

DISORDERS OF GLOBIN SYNTHESIS

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LEARNING OBJECTIVES

- To review the normal structure and function of hemoglobin.
- To understand the mechanisms by which the thalassemias arise.
- To appreciate the clinical presentations and complications of thalassemia.
- To appreciate the contribution of hemolysis and ineffective erythropoiesis to the pathophysiology of thalassemia.
- To understand the pathophysiology of sickle cell anemia and complications of sickle cell anemia.
- To understand the role of hemoglobin electrophoresis in the investigation of globin disorders.
- To appreciate the many other hemoglobin variants associated with disease.

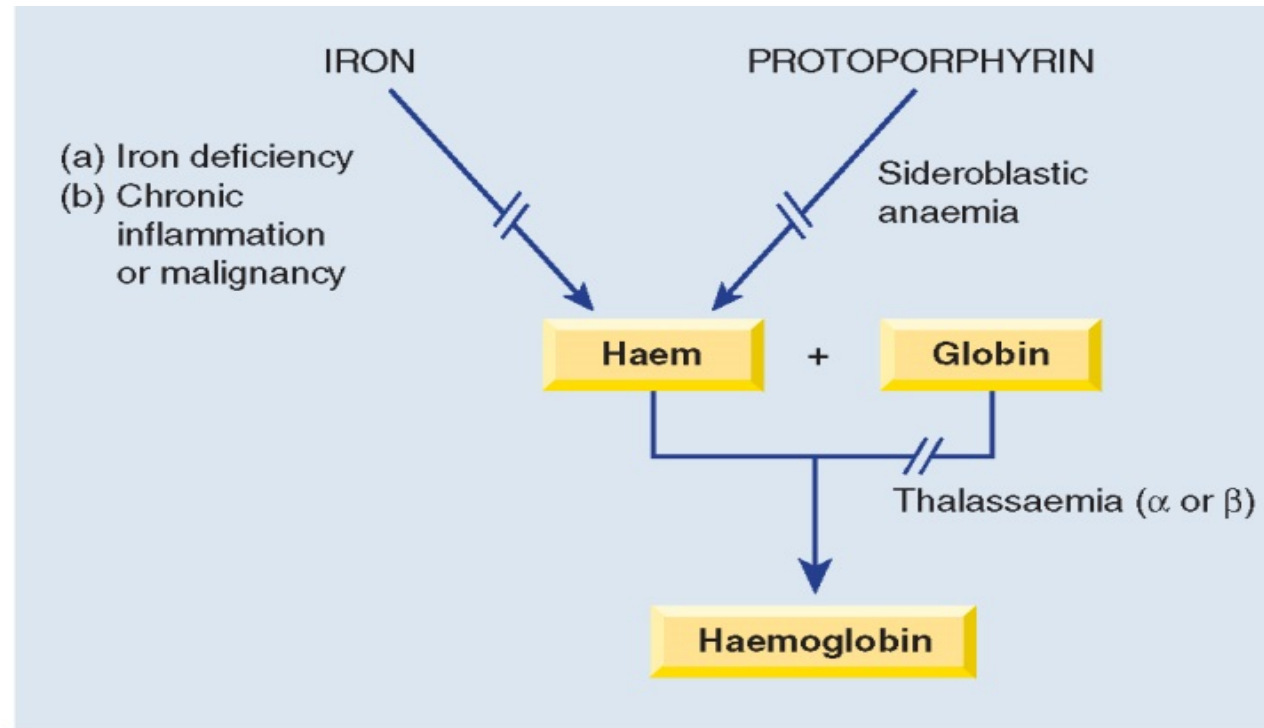


Figure 3.1 The causes of a hypochromic microcytic anaemia. These include lack of iron (iron deficiency) or of iron release from macrophages to serum (anaemia of chronic inflammation or malignancy), failure of protoporphyrin synthesis (sideroblastic anaemia) or of globin synthesis (α - or β -thalassaemia). Lead also inhibits haem and globin synthesis.

Normal Structure and function of Hemoglobin

- Hemoglobin is critical to the normal function of the red cell, the fundamental role of which is the **transport of oxygen** from the lungs to the tissues.
- The normal **tetramer** hemoglobin molecule comprises **two 'alpha-like'** globin polypeptide chains and **two 'beta-like'** globin chains; each globin molecule is **associated with a hem group**, which comprises a **porphyrin ring with iron** in its ferrous form at the center.
- The **α chains** are encoded on **chromosome 16**.
- The **β and its like globins** are encoded on **chromosome 11**.

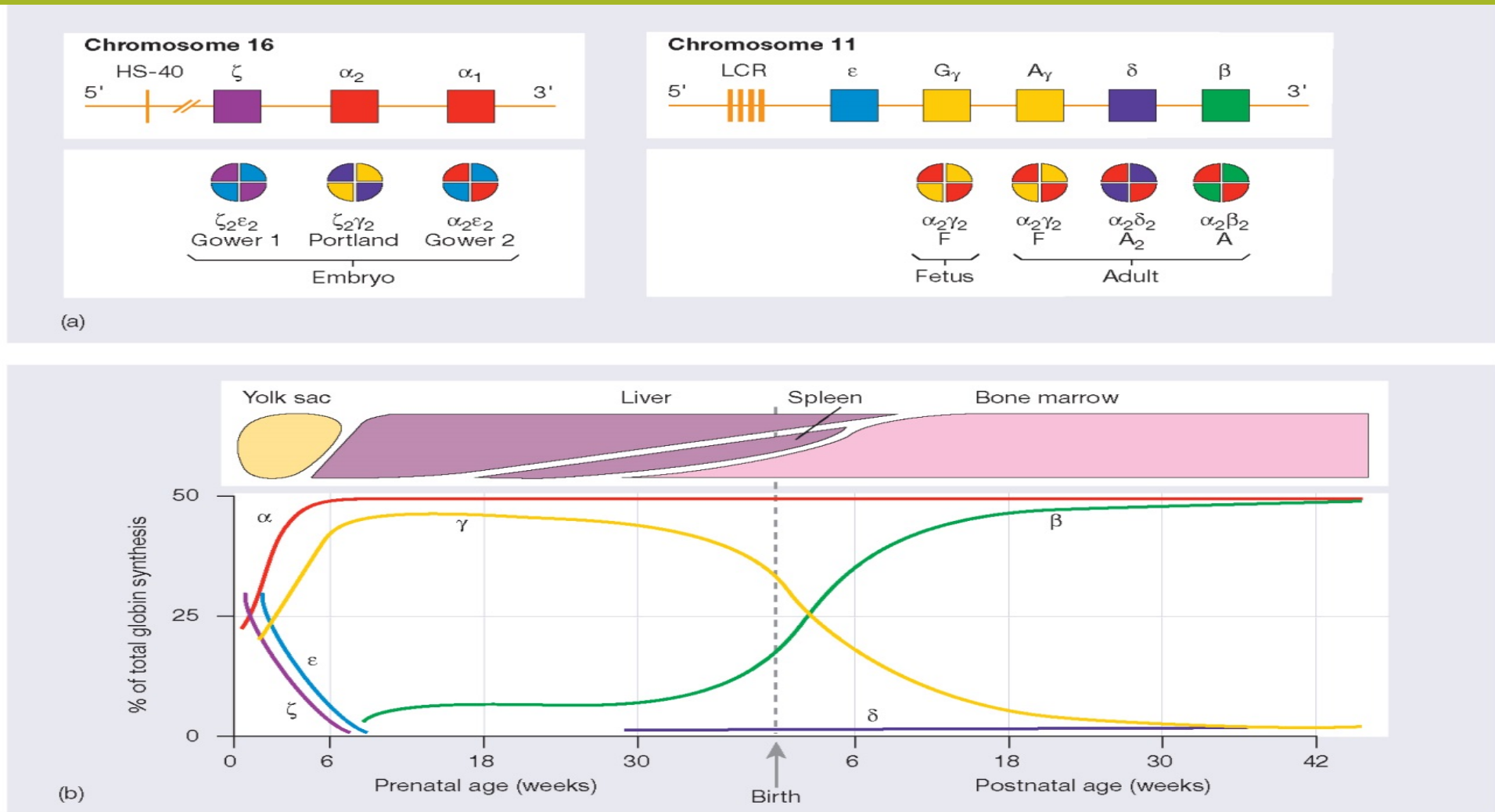


Figure 7.1 (a) The globin gene clusters on chromosomes 16 and 11. In embryonic, fetal and adult life different genes are activated or suppressed. The different globin chains are synthesized independently and then combine with each other to produce the different haemoglobins. The γ gene may have two sequences, which code for either a glutamic acid or alanine residue at position 136 (G_γ or A_γ , respectively). LCR, locus control region, HS-40, see text. **(b)** Synthesis of individual globin chains in prenatal and postnatal life.

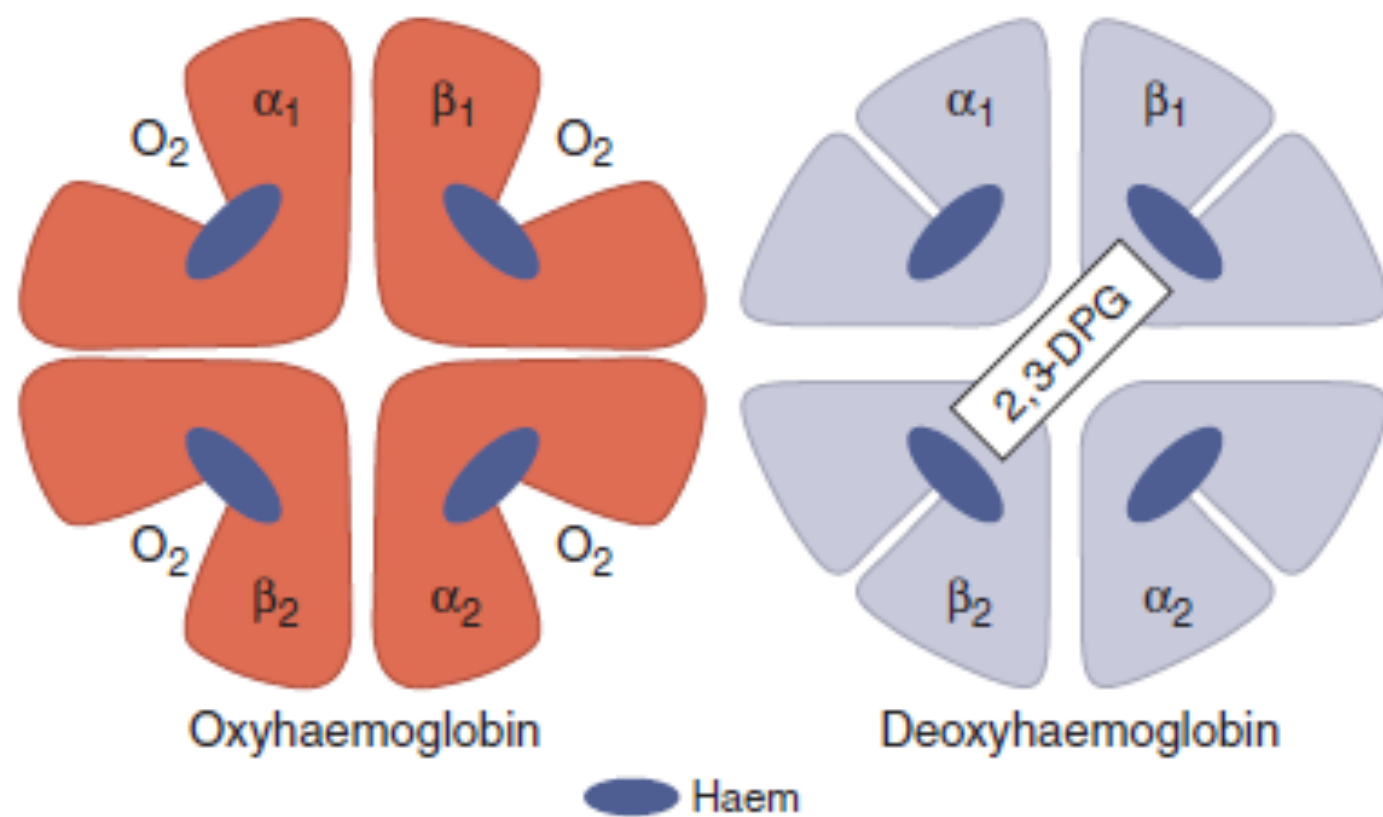


Figure 2.9 The oxygenated and deoxygenated haemoglobin molecule. α , β , globin chains of normal adult haemoglobin (Hb A). 2,3-DPG, 2,3-diphosphoglycerate.

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- Fetal hemoglobin HbF ($\alpha_2\gamma_2$)
- The major adult hemoglobin is HbA ($\alpha_2\beta_2$) with a much smaller contribution from HbA2 ($\alpha_2\delta_2$ - usually 1.5-3.5% of adult hemoglobins).
- The fetal hemoglobin HbF has a higher oxygen affinity than the adult hemoglobins, facilitating transfer of oxygen from the maternal to the fetal circulation.

- Normally, the synthesis of α -like and β -like chains is balanced.
- An imbalance between the production of α and β chains is the pathophysiological basis of the thalassemias (a **quantitative issue**).

Thalassemia

- The thalassemias are divided into two main groups, the α -thalassemias and the β -thalassemias, depending on whether the defect lies in the synthesis.
- Its **pathophysiology** includes the chains which are present in excess will precipitate in the precursor red cells, leading to their **premature death** prior to release from the bone marrow (**ineffective erythropoiesis**) resulting to an increased erythroid drive and further expansion of the marrow into bones not typically used for hemopoiesis, and into the **spleen**.
- Thus, the **long-term consequences** of thalassemia (major, mainly) therefore include splenomegaly, bony deformities and iron overload due to transfusion and ineffective erythropoiesis as well as chronic anemia.

α -Thalassemia

- ❖ α -Thalassemia is seen with the greatest frequency in **south-east Asia** (Thailand, the Malay Peninsula and Indonesia) and **west Africa but also in the Middle East**.
- ❖ Each chromosome 16 has an α -globin locus consisting of **two α -globin** (i.e; 4 genes) genes plus the regulatory sequences essential for their normal expression.
- ❖ In most patients with α -thalassemia, there is a **deletion** of one or more of the α -globin genes; there are occasional cases that are the consequence of non-deletional defects.

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- ❖ Deletion of one or two genes causes an asymptomatic condition with minor hematological features; deletion of **three of the four** α -globin genes causes a more severe imbalance of $\alpha:\beta$ -globin chains and results in **hemoglobin H**, four beta globins, disease; and loss of **all four α -globin** genes causes **Hb Bart's**, four gamma globins, (**hydrops fetalis** syndrome).
- ❖ 0.4% of deliveries are stillbirths due to Hb Bart's hydrops fetalis syndrome.

α^+ -Thalassemia trait (deletion of one α globin gene)

- This is seen when an individual inherits the α^+ -thalassemia allele from one parent and a normal chromosome 16 from the other parent (i.e. heterozygotes for the α^+ determinant).
- Affected individuals are asymptomatic, although they have minor hematological changes such as slight reductions in mean cell volume (MCV) and mean cell hemoglobin (MCH).

α^0 -Thalassaemia trait (deletion of both α -globin genes in same allele on ch 16)

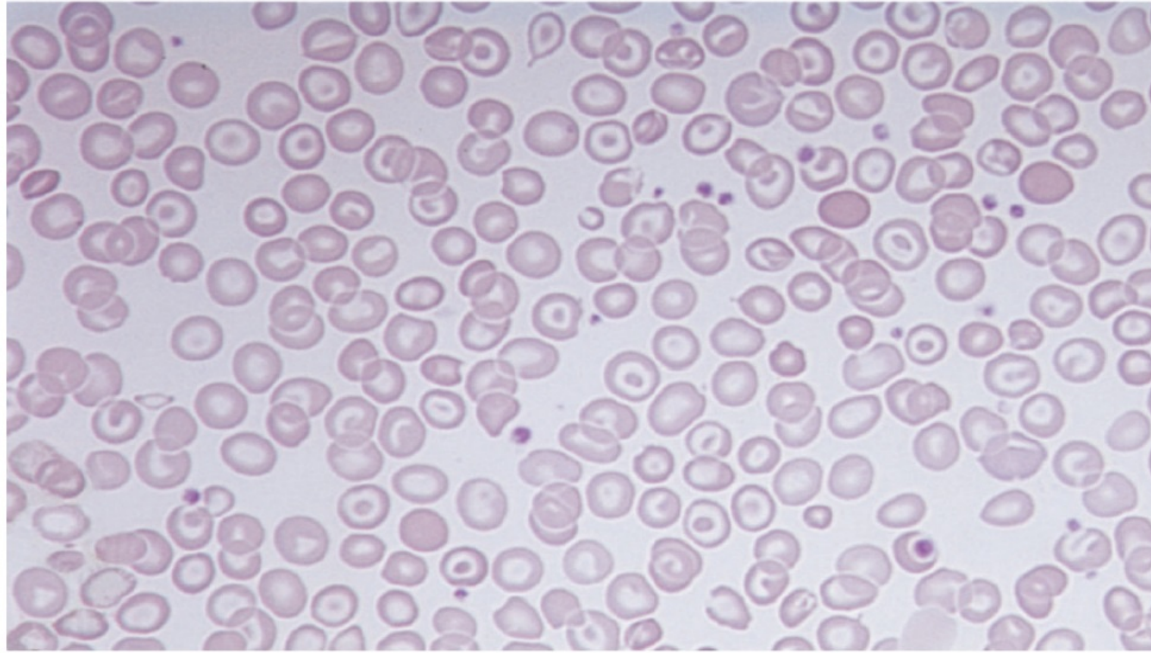
The Hb is either normal or slightly reduced and the MCV and MCH are low. However, RBC count is elevated and RDW is not affected (Thalassemic picture) and frequently form target cells (why?).

Hemoglobin H disease (deletion of three α -globin genes)

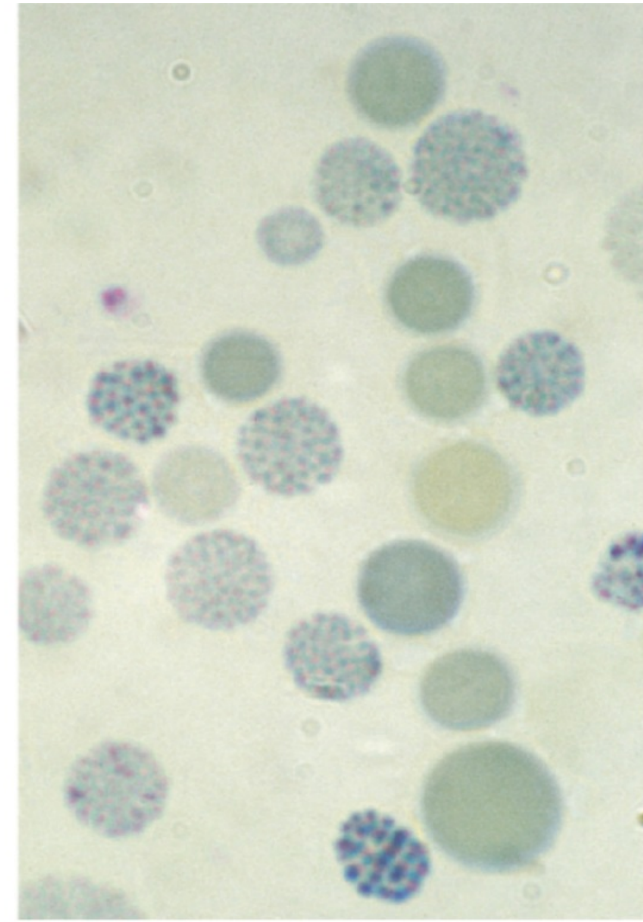
- This chronic hemolytic anemia results from the inheritance of both the α^+ - and α^0 -thalassaemia alleles, leaving one functioning α -globin gene per cell.
- α -globin chains are produced at very low rates, leaving a considerable excess of β -chains, which combine to **form tetramers (β_4)**. This tetramer is known as HbH.
- HbH is unstable and precipitates as the erythrocytes age, forming rigid membrane-bound inclusions that are removed during the passage of affected red cells through the spleen.
- The damage to the membrane brought about by this removal results in a shortened red cell lifespan.

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- Most patients are moderately affected, with a mild anemia of 7-11g/dl and markedly hypochromic, microcytic indices.
- Supravital staining of the blood film demonstrates cells with many HbH inclusions, giving a characteristic '**golf-ball**' appearance.
- Most patients will be transfusion independent.
- Splenomegaly is seen in most patients.



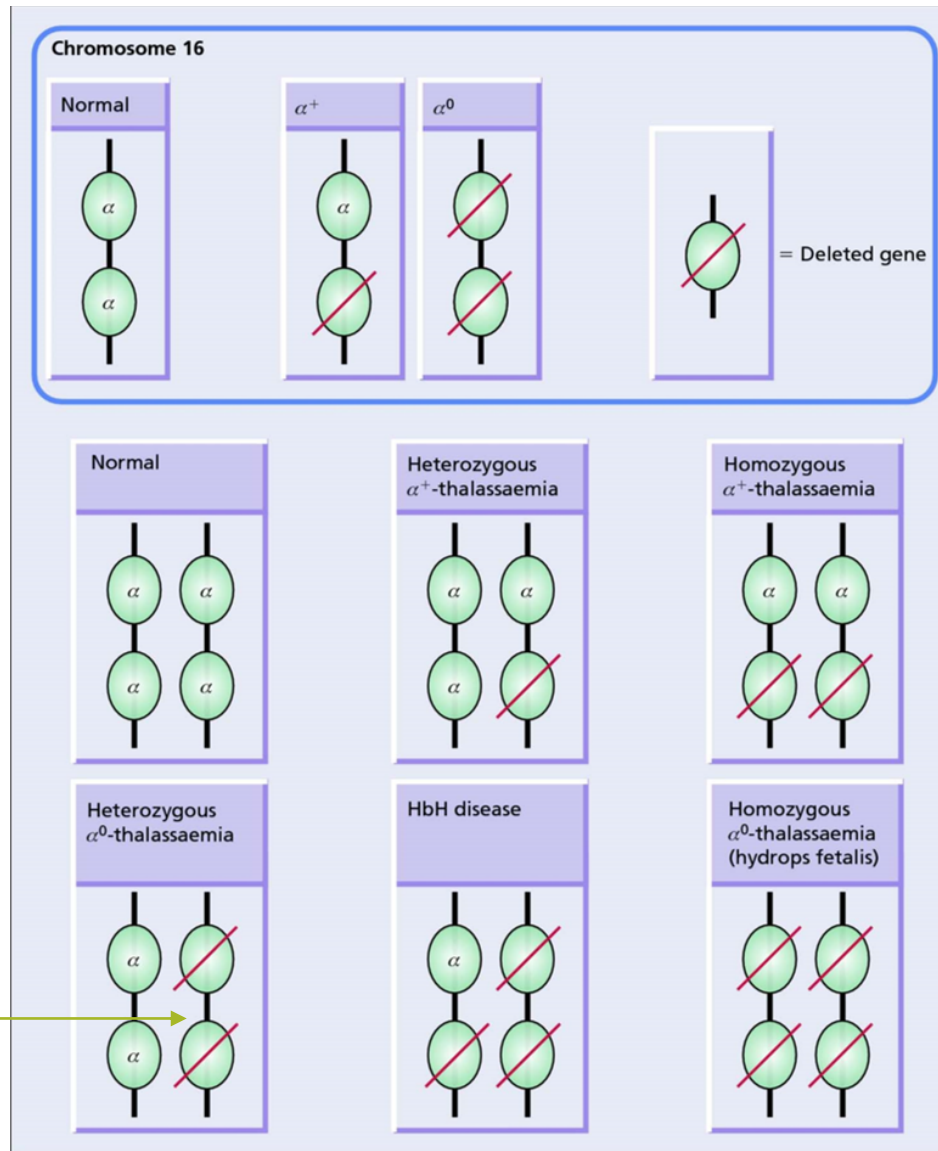
(a)



(b)

Figure 7.7 (a) α -Thalassaemia: haemoglobin H disease (three α -globin gene deletion). The blood film shows marked hypochromic, microcytic cells with target cells and poikilocytosis. **(b)** α -Thalassaemia: haemoglobin H disease. Supravital staining with brilliant cresyl blue reveals multiple fine, deeply stained deposits ('golf ball' cells) caused by precipitation of aggregates of β -globin chains. Hb H can also be detected as a fast-moving band on haemoglobin electrophoresis (see Fig. 7.12).

Cis



Cis or trans,
which is worse
to have?

Figure 4.3 A diagram to show how the two forms of abnormal chromosome 16 (α^+ and α^0) are arranged to give the different forms of α -thalassaemia. Homozygotes for α^0 -thalassaemia die from Hb Bart's hydrops fetalis syndrome.

Hb Bart's hydrops fetalis syndrome (deletion of all four α -globin genes)

No α -chains can be formed, and the fetal β -like chain γ -globin forms tetramers known as Hb Bart's. This hemoglobin is not useful for oxygen transport and, despite the persistence of the embryonic hemoglobin Hb Portland ($\zeta_2\gamma_2$), there is intrauterine or neonatal death due to hydrops.

β -Thalassemia

- The World Health Organization estimates that 1.5% of the world's population are carriers of β -thalassemia. The prevalence of the β -thalassemia trait is particularly high in **southern Europe** (10-30%) and **south-east Asia** (5%), common in Africa, the **Middle East**, India, Pakistan and southern China.
- α -thalassaemia typically arises **from gene deletions**.
- β -thalassaemia usually results from a multiplicity of different single nucleotide substitutions, insertions or small deletions affecting the β -gene itself or occasionally in promoting regions (**mutations**).

Heterozygous β -thalassemia (Beta-thalassemia trait)

- Most affected subjects with beta thalassemia trait are asymptomatic.
- The Hb concentration is either normal or slightly reduced, hypochromic and microcytic red cell indices are seen.
- Examination of peripheral blood film may show red cell abnormalities such as **target cells** and poikilocytes.
- β -thalassemia HbA2 levels will be raised above the normal range to 3.5-7.0%. Sometimes, a slightly increased Hb F levels, in the range of 1-5%.

Homozygous β -Thalassemia

- Defects of β -globin on both copies of chromosome 11.
- Marked anemia.
- Transfusion dependent.

Clinical classification of the thalassemias

- 1) *Thalassemia minima* describes the presence of a thalassemia mutation that is without clinical consequences.
- 2) *Thalassemia minor* describes patients with microcytosis and hypochromic red cells secondary to thalassemia mutations, but with only mild anemia or a normal hemoglobin. Patients who inherit a single affected allele are usually in this category.

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- 3) *Thalassemia intermedia* patients will also have a microcytic hypochromic anemia, increased erythroid drive to maintain their hemoglobin, packed bone marrow with a decreased myeloid to erythroid ratio, and extramedullary hematopoiesis, giving splenomegaly. Transfusion may be required to maintain the hemoglobin at times of additional physiological stress.
- 4) *Thalassemia major* have severe anemia and are transfusion dependent. Their increased erythroid drive leads to a packed erythroid marrow and splenomegaly, development of bony abnormalities secondary to unchecked marrow expansion. Patients in this category are those with complete loss of β -globin expression from both copies of Ch11.

The clinical course and complications of thalassemia major

Anemia - is the principal feature of thalassemia major, the massive expansion of erythroid activity results in several complications:

- a) **Splenomegaly.**
- b) **Bony deformities.**
- c) **Growth retardation.**
- d) **Transfusion dependence.**
- e) **Marked iron overload.**

Iron deposition due to overload occurs in the myocardium, resulting in congestive cardiac failure and potentially fatal arrhythmias; in the liver, leading to cirrhosis; in the pancreas, causing diabetes mellitus; and in other endocrine organs, leading to delayed puberty.

Treatment of β -thalassemia major

- 1) Transfusion are planned to maintain the pre-transfusion Hb concentration at 9-10g/dL or above.
- 2) Splenectomy can be performed.
- 3) **Iron chelator**, required subcutaneous infusion treatment over several hours on five days of the week.
- 4) Hematopoietic stem cell transplantation (HSCT) is curative.
- 5) **Gene therapy** (see the NEJM quick tack, <https://www.nejm.org/doi/10.1056/NEJMdo005273/full/>).



Genetic counselling and antenatal diagnosis of β -thalassemia major

- Antenatal diagnosis can be made early during pregnancy from an analysis of **chorionic villous DNA** (at 9-12 weeks) or **amniocyte DNA** (at 13-16 weeks), or later using DNA from blood obtained from an 18-20-week-old fetus.
- Newer techniques focus on the non-invasive analysis of **fetal DNA** in the maternal circulation.
- A pre-marital screening, national program, is one of the major intervention leading to reduced incidence of beta thal major & SCD.

Structural Hemoglobin Variants

- **Over 1000 abnormal hemoglobin variants** have been reported. The majority of structural Hb variants are the consequence of a single-point mutation with a single amino acid substitution in the affected globin chain (e.g. HbS, HbE, HbC and HbD).
- When the **amino acid substitution** results in an overall change in the charge of the hemoglobin molecule, its migration in a voltage gradient is altered and this can be demonstrated by standard electrophoretic techniques. The speed of migration is characteristic for each abnormal hemoglobin.
- Abnormal hemoglobin variants are now usually detected by **high-performance liquid chromatography (HPLC)**. The most common structural Hb variant is hemoglobin S (HbS).

Hemoglobin	Original Geographic Association	Amino Acid Substitution	% Present in Heterozygotes
S	Africa	$\beta 6$ Glu \rightarrow Val	40%
C	Africa	$\beta 6$ Glu \rightarrow Lys	40%
E	Asia	$\beta 26$ Glu \rightarrow Lys	30%
G Philadelphia*	Africa	$\alpha 68$ Asn \rightarrow Lys	20, 30, 40% **
D Los Angeles***	Europe/India	$\beta 121$ Glu \rightarrow Gln	40%
O Arab	Africa	$\beta 121$ Glu \rightarrow Lys	40%
Lepore#	Mediterranean	$\delta(1-87)$ $\beta(116-146)$ #	10-15%

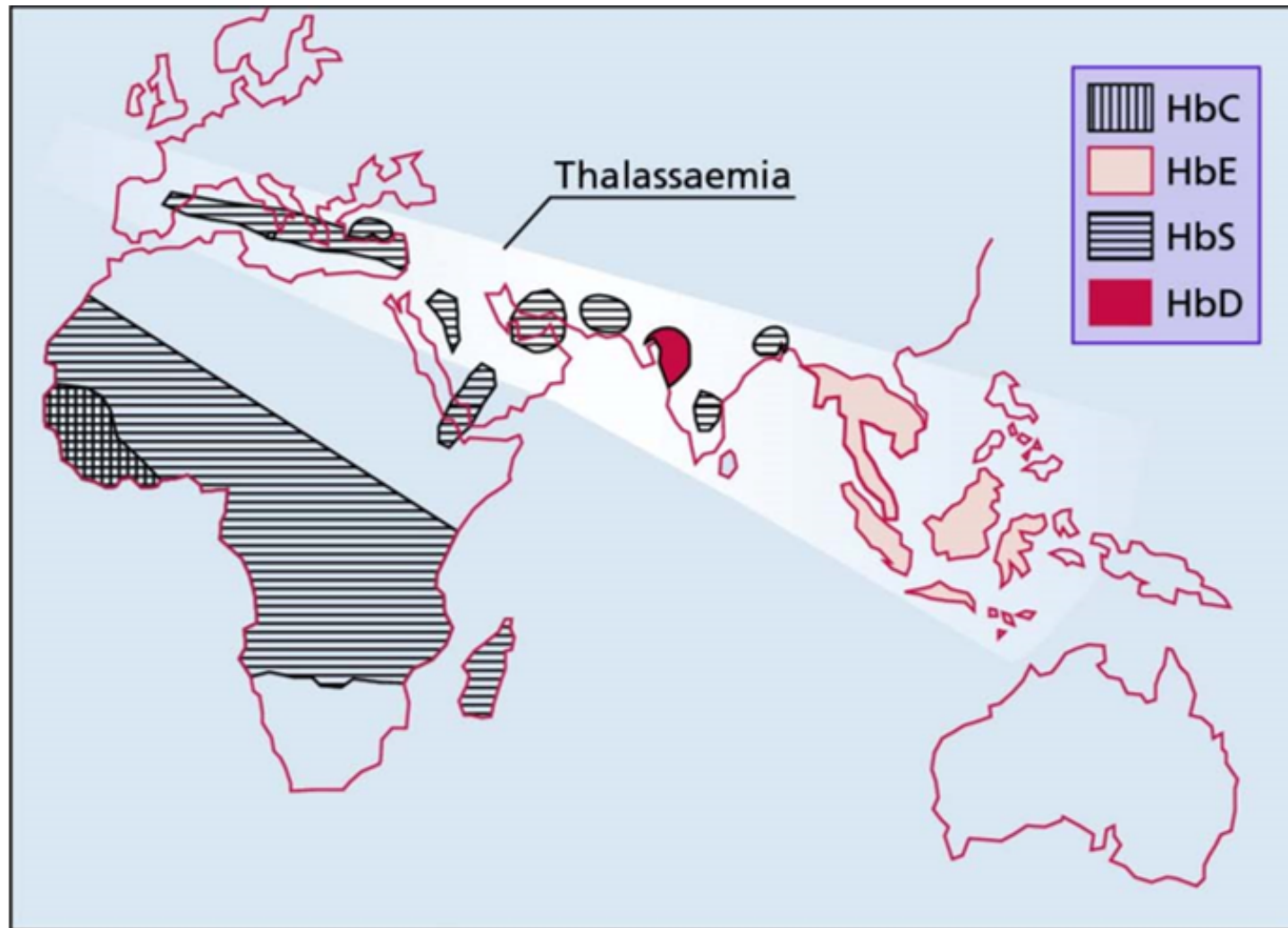


Figure 4.2 Distribution of the genes for the major Hb variants (S, E, C, D) and thalassaemia.

Table 4.1 Different clinical and haematological abnormalities associated with some structural haemoglobin variants.

Variant	Clinical and haematological abnormalities
HbS	Recurrent painful crises (in adults) and chronic haemolytic anaemia, both related to sickling of red cells on deoxygenation
HbC	Chronic haemolytic anaemia due to reduced red cell deformability on deoxygenation; deoxygenated HbC is less soluble than deoxygenated HbA
Hb Köln, Hb Hammersmith	Spontaneous or drug-induced haemolytic anaemia due to instability of the Hb and consequent intracellular precipitation
HbM Boston, HbM Saskatoon	Cyanosis due to congenital methaemoglobinemia as a consequence of a substitution near or in the haem pocket
Hb Chesapeake, Hb Radcliffe	Hereditary polycythaemia due to increased O ₂ affinity
Hb Kansas	Anaemia and cyanosis due to decreased O ₂ affinity
Hb Constant Spring, Hb Lepore, HbE	Thalassaemia-like syndrome due to decreased rate of synthesis of abnormal globin chain
Hb Indianapolis	Thalassaemia-like syndrome due to marked instability of Hb

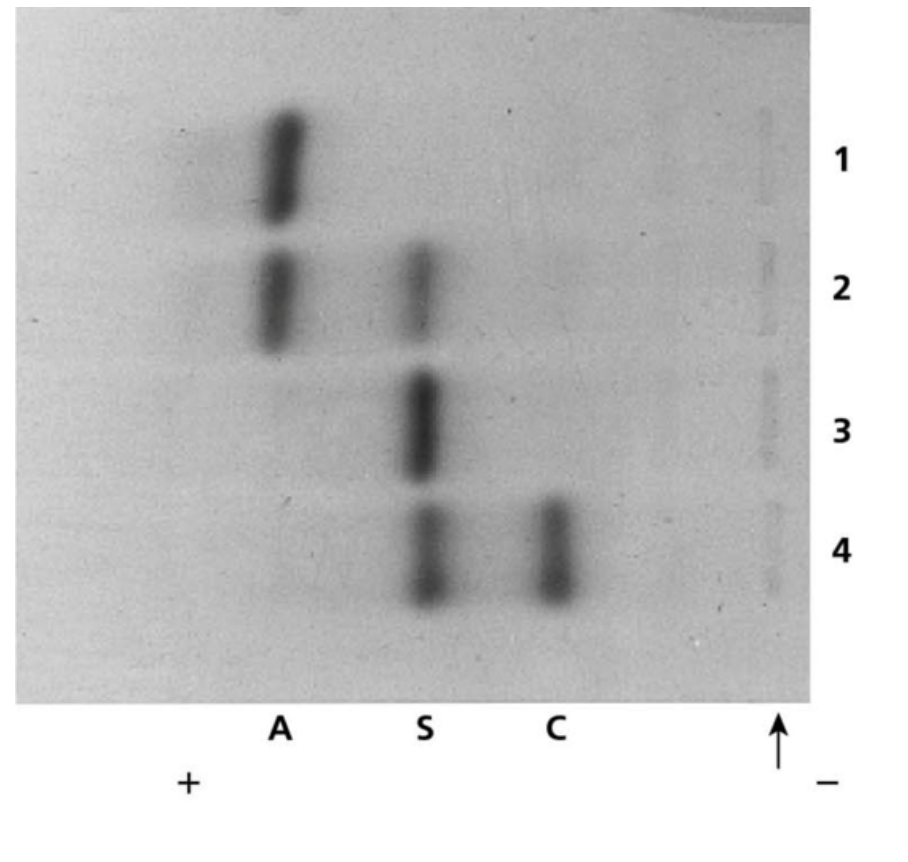


Figure 4.5 Electrophoresis of haemolysates on cellulose acetate (pH 8.5). The arrow marks the site of application of the haemolysate. (1) Normal adult. (2) Individual with sickle-cell trait; 35% of the Hb consists of HbS and most of the remainder is HbA. (3) Patient with sickle cell anaemia; most of the Hb is S and there is no A. (4) Double heterozygote for HbS and HbC. This results in a disease that is usually milder than that in homozygotes for HbS.

Hemoglobin S

- A mutation in the β -globin gene results in the charged **glutamic acid** residue in position 6 of the normal β -chain being replaced by an uncharged **valine** molecule.
- The interaction of sickle β -globin chains with normal α -globin chains forms HbS.
- When deoxygenated, HbS is much less soluble than deoxygenated HbA, and HbS molecules polymerize, eventually forming long fibers. These result in the deformation of the cell into the well-recognized **sickle shape**.

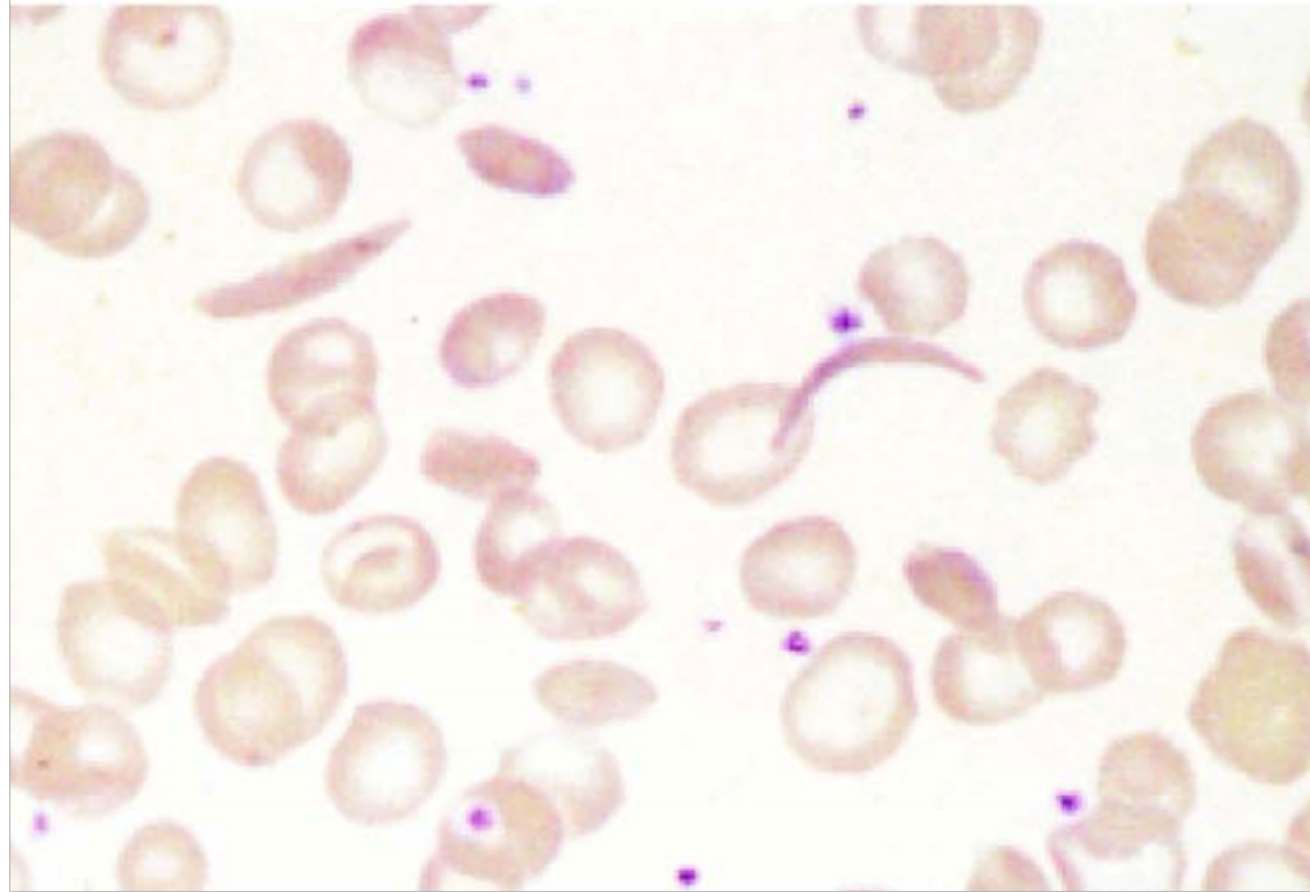


Figure 4.7 Two sickle-shaped red cells with pointed ends and some partially sickled red cells from the blood film of a patient with sickle cell anaemia (homozygote for HbS).

Sickle Cell Trait

- Heterozygotes (one gene for normal β -globin and one for β^S) are described as having sickle cell trait. Their red cells contain between 20% and 45% HbS, the rest being mainly HbA.
- Individuals with sickle cell trait are usually asymptomatic. However, **spontaneous hematuria** may occur occasionally due to microvascular infarctions in the renal medulla. Renal **papillary necrosis** may rarely occur. The red cells **do not sickle** until the O_2 saturation falls below 40%.

Sickle Cell Anemia

- ❖ It is a **descriptive** name when have at least a **copy of beta globin being S and another beta harbor any mutations** (beta that/S, S/S, S/D, ect).
- ❖ **Homozygotes for sickle β -globin** are described as having sickle-cell anemia. Their red cells contain almost **exclusively HbS** and NO HbA; there is a small but variable percentage of fetal hemoglobin.
- ❖ Sickled red cells then occlude the microvasculature, with poor downstream perfusion and oxygenation. They may be lysed directly in the circulation, where the resulting free hemoglobin scavenges nitric oxide.
- ❖ HbS are less deformable than normal red cells and this results in a chronic, extravascular, hemolytic anaemia. The Hb usually varies between 6 and 9 g/dL.

Diagnosis

- Sickled cells are invariably present on the **blood films** of patients with HbSS. HbSS is made by finding;
 - (i) a positive result with a screening test for HbS (**Sickle solubility test**); and
 - (ii) a peak at an appropriate position on an **HPLC trace**, confirmed by isoelectric focusing or hemoglobin electrophoresis.
- In young children, a classic acute painful presentation is with dactylitis, or the '**hand-foot syndrome**', in which there is occlusion of the nutrient arteries to the metacarpals and metatarsals (Figure 4.8) and painful swelling of the hands and feet.
- In the central nervous system, cerebral infarction occurs in approximately 10% of patients under the age of 20, and is a cause of significant morbidity in sickle cell patients. It has been found that children with an **increased velocity of blood flow** in the major cerebral vessels are at **particular risk of stroke**.
- **Family History and Molecular testing.**

Table 4.2 Clinical manifestations of sickle-cell anaemia.

Chronic haemolytic anaemia and consequent cholelithiasis

Splenic sequestration syndrome; rarely, hepatic Sequestration

Acute chest syndrome

Cerebral infarction, TIA, intracranial haemorrhage

Widespread painful vaso-occlusive crises

Bone infarction (osteonecrosis)

Osteomyelitis (*Salmonella*, *Staphylococcus*)

Chronic leg ulcers

Priapism

Chronic pulmonary disease and pulmonary hypertension

Haematuria, proteinuria, chronic renal failure

Pregnancy: increased peripartum fetal loss, preterm births, babies small for gestational age

Aplastic crises due to parvovirus infection

Proliferative sickle retinopathy (more common in HbSC disease)

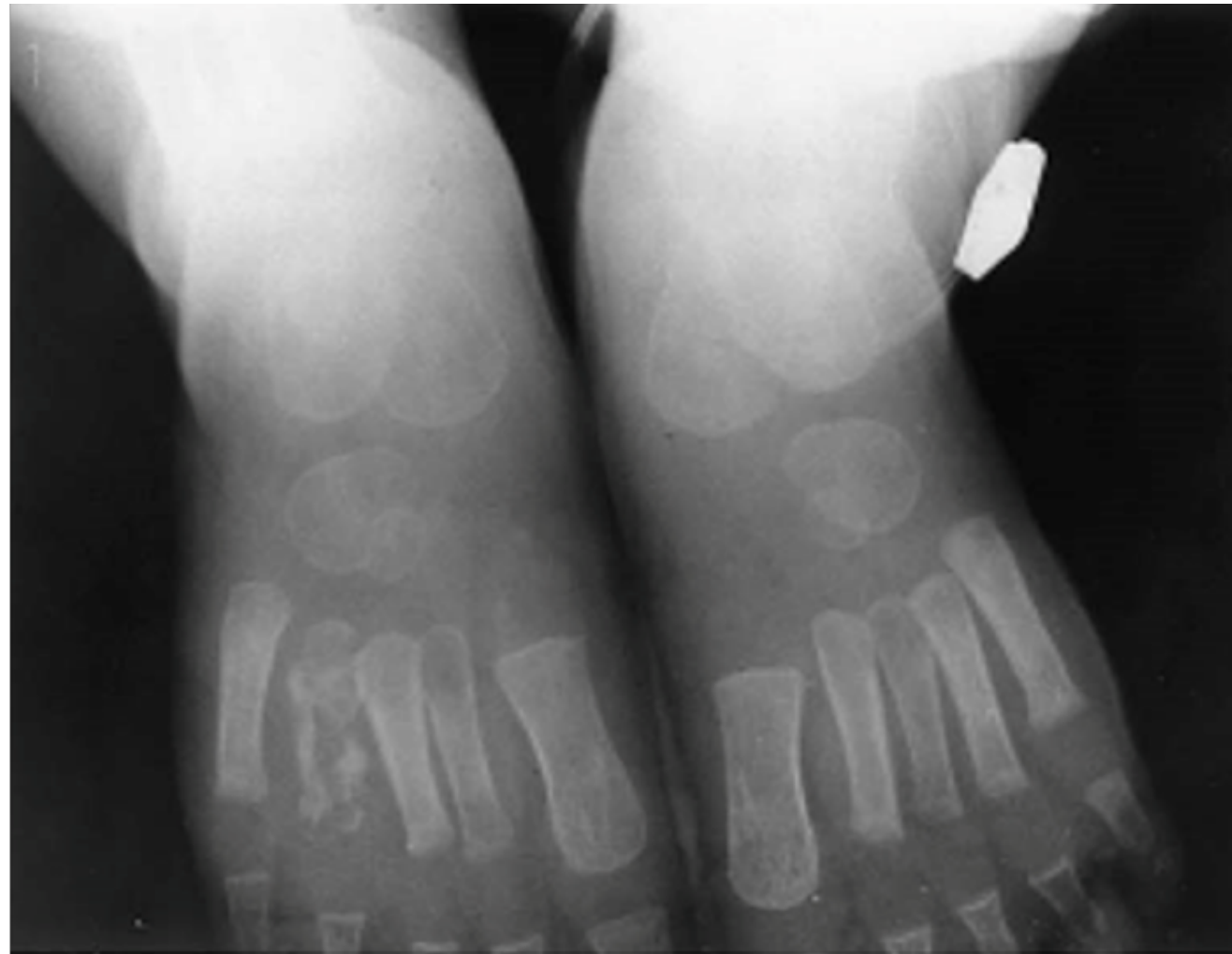


Figure 4.8 An X-ray of the feet of a child with sickle cell anaemia two weeks after the onset of hand-foot syndrome, showing necrosis of the right fourth metatarsal.



Figure 4.9 A chronic leg ulcer with increased pigmentation of the surrounding skin in a woman with sickle cell anaemia.

Treatment

The principles of the management of sickle cell anemia include:

- 1) Management of the increased infection risk by **immunization**.
- 2) Administration of **folic acid** daily to prevent secondary folate deficiency.
- 3) Avoidance of **factors precipitating painful crises** such as dehydration, hypoxia, circulatory stasis.
- 4) Treatment of painful crises with oral or intravenous **fluids and analgesics**, including opiates when necessary.
- 5) Early detection of the **acute chest syndrome** (blood gas measurements and chest X-ray). Exchange transfusions are often needed to lower the patient's HbS levels and limit ongoing sickling.
- 6) **Blood transfusion** when necessary.
- 7) **SCT and gene therapy**.

Hemoglobin E and C

- Among the commonest of structural hemoglobinopathy are **HbE and HbC**, both of which result from **single amino acid substitutions in the β -chains**.
- HbE is very common in **south-east Asia** (~50% in some parts of Thailand). It gives **thalassemic picture** of CBC.
- HbC is the consequence of a **glutamine to lysine** substitution in the 6th amino acid of β -globin chain.
- HbCC does not polymerize as with HbSS, but **can crystallize**, with a resulting reduction in the flexibility of the red cell and a reduction in its survival. HbC is found in patients of **West African origin**.
- When one allele being S and other being C or E, it is an example of a sickle cell disease (the most benign form is S/E).

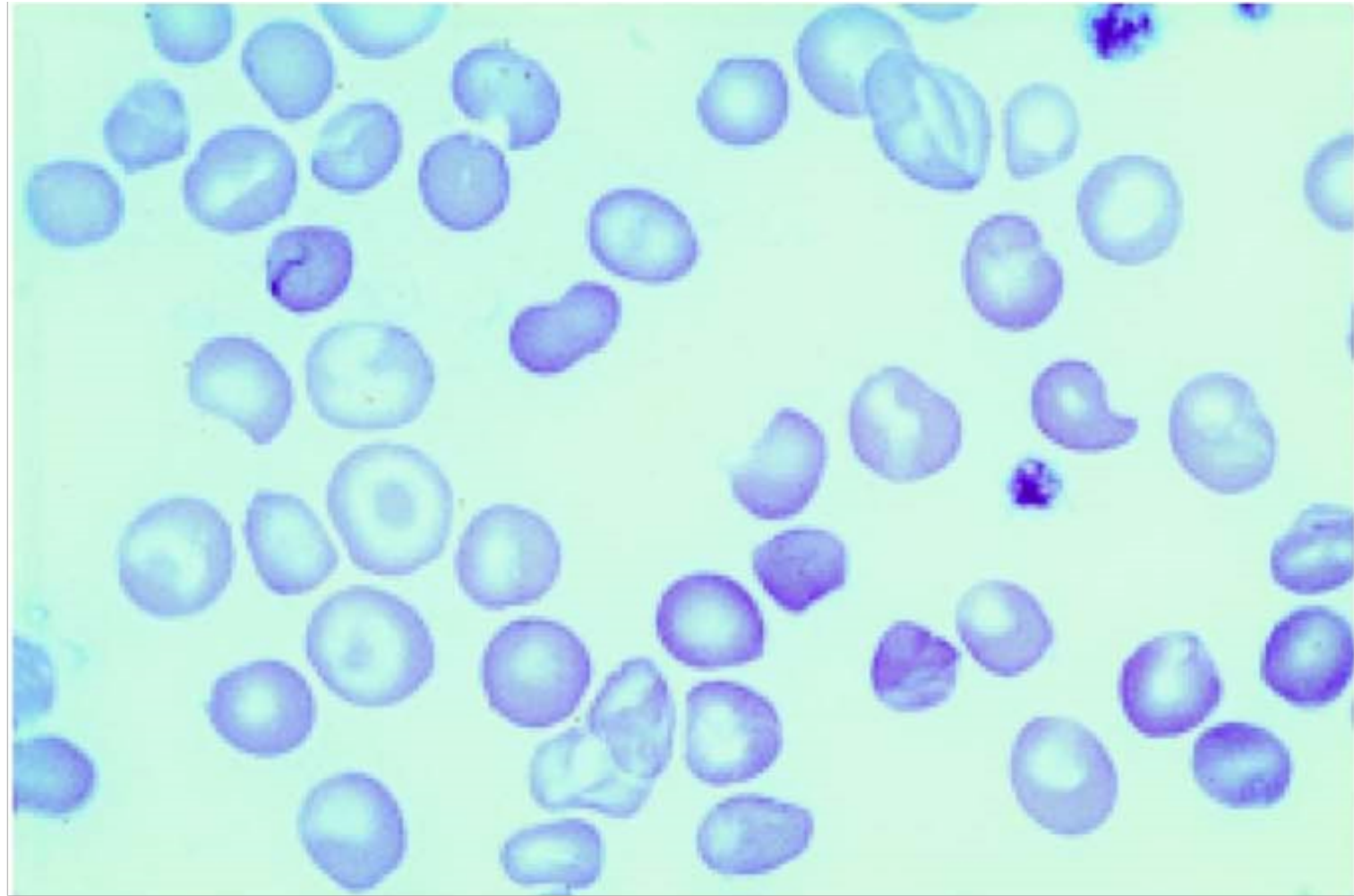


Figure 4.10 Target cells and irregularly contracted cells in the blood film of a homozygote for HbC.

Q1)

Which ONE of the following statements is TRUE about sickle cell anemia?

- A) The oxygen dissociation curve is shifted to the left.
- B) It may cause ankle ulcers.
- C) It is NOT associated with stroke.
- D) It is NOT associated with atrophy of the spleen

Q2)

Which ONE of the following statements is TRUE about β -thalassemia trait?

- A) It is associated with a raised hemoglobin A₂ level with normal CBC indices.
- B) It is associated with iron overload.
- C) It is associated with a normal reticulocyte index.
- D) It is associated with splenomegaly.

Q3)

Which ONE of the following statements is TRUE about beta-thalassemia?

- A) It may cause hemoglobin H disease.
- B) It causes a microcytic hypochromic blood picture.
- C) It frequently causes a hydrops fetalis.
- D) It is very common in the Far East.

Q4)

Which ONE of these statements is TRUE about β -thalassemia major?

- A) It presents at birth.
- B) It is usually caused by deletion of β globin genes.
- C) It is associated with an increased risk of bone infarction.
- D) It is associated with stunted growth.

Q5)

Which ONE of these statements is TRUE concerning sickle cell trait?

- A) It is a cause of anemia.
- B) It protects against malaria.
- C) It is usually associated with splenomegaly.
- D) It is a cause of frequent sickle cells in the peripheral blood.

Q6)

Sickle cell trait contains:

- A. 20% - 45% HBS
- B. 20% - 45% HBC
- C. 70% HBS
- D. 70% HBC

Thank You!!!