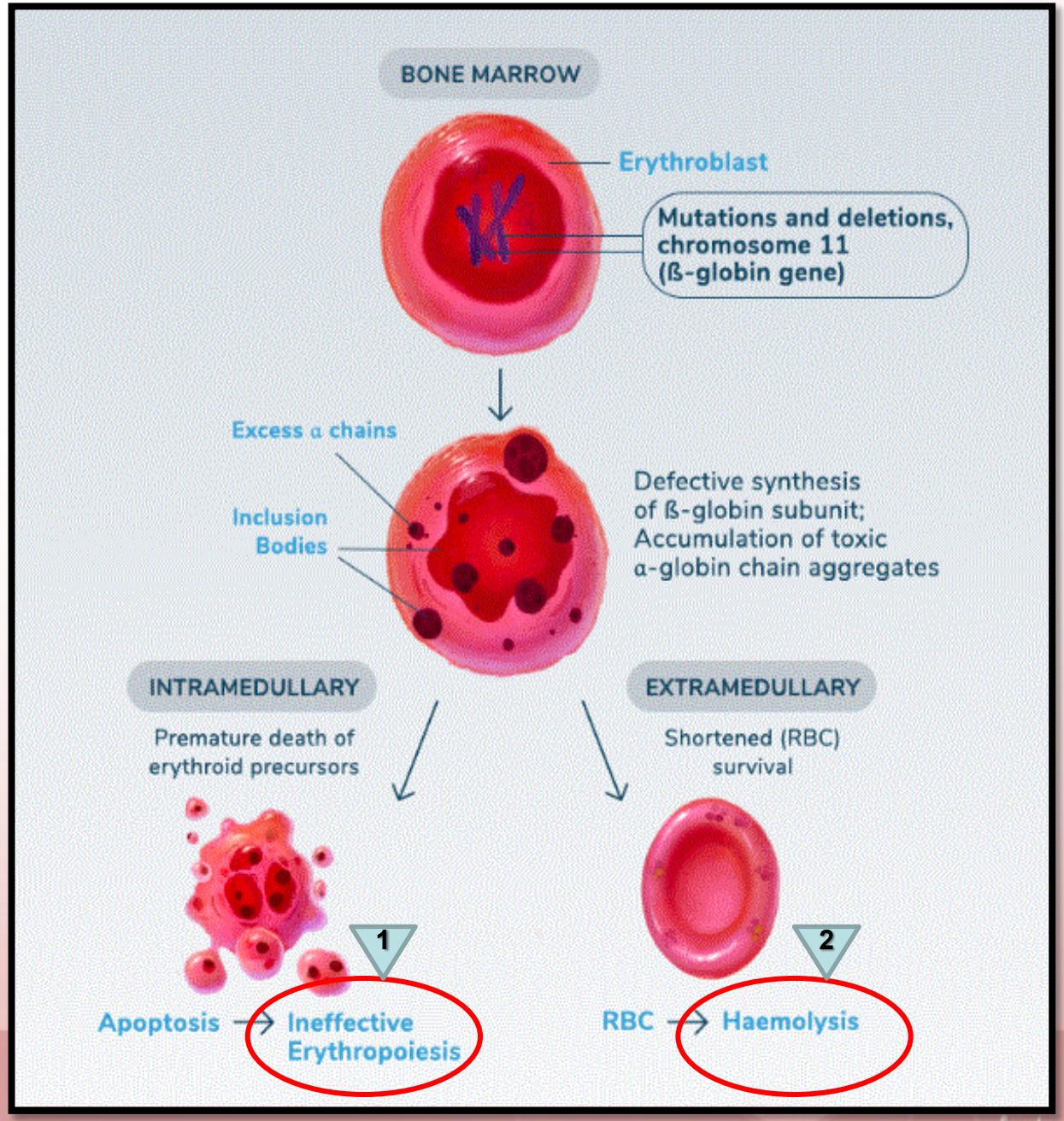
(Mediterranean, or Cooley's anemia)

- Transfusion-dependent hereditary hemolytic anemia with iron overload, due to complete absence of β chain (β^0) or small amount synthesized (β^+).
- Excess α chains lead to more susceptible cells for destruction in a form of ineffective erythropoiesis and hemolysis. With Increased level of HB F because of the γ.
- Over 400 genetic defects reported, certain defects are more common in some countries, which is helpful for the antenatal diagnosis.

Pathophysiology of Anemia in β-thalassemia;

1- Ineffective erythropoiesis within the BM.

2- Extravascular hemolysis.



Clinical Features:

Presented within first year (between 6 and 24 months) of age (due to change from γ to β globin chain production).

- **Severe anemia;**

a- Hemolysis.

b- In effective erythropoiesis.

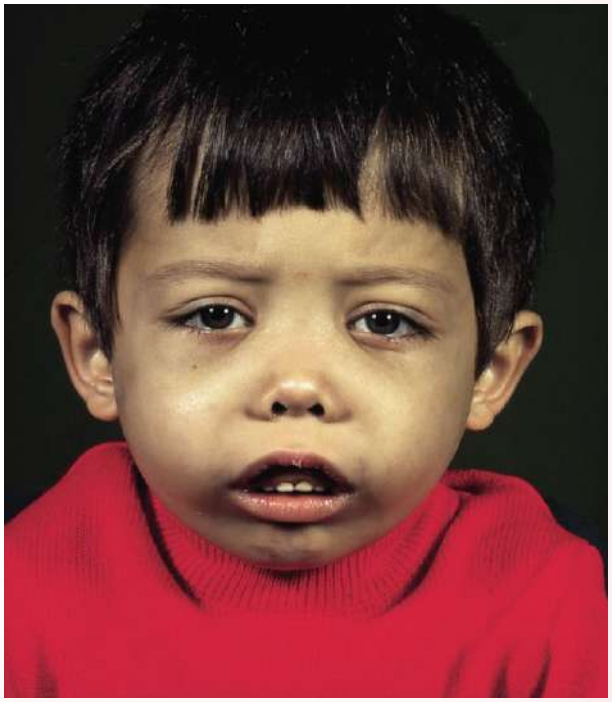
c- Large spleen cause more destruction and plasma volume expansion.

- **Jaundice.**
- **Abdominal distension;**

Hepatosplenomegaly; due to hemolysis (RBC destruction) and extramedullary erythropoiesis.

- **Bones expansion** (marrow hyperplasia), with increased metabolic rate (wasting, gout and folate deficiency).

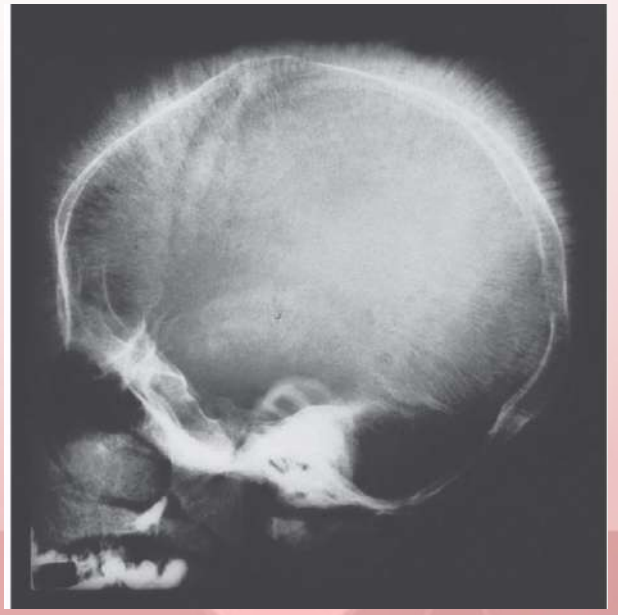
- Thalassemic facies; skull bossing and enlarge maxilla.
- Tendency to fractures.
- X-ray of skull shows "hair-on-end" appearance.



➢ **Infections;**

a- Mostly bacterial in splenectomized and in iron overloaded patients.

b- Viral; transfusion related (Hepatitis and HIV).

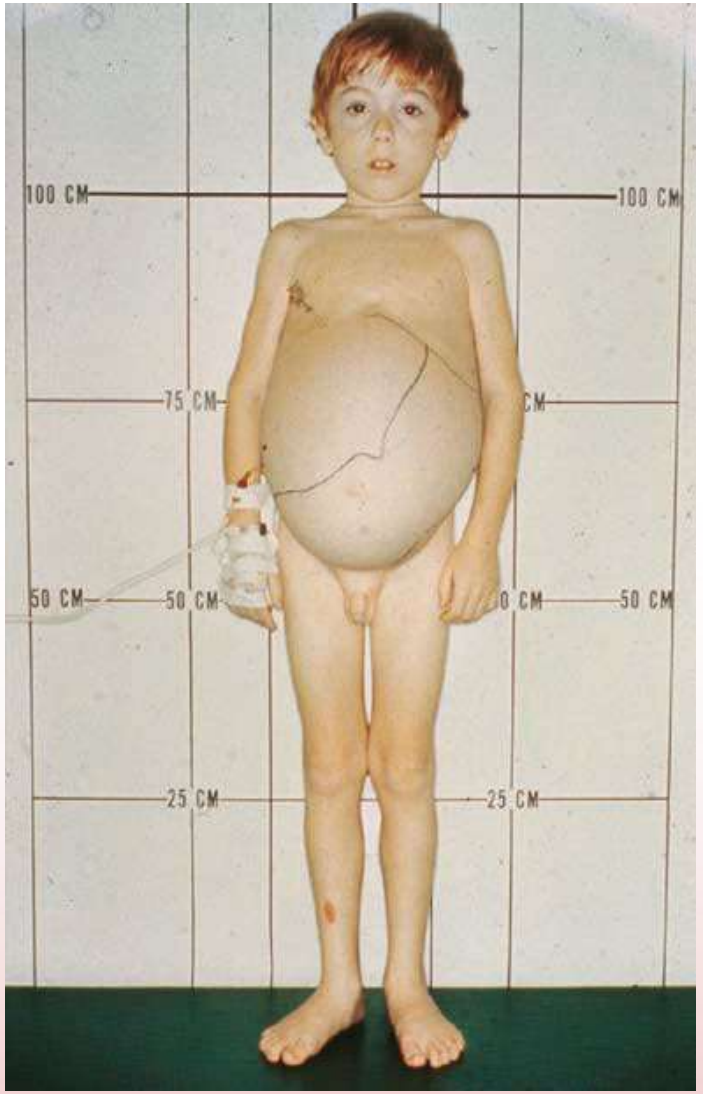


➢ **Iron overload;**

a- Frequent transfusion.

b- Increase iron absorption; ineffective erythropoiesis that leads to reduced Hepcidin level.

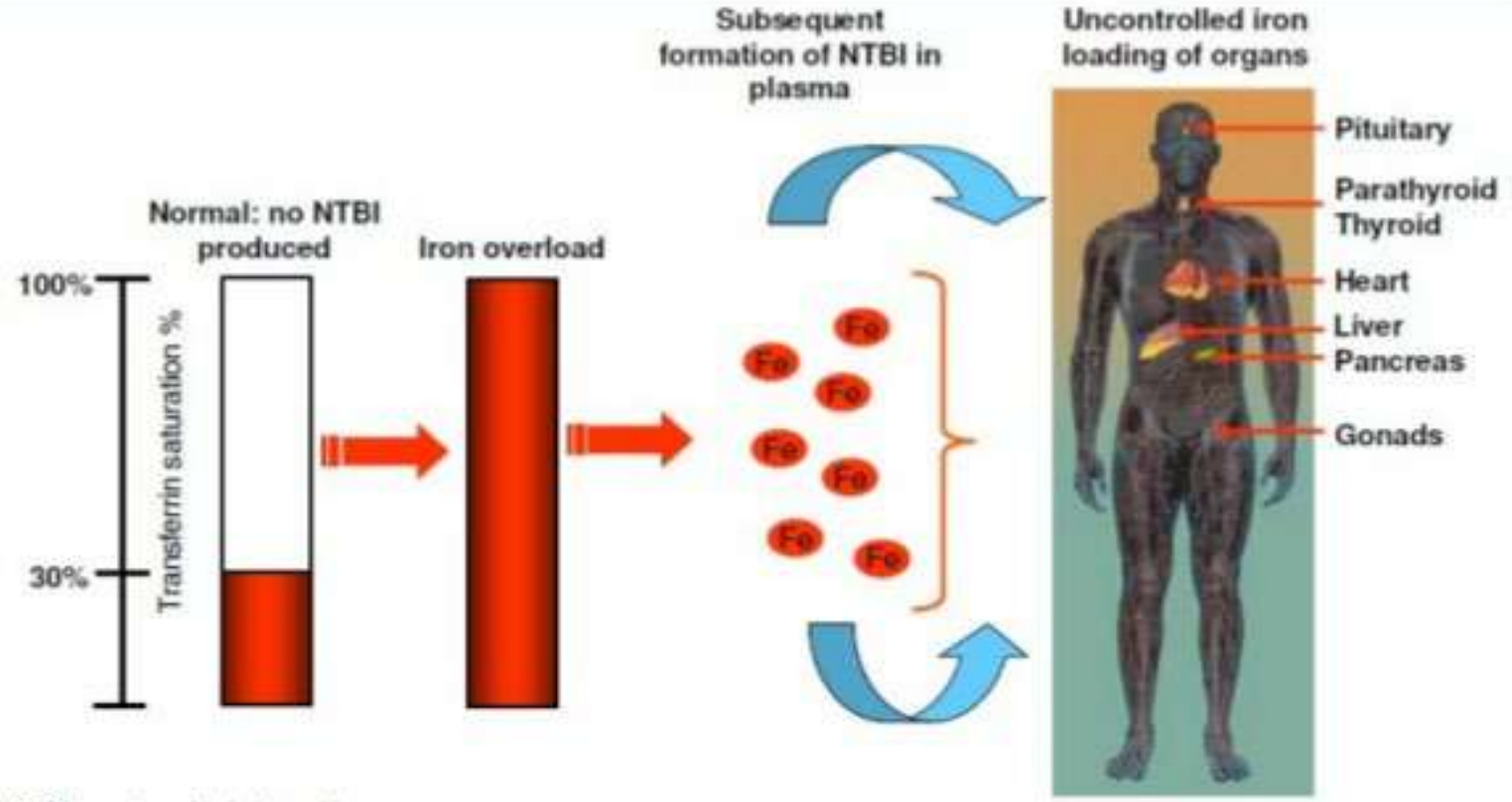
Thalassemia facial bone abnormalities; bossing of the skull, hypertrophy of the maxilla, exposing the upper teeth, depression of nasal bridge and periorbital puffiness.



Undertransfused β-thalassemia major; pallor, short stature, massive hepatosplenomegaly, and wasted limbs

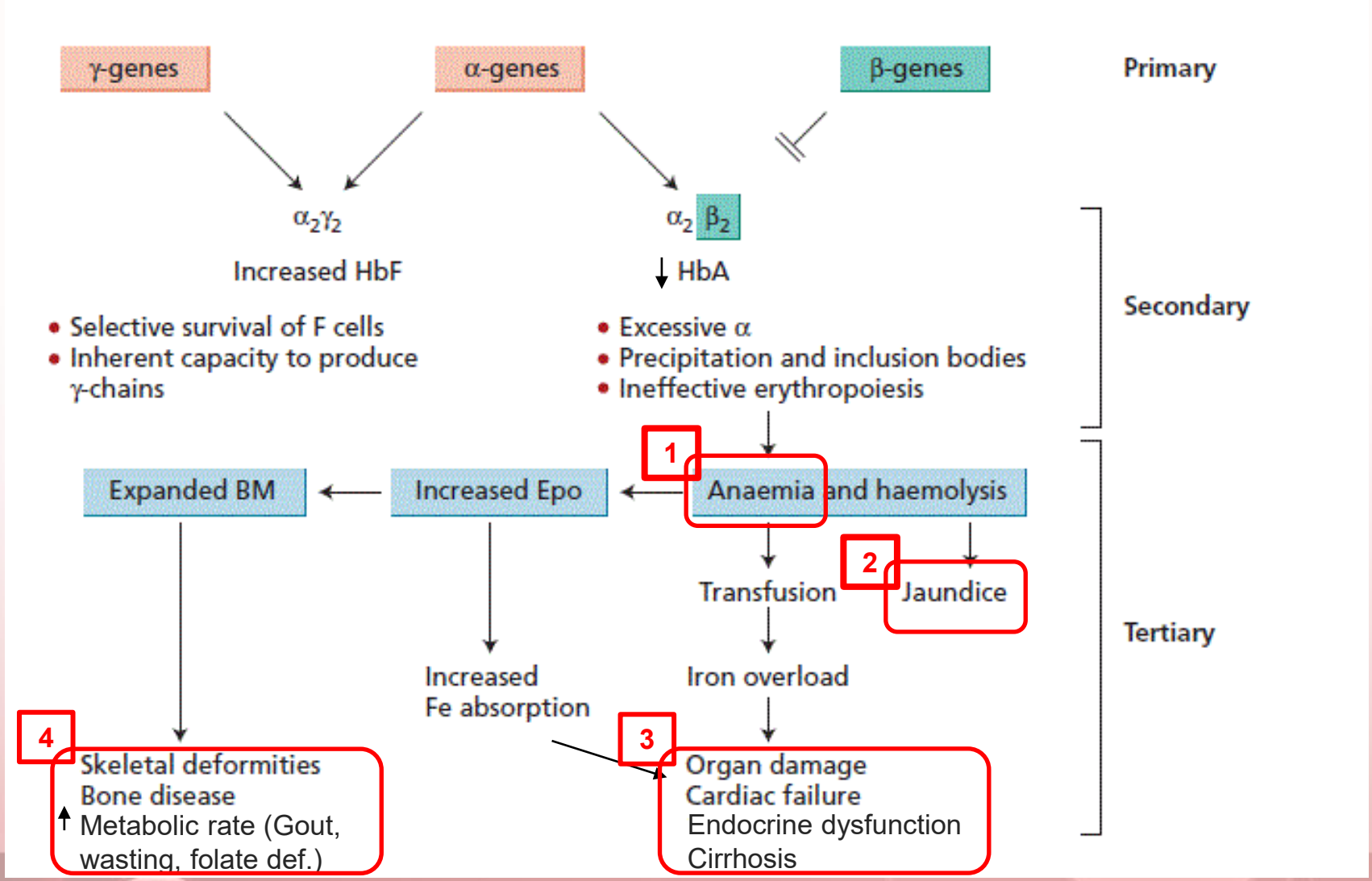
- **Organ Damage and iron overloading;**
- Pancreatic often leads to diabetes mellitus.
- Liver damage (Cirrhosis) may also occur due to viral hepatitis from repeated transfusions as well as from iron overload.
- Myocardium; progressive cardiac damage. Ultimately, these patients die either in protracted cardiac failure or suddenly due to an acute arrhythmia, often precipitated by infection.
- Damage to the other endocrine organs, particularly the hypothalamus, pituitary, thyroid and parathyroid.

Iron overload leads to formation of NTBI and organ loading



NTBI, non-transferrin-bound iron.

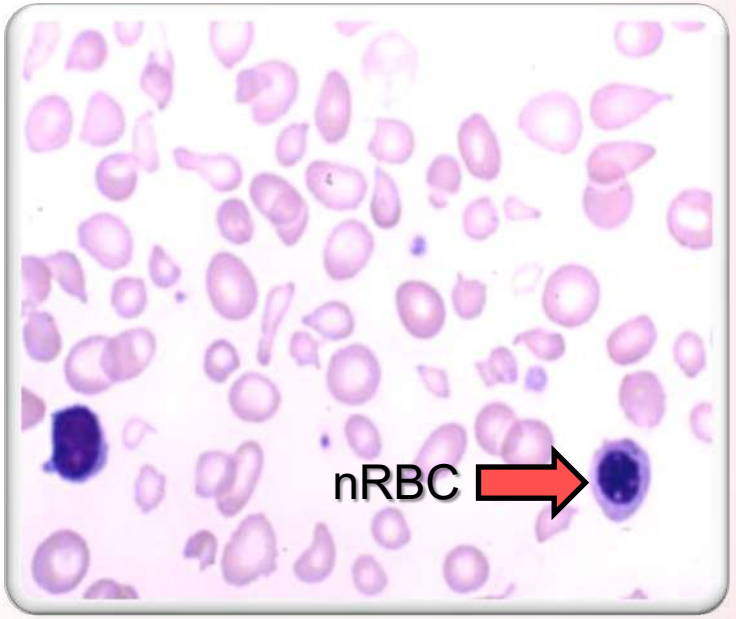
❖ Pathophysiology of β-thalassemia Features



BM; bone marrow, Epo; erythropoietin

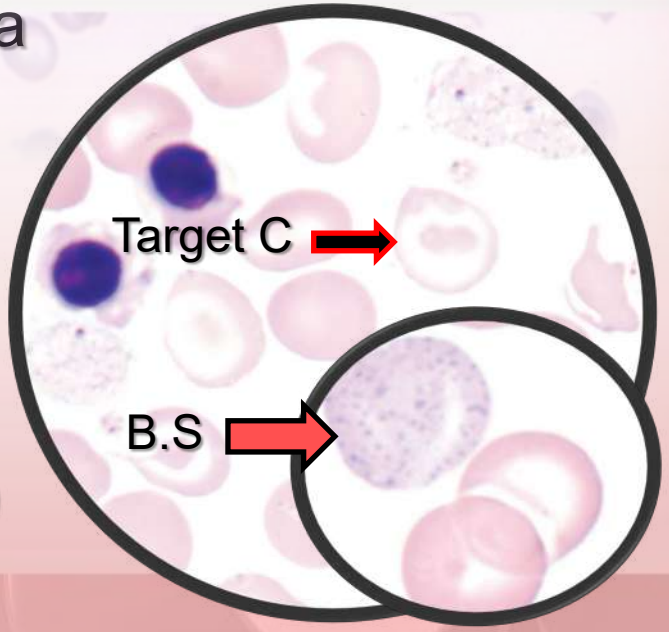
Laboratory Features:

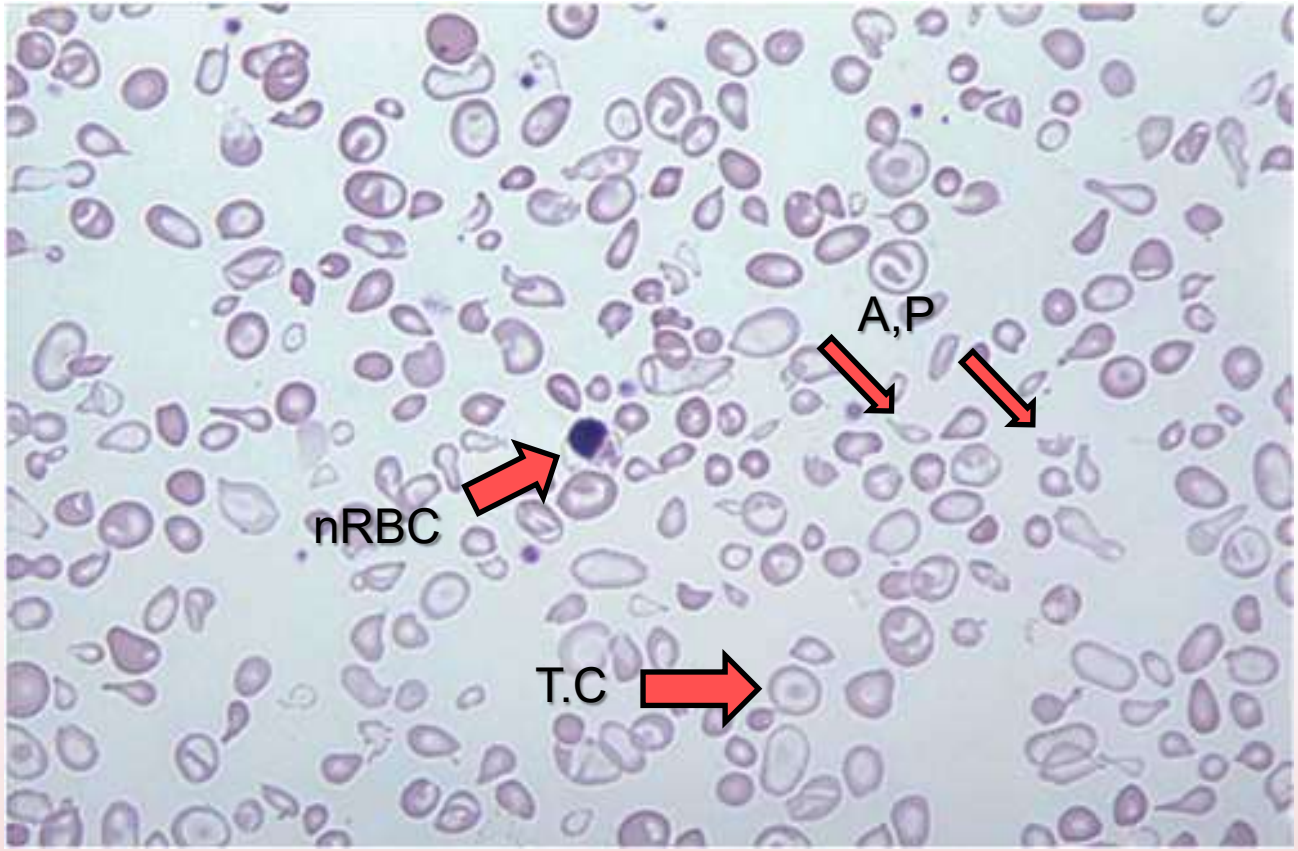
- Severe anemia (Hb may be less than 5.0 g/dL).
- Low MCV and MCH with high RDW.
- High WBC count due to nRBCs.



Blood film;

- Severe hypochromic microcytic anemia
- Anisocytosis (difference in size) and poikylocytosis (difference in shape).
- Target cells.
- Basophilic stippling (B.S)
- Normoblastemia (nRBC in the Bd film)
- Reticlocytosis.





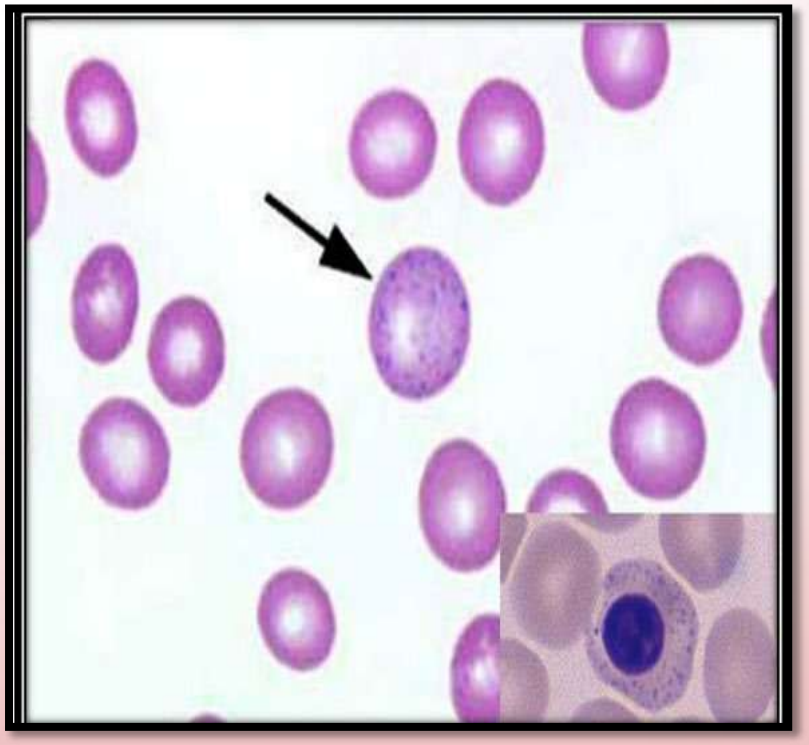
Severe hypochromic microcytic cells, anisocytosis (A), poikylocytosis (P), target cells (T.C) and normoblastemia (nRBC)

Basophilic stippling

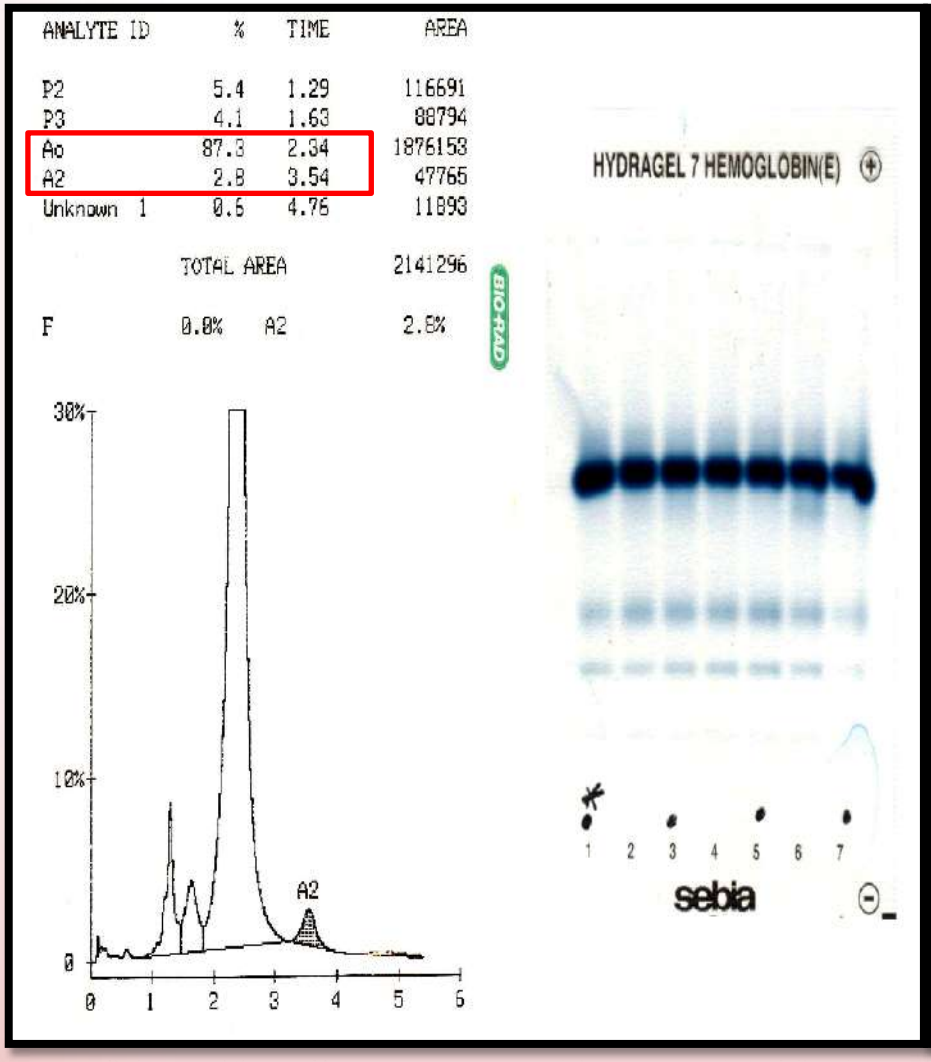
Number of small basophilic inclusions within the red blood cells.

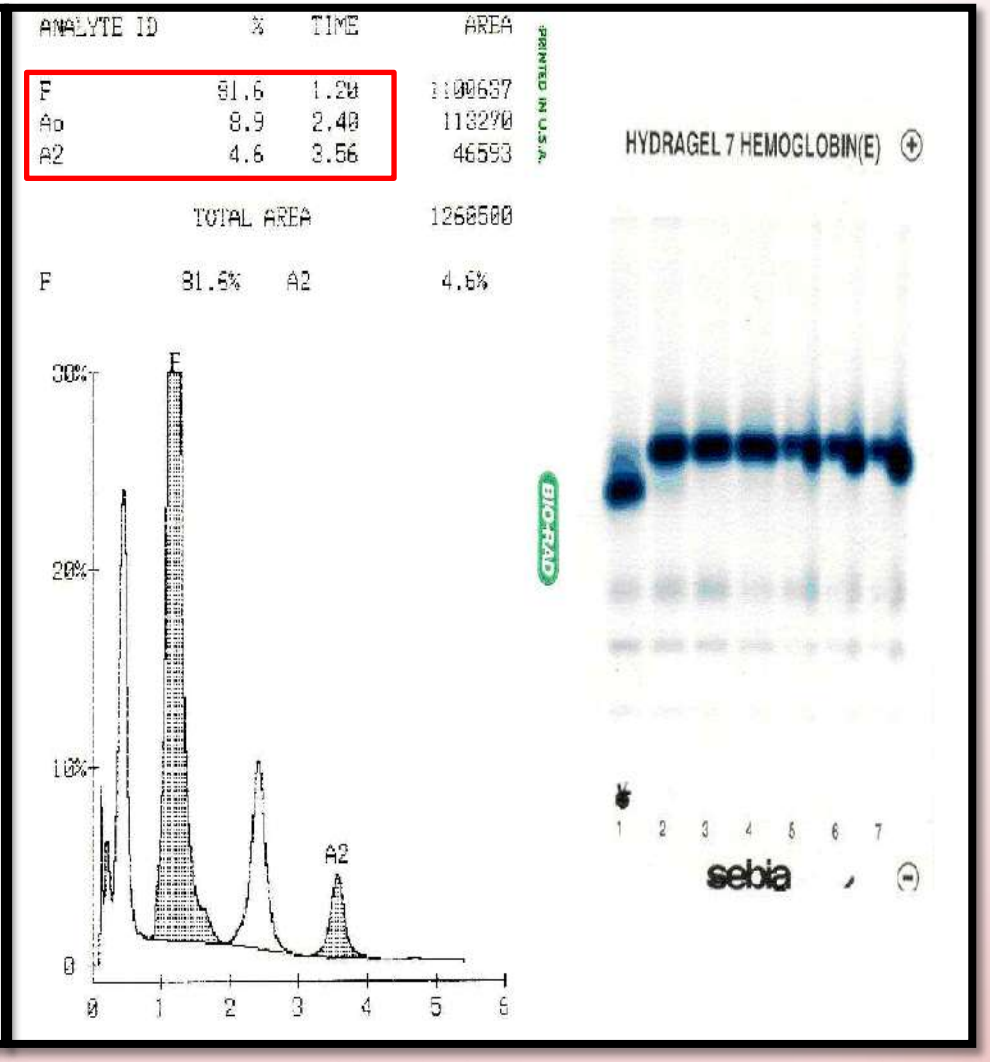
Seen in;

1. Thalassemias
2. Megaloblastic Anemia
3. Hemolytic Anemias
4. Heavy metals poisoning (Lead)



- **Iron status** of iron overload with very high serum ferritin and transferrin saturation, due to Increase iron absorption and the ineffective erythropoiesis that leads to **reduced Hepcidin level**.
- **Hb electrophoresis;** reveals nearly all circulating hemoglobin of Hb F with complete absence of Hb A. variable level of Hb A_2.
- **High performance liquid chromatography** (HPLC); can be the first line in diagnosis in well-equipped laboratories.
- **Molecular Genetic (DNA) Analysis;** identify the genetic defect, usually by PCR.





Hb electrophoresis and High performance liquid chromatography (HPLC) for normal (left) and β-thalassemia major (right).

Treatment:

- Blood transfusion; packed and filtered.
- Hb around 10mg/dL.
- 2-3 units 4-6 wks.
- Folic acid.
- Iron chelating;

✓ IV or SC; Desferoxamine (DFO).

✓ Oral; Deferiprone (DFP), new Deferasirox (DFX) and others.

- Splenectomy.
- Allogenic stem cell transplantation (ASCT).

- Much of the bone abnormality can be prevented by regular transfusions from the age of presentation (usually 6 months) to maintain the hemoglobin at all times at a level above 9–10 g/dl.
- However, these regular transfusions, together with increased iron absorption, lead to iron overload.
- Each unit of blood contains 200–250 mg iron.
- After 50 units have been transfused, or earlier in children, siderosis develops, with increased pigmentation of skin exposed to light and susceptibility to infection reduced growth, and delayed sexual development and puberty .

Prognosis

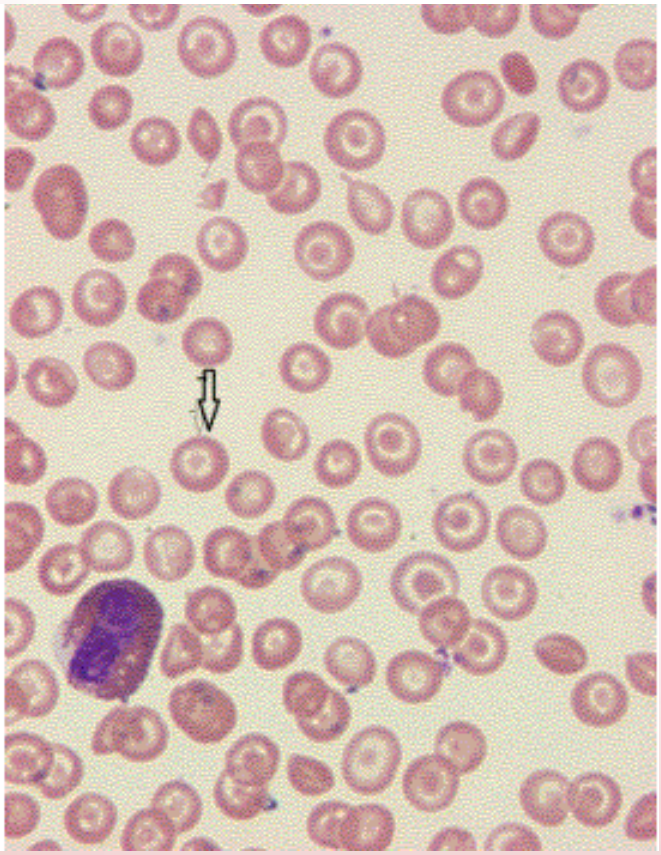
- Survival of individuals who have been regularly transfused and treated with appropriate chelation extends beyond age of 40 years.
- Cardiac disease caused by myocardial siderosis is the most important life-limiting complication of iron overload.
- Cardiac complications are the cause of the deaths in 71% of the patients with beta-thalassemia major.

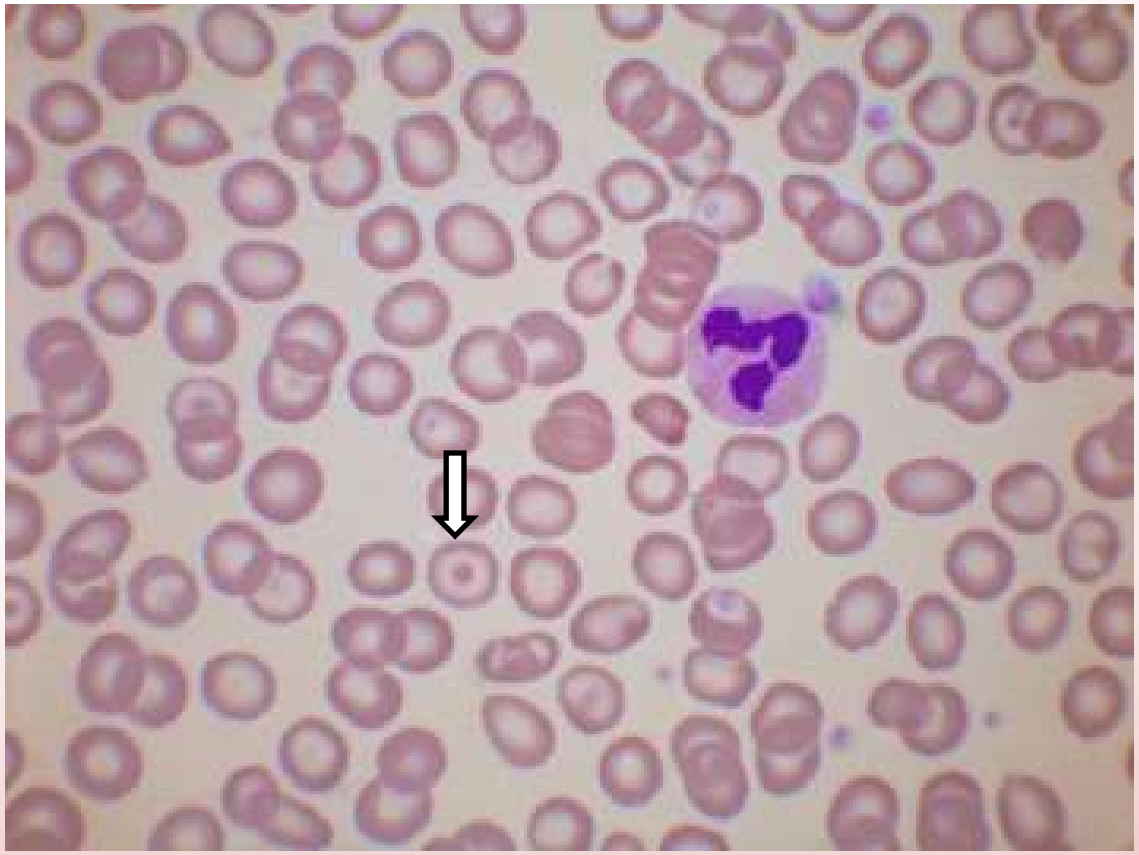
Antenatal diagnosis

- If both parents are carriers, fetal diagnosis is carried out, either by using fetoscopy to obtain blood and measuring the α/β chain synthesis ratio.
- Amniocentesis or trophoblast biopsy to obtain DNA for hybridization with relevant DNA probes or for analysis by one or other polymerase chain reaction (PCR) technique.
- These include analysis by direct restriction fragment length polymorphism (RFLP) analysis, using of oligonucleotide probes with radioactivity or horseradish peroxidase labeling.

β-thalassemia trait (minor):

- Heterozygous for β gene.
- Asymptomatic.
- Mild anemia (10-12 g/dL).
- Low MCV and MCH with high RBC count (>5.5X10^{12} /L)
- High Hb A_2 (>3.5%).
- Important for the prenatal counselling for the possibility of inheritance.





β thalassaemia heterozygosity; shows hypochromia, microcytosis and several target cells (arrows).

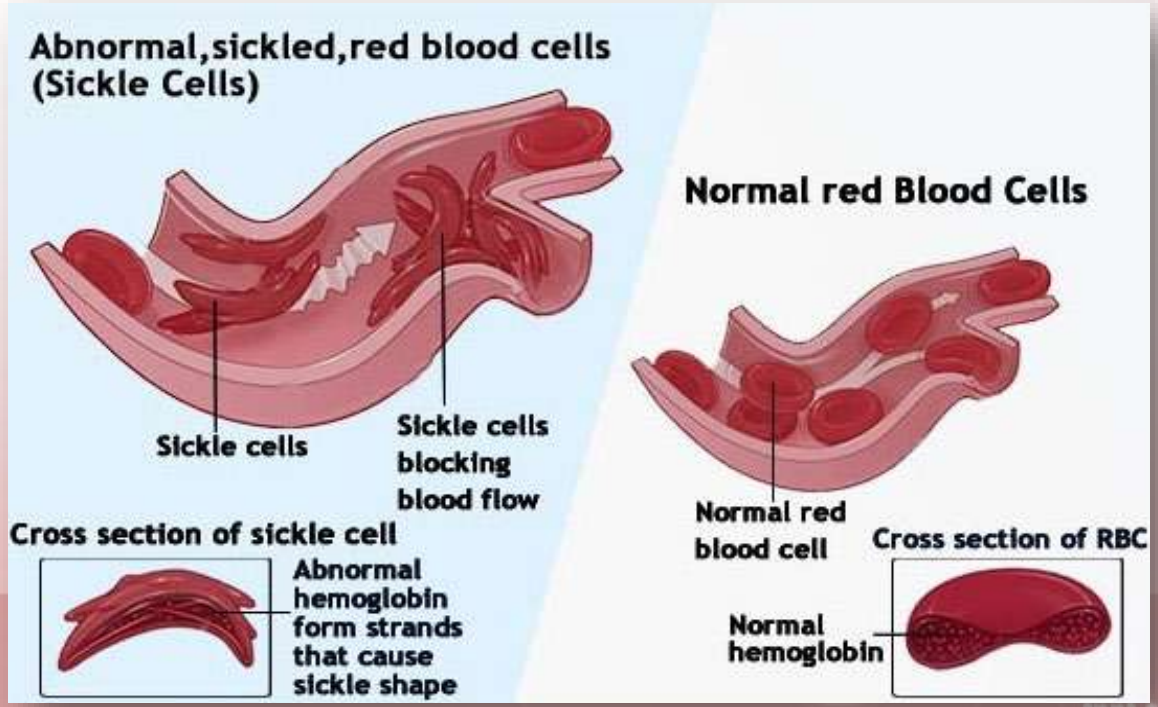
Thalassemia Intermedia:

The term thalassemia intermedia is used to describe patients with the clinical picture of thalassemia which, referred as non-transfusion-dependent thalassemia (NTDT) is associated with a much more severe degree of anemia than is found in carriers for α- or β-thalassemia.

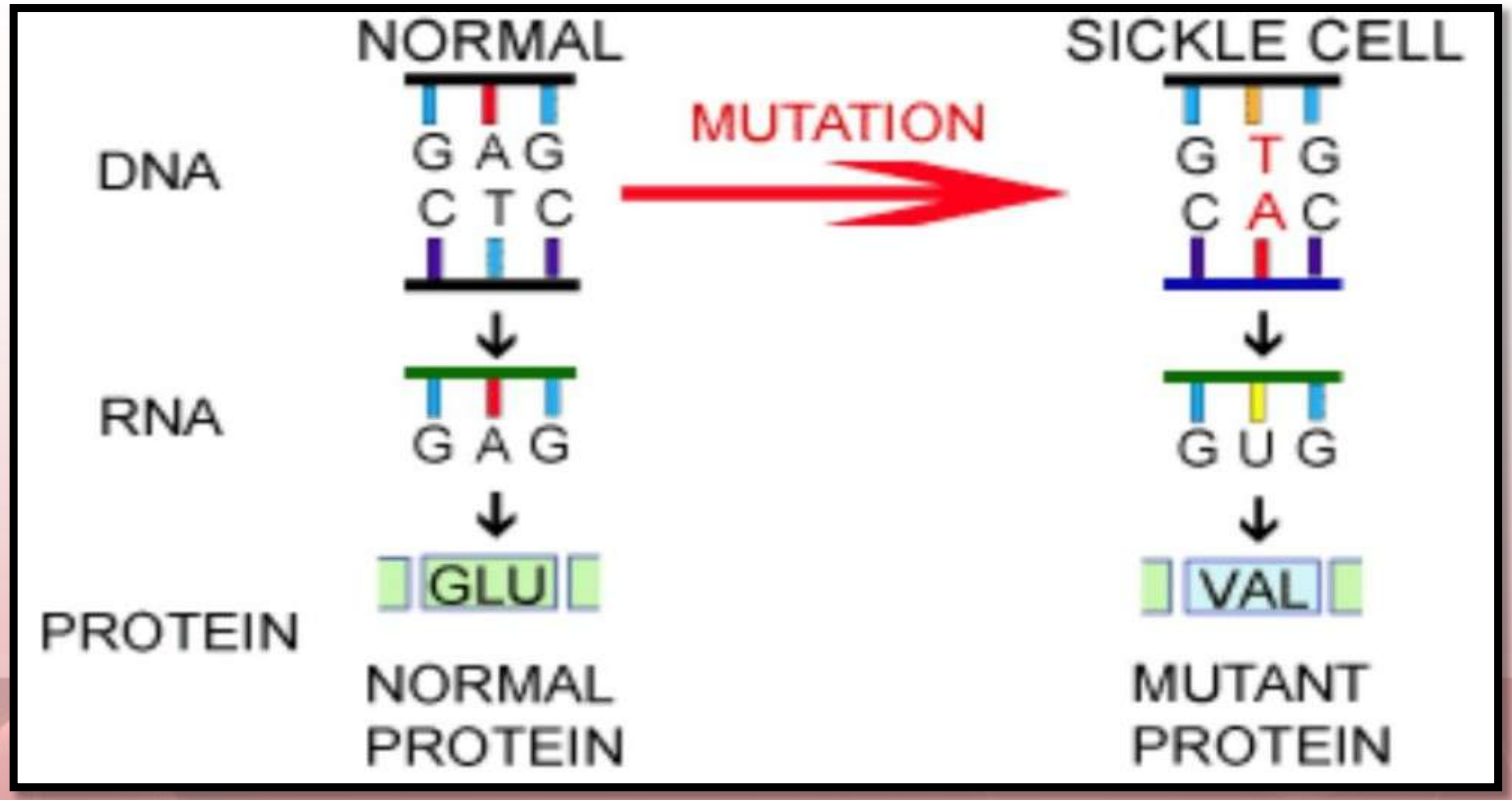
- Present between the ages of 2 and 6 years, or even at adult age.
- Wide variability of features; bone deformities, and hepatosplenomegaly may be a feature.
- Showing mild to moderate anemia (7-10 mg/dL) without the need for transfusion or occasionally.
- Different genetic abnormalities of α and/or β.
- Rx; Oral chelating agents for iron overload or Drugs to increase HbF may be of a value.

Sickle Cell Anemia:

- Group of Hb disorders resulting from sickle β-globin (β^s) gene inheritance in chromosome 11.
- The RBCs are rigid sickle-like (crescent shape) due to insoluble Hb with crystal formation by its abnormality.
- HbS is an insoluble forming crystals in low O2 tension leading to occlusion of the Bd vessels.



- The gene abnormality mostly distributed in Africa and the carrier develops protection against malaria protect Invasion and replication of Plasmodium parasite
- Result of substitution of glutamic acid (hydrophilic) to valine (hydrophobic) in the sixth position of the β chain, due to a single base change in the corresponding portion of DNA .



- The term 'sickle cell anemia' refers specifically to homozygosity for the sickle cell (β^S) gene, the patient having the genotype β^S β^S.
- The oxygen is given up to tissues relatively easily, therefore the patient has few symptoms of anemia with mild jaundice, despite a hemoglobin level in the steady state of 6–8 g/dl, and has a chronic hemolytic anemia punctuated by sickle crises.
- Fragile cells with survival of 1/10th of the normal RBC life, giving the picture of hemolysis in addition to occlusive effect.

Clinical Forms;

1. **Homozygous** (Hb SS); Sickle cell anemia (disease); the most severe type.
2. **Trait** (Hb AS); is a benign condition that has no hematological manifestations and is associated with normal growth and life expectancy, may develop painless hematuria. Special care needed in certain conditions of hypoxia; eg; anesthesia, pregnancy and high altitude or intensive exercise.
3. **Heterozygous**; combined with other type of abnormal globin gene (Hb SC, Hb Sβthal, or Hb S αthal, …).

Sickle Cell Disease (SCD):

- It's a severe hereditary hemolytic anemia punctuated by crisis.
- The presence of HbS underlies the major pathologic manifestations:

1- Chronic hemolysis,

2- Microvascular occlusions, and

3- Tissue damage.

- Several variables affecting the **rate and degree** of sickling:

1- **Interaction** of HbS with the other types of hemoglobin in the cell;

- In heterozygotes HbA, which interferes with HbS polymerization, unless there is profound hypoxia.

- HbF inhibits the polymerization of HbS even more than HbA; hence, infants do not become symptomatic until they reach 5 or 6 months of age .

2- Mean cell hemoglobin concentration **(MCHC);**

- Intracellular dehydration; which increases the MCHC, facilitates sickling.

- Conditions that decrease the MCHC reduce disease severity, as in coexistent α-thalassemia, leads to milder disease.

3- Intracellular **pH**; decreased pH reduces the Hb oxygen affinity, with increasing the fraction of deoxygenated HbS augment sickling.

Clinical Features:

- Mild in relation to the severity of the anemia, because of low O2 affinity (O_2 dissociation right shift) of Hb S.
- Normal life in some with free of crisis.
- Early death due to recurrent crisis (vaso-occlusive, visceral and aplastic or hemolytic anemia).
- Precipitated by; infection, hypoxia, dehydration and acidosis.

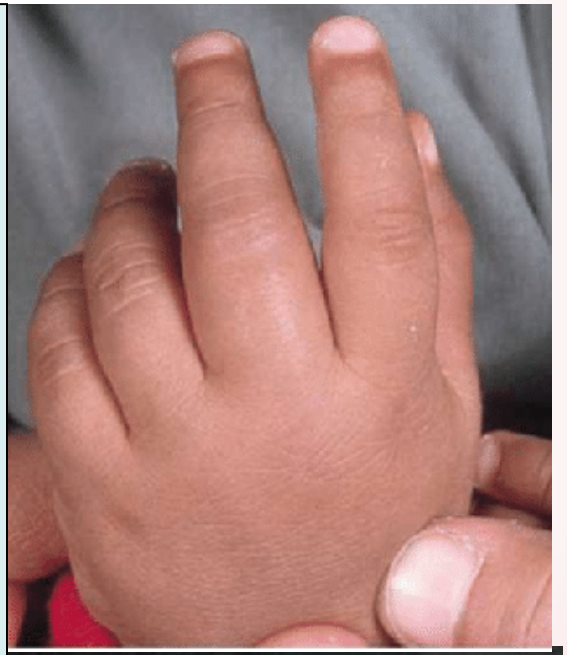
1. **Vaso-occlusive Crisis;**

- Infarctions of bone vessels lead to severe bone pain.
- Infarctions of small bones; painful swelling (*dactylitis)* and with digits of variable

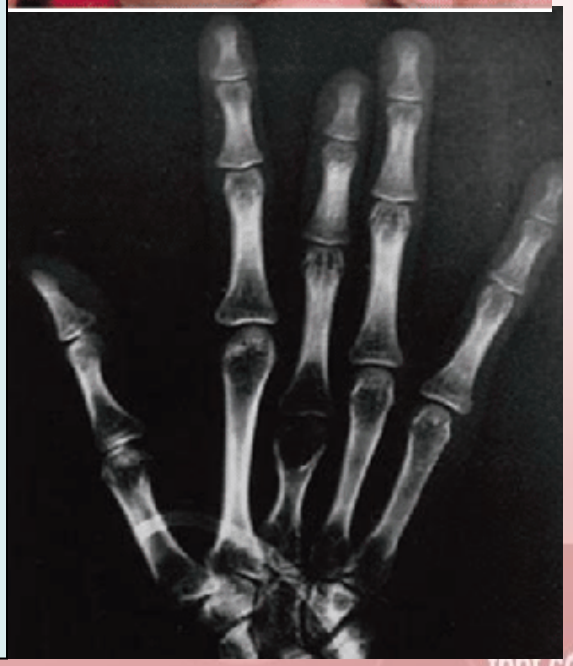
lengths; 'Hand-foot' syndrome

- Brain infarcts (CVA); most serious effect seen in 75% of patients.
- Splenic infarctions lead to splenic atrophy (autosplenectomy)

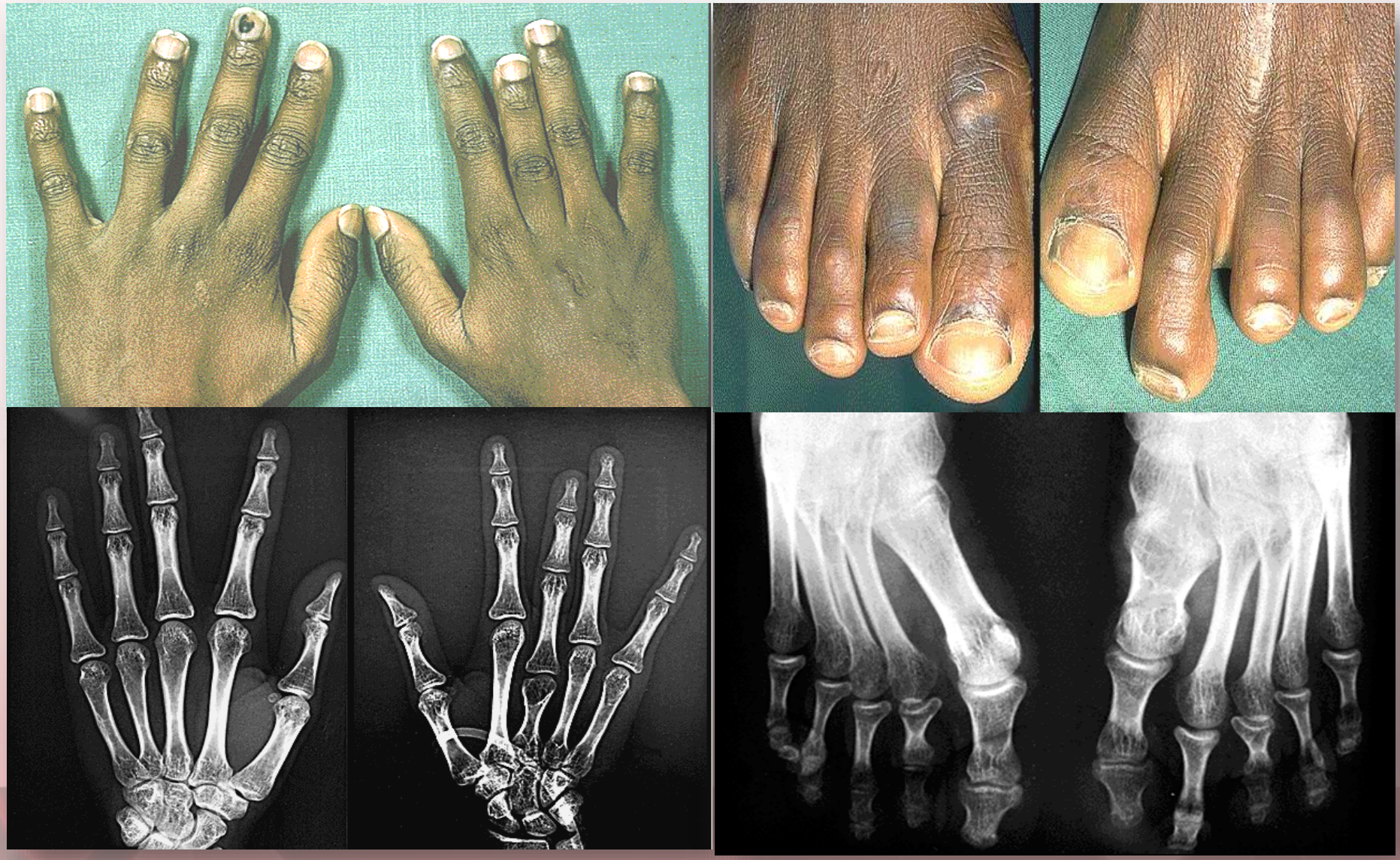
Painful swollen fingers ***(dactylitis)*** *in a child.*



'Hand-foot' syndrome*. of an 18-year-old Nigerian boy. Marked shortening of the right middle finger because of dactylitis in childhood affecting the growth of the epiphysis.*



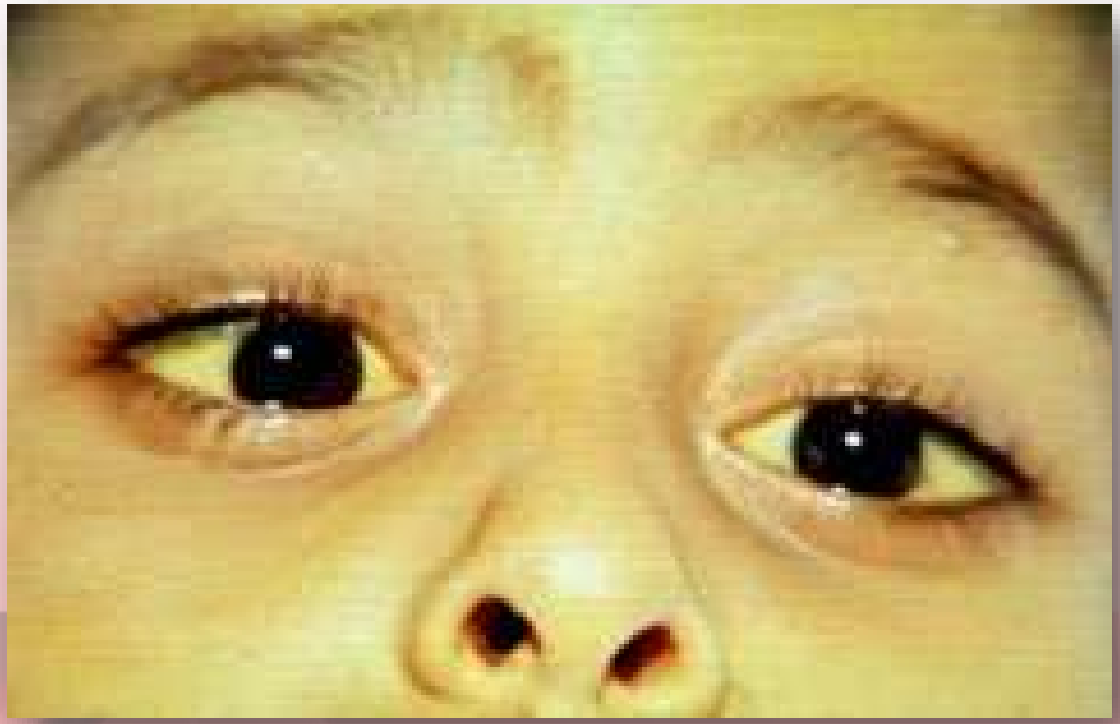
'Hand-foot' syndrome;

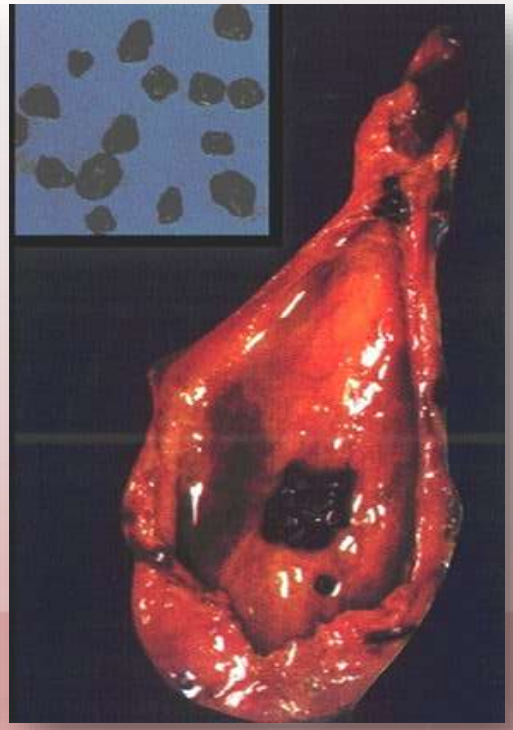


2. Visceral crisis; Sickling within organs; acute chest syndrome, splenic enlargement (splenomegaly).

3. Aplastic crisis; usually precipitated by viral infection or folic acid deficiency.

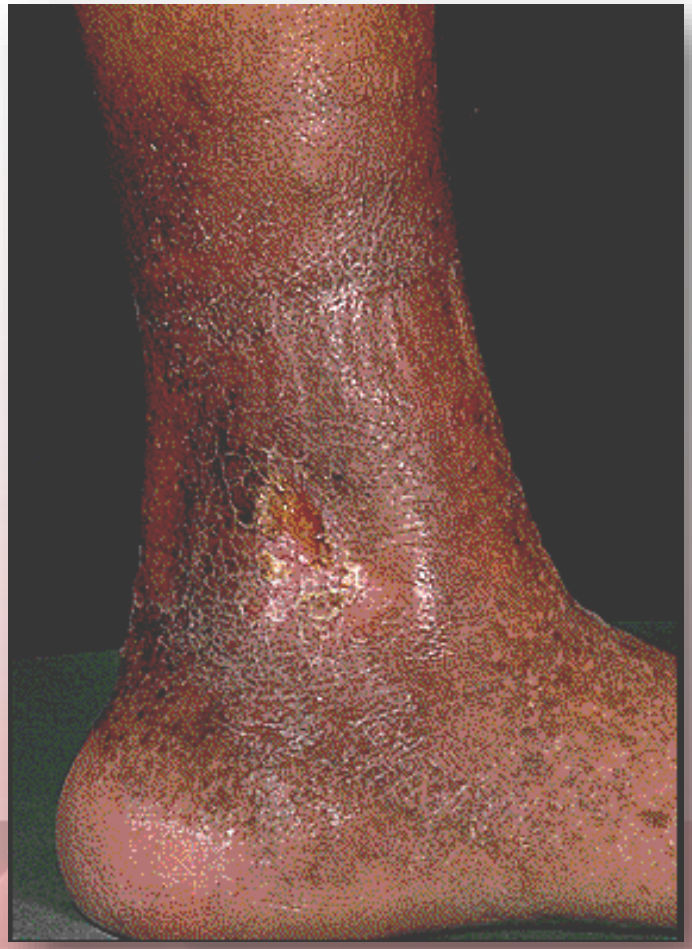
4. Hemolytic crisis (pallor and jaundice); of intra- and extravascular, with gallstones formation.

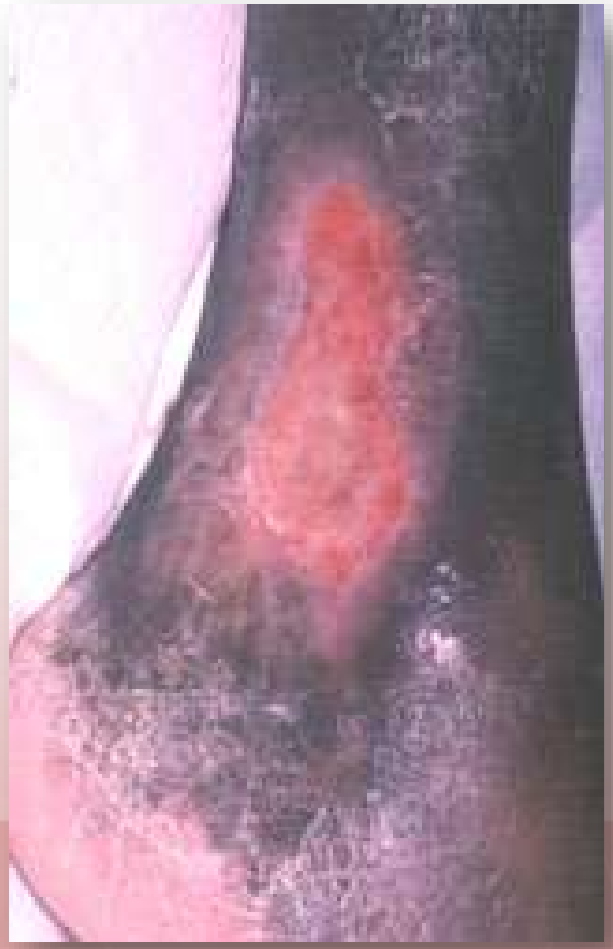




5. Leg ulcer, mostly at ankle area. Ulceration follows vessel occlusion by sickle cells with resultant infarction, .

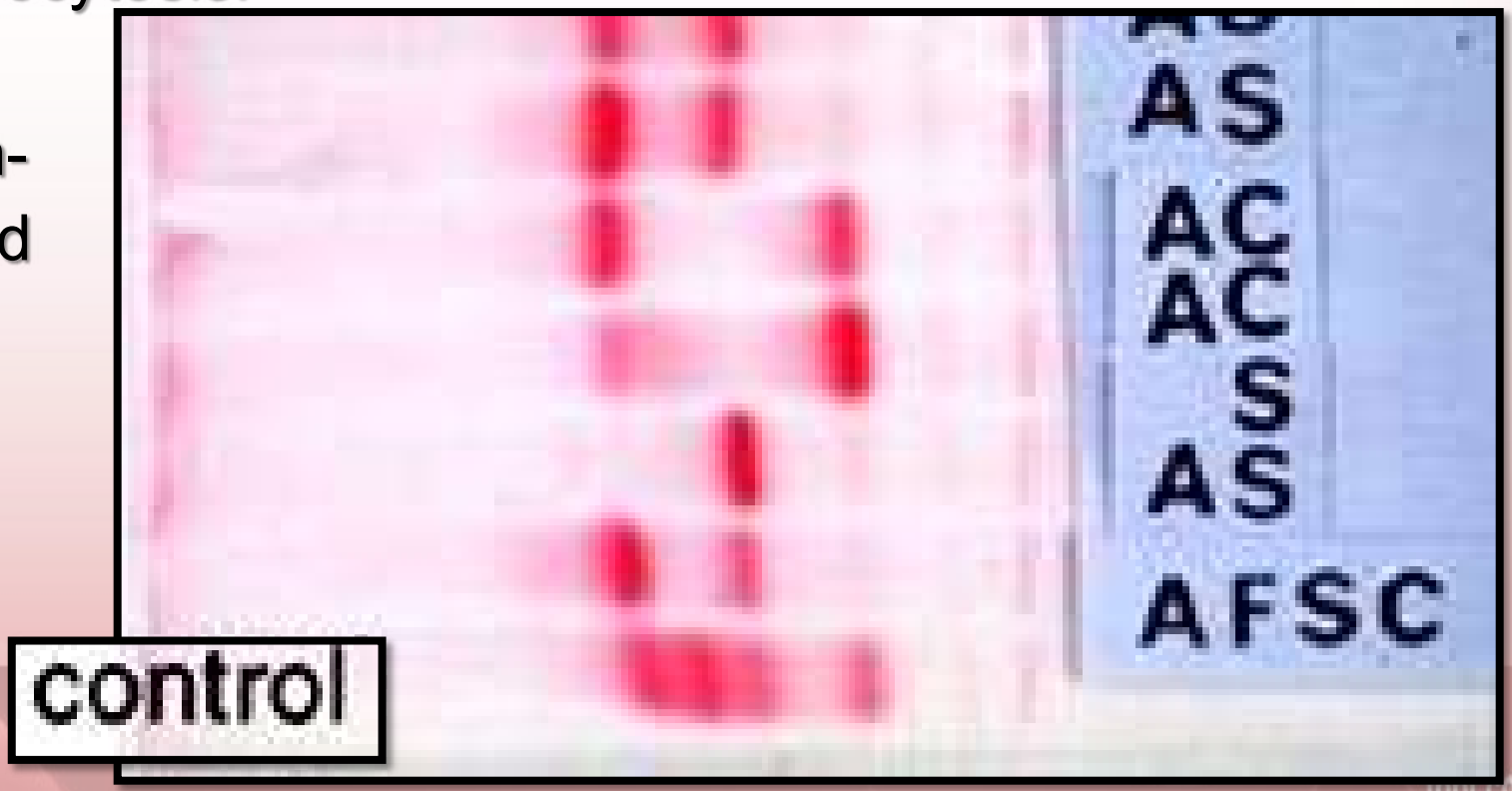
6. Repeated tissue and organ infarctions lead to; pulmonary hypertension, chronic liver and renal damages and osteomyelitis.

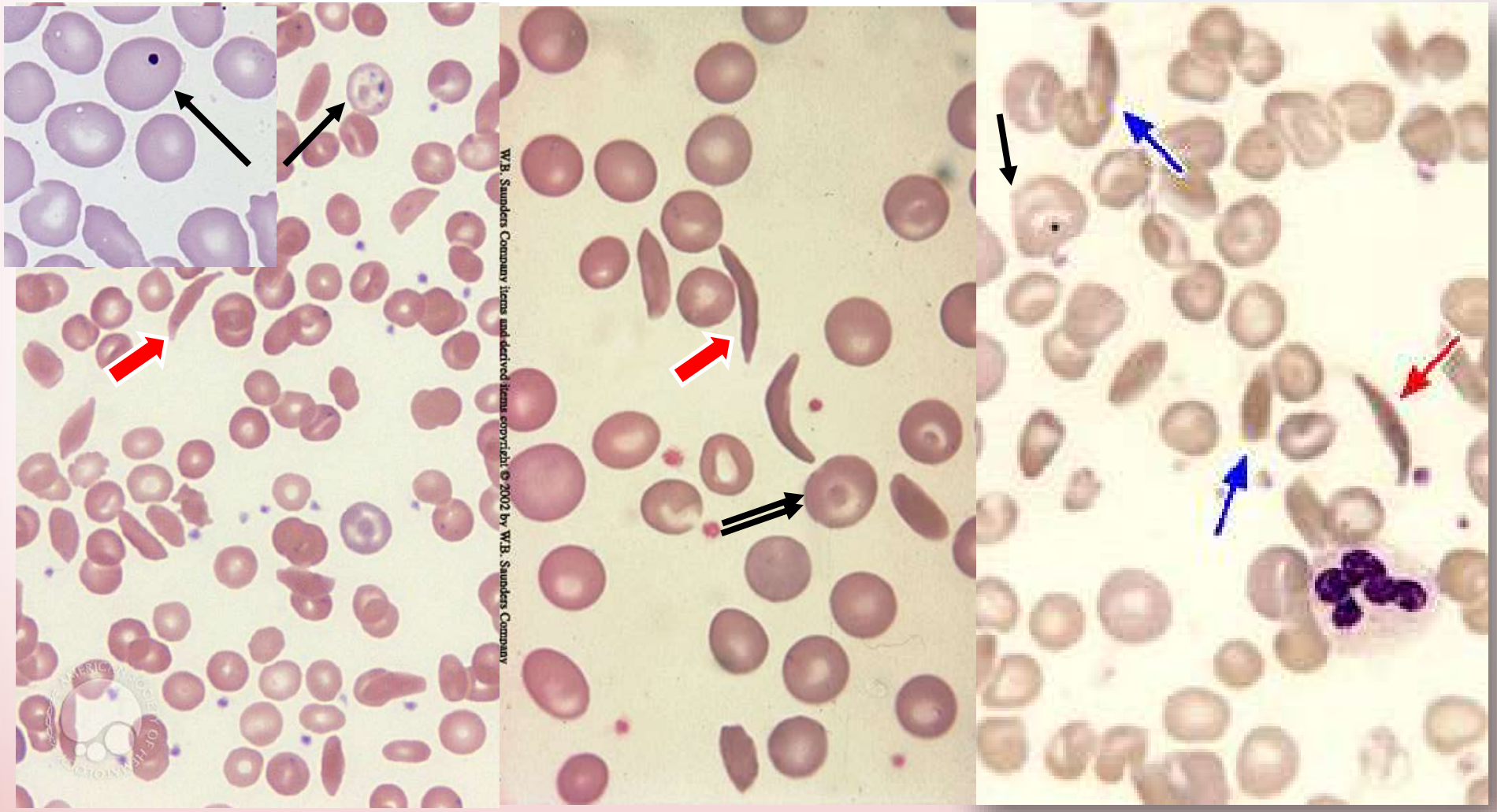




Laboratory findings:

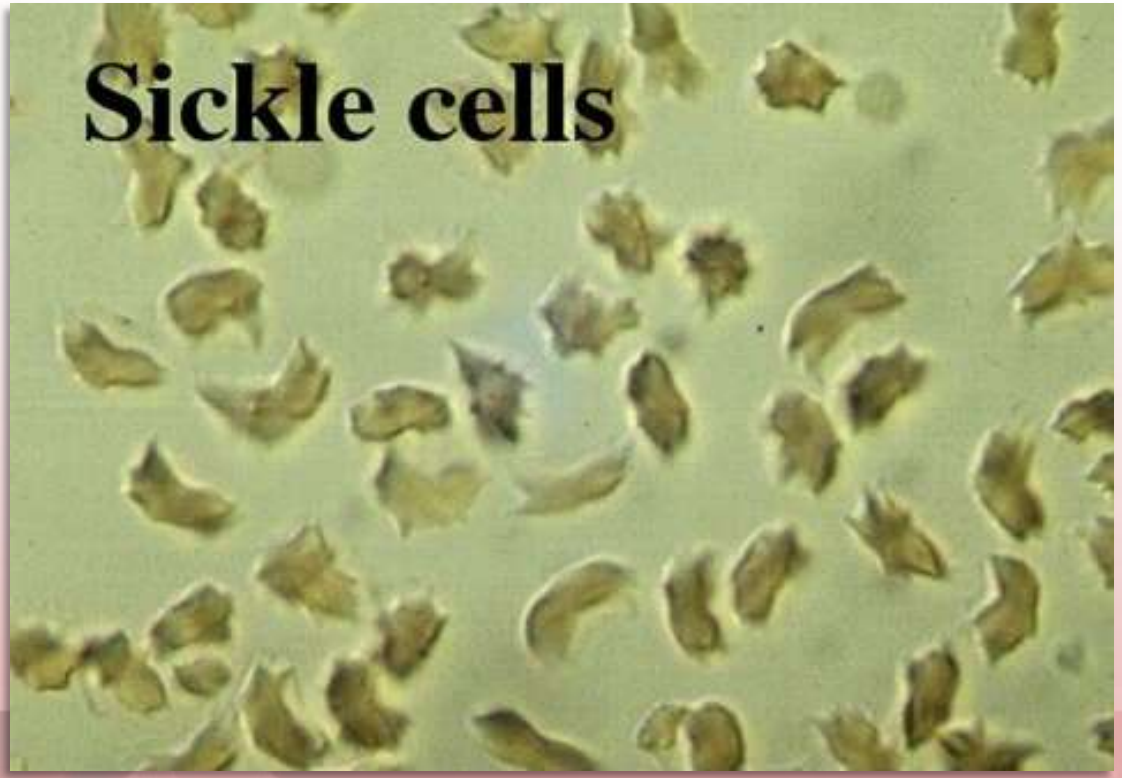
- Hb usually low (6-9 g/dL).
- Blood film shows; sickle (crescent shape), target and polychromatic cells. Howell-Jolly bodies (nuclear DNA remnants), due to hyposplenism.
- Reticulocytosis.
- Hb Electroph-oresis and HPLC.

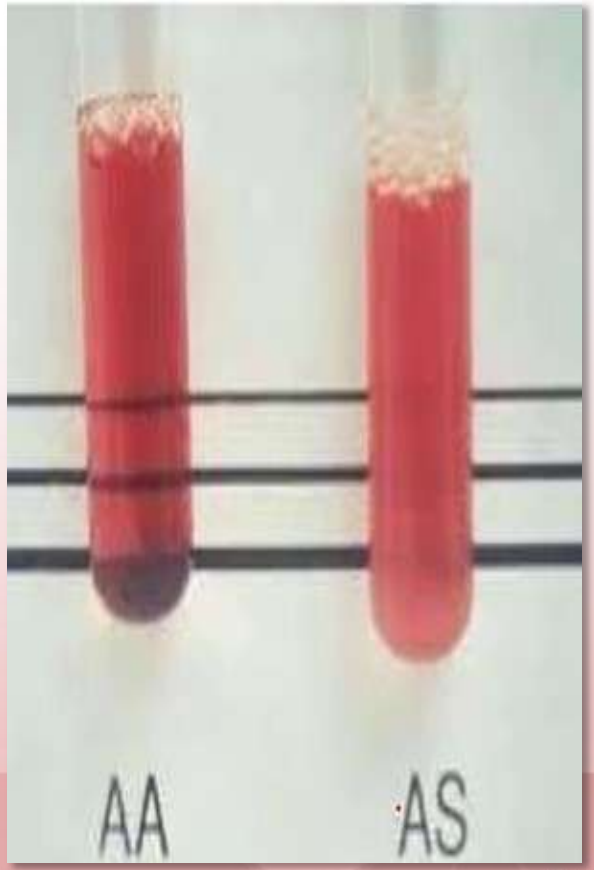




Blood film of a patient with sickle cell anemia shows a sickle cell (red arrow), several boat-shaped cells (blue arrows), target cells (double arrows) and a red cell containing a Howell-Jolly body (black arrow).

- Sickling test; using Sodium metabisulphite reduces the oxygen tension with blood sample deoxygenation.
- Solubility test; mixture of Hb S in reducing solution gives a cloudy appearance (precipitation Hb S), while the normal gives clear solution.





Treatment;

- Avoid precipitating factors.
- Folic acid.
- Good hygiene and nutrition.
- Vaccination and proper antibiotic prophylaxis.
- Treat crisis urgently; with rehydration, oxygenation, antibiotics and analgesia.
- Transfusion; in severe cases to reduce Hb S level.
- Pregnant; needs special care.
- Hydroxycarbamide (hydroxyurea); increase Hb F level.
- Stem cell transplantation (SCT).
- Gene therapy (under experiment).

Sickle cell trait

Usually gives a normal blood appearance, with targeting and possibly an occasional sickle cell present, unless a crisis is induced, for example by anoxia or severe infection.

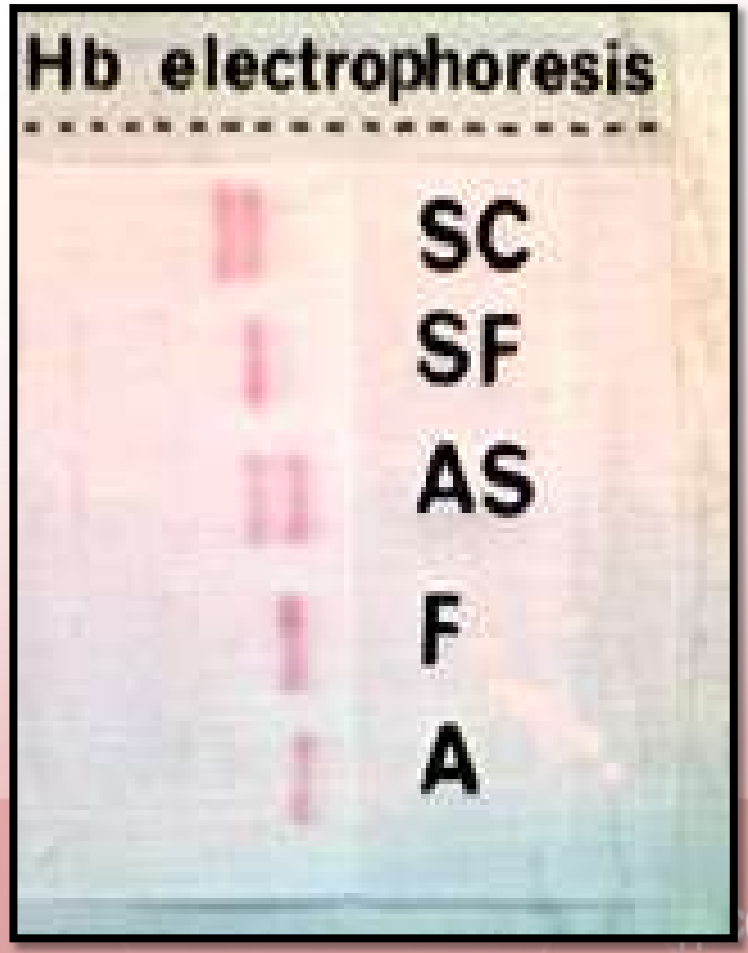
- Recurrent hematuria due to renal papillary necrosis is an occasional problem.

- Usually, combinations of sickle trait with other hemoglobin defects, such as β-thalassemia trait, give rise to mild forms of sickle cell disease.

Hemoglobin electrophoresis at alkaline pH in sickle cell trait;

- Hemoglobin electrophoresis on cellulose acetate at alkaline pH in sickle cell trait (AS) (middle strip) showing the separation of the bands when compared with normal (control).

- The other electrophoretic patterns, from above downwards, are heterozygosity sickle cell/hemoglobin C (SC) sickle cell anemia (SF), a normal neonate (Hb F) and a normal adult (Hb A).




thank you
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