# Genetic disorders of hemoglobin


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# Topics

- Hemoglobin synthesis.
- Switch from fetal to adult hemoglobin.
- α-Thalassemia.
- β-Thalassemia.
- Sickle cell disease.
- Hemoglobin C disease.
- Hemoglobin D disease.
- Hemoglobin E disease.
- Prenatal diagnosis of genetic hemoglobin disorders.

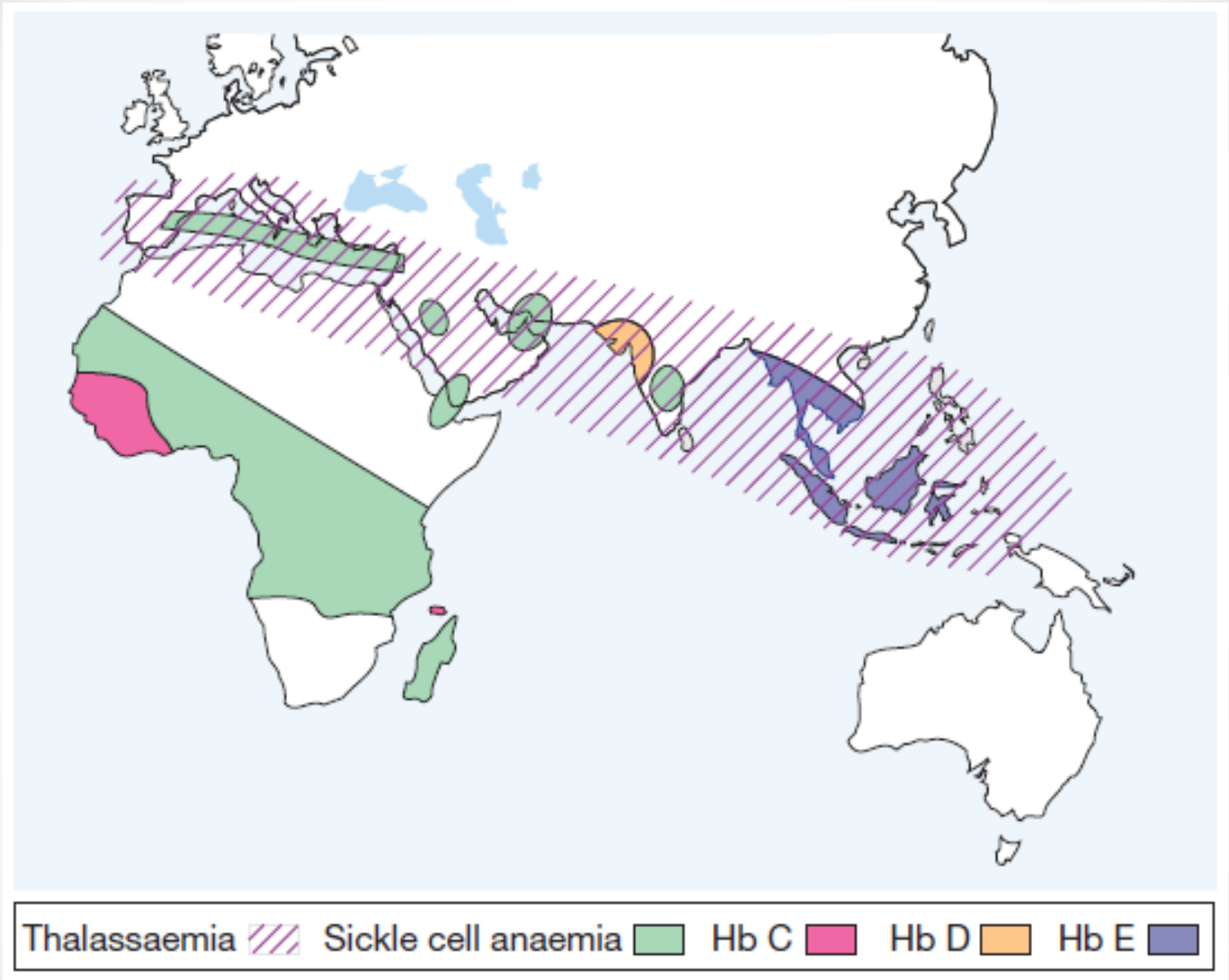
# Objectives

➢ **At the end of the lecture, you will be able to:**

- Define and describe the hemoglobiopathies (thalassemias and hemoglobin variants).

# Genetic disorders of hemoglobin

- They are the commonest single gene disorders, mutations in the globin genes are the most prevalent monogenic disorders worldwide and affect approximately 7% of the world's population.
- They occur at particularly high frequencies in populations of the tropical and subtropical areas, and most appear to have been selected because the carrier state affords some protection against malaria
- These a diseases caused by reduced or abnormal synthesis of globin chains, and consist mainly of the α- and β- thalassemias, and the hemoglobin variants S, C and E.


Thalassaemia
Sickle cell anaemia
Hb C
Hb D
Hb E

# Hemoglobin synthesis

Normal adult blood contains three types of hemoglobin. The major component is hemoglobin A with the molecular structure of four polypeptide chains $\alpha_2\beta_2$. The minor Hb F (fetal Hb ) and $HbA_2$, also contain α chains but with γ and δ chain respectively instead of β chains.

In the embryo and fetus, Gower 1, Portland, Gower 2

and fetal Hb dominate at different stages.

The genes for the globin chains occur in two clusters: β on chromosome 11 and α on chromosome 16.

The α- chain gene is duplicated and both α genes ($\alpha_1$ and $\alpha_2$) on each chromosome are active.

**Table 2.3** Normal haemoglobins in adult blood.

| | Hb A | Hb F | Hb $A_2$ |
|---|---|---|---|
| Structure | $\alpha_2\beta_2$ | $\alpha_2\gamma_2$ | $\alpha_2\delta_2$ |
| Normal (%) | 96–98 | 0.5–0.8 | 1.5–3.2 |

# Switch from fetal to adult hemoglobin

- Hemoglobin F is the predominant Hb in fetal and neonatal life.
- At term, Hb F represents 53%–95% of all hemoglobin with Hb A levels reaching, on average, 20%–30%.
- The β-globin gene is expressed at a low level in early fetal life, but the main switch to adult hemoglobin occurs 3–6 months after birth when synthesis of the γ chain is replaced by β chains.
- BCL11A is a major transcriptional regulator of the switch.

# Hemoglobin abnormalities

**These result from the following:**

1. Reduced rate of synthesis of normal α- or β-globin chains (the α- and β-thalassemias).
2. Synthesis of an abnormal hemoglobin (hemoglobin variants S, C, D and E).

**The genetic defects of hemoglobin are the most common genetic disorders worldwide.**

# Thalassemias

- These are a heterogeneous group of genetic disorders that result from a reduced rate of synthesis of α or β chains.
- β-Thalassemia is more common in the Mediterranean region while α-thalassemia is more common in the Far East.
- Clinically the main syndromes are **transfusion dependent thalassemia major**, non-transfusion dependent thalassemia (**thalassemia intermedia**) with a moderate degree of anemia due to a variety of genetic defects and **thalassemia minor**, usually due to a carrier state for α- or β-thalassemia.

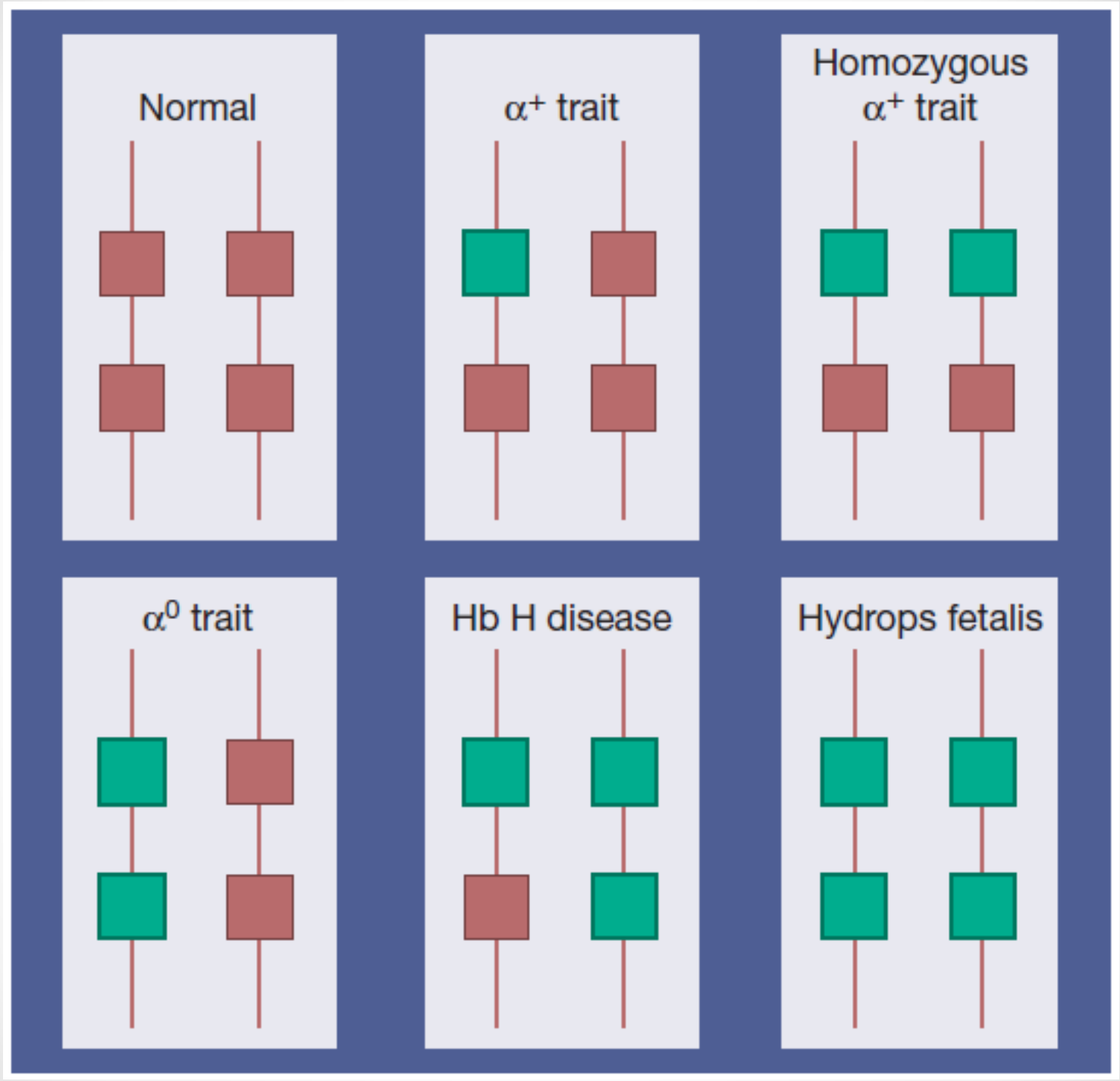
# α-Thalassemia syndromes

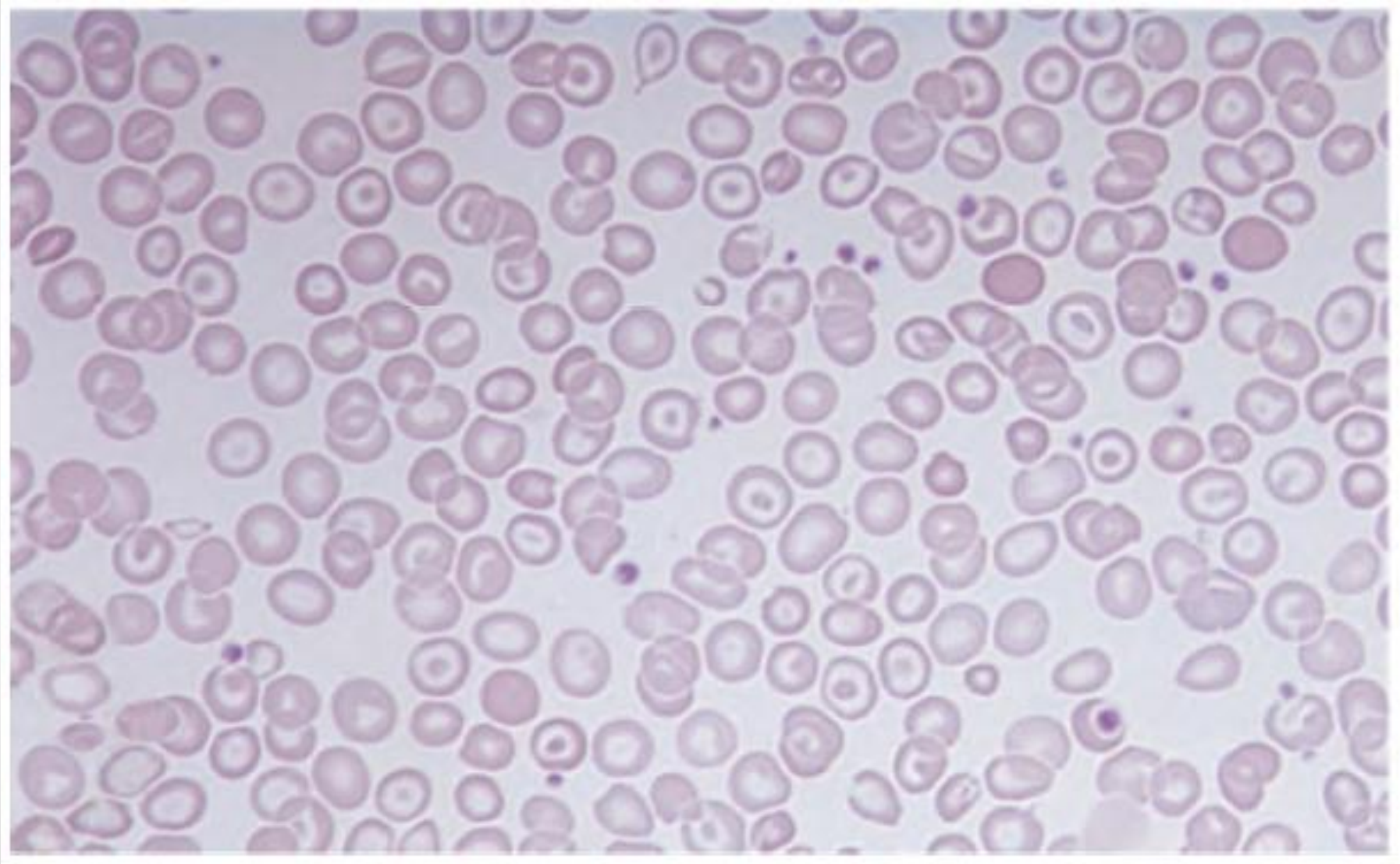
These are caused by α-globin gene deletions or less frequently mutations.

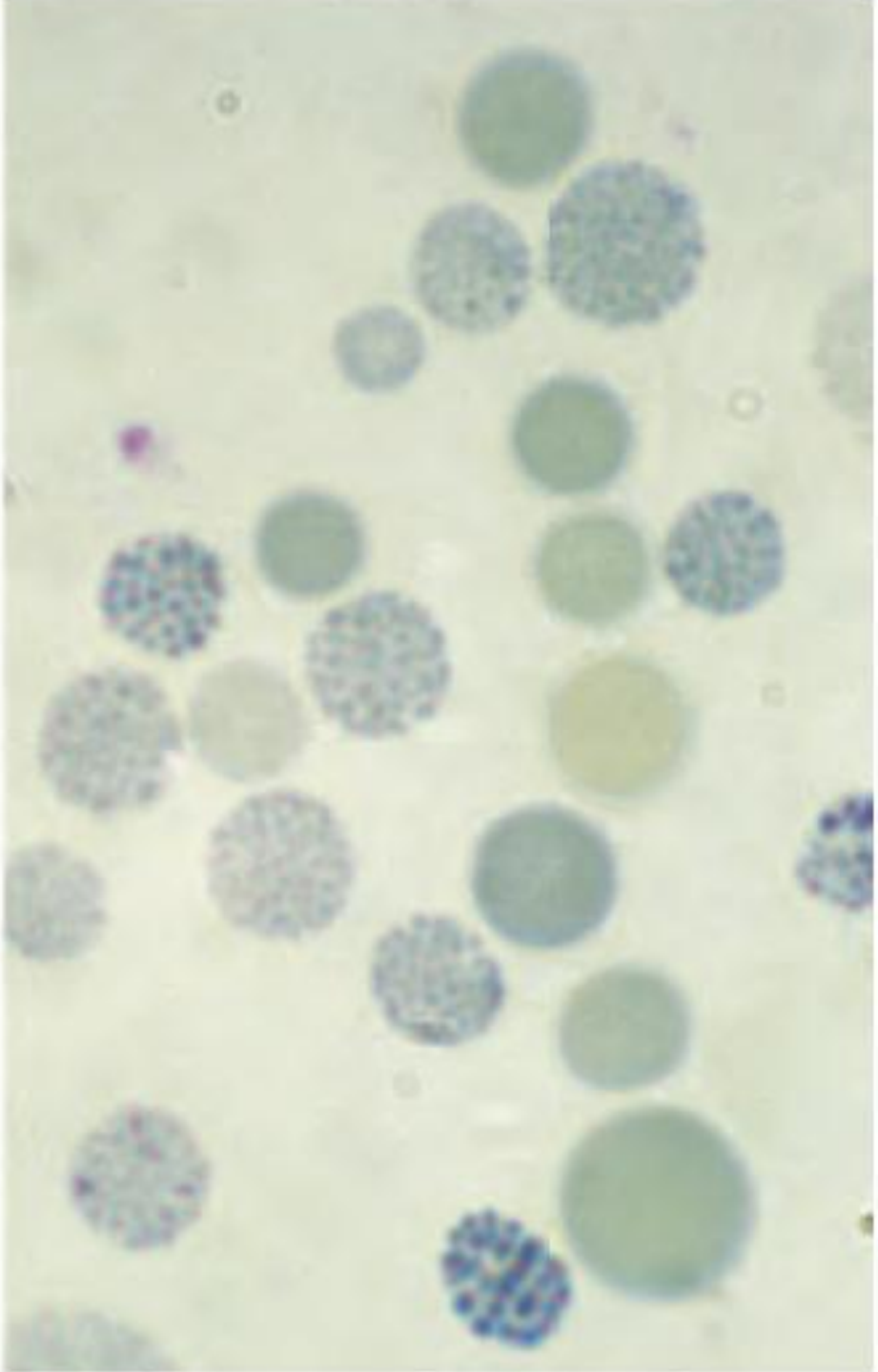
The clinical severity is related to the number of the four α-globin genes missing or inactive.

- Loss of all four genes completely suppresses α-chain synthesis and this is incompatible with life and leads to death in utero (**hydrops fetalis**).
- Three α gene deletion leads to a moderately severe microcytic hypochromic anemia (hemoglobin 7–11 g/dL) with splenomegaly. This is known as **Hb H disease** because hemoglobin H ($\beta_4$) can be detected in red cells of these patients by electrophoresis or in Hb H preparations.

- The **α-thalassemia traits** are caused by loss of one or two genes and are usually not associated with anemia. The mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) are low and the red cell count is over $5.5 \times 10^{12}$/L. Hemoglobin electrophoresis is normal and DNA analysis is needed to be certain of the diagnosis.
- Uncommon non-deletional forms of α-thalassemia are caused by point mutations producing dysfunction of the genes or rarely by mutations affecting termination of translation which give rise to an elongated but unstable chain (e.g. Hb Constant Spring).
- Two rare forms of α-thalassemia are associated with mental retardation. They are caused by mutation in a gene on chromosome 16 (ATR-16) or on chromosome X (ATR-X).


Normal
α+ trait
Homozygous α+ trait
α0 trait
Hb H disease
Hydrops fetalis





# β thalassemia syndromes

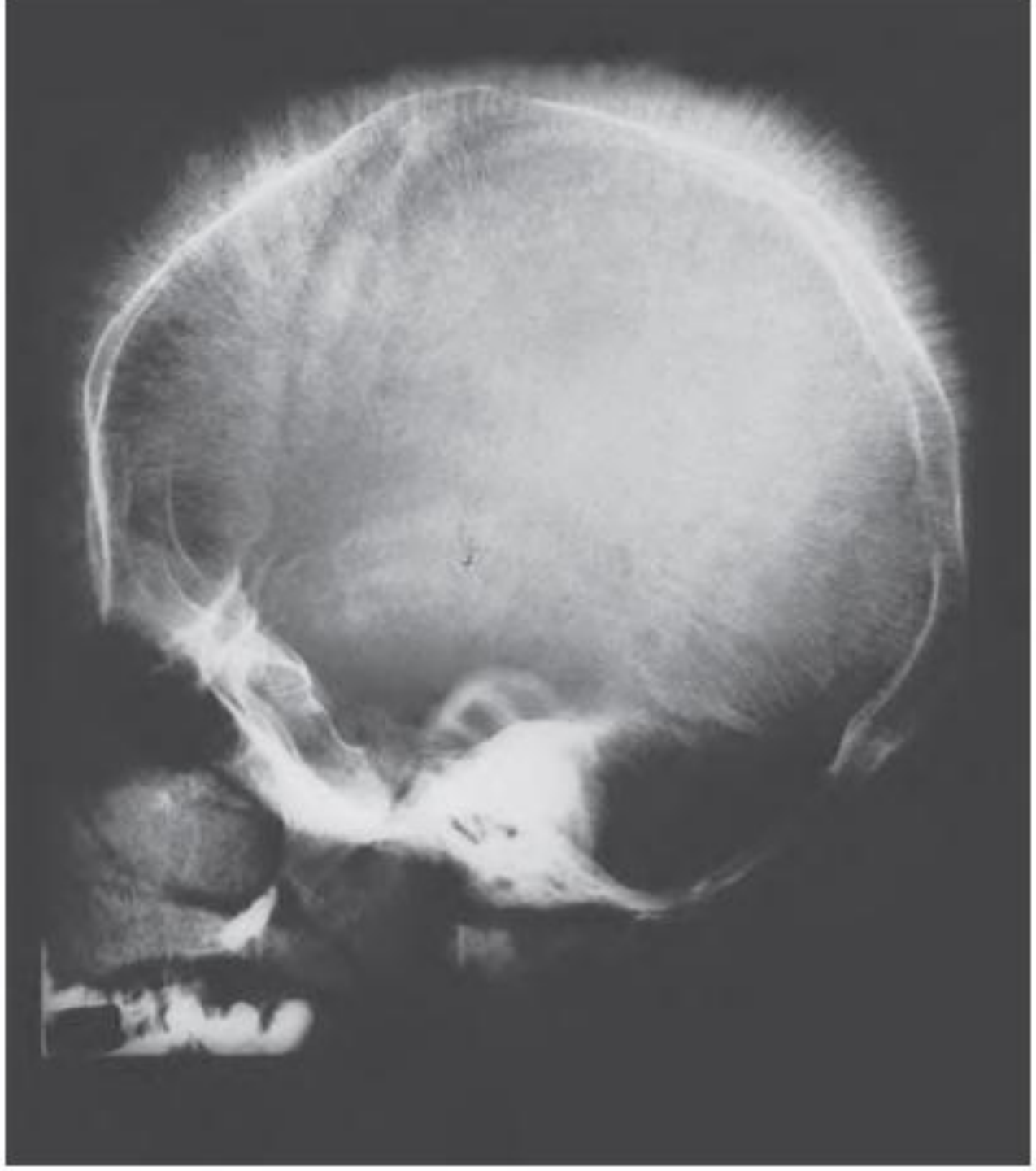
## β-thalassemia major

- This condition occurs in one of four offspring if both parents are carrier of the β thalassemia trait.
- Either no β chain ($\beta^0$) or small amounts ($\beta^+$) are synthesized, excess α chains precipitate in erythroblasts and in mature red cells causing severe ineffective erythropoiesis and hemolysis that are typical of this disease. The greater the α-chain excess, the more severe the anemia.
- Unlike α-thalassemia, the majority of genetic lesions are point mutations rather than gene deletions.

# Clinical features

1. **Severe anemia** becomes apparent at 3–6 months after birth when the switch from γ- to β-chain production take place.
2. **Enlargement of the liver and spleen** occurs as a result of excessive red cell destruction, extramedullary hemopoiesis and later because of iron overload.
3. **Expansion of bones** caused by intense marrow hyperplasia leads to a *thalassemic facies* and to thinning of the cortex of many bones with a tendency to fractures and bossing of the skull with a '*hair-on-end*' appearance on X-ray as a result of expansion of the bone marrow into cortical bone.

The facial appearance of a child with β-thalassemia major. The skull is bossed with prominent frontal and parietal bones; the maxilla is enlarged.



4. **Iron overload** secondary to repeated transfusion is inevitable unless chelating therapy is given.

Regular transfusions are usually commenced in the first year of life and unless the disease is cured by stem cell transplantation, are continued for life. Also iron absorption is increased.

Iron damages heart, liver and endocrine organs causing heart and liver failure, diabetes mellitus, hypothyroidisms and hypoparayhyroidism. In the children, failure of growth and delayed puberty are frequent, and without iron chelation, death from cardiac damage usually occurs in teenagers.

Skin pigmentations as a result of excess melanin and hemosidrin gives a gray appearance at an early stages of iron overload.

**5. Infections** occur frequently.

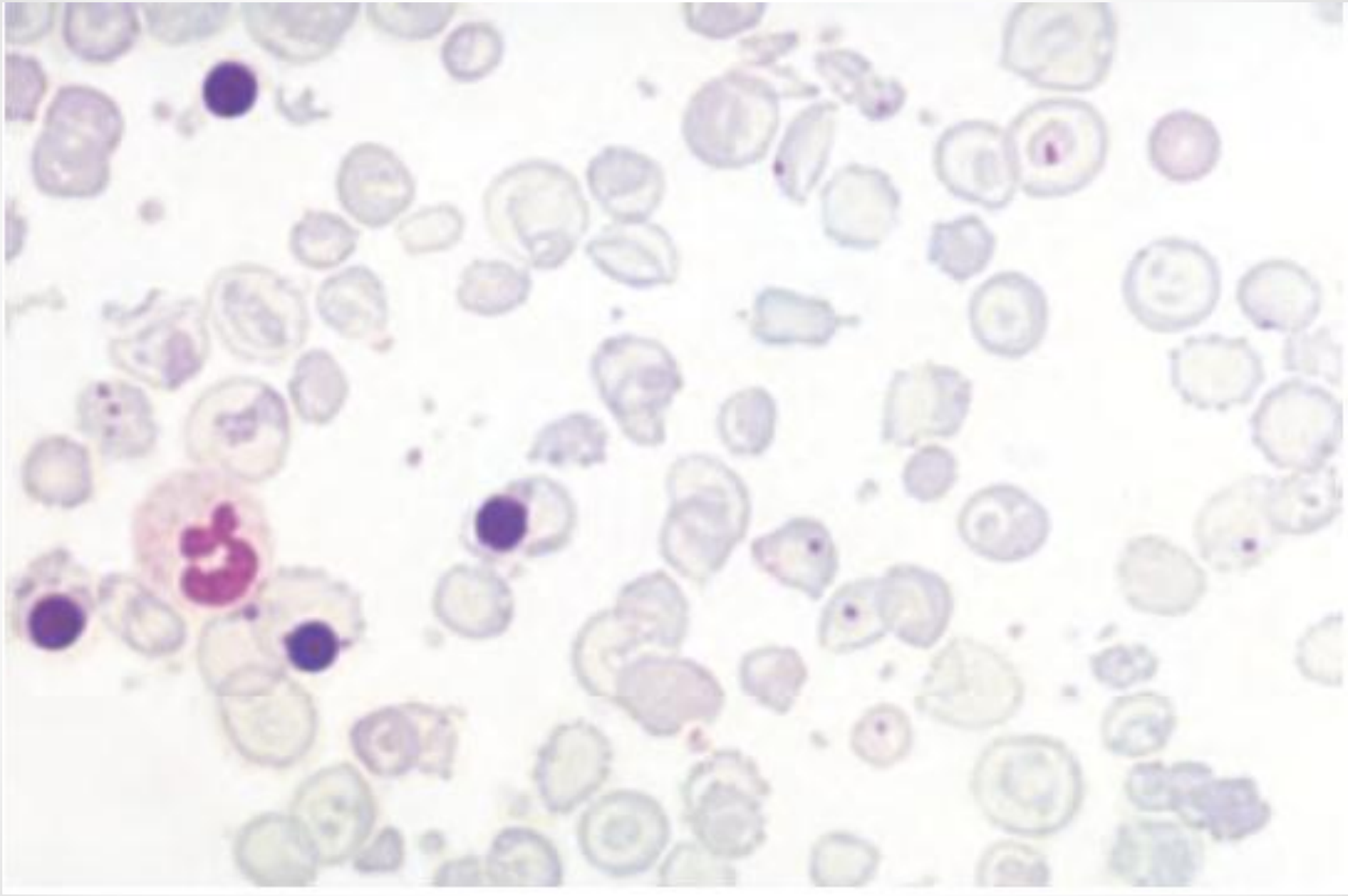
- In infancy, without adequate transfusion, anemia predisposes to bacterial infections.
- if splenectomy has been carried out, Pneumococcal, Hemophilus and meningococcal infections are likely.
- Yersinia enterocolitica occurs, particularly in iron-loaded patients being treated with deferoxamine; it may cause severe gastroenteritis.
- Iron overload itself also predisposes to bacterial infection,
- and to fungal infection.
- Transfusion of viruses by blood transfusion may occur.
- As a result of reduction of deaths from cardiac iron overload by improved chelation therapy, infections now account for an increasing proportion of deaths in thalassemia major.

6. **Liver disease** in thalassemia is most frequently a result of hepatitis C but hepatitis B is also common where the virus is endemic. Iron overload may also cause liver damage.
7. **Osteoporosis** may occur in well-transfused patients. It is more common in diabetic patients with endocrine abnormalities.
8. **Hepatocellular carcinoma** incidence is increased in those with iron overload and chronic hepatitis B or C.

**Typically the infant presents in the first year with failure to thrive, pallor and a swollen abdomen.**

# Laboratory diagnosis

1. Severe hypochromic, microcytic anemia with reticulocytosis. NRBC, target cells and basophilic stippling in the blood film.
2. Hemoglobin HPLC or electrophoresis is now used as the first-line method to diagnose hemoglobin disorders, reveals complete or partial absence of Hb A, with almost all the circulating hemoglobin being Hb F. The Hb $A_2$ percentage is normal, low or slightly raised.
3. DNA analysis is used to identify the genetic defect, important in antenatal diagnosis.



# Treatment

1. **Regular blood transfusions** are needed to maintain the hemoglobin over 100 g/L at all times. This usually requires 2–3 units every 4–6 weeks.
2. **Iron chelation therapy** is essential.
3. **Regular folic acid** (e.g. 5 mg/day).
4. **Splenectomy** (with prophylactic vaccinations and antibiotics) may be needed to reduce blood requirements. This should be delayed until the patient is over 6 years old because of the high risk of dangerous infections postsplenectomy.

5. **Endocrine therapy** is given either as replacement because of end organ failure or to stimulate the pituitary if puberty is delayed.
6. **Immunization** against hepatitis B should be carried out in all non-immune patients. Treatment for transfusion-transmitted hepatitis C
7. **Allogeneic stem cell transplantation** offers permanent cure.

# β-Thalassemia trait (minor)

This is a common, usually symptomless.

like α-thalassemia trait characterized by a hypochromic, microcytic blood picture (MCV and MCH very low) but high red cell count (>5.5 × $10^{12}$/L) and mild anemia (Hb 10–12 g/dL). It is usually more severe than α thalassemia trait.

A raised Hb $A_2$ (>3.5%) confirms the diagnosis.

The diagnosis allows the possibility of prenatal counselling. If the partner also has β-thalassemia trait there is a 25% risk of a thalassemia major child.

# Non-transfusion dependent thalassaemia (thalassaemia intermedia)

This is thalassemia of moderate severity (Hb 7–10 g/dL) without the need for regular transfusions.

- It is a clinical syndrome caused by a variety of genetic defects: homozygous β-thalassemia with production of more Hb F than usual or with mild defects in β-chain synthesis, by β-thalassemia trait alone of unusual severity (‘dominant’ β-thalassemia), or by β-thalassemia trait in association with mild globin abnormalities such as Hb Lepore.

- The coexistence of α-thalassemia trait improves the hemoglobin level in homozygous β-thalassemia by reducing the degree of α:β chain imbalance and thus ofα-chain precipitation and ineffective erythropoiesis. Conversely, patients with β-thalassemia trait who also have excess (five or six) α genes tend to be more anemic than usual.
- The patient with thalassemia intermedia may show bone deformity, enlarged liver and spleen, extramedullary erythropoiesis, and features of iron overload caused by increased iron absorption and occasional transfusions (during pregnancy or infections or given to reduce bone deformity).
- **Hb H disease** (three-gene deletion α-thalassemia) is a type of thalassemia intermedia without iron overload or extramedullary hemopoiesis.

# Association of β-thalassemia trait with other genetic disorders of hemoglobin

- The combination of β-thalassemia trait with Hb E trait usually causes a transfusion-dependent thalassemia major syndrome, but some cases are intermediate.
- β-Thalassemia trait with Hb S trait produces the clinical picture of sickle cell anemia rather than of thalassemia.
- β-Thalassemia trait with Hb D trait causes a hypochromic, microcytic anemia of varying severity.

# Sickle cell disease

Sickle cell disease is a group of hemoglobin disorders resulting from the inheritance of the sickle β-globin gene.

The sickle β-globin abnormality is caused by substitution of valine for glutamic acid in position 6 in the β chain.

Homozygous sickle cell anemia (Hb SS) is the most common severe syndrome while the doubly heterozygote conditions of Hb S/C and Hb S/βthal also cause sickling disease.

The carrier state is widespread and is found in up to 30% of West African people, maintained at this level because of the protection against malaria that is afforded by the carrier state.

# Pathogenesis

Hb S (Hb $\alpha_2\beta_2^S$) is insoluble and forms crystals when exposed to low oxygen tension.

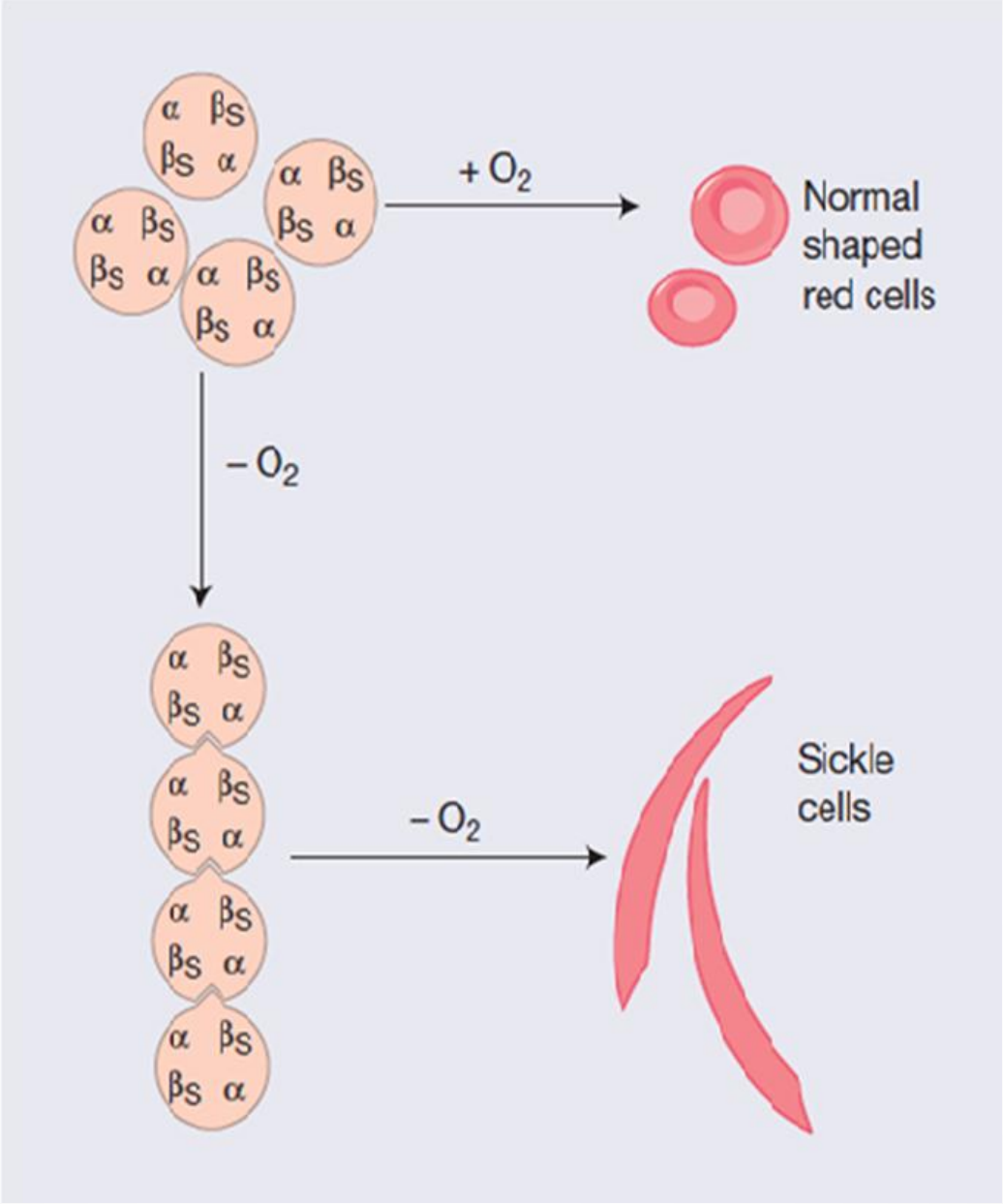
Deoxygenated sickle hemoglobin polymerizes into long fibres, and red cells become sickle-like, which may block different areas of the microcirculation or large vessels causing infarcts of various organs.

**Major pathologic manifestations:**

(1) Chronic hemolysis.

(2) Microvascular occlusions.

(3) Tissue damage.


α βS
βS α
+ O2
Normal shaped red cells
– O2
Sickle cells
– O2

# Homozygous sickle cell anemia

- Clinical features are of a severe hemolytic anemia punctuated by crises.
- The symptoms of anemia are often mild in relation to the severity of the anemia because Hb S gives up oxygen (O2) to tissues relatively easily compared with Hb A.
- The clinical expression of Hb SS is very variable, some patients having an almost normal life, free of crises, but others develop severe crises even as infants and may die in early childhood or as young adults.
- Crises may be vaso-occlusive (painful or visceral), aplastic or hemolytic. There may be serious damage to many organs.

# Vaso-occlusive crises

**Painful:**

- These are the most frequent. They may be sporadic and unpredictable or precipitated by infection, acidosis, dehydration or deoxygenation (e.g. altitude, operations, obstetric delivery, stasis of the circulation, exposure to cold, violent exercise).
- Infarcts causing severe pain occur in the bones (hips, shoulders and vertebrae are commonly affected) . The ‘hand–foot’ syndrome (painful dactylitis caused by infarcts of the small bones) is frequently the first presentation of the disease and may lead to digits of varying lengths.

# Vaso-occlusive crises

**Visceral:**

- These are caused by sickling within organs causing infarction and pooling of blood, often with a severe exacerbation of anemia.
- Acute sickle chest syndrome is the most common cause of death both in children and adults. It presents with dyspnoea, falling arterial PO2, chest pain and pulmonary infiltrates on chest X-ray.
- Hepatic and girdle sequestration.
- Splenic sequestration.
- Priapism and liver and kidney damage due to repeated small infarcts are other complications.

# Aplastic crises

These occur as a result of infection with parvovirus or from folate deficiency and are characterized by a sudden fall in hemoglobin and reticulocytes, usually requiring transfusion

# Hemolytic crises

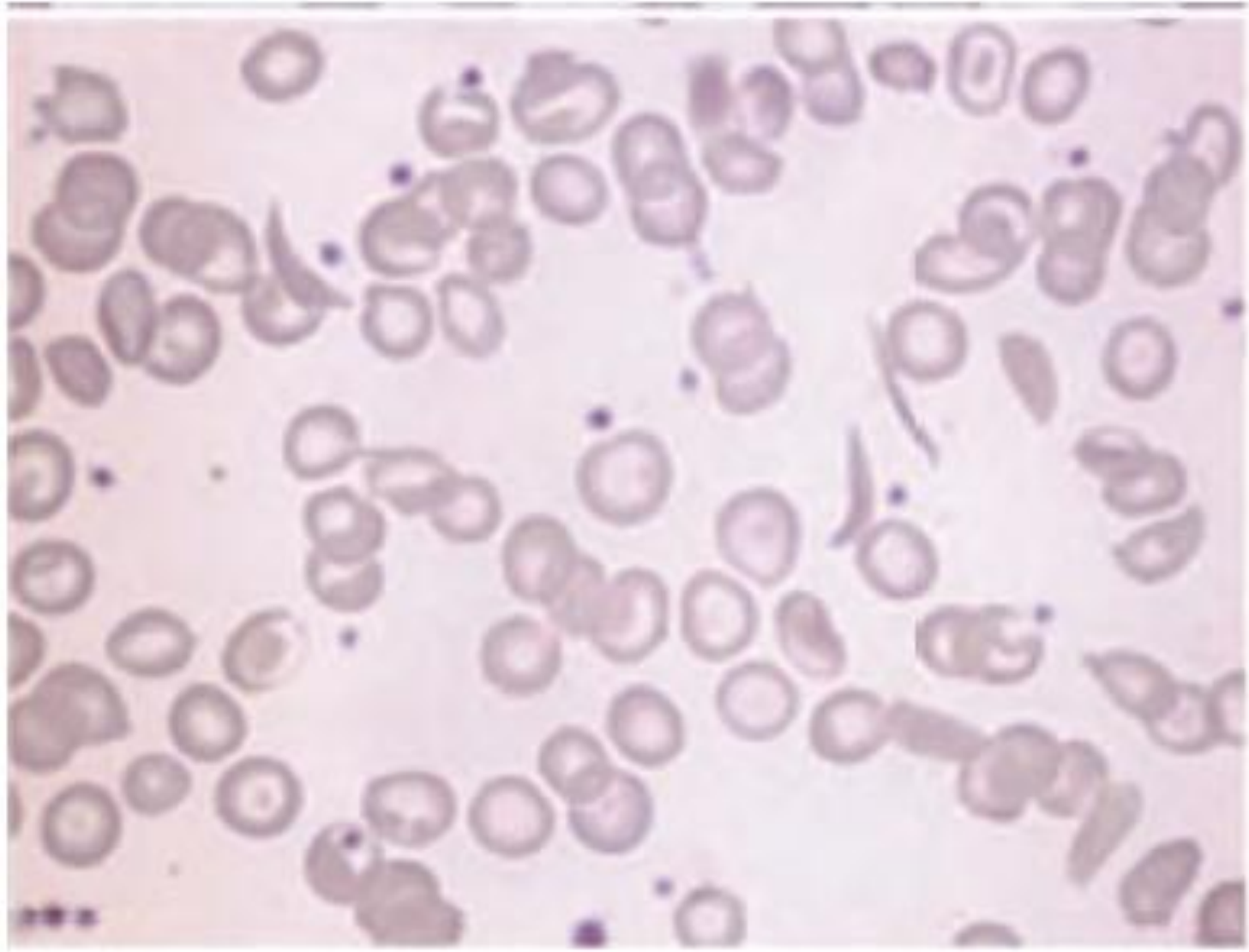
These are characterized by an increased rate of hemolysis and fall in hemoglobin but rise in reticulocytes and usually accompany a painful crisis.

# Other organ damage

- The most serious is of the brain (a stroke occurs in 7% of patients) or spinal cord.
- Ulcers of the lower legs are common, as a result of vascular stasis and local ischemia.
- The spleen is enlarged in infancy and early childhood but later is often reduced in size as a result of infarcts (autosplenectomy).
- Pulmonary hypertension
- Proliferative retinopathy.
- Pigment (bilirubin) gallstones are frequent.
- Infections are frequent partly due to hyposplenism.
- Osteomyelitis may also occur.

# Laboratory findings

1. The hemoglobin is usually 6–9 g/dL, low in comparison to mild or no symptoms of anemia.
2. Sickle cells and target cells occur in the blood. Features of splenic atrophy (e.g. Howell–Jolly bodies) may also be present.
3. Screening tests for sickling are positive when the blood is deoxygenated (e.g. with dithionate and $Na_2HPO_4$).
4. HPLC or hemoglobin electrophoresis: in Hb SS, no Hb A is detected. The amount of Hb F is variable and is usually 5–15%, larger amounts are usually associated with a milder disorder.



# Treatment

1. Prophylactic:  avoid those factors known to precipitate crises, especially dehydration, anoxia, infections, stasis of the circulation and cooling of the skin surface.
2. Folic acid (e.g. 5 mg once weekly).
3. Good general nutrition and hygiene.
4. Pneumococcal, Haemophilus and meningococcal vaccination and regular oral penicillin are effective at reducing the infection rate with these organisms.

5. Crises: treat by rest, warmth, rehydration by oral fluids and/or intravenous normal saline, analgesia and antibiotics if infection is present. Blood transfusion is given only if there is very severe anemia with symptoms. Exchange transfusion may be needed, if there is neurological damage, a visceral sequestration crisis or repeated painful crises. This is aimed at achieving an Hb S percentage of less than 30% in severe cases and, after a stroke, is continued for at least 2 years.
6. Routine transfusions throughout pregnancy are given to those with a poor obstetric history or a history of frequent crises.
7. Careful anesthetic and recovery techniques must be used to avoid hypoxaemia or acidosis.

8. Transfusions: sometimes given repeatedly as prophylaxis to patients having frequent crises or who have had major organ damage (e.g. of the brain) to suppress Hb S production
9. Hydroxycarbamide (Hydroxyurea) can increase Hb F levels and improves the clinical course of children or adults. It is given to those with severe or moderately severe disease, e.g. who are having three or more painful crises each year. It should not be used during pregnancy.
10. Stem cell transplantation can cure the disease. Transplantation is only indicated in the severest of cases whose quality of life or life expectancy are substantially impaired.
11. Research into other drugs (e.g. butyrates) to enhance Hb F synthesis or to increase the solubility of Hb S.

# Heterozygous sickle cell trait

- This is a benign condition with no anemia and normal appearance of red cells in a blood film.
- Hematuria is the most common symptom and is thought to be caused by minor infarcts of the renal papillae.
- Hb S varies from 25–45% of the total hemoglobin.
- Care must be taken with anesthesia, pregnancy and at high altitudes.

# Combination of hemoglobin S with other genetic defects of hemoglobin

- In Hb S/β-thalassaemia, the MCV and MCH are lower than in homozygous Hb SS. The clinical picture is of sickle cell anemia; splenomegaly is usual.
- Patients with Hb SC disease have a particular tendency to thrombosis and pulmonary embolism, especially in pregnancy. In general, when compared with Hb SS disease, they have a higher incidence of retinal abnormalities, milder anemia, splenomegaly and generally a longer life expectancy.
- Diagnosis is made by hemoglobin electrophoresis or HPLC, with family studies.

# Hemoglobin C disease

- This genetic defect of hemoglobin is frequent in West Africa and is caused by substitution of lysine for glutamic acid in the β-globin chain at the same point as the substitution in Hb S.
- Hb C tends to form rhomboidal crystals.
- In the homozygous state there is a mild hemolytic anemia with marked target cell formation, cells with rhomboidal shape and microspherocytes. The spleen is enlarged.
- The carriers show a few target cells only.

# Hemoglobin D disease

Heterozygotes show no hematological abnormality while homozygotes have a mild hemolytic anemia.

# Hemoglobin E disease

- In the homozygous state, there is a mild microcytic, hypochromic anemia.
- Hemoglobin E/$\beta^0$-thalssaemia, resembles homozygous $\beta^0$-thalassaemia both clinically and hematologically.

# Prenatal diagnosis of genetic hemoglobin disorders

- It is important to give genetic counseling to couples at risk of having a child with a major hemoglobin defect.
- If a pregnant woman is found to have a hemoglobin abnormality, her partner should be tested to determine whether he also carries a defect. When both partners show an abnormality and there is a risk of a serious defect in the offspring, particularly β-thalassaemia major, it is important to offer antenatal diagnosis.
- Several techniques are available, the choice depending on the stage of pregnancy and the potential nature of the defect.

# Any Question?

# Home work:

**What is the antenatal diagnosis?**

# THE END

THANK YOU FOR LISTENING