Haemoglobinopathy Diagnosis, Second Edition
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4 Sickle cell haemoglobin and its interactions with other variant haemoglobins and with thalassaemias

Sickle cell anaemia was first described in 1910 when a patient with severe anaemia was noted to have 'peculiar elongated and sickle shaped red blood corpuscles' [1]. Many years later, Linus Pauling and colleagues found that the sickling phenomenon was caused by haemoglobin with unusual characteristics [2] and, subsequently, Vernon Ingram and colleagues identified the causative amino acid in the β chain of haemoglobin [3]. Sickle cell haemoglobin, haemoglobin S, has a valine for glutamic acid substitution at position 6 of the β chain. The haemoglobin can be designated $\alpha_2\beta_2^{6Glu\rightarrow Val}$. Sickle cell haemoglobin can produce deleterious effects because, on deoxygenation, its solubility is reduced and polymerization occurs (Fig. 4.1). Both partially and fully deoxygenated haemoglobin S can be incorporated into a polymer. Long polymers distort the red cell into a holly-leaf or into a crescent or sickle shape that hinders blood flow through capillaries, because of both reduced deformability and increased adhesion to endothelial cells resulting from secondary changes in the red cell membrane. When fully oxygenated, haemoglobin S is as soluble as haemoglobin A. The presence of haemoglobin A in a red cell slows polymerization, although haemoglobin A can copolymerize with haemoglobin F. Haemoglobin F and haemoglobin A_2 are even more effective at retarding sickling whereas, in comparison with haemoglobin A, sickling is facilitated by the presence of haemoglobin C, haemoglobin D-Punjab or haemoglobin O-Arab. Haemoglobin F and haemoglobin A_2 cannot copolymerize with haemoglobin S and the hybrid tetramer, $\alpha_2\beta^S\gamma$, is similarly unable to polymerize. Because acidosis and a rise in temperature shift the oxygen dissociation curve to the right, they favour sickling. However, in clinical practice, exposure to cold can also provoke sickling because of slowed circulation through capillaries.

The sickle cell mutation appears to have arisen spontaneously at least five times in the history of mankind (Fig. 4.2). Such independent mutations can be recognized by their association with different β globin gene haplotypes, demonstrated by the analysis of restriction fragment length polymorphisms (RFLPs). There are three foci of haemoglobin S in Africa, associated with different haplotypes, the haplotypes being defined by RFLP analysis. They are in Senegal (Senegal type), the Central African Republic and southern Africa (Bantu or Central African Republic type) and Benin, Central, West and North Africa (Benin type) [4]. The Benin type has also spread to Spain, Portugal, Sicily (perhaps from Greece, perhaps from Sudanese soldiers in Arab armies) and southern mainland Italy, Greece (particularly Macedonia), Albania, Turkey, north-western Saudi Arabia and Oman. The Bantu type has spread to Kenya, Zambia and the Sudan. In addition to the three major foci, there may have been a further independent mutation amongst the Eton people in southern Cameroon. A fifth mutation is associated with further foci in eastern Saudi Arabia and in extensive areas of central and southern India, particularly amongst the scheduled tribes (a group living outside the caste system). It may have arisen initially in the Indus valley. The prevalence of the haemoglobin S gene is up to 25% in eastern Saudi Arabia and as high as 30% in some tribal populations in central India. The Indian/Saudi Arabian haplotype has also been found in Afghanistan, Oman, Kuwait, Bahrain and Iran and amongst Bedouin Arabs in Israel.

Migration from Africa has led to the sickle cell gene occurring also in Central and South America, in Afro-Americans and in Afro-Caribbeans in Canada, the UK and other European countries. There is a high prevalence in some populations in Mexico, Colombia, Venezuela, Guyana, Surinam, French Guyana, Brazil and Peru. All three major African haplotypes

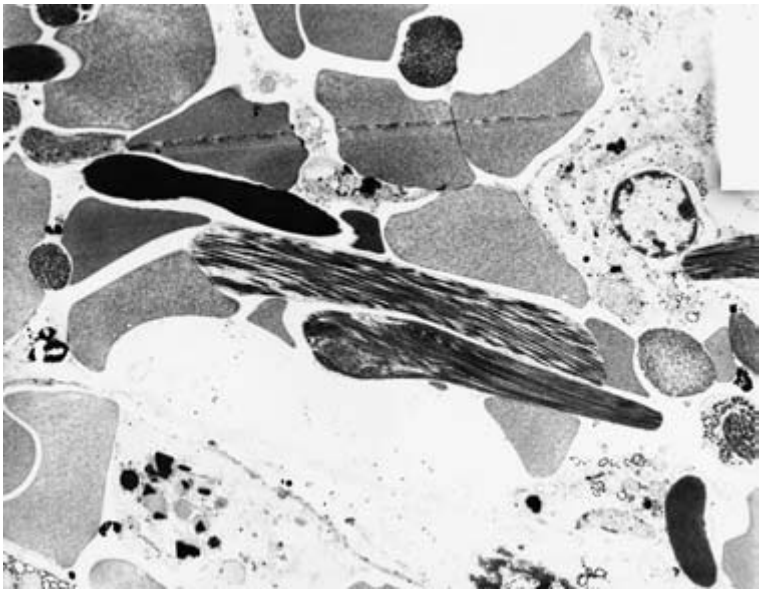


Fig. 4.1 Transmission electron micrograph showing polymerization of haemoglobin S in a patient with compound heterozygosity for haemoglobin S and haemoglobin D-Punjab. (With thanks to Mr S. Ladva, St Mary's Hospital.)

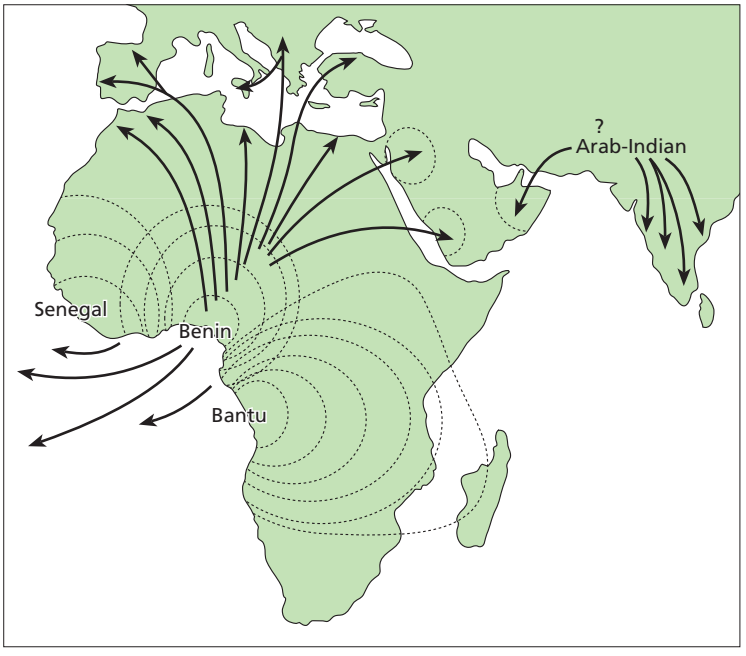


Fig. 4.2 Multifocal origin and spread of the β^S gene.

are represented in the USA, the Caribbean and the UK. The sickle cell gene is also found in Madagascar, Mauritius (both Bantu and Arab–Indian haplotypes), Abu Dhabi, United Arab Emirates, Lebanon, Iraq, the southern part of the former USSR and amongst North African Arabs.

The wide geographical spread of this potentially deleterious gene has been attributed to the protection of heterozygotes from premature death from falciparum malaria. In areas in which malaria is endemic, the β^S and β^A genes may exist as a balanced polymorphism, i.e. death or serious disability from

sickle cell anaemia before the age of reproduction is balanced by a decreased death rate from malaria amongst heterozygotes. The prevalence of haemoglobin S in various populations is shown in Table 4.1 [5–18].

A second mutation has occasionally occurred in a β^S gene leading to the synthesis of a variant haemoglobin with two amino acid substitutions. Such variant haemoglobins retain their ability to sickle, but may have a different electrophoretic mobility from haemoglobin S. In heterozygous, homozygous and compound heterozygous states, these mutations have a similar, although not necessarily identical, significance to haemoglobin S. At least 10 such double mutations are known (Table 4.2) [19–22]. The most common, haemoglobin C-Harlem (initially described under the name of haemoglobin C-Georgetown), $\alpha_2\beta_2^{6Glu\rightarrow Val,73Asp\rightarrow Asn}$, is less prone to polymerization than haemoglobin S itself. The rare double substitution haemoglobin, haemoglobin S-Antilles, is even more prone to sickle than haemoglobin S itself, as is haemoglobin Jamaica Plain.

There are other haemoglobins unrelated to haemoglobin S that can polymerize *in vitro*, e.g. haemoglobin I (α16 Lys→Glu) and haemoglobin Setif (α22 Asp→Tyr). Although they are not associated with any relevant clinical abnormality, haemoglobin Setif can cause a false positive sickle solubility test [23].

Homozygosity for haemoglobin S ($\beta^S\beta^S$) causes a serious condition referred to as 'sickle cell anaemia'. Heterozygosity for haemoglobin S ($\beta\beta^S$), referred to as sickle cell trait, is usually asymptomatic. The β^S gene may also be coinherited with another β chain variant. When there is deleterious interaction between the sickle cell haemoglobin and the second variant haemoglobins, as is the case, for example, with haemoglobin C and haemoglobin D-Punjab, a clinically significant sickling disorder occurs. Subjects who are heterozygous for β thalassaemia and haemoglobin S likewise suffer from the clinicopathological effects of sickle cell formation and consequent vascular occlusion. The term 'sickle cell disease' is often used as a generic term to include sickle cell anaemia and other conditions in which a clinically significant disorder results from sickle cell formation and the associated pathological processes. Some such conditions are shown in Table 4.3 [21,24–27].

Sickle cell trait

The term 'sickle cell trait' indicates heterozygosity for the sickle cell gene ($\beta\beta^S$). Sickle cell trait is asymptomatic in the great majority of individuals, but is of genetic importance. It gives partial protection against death from *Plasmodium falciparum* malaria.

If a patient with symptoms suggestive of sickle cell disease appears to have sickle cell trait on haemoglobin electrophoresis or high performance liquid chromatography (HPLC), further detailed investigation is indicated, as this may be the result of a second mutation in a β^S gene, e.g. haemoglobin Jamaica Plain (see below), or a second mutation in a β^C gene, e.g. haemoglobin Arlington Park (see below). The second mutation alters the characteristics of the variant haemoglobin so that it can be confused with haemoglobin A.

Clinical features

Subjects with sickle cell trait are usually asymptomatic. However, sickle cell formation leading to vascular occlusion can occur during high fever and under conditions of significant hypoxia, such as during travel by air (particularly but not only in unpressurized aircraft), mountain climbing, vigorous exercise and anaesthetic misadventures. Vascular occlusion in such circumstances can lead to splenic, pulmonary, pituitary, cerebral, retinal, renal and bone infarcts and also to priapism (persistent erection caused by sickling within blood vessels of the penis). Bone infarcts can lead to avascular necrosis. There is a low risk of sudden death associated with vigorous exercise, particularly exercise at a high altitude and exercise complicated by dehydration and acidosis [28]. Such circumstances can also lead to exertional rhabdomyolysis, disseminated intravascular coagulation and renal failure [29]. In a study of USA Air Force personnel, the rate of non-traumatic deaths in airmen was very low, but was 25-fold higher in those with sickle cell trait than in those without sickle cell trait [30]. Similar observations have been made in USA Army recruits. In sickle cell trait, spontaneous sickle cell formation can occur in renal papillae where oxygen tension is normally low, leading to renal papillary necrosis, episodes of haematuria and impairment of renal concentrating ability. Loss of

Table 4.1 Prevalence (%) of haemoglobins S and C in different populations. (From references [5–18] and other sources.)

Country or people	Haemoglobin S	Haemoglobin C
West Africa		
Senegal	3–15	<1–6
Gambia	6–28	<1–2
Guinea Bissau	<1–25	<1–1.5
Guinea	13–33	
Sierra Leone	22–30	
Liberia	<1–29	1–3
Ivory Coast	2–26	<1–50
Mali	5–17	
Burkina Faso (previously Upper Volta)	2–34	15–40
Ghana	3–25	8–40
Togo	6–28	7–17
Benin	5–31	7–27
Niger	5–23	1–8
Nigeria	10–41	<1–9
Central Africa		
Gabon	8–32	
Cameroon	<1–31	<1
Central African Republic	2–24	
The Republic of the Congo	7–32	
Democratic Republic of the Congo (previously Zaire)	1–46	
East Africa		
Kenya	<1–34	
Uganda	1–39	
Tanzania	1–38	
Rwanda		
Tutsi	<1–5	
Hutu	5–15	
Burundi	1.5–26	
Southern Africa		
Angola	8–40	
Zambia	<1–30	
Zimbabwe	<1–11	
Malawi	3–18	
Mozambique	<1–40	
Madagascar	<1–23	
Botswana	<1	
Namibia	0–15	
South Africa		
Bantu	<1–4	
Indian	2–10	
Cape Coloured	<1	<1
North Africa		
Morocco	<1–7	<1–6
Algeria	<1–15	<1–13
Tunisia	<1–2	
Libya	<1–70	
Egypt	<1*	
Sudan	<1–17	

Table 4.1 *Continued.*

Country or people	Haemoglobin S	Haemoglobin C
Horn of Africa		
Ethiopia	0–1	
Djibouti	≈0	
Somalia	≈0	
Afro-Americans	6–15	1–3.5
Afro-Caribbeans		
Jamaica	3.5–12	2–4
Bahamas	14	3
Barbados	4	3–5
Cuba	0–23	0–2.5
Haiti	7–17	1–3
Dominican Republic	6–12	3
Puerto Rica	<1–8	<1–2
Lesser Antilles	1–14	1–4.5
Guadeloupe	4.4	
Central America		
Mexico	<1–9	<1
Guatemala	<1–17	
Belize	0–25	
El Salvador	<1–2	
Honduras	<1–16	
Nicaragua	≈0	
Costa Rica	<1–8	
Panama	0–21	0–2.5
South America		
Colombia	0–15	0–6
Venezuela	0–9	0–3
Guyana	<1	
Surinam	0–22	0–6
French Guyana	0–18	0–7
Ecuador	≈0	≈0
Peru	<1	≈0
Bolivia	≈0	≈0
Brazil	0–16	0–4
Paraguay	≈0	
Argentina	<1	
Uruguay	≈0	
Chile	<1	<1
Europe		
Greece	0–32	
Turkey	<1–34	0.5–1
Cyprus	<1	
Italy		
Sicily	<1–13	
Sardinia	≈0	
Mainland southern Italy	0.5–1	
Portugal	<1–5	
Spain		0.12 (southern Spain)

Continued on p. 144.

Table 4.1 *Continued.*

Country or people	Haemoglobin S	Haemoglobin C
Middle East		
Turkey	<1–34	
Syria	<5–25	
Lebanon	<1	
Jordan	4–6	
Israel		
Arabs	1–38	
Jews	≈0	
Iraq	0–25	
Iran	≈0	
Saudi Arabia	<1–36	
Kuwait	2	
Bahrain	2.5	
Oman	5	Rare
Yemen	1–2†	
Abu Dhabi	2	
United Arab Emirates	2	
Asia		
India	0–35‡	
Pakistan	0.5–1	
Sri Lanka	Rare	
Thailand		Rare

*5–22% in various oases.
†23% in Western province.
‡5–35% in various tribal populations [17]; 15% in Orissa, Madhya Pradesh and Maharashtra states [18].

Table 4.2 Variant haemoglobins in which the mutation of haemoglobin S is one of two mutations. (Derived from references [9,19–22].)

Variant haemoglobin	Second substitution	Mobility on cellulose acetate at alkaline pH
C-Harlem	β73 Asp→Asn	C
C-Ziguinchor	β58 Pro→Arg	C
S-Travis	β142 Ala→Val	S
S-Antilles	β23 Val→Ile	S
S-Providence	β82 Lys→Asn	A
S-Oman	β121 Glu→Lys	Slower than C
S-Wake	β139 Asp→Ser	
Cameroon	β90 Glu→Lys	
Jamaica Plain	β68 Leu→Phe	S [21]
South End	β132 Lys→Asn	[22]

renal concentrating ability is less if thalassaemia trait coexists [31]. If the kidney is excluded, spontaneous episodes of vascular occlusion, i.e. episodes occurring in the absence of fever, dehydration, hypoxia or acidosis, are very rare but do occur. Splenic sequestration has likewise been described, but very rarely, in sickle cell trait [32]. During pregnancy, women with sickle cell trait may have an increased incidence of bacteriuria and pyelonephritis [33] and pregnancy-associated hypertension [34]. There is

also an unexpected but quite strong association between medullary carcinoma of the kidney and sickle cell trait [35].

Despite this list of potential complications, the great majority of patients with sickle cell trait are asymptomatic, and the main reason for seeking to identify the heterozygous state is the genetic implications. If both parents have sickle cell trait, there is a 25% probability of sickle cell anaemia in a child.

Table 4.3 Causes of sickle cell disease.

Sickle cell anaemia (homozygosity for haemoglobin S)
Compound heterozygous states
Sickle cell/haemoglobin C disease
Sickle cell/β thalassaemia
Sickle cell/haemoglobin D-Punjab
Sickle cell/haemoglobin C-Harlem
Sickle cell/haemoglobin S-Antilles
Sickle cell/haemoglobin O-Arab
Sickle cell/haemoglobin Quebec-Chori [24]
Sickle cell/haemoglobin S-Oman
Sickle cell/haemoglobin O-Tibesti
Haemoglobin S-Antilles/haemoglobin C
Sickle cell/haemoglobin Lepore
Haemoglobin C/haemoglobin C-Harlem [25]
Mutations leading to sickle cell disease in β^s heterozygotes
Haemoglobin S-Antilles [19]
Haemoglobin S trait plus haemoglobin Conakry trait (an α chain variant) [26]
Haemoglobin S-Oman [27]
Haemoglobin Jamaica Plain [21]

Laboratory features

Blood count

The haemoglobin concentration is normal, except in those with coexisting α thalassaemia trait who may be slightly anaemic [36]. Similarly, the mean cell volume (MCV) and mean cell haemoglobin (MCH) are reduced in those with coexisting α thalassaemia trait (Table 4.4) [9,36–40], but otherwise are normal. α thalassaemia trait is somewhat more prevalent in Africans and Afro-Americans with sickle cell trait than in those with normal β globin genes [41], so that it is not rare for subjects with sickle cell trait to have borderline anaemia or reduction of the MCV and MCH.

Blood film

The blood film may be completely normal or may show microcytosis or target cells (Fig. 4.3). If a subject with sickle cell trait develops iron deficiency, target cells are often prominent. Although classical sickle cells are not seen, small numbers of plump cells that are pointed at both ends have been reported [42]; such cells were described in about 96% of individuals with sickle cell trait in comparison with 4% of normal subjects.

During *P. falciparum* malaria, subjects with sickle cell trait have a blood film showing a lower percentage of parasitized cells than is seen in subjects without a haemoglobinopathy [43].

Table 4.4 Mean cell volume (MCV) and percentage of haemoglobin S reported in sickle cell trait with and without deletion of α globin genes.

	αα/αα	–α/αα	–α/–α	Reference
Haemoglobin S (%)	>38	31–38	<31	[37]
	35–39	29–34	24–27	[38]
	35–45*	30–35*	25–30*	[36]
	34–38	28–34	20–28	[9]
	31–43	29–35	22–29	[39]
MCV (fl) (range and	80–90	75–85	70–75	[36]
mean ± standard deviation)	84.9 ± 8.26	79.1 ± 5.8	67.8 ± 5.81	[39]

*These values are averages derived from published series in which the α chain deletion was $-\alpha^{3.7}$; the deletion $-\alpha^{4.2}$ leads to a greater reduction in haemoglobin S percentage [40].

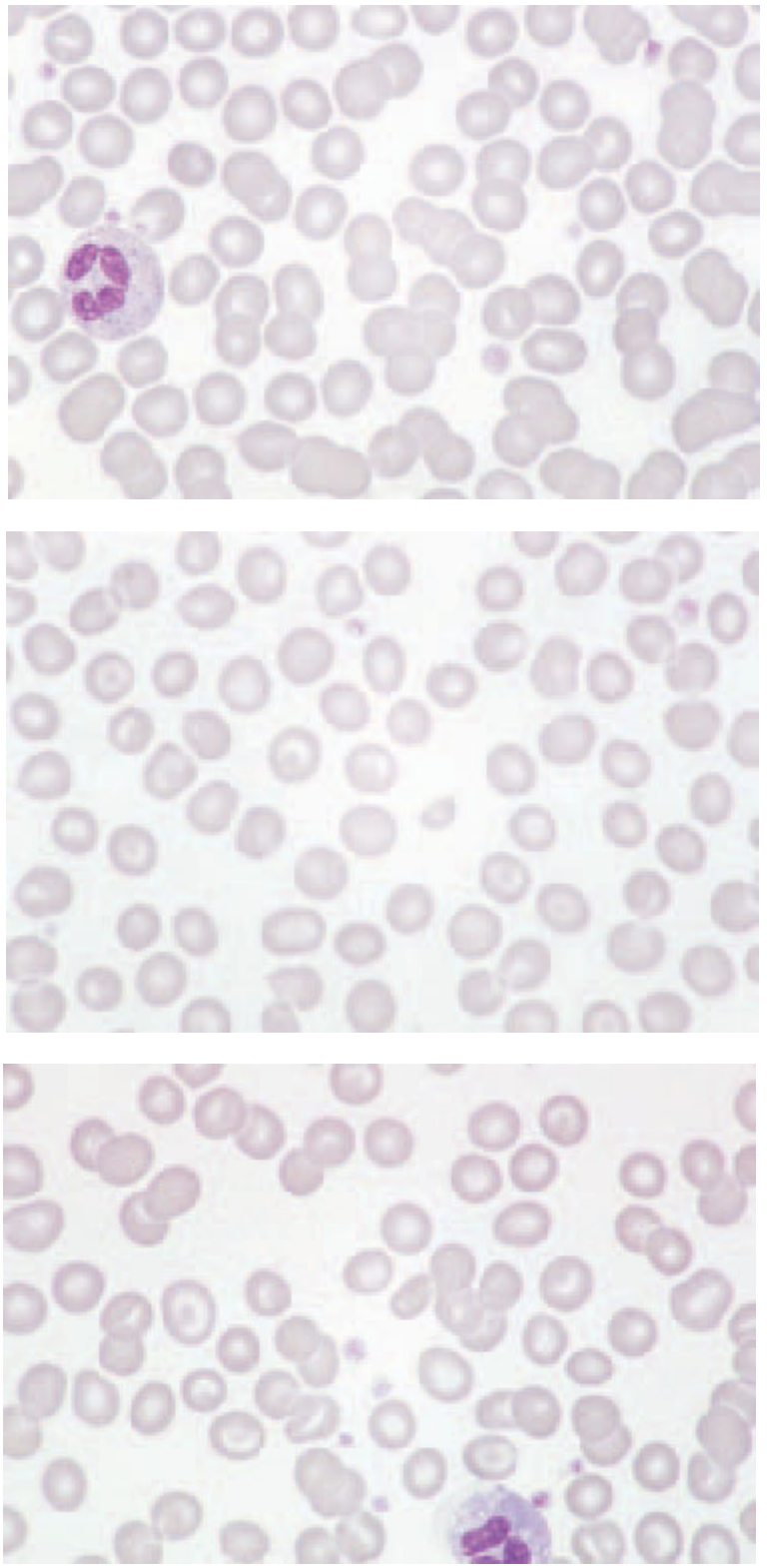


Fig. 4.3 Blood films from three patients with sickle cell trait showing the range of features observed: (a) normal film; (b) minimal anisocytosis and poikilocytosis with occasional target cells; (c) hypochromia with occasional target cells and other poikilocytes.

Other investigations

It might be expected that heterozygotes for haemoglobin S ($\beta\beta^S$) would have equal amounts of haemoglobin S and haemoglobin A. In fact, haemoglobin A is somewhat more than 50% and haemoglobin S is somewhat less, usually around 40%; this is because normal β chain has a greater affinity for β^S than for α chains. Haemoglobin S is readily detected by haemoglobin electrophoresis and other techniques. Haemoglobin electrophoresis at alkaline or acid pH shows a variant haemoglobin with characteristic mobility (Fig. 4.4). Haemoglobins D and G have the same electrophoretic mobility at alkaline pH, but can be distinguished by electrophoresis at acid pH, which usually shows mobility which is the same as or very similar to that of haemoglobin A. Haemoglobin S can also be distinguished from haemoglobins A, D and G by isoelectric focusing and HPLC (Fig. 4.5; see also Fig. 2.16). A sickle solubility test (see Fig. 2.19) should always be performed when the presence of a significant proportion of haemoglobin S is suspected. It will be positive, except in the early neonatal period when the percentage may be below the detection limit. It follows that a negative sickle solubility test in a neonate with a variant haemoglobin consistent with haemoglobin S does not exclude a diagnosis of sickle cell trait. Reagents suitable for a sickle solubility test are commercially available in kit form [44].

Haemoglobin S can also be distinguished from haemoglobin A and haemoglobin C by immunologi-

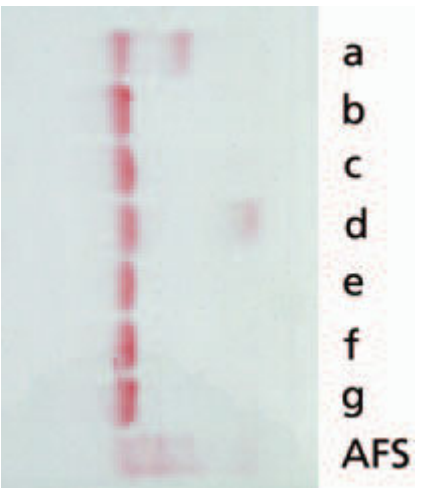


Fig. 4.4 Haemoglobin electrophoresis on cellulose acetate at alkaline pH showing haemoglobins A and S in a patient with sickle cell trait (lane a); AFS, control sample containing haemoglobins A, F and S.

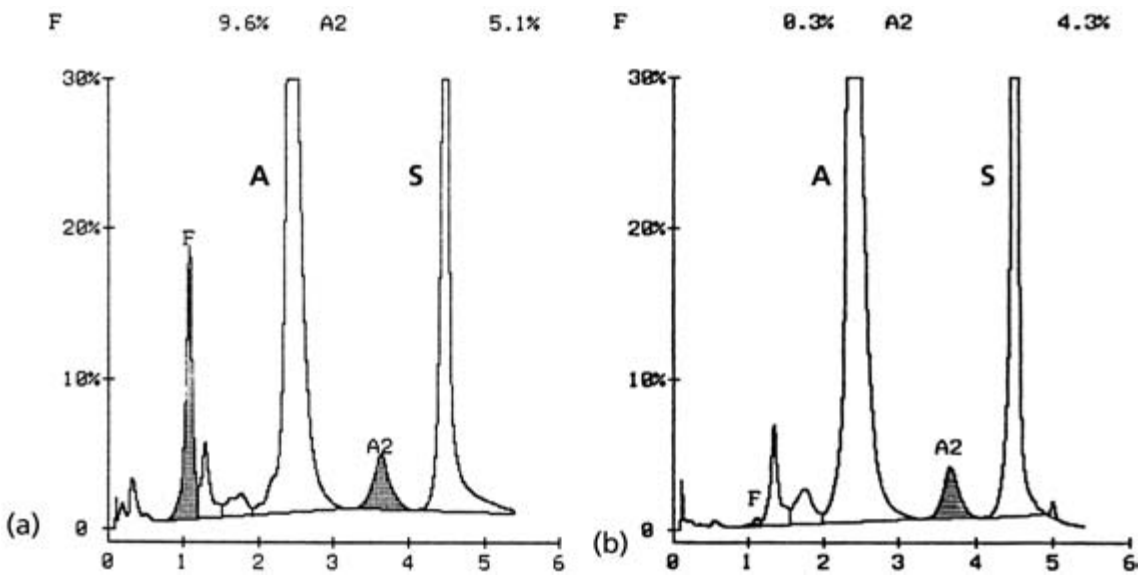


Fig. 4.5 High performance liquid chromatography (HPLC) chromatograms in sickle cell trait; both chromatograms show haemoglobins A, A_2 and S and chromatogram (a) shows, in addition, increased haemoglobin F; haemoglobin A that has undergone post-translational modification appears as two peaks to the left of haemoglobin A.

cal techniques based on monoclonal antibodies to sequences including the amino acids which are substituted in haemoglobin S and haemoglobin C. It is thus possible to distinguish sickle cell trait from sickle cell anaemia and sickle cell/haemoglobin C disease. However, sickle cell/β^+ thalassaemia may not be distinguished from sickle cell trait. Such monoclonal antibodies were previously commercially available in kit form as HemoCard A plus S and HemoCard C [44].

The proportion of haemoglobin S can be quantified by scanning densitometry of an electrophoretic strip, elution from an electrophoretic strip or HPLC. The percentage shows a trimodal distribution, depending on whether there is coexisting α thalassaemia trait (Table 4.4). If there is coexisting haemoglobin H disease, the percentage of haemoglobin S is even lower, around 10–25% [40]. Conversely, if there are five α genes (ααα/αα), the haemoglobin S percentage is somewhat higher than in those with a normal complement of α genes [45], about 45% rather than about 40%. The percentage of haemoglobin S correlates with the MCV and MCH as all of these variables are influenced by coexisting α thalassaemia trait. A rare cause of a low haemoglobin S percentage in sickle cell trait is coinheritance of a β thalassaemia determinant in *cis*, i.e. on the same chromosome [46] (see p. 149). Haemoglobin S may then be as low as 10%. The proportion of haemoglobin S is also reduced if there is coexisting iron deficiency [47]. It has been observed to fall markedly in megaloblastic anaemia [48] and may be low in lead poisoning [49].

The percentage of haemoglobin A_2 may be slightly elevated in sickle cell trait. However, this is not a particularly useful investigation to perform. Adult levels of haemoglobin F are reached by 2 years of age [50]. It is not clear whether, thereafter, the frequency of an elevated haemoglobin F percentage differs from normal [9].

In the neonatal period, haemoglobin F will be present in large amounts. There will be more haemoglobin A than haemoglobin S. However, if haemoglobins S and A are present in small amounts, precise quantification may be difficult. Unless there is clearly more haemoglobin A than haemoglobin S, sickle cell/β^+ thalassaemia is a possible alternative diagnosis. If necessary, the test should be repeated when the infant is a few months old.

Electrophoretic features suggestive of sickle cell trait despite clinical features of sickle cell disease should lead to investigation for an electrophoretically silent variant haemoglobin that may be interacting with haemoglobin S [24].

Diagnosis

The diagnosis rests on the demonstration of the presence of haemoglobin S and haemoglobin A, with the percentage of haemoglobin S being less than the percentage of haemoglobin A. The haemoglobin S identification must be supported by two independent tests.

Interactions of haemoglobin S heterozygosity with thalassaemias and haemoglobinopathies

The interaction of sickle cell trait and α thalassaemia trait has been discussed above. The coexistence of sickle cell trait and the genotype of haemoglobin H disease leads to a modification of the phenotype of haemoglobin H disease. There is a hypochromic microcytic anaemia with splenomegaly and erythroid hyperplasia, but with a normal reticulocyte count [51]. Haemoglobin S is lower than is usual in sickle cell trait with coexisting α thalassaemia [25,51]. Haemoglobin Bart's may be present in infancy, but haemoglobin H is not detected and only occasional inclusion-containing cells are found on a haemoglobin H preparation. Inclusions have been reported in bone marrow erythroblasts. It could be speculated that these represent β^S precipitates, β^A having combined preferentially with the reduced numbers of α chains.

The coinheritance of β^S and various β chain variants, β and δβ thalassaemias is discussed below. The interaction of sickle cell trait and α chain variants is generally clinically silent, but leads to extra bands on haemoglobin electrophoresis. For example, coinheritance of sickle cell trait and $\alpha^{G\text{-Philadelphia}}$ is associated, on electrophoresis at alkaline pH, with the presence of three bands with the mobility of haemoglobins A, S (representing S and G-Philadelphia) and

C (representing an S–G hybrid) (Fig. 4.6). On agarose gel at acid pH, there are two bands, a band with the mobility of haemoglobin A (representing A plus G-Philadelphia) and a band with the mobility of haemoglobin S (representing S and S–G hybrid). On HPLC, there are four fractions distinguished by their retention times (Fig. 4.7).

A β thalassaemia mutation occurring in *cis* to a β^S mutation differs considerably from sickle cell trait. Two individuals with this combination had haemoglobin S of 10–11%, haemoglobin A_2 of 6–7%, haemoglobin F of around 3% and a mild microcytic anaemia with a reticulocyte count of around 3% [46,52].

Interactions of haemoglobin S heterozygosity with other haematological conditions

The coexistence of sickle cell trait and hereditary spherocytosis has been reported in 19 instances. Four of these individuals suffered either splenic sequestration or splenic infarction [53]. It is likely that the increased haemoglobin concentration within red cells as a result of the hereditary spherocytosis favours sickling within the spleen.

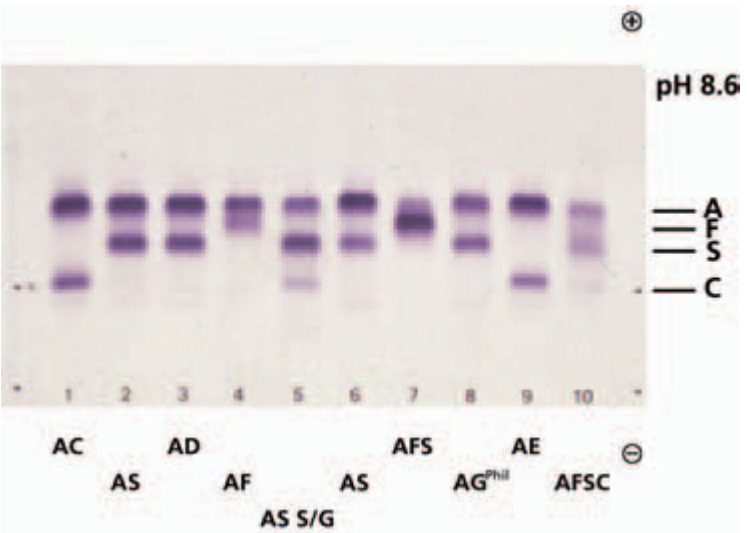


Fig. 4.6 Haemoglobin electrophoresis on agarose gel at pH 8.6 showing three bands with the mobilities of haemoglobins A, S and C in a patient with sickle cell trait and heterozygosity for haemoglobin G-Philadelphia (lane 5); AFSC, control sample containing haemoglobins A, F, S and C.

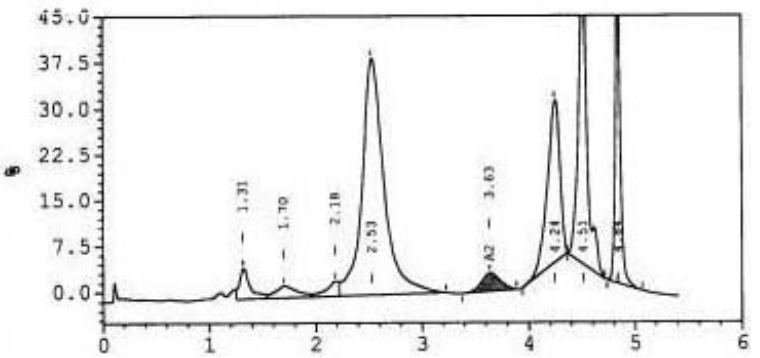


Fig. 4.7 HPLC chromatogram in a patient with heterozygosity for both haemoglobin S and haemoglobin G-Philadelphia; from left to right, the peaks are post-translationally modified haemoglobin A (two peaks) and haemoglobins A, A_2, S, G-Philadelphia and hybrid ($\alpha\alpha^G\beta\beta^S$).

Sickle cell anaemia

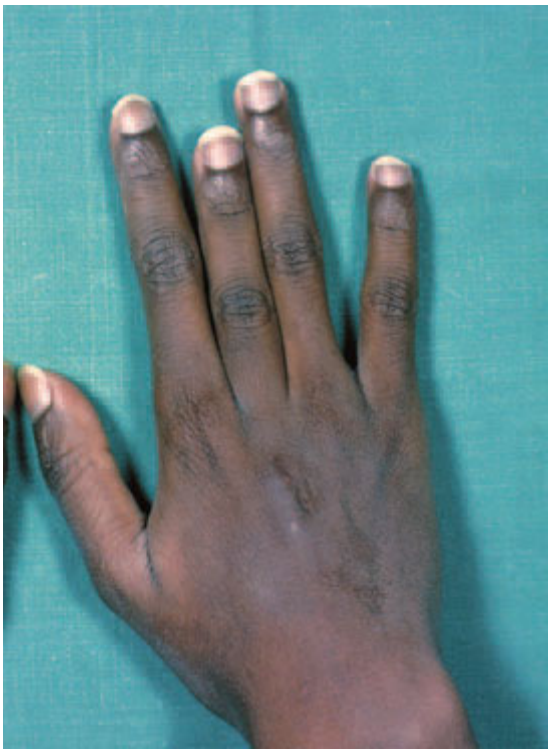
Sickle cell anaemia is the disease resulting from homozygosity for haemoglobin S. Individuals with sickle cell anaemia have haemoglobin S as the major haemoglobin component with a small proportion of haemoglobin A_2 and a variable proportion of haemoglobin F. As there is no synthesis of normal β chain, there is a total absence of haemoglobin A. Red cells sickle due to polymerization of haemoglobin S under conditions of low oxygen tension, but initially this process is reversible as the cells pass through the lungs. This process is cyclical, but eventually membrane damage leads to the red cell becoming irreversibly sickled. The irreversibly sickled cell has an increased calcium content, which triggers calcium-dependent potassium transport and loss of potassium and water. Potassium/chloride (K^+/Cl^-) cotransport is also increased. The dehydrated cell becomes even more rigid. The red cell membrane is damaged by oxidation and the effects of repeated polymerization with clustering of band 3 protein, binding of immunoglobulin G and phagocytosis by macrophages. Sickle cells also show increased interaction with endothelium, particularly when adhesion molecules are upregulated.

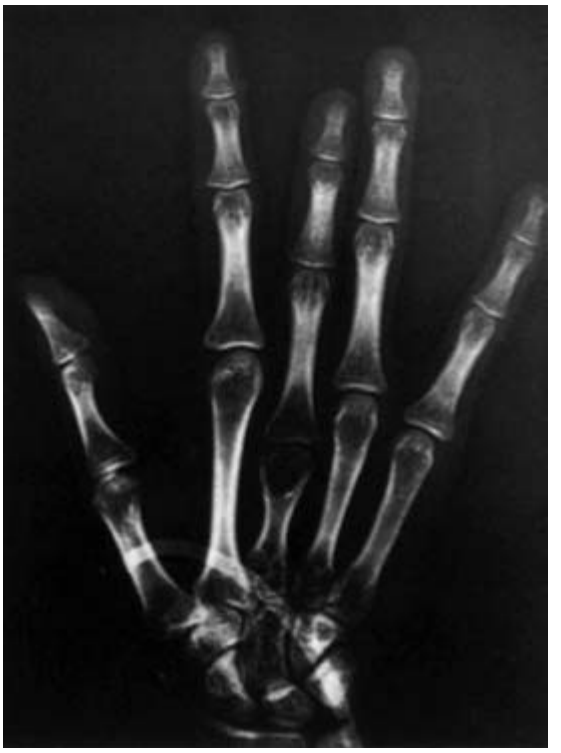
Clinical features

The clinicopathological features of sickle cell anaemia result directly or indirectly from vascular obstruction by sickled red cells, with consequent tissue infarction. In addition to the shape change, erythrocytes show increased adhesion to endothelium, which contributes to vascular occlusion. Neonates are asymptomatic as a major part of the total haemoglobin is haemoglobin F. As the synthesis of haemoglobin F decreases and that of haemoglobin S increases, symptoms start to appear, usually from 6 months of age. In infants, bony infarction leads to avascular necrosis of the small bones of the hands and feet which presents clinically as painful swelling of the fingers and toes (dactylitis or 'hand–foot syndrome'). This can lead to failure of growth of a phalanx and later shortening of a digit (Fig. 4.8). In children, there may be splenomegaly and, occasionally, hypersplenism. Young children can also suffer from splenic sequestration in which pooling of red cells in a rapidly enlarging spleen leads to acute anaemia. Hepatic sequestration also occurs, but is less common. Cerebral haemorrhage and infarction are particular features of children with sickle cell disease, as a result of prior endothelial damage.

In older children and adults, there continues to be infarction of bones, such as the ribs, vertebrae and long bones; osteonecrosis can be detected radiologically (Fig. 4.9a). In addition, there is infarction of internal organs, including the lungs (Fig. 4.9b), abdominal organs and brain. Pulmonary infarction can be associated with pulmonary sequestration of red cells and platelets and, if recurrent, leads to pulmonary hypertension in a significant minority of patients; inactivation of nitric oxide by reactive oxygen species and by free haemoglobin in the plasma may contribute to pulmonary hypertension [54]. Bone marrow infarction may be extensive and may be complicated by embolism of necrotic bone marrow to the lungs. Infarction of bones may also be complicated by osteomyelitis, usually caused by salmonella or staphylococcus. Recurrent infarction of the spleen leads to hyposplenism, which, in turn, causes increased severity of various infections, including malaria and pneumococcal septicaemia. Splenic phagocytic function is lost first and then splenic filtering function [55]. Infarction of the skin can result in ulceration of the legs. The increased breakdown of red cells means that patients are intermittently jaundiced (Fig. 4.10). There is a high incidence of pigment gallstones (Fig. 4.11) consequent on this chronic haemolysis. Increased erythropoiesis occurs as a response to haemolytic anaemia, leading to overexpansion of the bone marrow cavity. In some patients, this causes frontal bossing of the skull and malpositioned teeth. On skull radiology, there may be thickening of the cranial bones (Fig. 4.12) and a 'hair-on-end' appearance. Patients with sickle cell anaemia may suffer rapid worsening of anaemia during infection by parvovirus B19. The mechanism is pure red cell aplasia, which is transient but, because of the shortened red cell life span, rapidly leads to anaemia. In some countries, patients with sickle cell anaemia show an increased incidence of megaloblastic anaemia, which has been attributed to inadequate intake of folic acid in the face of an increased need for this vitamin.

(a)

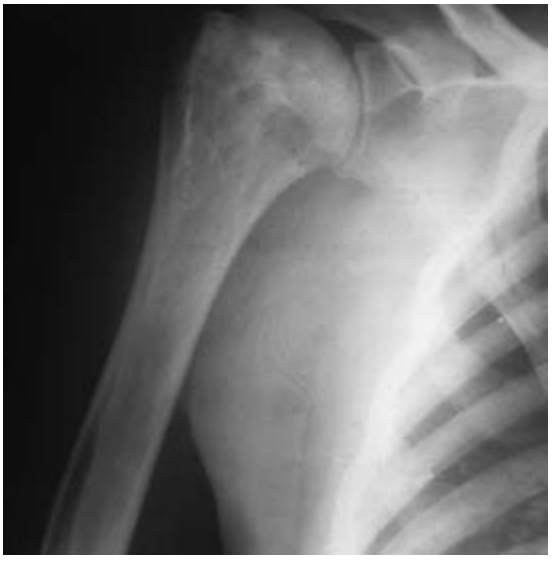


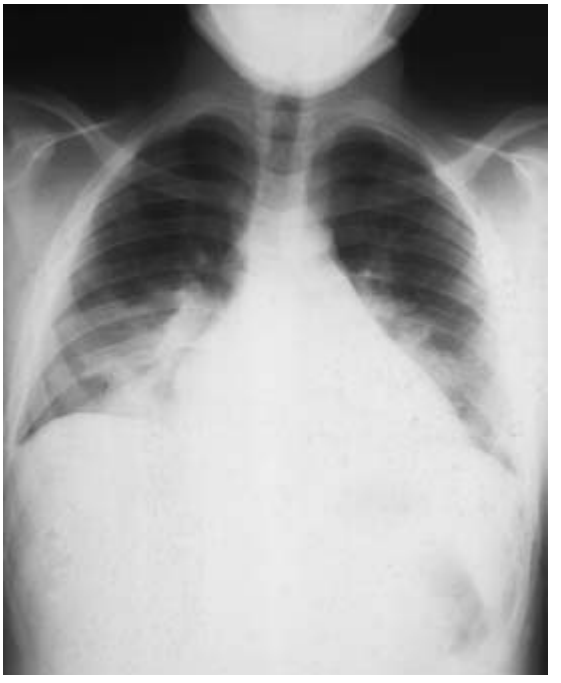


(b)

Fig. 4.8 Long-term result of 'dactylitis' in sickle cell anaemia: (a) the hand of an 18-year-old Nigerian man; (b) X-ray of the hand. (Reproduced from Hoffbrand AV and Pettit JE. *Essential Haematology*, 3rd edn. Blackwell Scientific Publications, Oxford, 1993, by kind permission of Professor A. V. Hoffbrand.)

(a)





(b)

Fig. 4.9 (a) Radiograph of the head of the humerus showing areas of reduced radiodensity consequent on previous infarction. (By courtesy of Professor I. Roberts.) (b) Chest radiograph showing opacities in the lower half of both lung fields representing pulmonary infarction as a result of sickle cell formation and vascular occlusion. (By courtesy of Professor I. Roberts.)

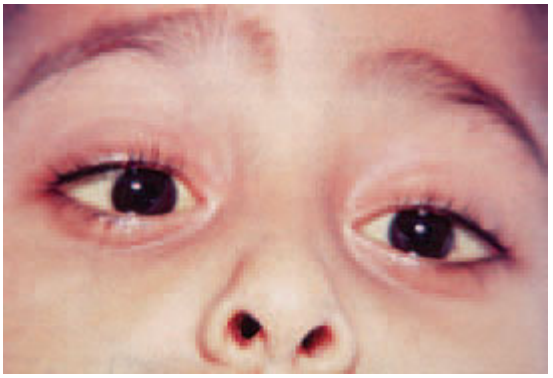


Fig. 4.10 Face of a child with sickle cell anaemia showing pallor and jaundice. (By courtesy of Professor I. Roberts.)

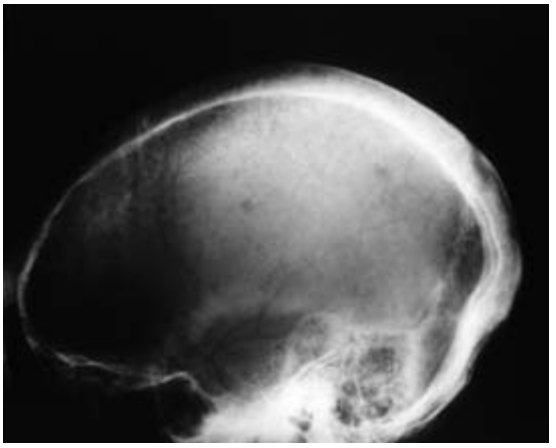


Fig. 4.12 Skull radiograph in sickle cell anaemia showing expansion of the bony cavity resulting from hyperplastic erythropoiesis.

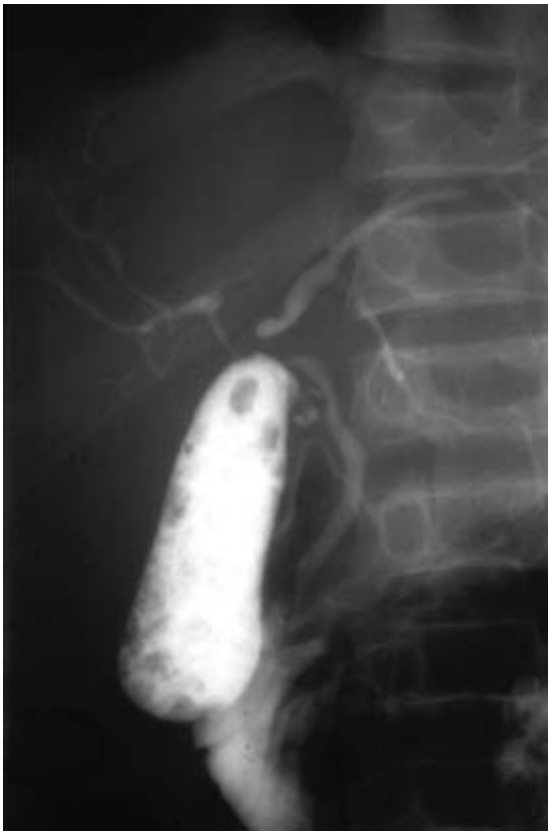


Fig. 4.11 Cholecystogram showing gallstones (negative images) caused by increased bilirubin production resulting from haemolysis. (By courtesy of Professor I. Roberts.)

Patients who require regular or intermittent blood transfusions often develop red cell alloantibodies. If a delayed transfusion reaction occurs, the haemoglobin concentration may fall rapidly to levels below the pre-transfusion level. This is due mainly to the destruction of transfused red cells while haemopoiesis is suppressed, but, in some patients, there is also 'bystander' destruction of the patient's own red cells [56]. In addition, transfusion may be followed by hyperhaemolysis without any evidence of red cell incompatibility [57].

The causes of anaemia in homozygotes for haemoglobin S are summarized in Table 4.5.

Death in sickle cell anaemia is most often attributable to infection, cerebrovascular accidents or respiratory failure, the latter consequent on extensive sickling within pulmonary blood vessels. In the absence of parental education and vigilance, death of infants can result from splenic sequestration. In African countries, it is likely that many deaths in infants and children with sickle cell anaemia are attributable to malaria and some to severe anaemia. Patients with sickle cell anaemia who survive childhood and adolescence may die from end-organ failure consequent on recurrent tissue infarction, e.g. from renal or hepatic failure.

The survival of individuals with sickle cell anaemia has improved greatly in recent decades. In one study in the USA, the median life expectancy was

Table 4.5 Causes of anaemia in sickle cell anaemia.

Causes of steady state anaemia
Haemolysis
Reduced oxygen affinity leading to reduced erythropoietic drive
Causes of worsening of anaemia
Splenic, hepatic or pulmonary sequestration
Hypersplenism (usually only in infants and children)
Parvovirus B19 infection
Suppression of erythropoiesis in other infections
Megaloblastic anaemia resulting from folic acid deficiency
Bone marrow infarction
Hyperhaemolysis following blood transfusion
Renal failure

Table 4.6 Factors ameliorating sickle cell anaemia or some features of the disease.

Coinheritance of hereditary persistence of fetal haemoglobin, or other factors either linked or unlinked to the β globin locus, leading to a high percentage of haemoglobin F; the haemoglobin F level is highest in the Arab–Indian and Senegal haplotypes, lowest in the Central African Republic haplotype and intermediate in the Benin haplotype
Coinheritance of certain α chain variant haemoglobins, e.g. haemoglobin Memphis or haemoglobin Hopkins II
Coinheritance of α thalassaemia trait — ameliorates haemolysis [61], ameliorates soft tissue end-organ damage [4], reduces leg ulcers [64], reduces the frequency of stroke [60,64] and is associated with longer preservation of splenic function [55]; however, does not ameliorate painful crises [61] and possibly increases their frequency [60], increases the frequency of retinopathy [64] and aggravates osteonecrosis [4,64]; does not improve survival [58]
Iron deficiency (ameliorates haemolysis) [62]

42 years for men and 48 years for women [58], and in another in Jamaica it was 58 years for men and 66 years for women [59]. Despite the severity of the disease in many patients, there are a significant minority who are asymptomatic for prolonged periods. For example, in one French study of 299 patients, 9% were asymptomatic for 3 years or more [60].

Ameliorating factors and interaction with α thalassaemia trait

The clinical course of sickle cell anaemia is very variable. This is largely unexplained, although some factors have been identified that appear to ameliorate the condition and lead to later presentation, milder symptoms and a better life expectancy. Some of these are shown in Table 4.6 [4,55,58,60–64]. Elevation of haemoglobin F to more than 20% is usually sufficient to render sickle cell anaemia largely asymptomatic. Coexisting α thalassaemia trait is common in sickle cell disease. For example, 30% of Afro-Americans with homozygosity for haemoglobin S have a single α gene deletion and 5% have two α genes deleted [63]. The effect of coexisting α thalassaemia trait is complex, with some features being ameliorated and others being worsened. In a study of sickle cell anaemia associated with the Arab–Indian haplotype, a tribal Indian group with a very high incidence of α thalassaemia trait had significantly fewer painful crises, infections and episodes of hospitalization than a non-tribal group with a much lower incidence of α thalassaemia trait [65]. In another study, deletion of two α genes was associated with an increased prevalence of avascular necrosis, retinopathy and splenomegaly and a decreased prevalence of leg ulcers and cerebrovascular accidents [64]. For the effects demonstrated in other studies, see Table 4.6. The complex effects of the presence of α thalassaemia in patients with sickle cell anaemia may be the result of two conflicting factors: (i) reduced polymerization, leading to less membrane damage, fewer dehydrated and irreversibly sickled cells and improved red cell survival; and (ii) higher haemoglobin concentration, leading to increased blood viscosity. In a large study of sickle cell anaemia in a population with the β^S gene associated with a variety of haplotypes, overall life expectancy was not altered by coexisting α thalassaemia [58]; presumably, therefore, the beneficial and adverse effects of coexisting thalassaemia trait balance out.

Laboratory features

Blood count

The blood count is normal at birth. During the first year, as haemoglobin F is replaced by haemoglobin S,

there is a fall in haemoglobin concentration and a rise in the reticulocyte count. Mean values differ from controls by 1–2 months of age [66,67]. Anaemia and reticulocytosis continue throughout childhood, adolescence and adult life. The haemoglobin concentration reported in adults is most often between 6 and 10 g/dl, but can range from 5 to 12 g/dl or even higher. In a personally observed series of 29 mainly Afro-Caribbean patients, the haemoglobin concentration ranged from 7.6 to 13.8 g/dl, with a mean of 9 g/dl. In males, there is a significant post-pubertal rise in the haemoglobin concentration, averaging between 1 and 2 g/dl [9]. Patients with a higher percentage of haemoglobin F tend to have a higher haemoglobin concentration [9]. The haemoglobin concentration is of some prognostic significance [58]. During complications such as splenic sequestration, parvovirus infection or megaloblastic anaemia, the haemoglobin concentration may fall to as low as 1.5–3 g/dl. Bacterial infection is also associated with some worsening of the anaemia. In older patients with sickle cell anaemia, a slow fall in the haemoglobin concentration without any alteration in the red cell indices may be found to be consequent on the onset of renal failure. Although the reticulocyte count is elevated in sickle cell anaemia, usually to 5–20%, it is not increased in proportion to the reduction in haemoglobin concentration. This is because haemoglobin S has a lower oxygen affinity than haemoglobin A and the drive to erythropoiesis is therefore less than would be anticipated from the haemoglobin concentration. For the same reason, the serum erythropoietin concentration is lower than would be expected for the degree of anaemia [68]. In patients with no associated α thalassaemia, the red cell indices are normal [38, 69]. However, the MCV and MCH are not elevated in keeping with the reticulocyte count, suggesting a relative microcytosis. The MCV tends to be higher in those with a higher haemoglobin F percentage [9]. The mean cell haemoglobin concentration (MCHC) may be slightly increased and the proportion of cells with a high haemoglobin concentration is increased (Fig. 4.13). The red cell distribution width (RDW) is generally markedly increased and correlates with

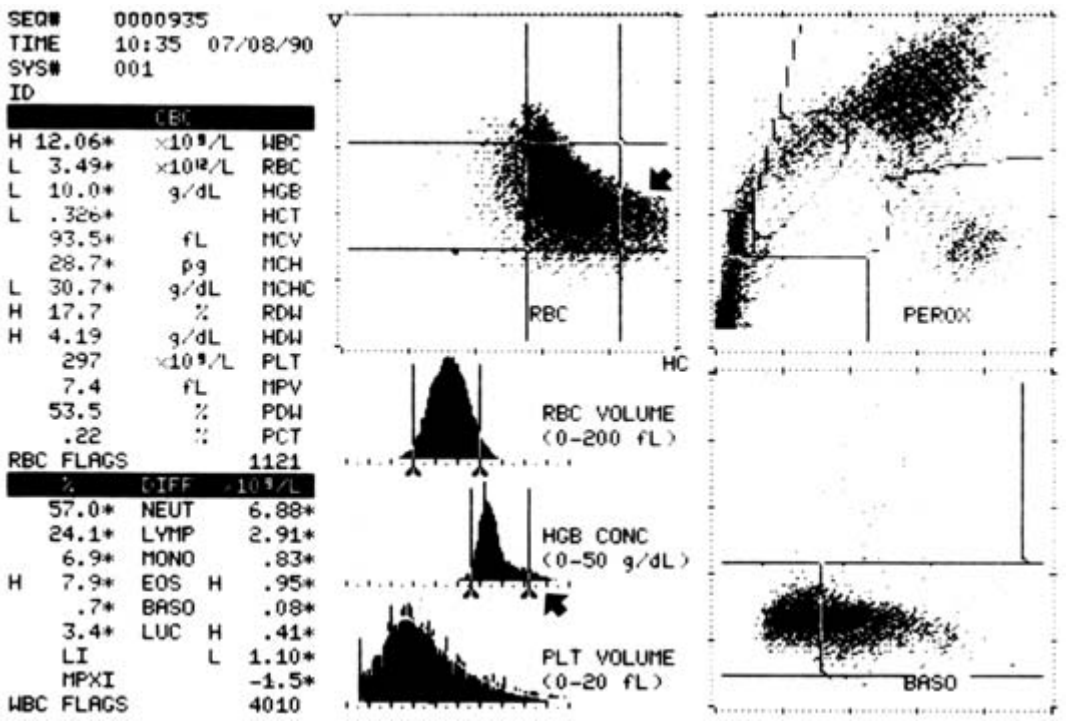


Fig. 4.13 Red cell cytogram and histograms showing an increase in hyperchromic cells (arrows), representing irreversibly sickled cells, and increased hypochromic macrocytes, representing reticulocytes.

disease severity [70]. The total nucleated cell count may be increased as a consequence of significant numbers of circulating erythroblasts. The neutrophil count may be increased between as well as during crises. The baseline white blood cell count (WBC) has been found to correlate with the frequency of acute chest syndrome [71] and to be predictive of earlier death from sickle cell disease [58]. The monocyte count and the lymphocyte count are also increased [72], the latter possibly as a feature of hyposplenism. The platelet count is increased and there is an increased proportion of large platelets. Both of these features are attributable to hyposplenism.

Patients with sickle cell disease who also have α thalassaemia trait have a lower MCV, MCH and MCHC than those with four α genes [38]. The haemoglobin concentration is, on average, 1–2 g/dl higher and the reticulocyte count is lower [40,73]. The percentage of hyperdense cells is reduced. Patients with sickle cell anaemia with a high haemoglobin F percentage tend to have a higher haemoglobin concentration and MCV and a lower percentage of hyperdense cells. Those with the highest haemoglobin F levels, e.g. patients with the Saudi/Indian haplotype, also have a lower reticulocyte count.

Coexisting iron deficiency leads to a lower haemoglobin concentration, MCV, MCH and MCHC. There is an associated amelioration of haemolysis [62]. Patients who are maintained on folic acid have an MCV, on average, 4 fl lower than patients not so maintained [74].

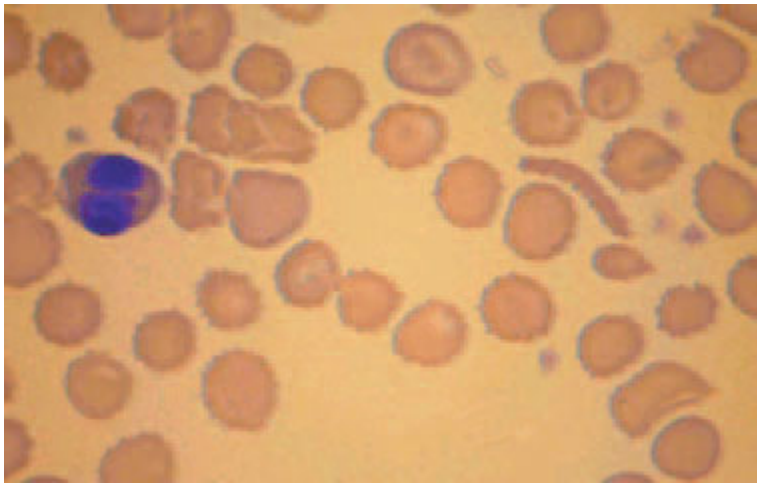
Changes occur in the blood count quite early in sickle cell crisis. There is a fall in the haemoglobin concentration, a rise in the reticulocyte percentage and a rise in the MCHC, RDW, haemoglobin distribution width (HDW) and percentage of hyperdense cells [75]. The HDW is a measurement of the variation in haemoglobin concentration between individual red cells; its increase is a reflection of the increased number of hyperdense cells. Later in a crisis, there is a return of the RDW, HDW and percentage of hyperdense cells towards baseline values; the percentage of hyperdense cells may fall below baseline values, probably because the densest, least deformable cells are being preferentially trapped in the spleen and destroyed. The WBC and the neutrophil count increase during painful crises and the platelet count may also increase. When a sickle cell crisis is complicated by an acute chest syndrome caused by pulmonary fat embolism, there is leucocytosis and usually a marked fall in haemoglobin concentration and platelet count [76]. Irregularly contracted cells may appear in quite significant numbers.

Patients whose sickle cell anaemia is treated with hydroxycarbamide, with a consequent increase in the haemoglobin F percentage, show characteristic changes in the haemoglobin concentration and red cell indices. The haemoglobin concentration and the MCV rise, while the MCHC, percentage of dense cells and reticulocyte count fall. The WBC, neutrophil count and platelet count may fall as a consequence of the cytotoxic effect of hydroxycarbamide.

Blood film

The blood film is usually normal at birth and in the early neonatal period as the haemoglobin S percentage is relatively low, but this is not necessarily so (Fig. 4.14). Abnormalities are usually detectable around 6 months of age (Fig. 4.15) when occasional sickle cells, target cells and Howell–Jolly bodies start to appear [66]. The majority of infants have features of hyposplenism by 1 year of age [66] and circulating erythroblasts, sickle cells and Howell–Jolly bodies are much more common thereafter. In an adult with sickle cell disease, the blood film shows a variable number of crescent or sickle-shaped sickle cells (Fig. 4.16a). These represent irreversibly sickled cells, which have not corrected their shape on exposure to atmospheric oxygen. The number of sickle cells is very variable, ranging from only occasional cells to 30–40%. They are less numerous in those with a lower MCHC [9]. In addition to classical sickle cells, there are elongated cells pointed at one or both ends (Fig. 4.16b) [77]; these have been referred to as boat-shaped or oat-shaped cells or as plump sickle cells. There is polychromasia and, in some patients, microcytosis and hypochromia. Small numbers of irregularly contracted cells may be seen (Fig. 4.16c) and sometimes there are cells in which the haemoglobin appears to have retracted into one half of the cell ('hemi-ghosts' or 'blister cells'); both of these features are particularly common in patients with widespread pulmonary infarction and hypoxia; these abnormal red cells have increased density; their formation has been attributed to oxidant damage

(a)



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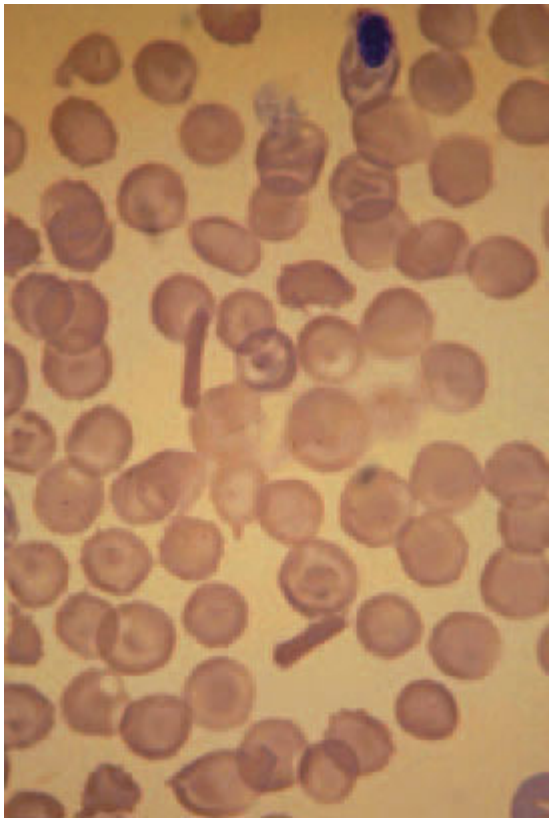


Fig. 4.14 Blood film of a neonate with sickle cell anaemia showing one sickle cell (a) and other poikilocytes consistent with reversibly sickled cells (b).

leading to transcellular bonding of damaged regions of the red cell membrane with trapping of haemoglobin within pseudovacuoles [78]. Linear red cell fragments may be present; these were first described in a patient with cold agglutinins and a positive antiglobulin test [79], but in fact they are not rare if specifically looked for. There are features of hyposplenism (Fig. 4.16d), specifically Howell–Jolly bodies, target cells, Pappenheimer bodies, an increased platelet count, increased platelet anisocyto-

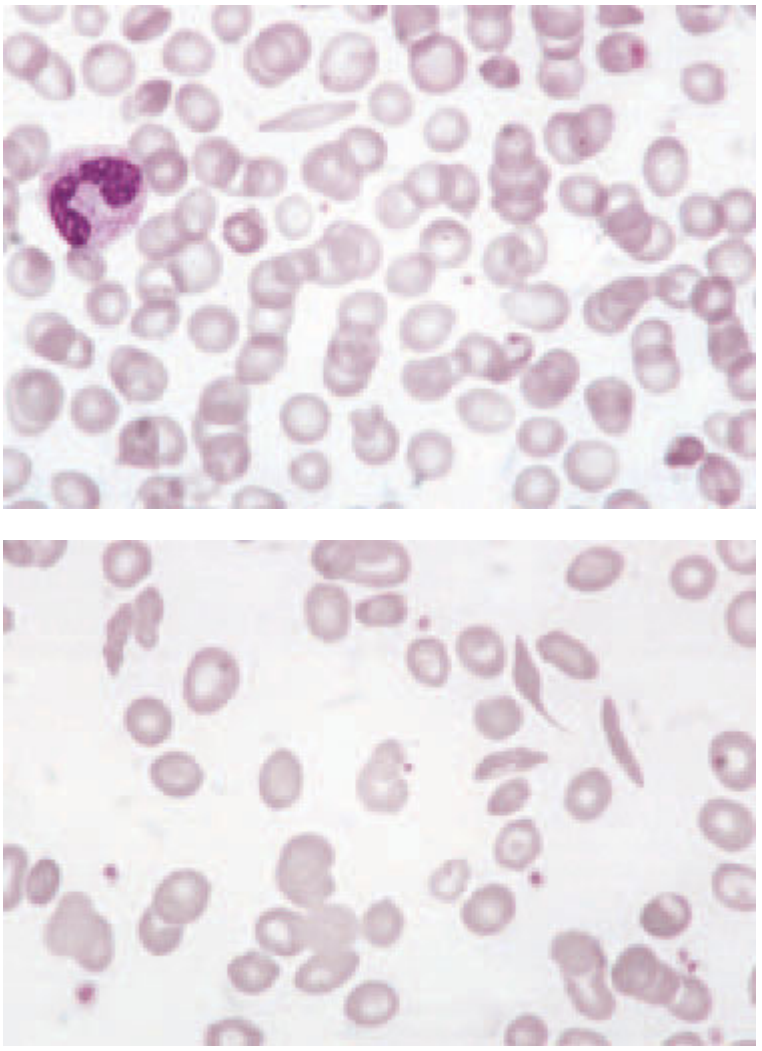


Fig. 4.15 Blood films of a child with sickle cell anaemia: (a) at the age of 5 months showing mild anisopoikilocytosis and one sickle cell; (b) at the age of 13 years showing more marked anisocytosis and sickle cell formation.

sis and sometimes an increased lymphocyte count. Acanthocytes, which are usually present in small numbers in hyposplenic individuals, are not usually a feature of hyposplenism in sickle cell disease. There are variable numbers of nucleated red cells. The neutrophil count may be increased. Phagocytosis of erythrocytes by monocytes or neutrophils may be observed, but is quite uncommon.

In patients with sickle cell anaemia with a high haemoglobin F, the abnormalities in the blood film are much less (Fig. 4.17). Sickle cells are less frequent and polychromasia and anaemia are less. The onset of features of hyposplenism is delayed. Coexisting α thalassaemia trait has also been observed to protect against the loss of splenic function [55,80], although, surprisingly, hyposplenism was not found to be related to age or haemoglobin F concentration [80]. Coexisting α thalassaemia trait (particularly homozygous α^+ thalassaemia trait) is associated with a blood film showing more target cells but fewer sickle cells [9].

Therapy with hydroxycarbamide leads to macrocytosis, a reduction in the number of sickle cells and boat-shaped cells and lessening of polychromasia.

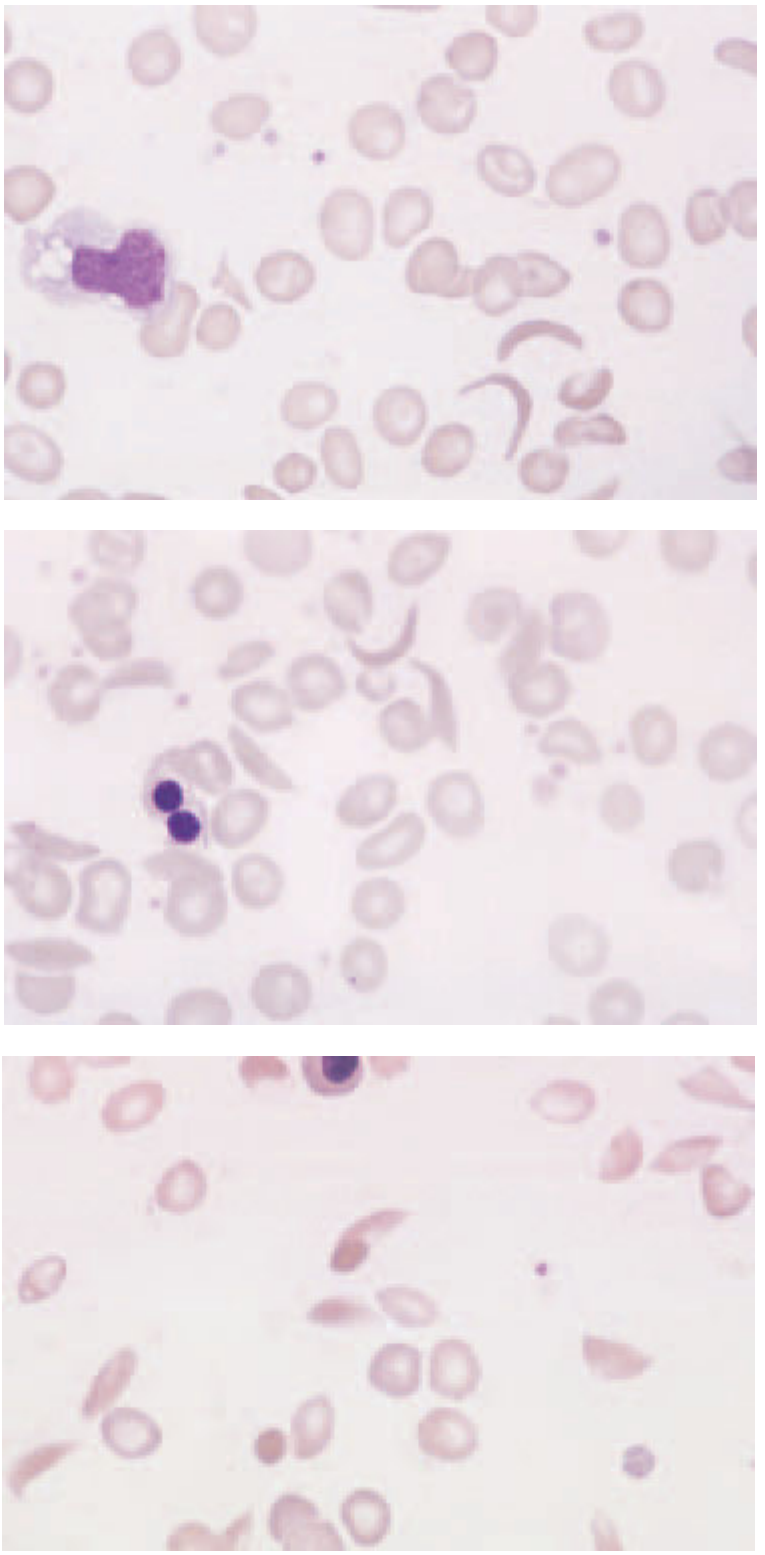


Fig. 4.16 Blood films of four patients with sickle cell anaemia showing the range of abnormality observed: (a) sickle cells and other poikilocytes; (b) sickle cells, a boat-shaped cell and nucleated red blood cells; (c) blood film during sickle cell crisis with pulmonary infarction and severe hypoxia showing one sickle cell, irregularly contracted cells and a 'hemi-ghost'; (d) minimal sickling but features of hyposplenism—a Howell–Jolly body, a large platelet and a target cell.

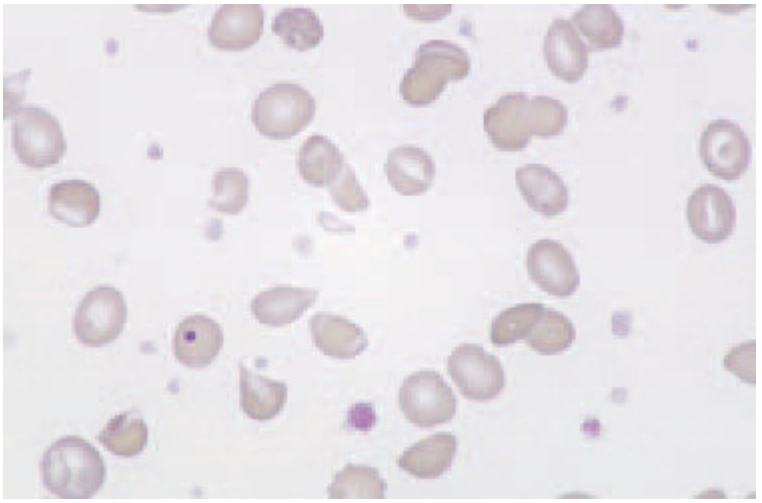


Fig. 4.16 *Continued.* (d)

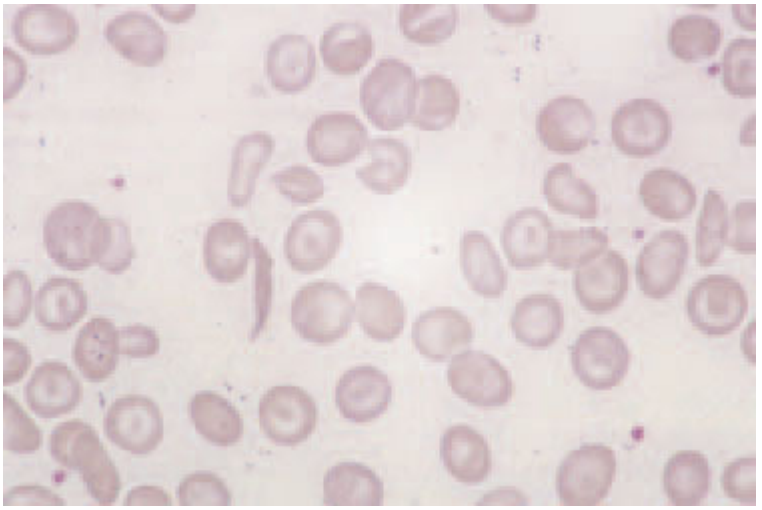


Fig. 4.17 Blood film from an Arab patient with a high haemoglobin F percentage and clinically mild disease; there were numerous target cells but only occasional sickle cells.

During sickle cell crisis, there is a slight worsening of anaemia. There may be a further elevation of the neutrophil count, left shift and an increase in the numbers of nucleated red blood cells. An increase in the number of sickle cells, in comparison with the same individual's blood film when not in crisis, has been observed, but not all investigators confirm this observation [81]. An increase in the number of spiculated or echinocytic sickle cells has also been noted [81]. Irregularly contracted cells and 'hemi-ghosts' can increase in number, particularly in those with severe hypoxia and extensive sickling within the pulmonary vasculature (Fig. 4.16c). In one scanning electron microscopy study, sickle cell crisis was associated with the presence of echinocytes, echinocytic sickle cells, 'blister cells' and macrocytes, but there was no increase in the number of non-echinocytic irreversibly sickled cells [82]. When sickle cell crisis is complicated by extensive bone marrow infarction, there is a greater fall in the haemoglobin concentration and platelet count, together with the appearance of increasing numbers of nucleated red cells.

Various other complications of sickle cell anaemia may be apparent from the full blood count (FBC) and the blood film. Parvovirus B19 infection may be suspected when there is worsening anaemia with a lack of polychromasia. The platelet count is also often reduced. When recovery occurs, there is initially the appearance of numerous nucleated red cells in the peripheral blood, followed by reticulocytosis and thrombocytosis. In splenic sequestration, there is an acute fall in the haemoglobin concentration with reticulocytosis and increasing numbers of nucleated red blood cells. The platelet count may also be reduced. In chronic hypersplenism, there is worsening anaemia, thrombocytopenia and reticulocytosis. When there is complicating megaloblastic anaemia, there may be macrocytes, oval macrocytes and hypersegmented neutrophils; polychromasia is less than would otherwise be expected in a patient with sickle cell anaemia. Patients with coexisting sickle cell anaemia and homozygosity for α^+ thalassaemia who develop megaloblastic anaemia may show an increase in the MCV and MCH, but with both values remaining within the normal range rather than exceeding it. Because of the shortened red cell life span, megaloblastic anaemia may also have an acute onset with pancytopenia and a rapidly falling haemoglobin concentration without macrocytosis. In patients with a delayed transfusion reaction, some spherocytes are seen, but it can be difficult to recognize the morphological features of a transfusion reaction in a patient with sickle cell anaemia. The direct antiglobulin test is positive. Patients with sickle cell disease may develop hyperhaemolysis following blood transfusion with both homologous and autologous cells being destroyed. During intercurrent infections, the patient with sickle cell anaemia is likely to show neutrophilia, left shift, toxic granulation and sometimes an increase in the platelet count; rarely organisms, e.g. pneumococci, are found within neutrophils.

Other investigations

In the adult, haemoglobin electrophoresis and HPLC show haemoglobins S, F and A_2 (Fig. 4.18). Haemoglobin S is the major haemoglobin present, usually comprising 90–95% of total haemoglobin. Haemoglobin A is totally absent. Haemoglobin A_2 may be present in normal amounts or may be slightly elevated (usually 2–4%) [9,38]. Higher percentages are seen in those who also have α thalassaemia trait, although there is considerable variation in the mean levels reported in different series of patients (Table 4.7) [9,50,63,83,84]. Haemoglobin F is usually only slightly elevated (see below). Inevitably, the percentage of haemoglobin S correlates inversely with the percentage of haemoglobin F. The percentage of haemoglobin S also varies with the number of α genes and with the MCV and MCH. Looked at in another way, the coexistence of α thalassaemia trait with sickle cell anaemia leads to a reduction in the MCV and MCH and a slight reduction in the haemoglobin S percentage.

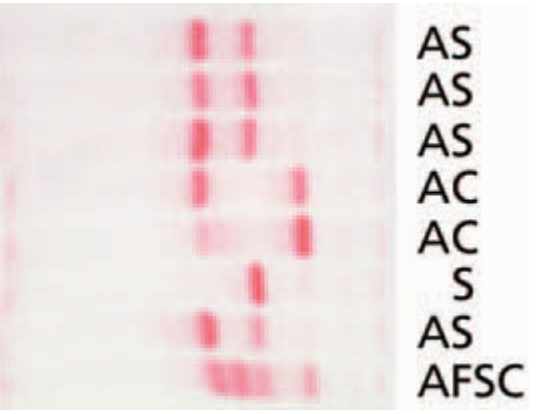


Fig. 4.18 Haemoglobin electrophoresis on cellulose acetate at alkaline pH showing a patient with sickle cell anaemia (third lane from bottom) with almost all the haemoglobin being haemoglobin S; AFSC, control sample containing haemoglobins A, F, S and C; AS, sickle cell trait; AC, haemoglobin C trait.

Neonates with sickle cell anaemia usually have predominantly haemoglobin F with haemoglobin S comprising only a low percentage of total haemoglobin (Fig. 4.19). Sometimes only haemoglobin F is present and repeat testing when the baby is a few months of age is then necessary for diagnosis. In the neonatal period, diagnostic confusion can occur not only with sickle cell/β^0 thalassaemia, but also with sickle cell/β^+ thalassaemia [85] (see below). The postnatal fall in haemoglobin F is slower in babies with sickle cell anaemia than in normal babies, with mean levels of about 20% at 1 year of age [66].

Table 4.7 Haematological characteristics and percentages of various haemoglobins in adults with sickle cell anaemia and other conditions with haemoglobins S, F and A_2 only. (Derived from references [9,50,63,83,84] and other sources.)

Genotype	Usual Hb (g/dl)	Usual MCV (fl)	Usual reticulocyte count (%)	Usual haemoglobin F (%)	Usual haemoglobin A_2 (%)
SS	6–10	70–100	5–20	Usually 5–10 but up to 40*	1.6–3.6† (occasionally up to 5)‡
$S\beta^0$	7–11	60–80	8–9	5–15	4–5.6†
$S/\delta\beta^0$	10–12	76–83	2–4	15–25	1.9–2.3
S/HPFH	>12	68–88§	Normal	20–30	1.1–2.2 in the majority

Hb, haemoglobin concentration; HPFH, hereditary persistence of fetal haemoglobin; MCV, mean cell volume.
* Influenced by coinheritance of non-deletional HPFH as well as by the haplotype associated with the β^S gene [83,84]: Arab–Indian haplotype, 10–25% haemoglobin F; Senegal haplotype, 7–10% haemoglobin F; Benin or Bantu haplotype, 6–7% haemoglobin F; Cameroon haplotype, 5–6% haemoglobin F. Mean ± standard deviation of 6.06 ± 4.23% for 120 SS adults in the UK [50].
† Some overlap occurs, particularly when coexisting homozygous α^+ thalassaemia raises the A_2 percentage in cases of SS [9]; in one series, the reported mean haemoglobin A_2 levels were 2.8% with four α genes, 3.3% with three α genes and 3.8% with two α genes [83]; in another series, the reported levels were higher — 3.5%, 3.7% and 4.9%, respectively [63].
‡ High levels are characteristically seen in the Arab–Indian mutation.
§ Normal if there is no coexisting α thalassaemia trait.

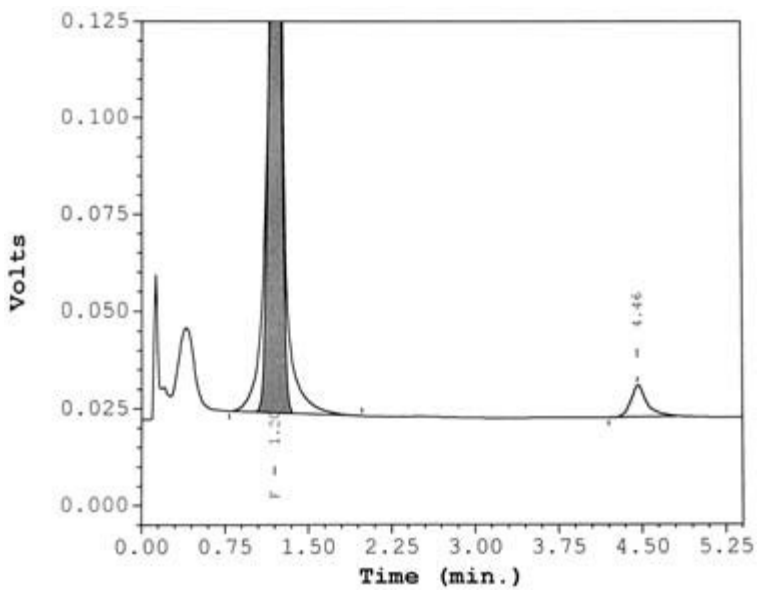


Fig. 4.19 HPLC chromatogram in a neonate with sickle cell anaemia showing mainly haemoglobin F, total absence of haemoglobin A and presence of haemoglobin S; haemoglobin S was 5.5% and the retention time was 4.46 min; the peaks on the left of the chromatogram represent altered F.

The sickle solubility test is positive and immunoassays [86] demonstrate the presence of haemoglobin S with no haemoglobin A.

The oxygen dissociation curve shows reduced oxygen affinity, i.e. a right-shifted curve and an increased P_{50} (the P_{O_2} at which haemoglobin is 50% saturated) [87]. The right shift is less in those with a high haemoglobin F percentage, either as a feature of the disease or as a consequence of hydroxycarbamide therapy. Resting arterial oxygen saturation when not in crisis is usually greater than 95%, but in patients with significant pulmonary damage may be reduced, e.g. to 80–95%.

Studies of globin chain synthesis show balanced synthesis of α and β^S globin chains unless there is coexisting α thalassaemia trait.

The bilirubin concentration is increased, the bilirubin being mainly unconjugated. Lactate dehydrogenase (LDH) is increased approximately two-fold. Hyperuricaemia is common. Serum haptoglobin is usually absent and Schumm's test for methaemalbumin may be positive. Red cell survival studies show a half-life of about 7–14 days, less if there is splenomegaly. Heterozygous α^+ thalassaemia is associated with longer red cell survival. Coexisting iron deficiency leads to a considerable improvement in red cell survival, associated with a fall in bilirubin concentration and LDH [62]. Hydroxycarbamide therapy also leads to improved red cell survival and reduced biochemical evidence of haemolysis [88].

A bone marrow aspirate shows erythroid hyperplasia and the presence of sickle cells (Fig. 4.20). Macrophages are increased and may contain sickled cells (Fig. 4.21). Foamy macrophages and sea-blue histiocytes may be increased. A trephine biopsy like-

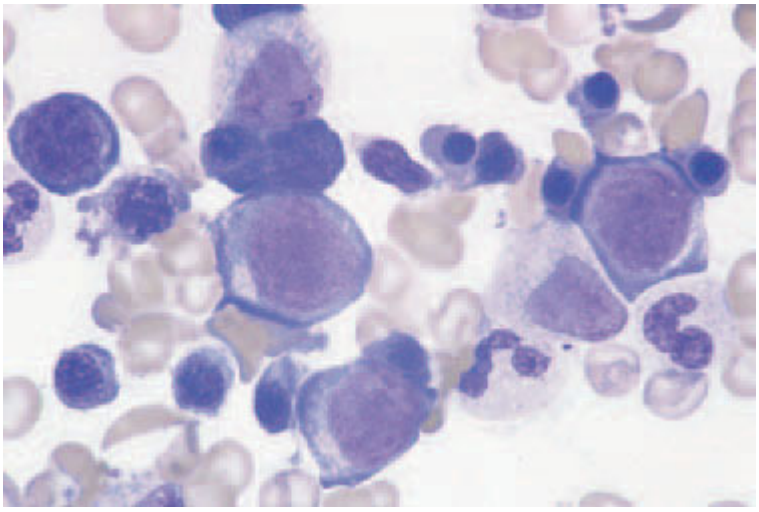


Fig. 4.20 Bone marrow aspirate in sickle cell anaemia showing erythroid hyperplasia and two sickle cells.

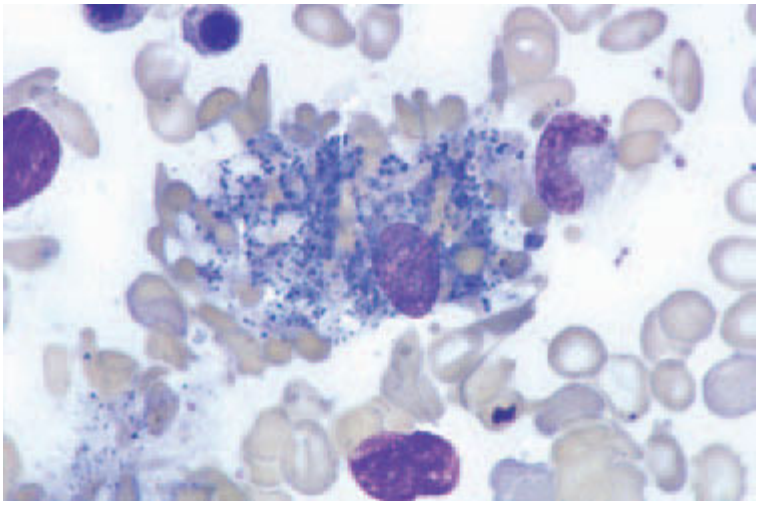


Fig. 4.21 Bone marrow aspirate in sickle cell anaemia showing a sea-blue histiocyte packed with sickle cells.

wise shows erythroid hyperplasia and sickle cells inside macrophages and within blood vessels (Fig. 4.22).

Haemoglobin F percentage

In sickle cell anaemia, haemoglobin F is usually around 5–10% but may be higher, sometimes comprising up to 40% of total haemoglobin (Table 4.7). The level is higher in infancy and tends to be higher in women than in men [70]. The switch from γ to β^S synthesis is delayed compared with the γ to β switch in normal subjects. The percentage of haemoglobin F falls most rapidly in the first 3 years of life and then more slowly until the age of 10 years; by this time, levels approximate those in adult life, although there may be a continued slow fall up to the age of 20 years. The percentage of haemoglobin F in an individual is determined by factors related and unrelated to the β globin gene cluster. There is a clear relationship to the haplotype of the chromosome carrying β^S. The Arab–Indian haplotype is usually associated with a haemoglobin F level of 10–25% [84]. The Senegal haplotype is also associated with a relatively high haemoglobin F level, e.g. 7–10% in adults, whereas the Bantu and Benin haplotypes have lower levels, in adults averaging around 6–7%, but with a wide range [83,84]. The Cameroon haplotype tends to be associated with the lowest haemoglobin F level, averaging 5–6% [83]. The relationship of haplotype to haemoglobin F percentage appears to result from an association between haplotype and determinants of non-deletional hereditary persistence of fetal haemoglobin. Both the Arab–Indian haplotype and the Senegal haplotype are linked with the common –158 $^G\gamma$ C→T polymorphism [89], whereas the Benin haplotype, which has a low percentage of haemoglobin F, is not linked with –158 $^G\gamma$ C→T. A polymorphism at $^A\gamma$ IVS2 has also been linked to the high haemoglobin F level observed when the β^S gene is associated with the Senegal and Arab–Indian haplotypes [90]. In addition, the Arab–Indian haplotype is associated with a polymorphism at –530 base pairs (bp), where there is $(AT)_9T_5$ rather than $(AT)_7T_7$, causing increased affinity for BP-1 (a negative *trans*-acting factor) and repression of β^S synthesis. The higher haemoglobin F in sickle cell anaemia associated with the Arab–Indian haplotype, in comparison with that in the Senegal haplotype, may be related to the combined effect of the $(AT)_x(T)_y$ polymorphism and the –158 $^G\gamma$ C→T and $^A\gamma$ IVS2 polymorphisms. The $^G\gamma$:$^A\gamma$ ratio is increased in individuals with a high haemoglobin F in association with the Saudi Arabian or Senegal haplotype [81]. The $^G\gamma$ promoter associated with the Bantu haplotype has been shown to be associated with low $^G\gamma$ synthesis [91].

The percentage of F cells (i.e. cells containing haemoglobin F) is increased in sickle cell anaemia. In

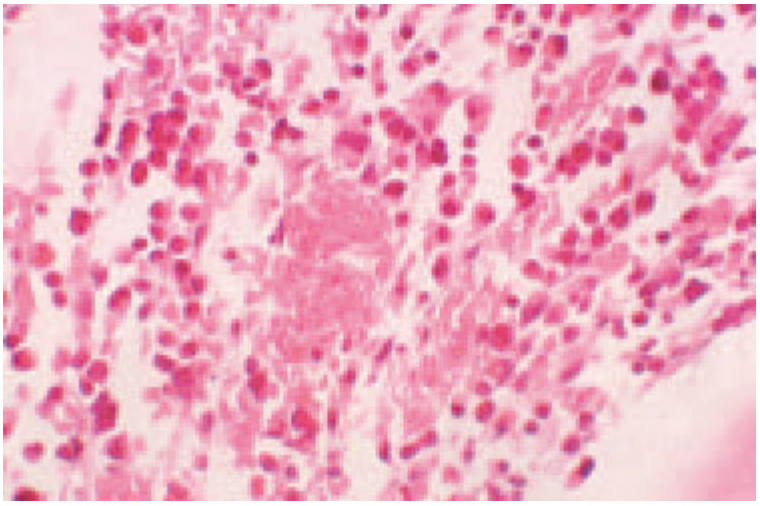


Fig. 4.22 Trephine biopsy in sickle cell anaemia showing erythroid hyperplasia and two vessels packed with sickle cells.

one study, the mean count was 55% (range 17–94%), the normal level being 0.5–7% [92]. The logarithm of the haemoglobin F concentration correlated with the percentage of F cells. In one study, the X-linked F-cell production locus was found to be the major determinant of haemoglobin F percentage in patients with sickle cell anaemia in association with the three major African haplotypes [93]. Factors linked to the β gene haplotype were next most important. The effect of the X-linked F-cell locus may be the reason why women with sickle cell anaemia, like haematologically normal women, tend to have a higher haemoglobin F level than men.

Individuals with coexisting α thalassaemia trait have been observed to have a significantly higher proportion of haemoglobin F in the first decade of life [73], but thereafter have a somewhat lower proportion, than those with four α genes [38,94].

The haemoglobin F percentage in sickle cell anaemia is of prognostic significance [58], the prognosis being more favourable when the percentage is high. The haemoglobin F percentage is increased 2–16-fold by hydroxycarbamide therapy [88].

Diagnosis

The diagnosis rests on the demonstration of haemoglobins S, F and A_2 only, with the presence of haemoglobin S as the sole variant haemoglobin being confirmed by at least two independent techniques. It is important not to misdiagnose compound heterozygous states for haemoglobin S and haemoglobins D, G, Korle Bu or Lepore as sickle cell anaemia. Recognizing the presence of haemoglobins D, G, Korle Bu or Lepore in compound heterozygous states with haemoglobin S is more complex than recognizing the simple heterozygous state, as all of these have a single band on cellulose acetate electrophoresis and a positive sickle solubility test (whereas the simple heterozygous state for any of these variant haemoglobins may simulate sickle cell trait on cellulose acetate electrophoresis at alkaline pH, but is easily distinguished as the sickle solubility test is negative). In patients with microcytosis or with a significant increase in haemoglobin F, the possibility of compound heterozygosity for haemoglobin S and β^0 or $\delta\beta^0$ thalassaemia or haemoglobin S and deletional hereditary persistence of fetal haemoglobin, respectively, must also be considered before a diagnosis of sickle cell anaemia is made.

Interactions of haemoglobin S homozygosity with other thalassaemias, haemoglobinopathies and other inherited erythrocyte abnormalities

The modification of sickle cell anaemia by coinheritance of α thalassaemia trait or non-deletional hereditary persistence of fetal haemoglobin has been discussed above.

The coinheritance of certain α chain variants, including haemoglobin Korle Bu, haemoglobin Memphis and haemoglobin Hopkins II, ameliorates sickle cell anaemia.

The coinheritance of other α chain variants, e.g. haemoglobin G-Philadelphia and haemoglobin Stanleyville II, has no significant effect on the clinical or haematological features of sickle cell anaemia [95,96]. The results of haemoglobin electrophoresis may be complex. With sickle cell anaemia and haemoglobin G-Philadelphia, there are two bands, an S band and a G-Philadelphia–S hybrid band (which has the same mobility at alkaline pH as haemoglobin C). The proportion of haemoglobin S is greater than the proportion of the hybrid band [95]. At acid pH, there is a single band with the mobility of haemoglobin S as, at this pH, the hybrid has the same mobility as S. Coinheritance with the α chain variant, haemoglobin Montgomery, also produces a hybrid band which has characteristics resembling those of haemoglobin C on both cellulose acetate electrophoresis and HPLC [97].

Glucose-6-phosphate dehydrogenase deficiency is common in many of the ethnic groups who carry the β^S gene. However, it has no effect on the clinical or haematological features of sickle cell anaemia [81].

Sickle cell/haemoglobin C disease

Sickle cell/haemoglobin C disease is consequent on the coinheritance of the β^S and β^C genes. There is no normal β gene and therefore no haemoglobin A. This compound heterozygous state leads to a sickling disorder that is similar to sickle cell anaemia but, on average, is somewhat less severe. The degree of haemolysis is less, with red cells surviving around

27 days, in comparison with around 17 days in sickle cell anaemia. The life expectancy is considerably better than that of sickle cell anaemia. In the USA, the average survival is 60 years for men and 68 years for women [58].

Sickle cell/haemoglobin C disease is characterized by an increased density of red cells, which is attributable to increased K^+/Cl^- cotransport with the loss of intracellular potassium and resultant cellular dehydration [98]. This, in turn, increases the likelihood of polymerization of haemoglobin S and, together with the higher haemoglobin S percentage (averaging 50% rather than 40%), helps to explain why the compound heterozygous state generally causes significant disease, whereas sickle cell trait does not [98].

Variant haemoglobins in which there is a second mutation in the β^C gene are likely to interact with haemoglobin S in a similar manner to haemoglobin C itself. One such haemoglobin is haemoglobin Arlington Park ($\beta^{6Glu\rightarrow Lys}$, $\beta^{95Lys\rightarrow Glu}$), which will be missed on cellulose acetate electrophoresis at alkaline pH as there is no net charge change in comparison with haemoglobin A [99,100].

Clinical features

Sickle cell/haemoglobin C disease leads to a chronic haemolytic anaemia and to intermittent sickle cell crises, similar to those of sickle cell anaemia but less frequent. Dactylitis is quite uncommon [101]. The haemoglobin concentration is higher than in sickle cell anaemia and the degree of haemolysis is less; the higher haemoglobin concentration is mainly due to a smaller reduction in the oxygen affinity rather than to less severe haemolysis. Aseptic necrosis (Fig. 4.23) and probably also bone marrow infarction with embolism of necrotic bone marrow to the lungs are more common than in sickle cell anaemia. In one series of patients, 15% suffered osteonecrosis of the femoral or humoral heads or vertebral bodies and two of 284 patients died of bone marrow embolism (two of 25 deaths) [101]. Retinal disease (retinitis proliferans and vitreous haemorrhage) is more frequent and more severe; in one series of patients, it was seen in 21% and in another in 23% [101]. Splenomegaly persists for longer, so that splenic infarction and splenic sequestration can occur in adults as well as in children, while the onset of hyposplenism, consequent

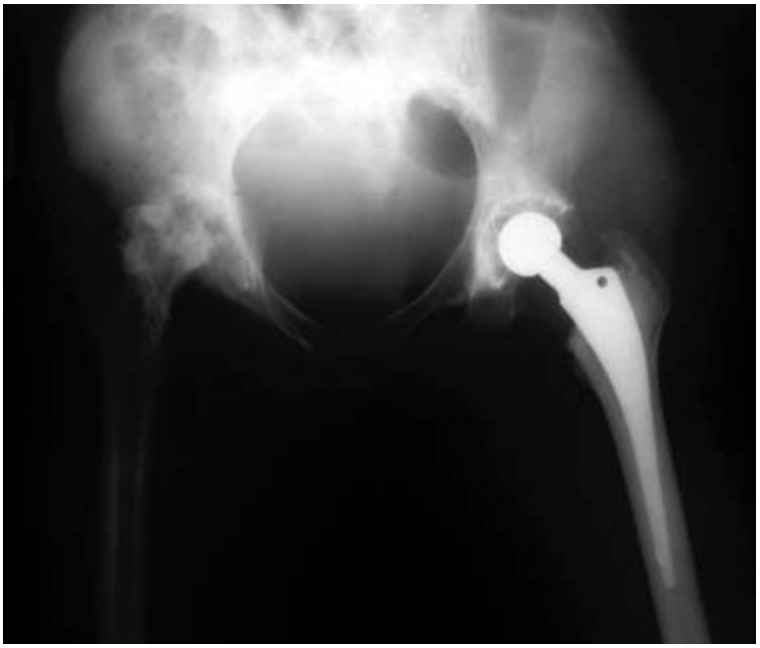


Fig. 4.23 Radiograph of the hips and pelvis in a patient with sickle cell/haemoglobin C compound heterozygosity showing osteonecrosis of one hip resulting from vascular occlusion by sickle cells; the other hip had already been replaced because of the same process. (By courtesy of Professor I. Roberts.)

on recurrent splenic infarction, is delayed. In contrast with patients with sickle cell anaemia, in whom the spleen is atrophic as a result of infarction, patients with sickle cell/haemoglobin C disease occasionally suffer splenic infarction during aeroplane flights [102]. As a consequence of the delay in the development of hyposplenism, life-threatening infections are less common than in sickle cell anaemia. Splenomegaly can lead to hypersplenism with chronic thrombocytopenia [103]. Because the red cell life span is considerably longer than in sickle cell anaemia, clinically apparent parvovirus-induced aplastic crises are uncommon and gallstones are less common.

Laboratory features

Blood count

The haemoglobin concentration is higher than in sickle cell anaemia, ranging from about 8 g/dl up to the top of the normal range [104]. In a personally observed series of 29 patients, the range was 8.9 to 15.6 g/dl with a mean of 12.2 g/dl. A concentration of 10 g/dl gives a fairly good separation between sickle cell anaemia and sickle cell/haemoglobin C disease. In one large study of adult patients originating in North Africa, West Africa and the Caribbean area, there were no individuals with sickle cell/haemoglobin C disease with a haemoglobin concentration of less than 11 g/dl [105]. In children, splenomegaly has been associated with a lower haemoglobin concentration and platelet count [103]. The MCV is lower than in sickle cell anaemia with a mean level around the lower limit of the normal range [104,106]. The MCH is similar, whereas the MCHC is more often elevated and the percentage of hyperdense cells is higher. The RDW is increased, but generally less than in sickle cell anaemia [70,107]. The HDW is increased [107]. The reticulocyte count is less markedly elevated than in sickle cell anaemia, with a mean level around 3–6%. The accuracy of measurement of red cell indices in sickle cell/haemoglobin C disease is dependent on the automated instrument used; cells in this disease are less deformable than normal, leading to a false elevation of the MCV and reduction of the MCHC on impedance counters and on some earlier light-scattering instruments [107].

Splenic sequestration is associated not only with a fall in the haemoglobin concentration, but also with a fall in the platelet count [103].

Individuals of African descent with sickle cell/haemoglobin C disease show a similar prevalence of α thalassaemia trait to those without this condition. The prevalence has varied between 20% and 35% in different series of patients [101]. Coexisting α thalassaemia trait leads to a higher red cell count and a lower MCV and MCH in comparison with other patients with sickle cell/haemoglobin C disease. In contrast with sickle cell anaemia, concomitant α thalassaemia trait does not alter the haemoglobin concentration [40,101,105,108], but a lower reticulocyte count and lower LDH indicate that there is less haemolysis [105].

The WBC, neutrophil count and monocyte count are elevated in sickle cell/haemoglobin C disease, but less so than in sickle cell anaemia [72].

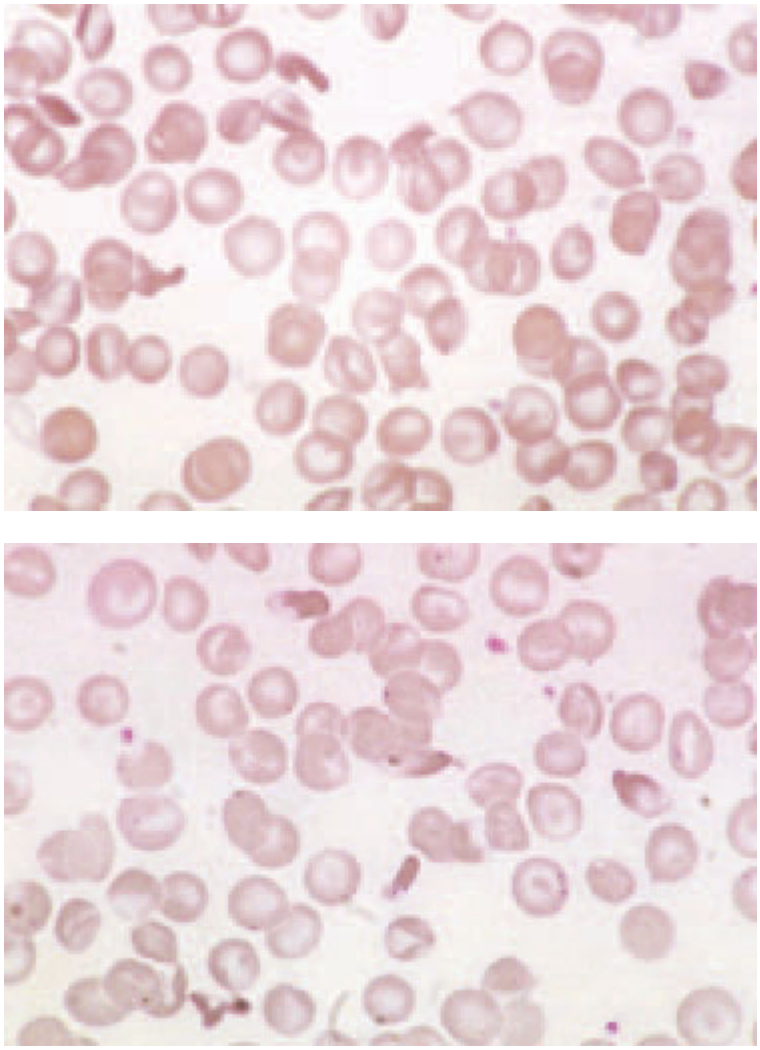
When sickle cell/haemoglobin C disease is treated with hydroxycarbamide, there is an increase in the MCV and a fall in the MCHC and the proportion of hyperdense cells. The reticulocyte count falls.

Blood film

The peripheral blood features of sickle cell/haemoglobin C disease are compared with those of sickle cell anaemia and haemoglobin C disease in Table 4.8 [77]. In contrast with sickle cell anaemia, the blood film does not often show classical sickle cells. Boat-shaped cells are more common than classical sickle cells, but they are less common than in sickle cell anaemia. Occasional cells may contain straight-edged six-sided haemoglobin C crystals. Around one-half of patients with sickle cell/haemoglobin C disease show characteristic poikilocytes (Fig. 4.24), which are not seen in either sickle cell anaemia or haemoglobin C disease [72,109]. These misshapen cells have complex forms. Some have crystals of varying shape and size jutting out at various angles. Others are curved, thus resembling sickle cells, but also appear to contain crystals with straight edges or with blunt-angled rather than pointed ends. Haemoglobin C will copolymerize with haemoglobin S (as occurs in the rare sickle cells and in the more common boat-shaped cells in the compound heterozygous state) [110]. Haemoglobin S will cocrystallize with

Table 4.8 Blood film features of sickle cell anaemia, sickle cell/haemoglobin C disease and haemoglobin C disease or $C\beta^0$ thalassaemia. (From reference [77].)

Genotype	SS	SC	CC or $C\beta^0$ thalassaemia
Number of cases	29	29	10
Sickle cells	24	6	0
Boat-shaped cells	24	16	1
Haemoglobin C crystals	0	4	5
SC poikilocytes	0	16	0
Irregularly contracted cells	5	25	9
Howell–Jolly bodies	29	5	0
Pappenheimer bodies	25	7	1
Target cells	27	29	9
Spherocytes	10	3	1
Polychromasia	24	9	3
Nucleated red blood cells	26	15	7

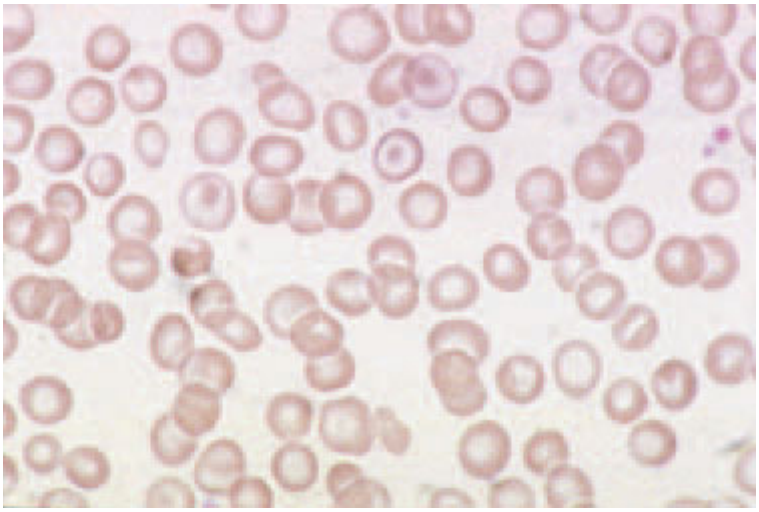


(a)

(b)

Fig. 4.24 Blood films in four patients with sickle cell/haemoglobin C compound heterozygosity showing the range of abnormalities observed: (a) target cells and SC poikilocytes; (b) SC poikilocytes and a boat-shaped cell. (*Continued on p. 168.*)

(c)



(d)

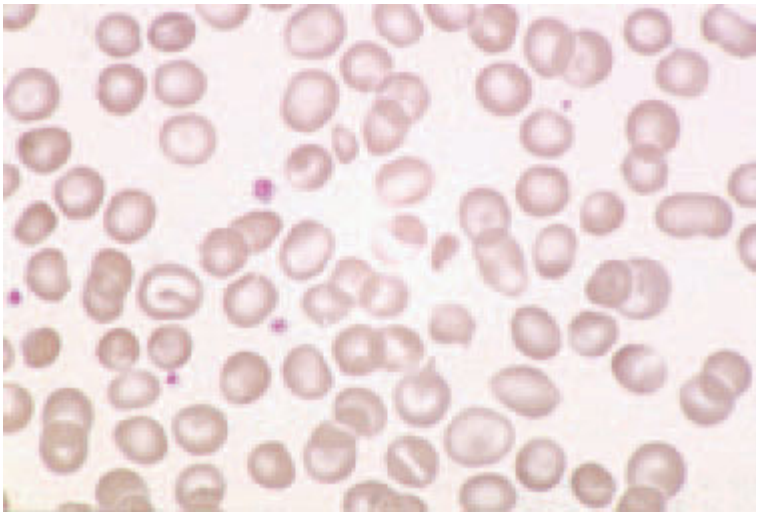


Fig. 4.24 *Continued*. (c) hypochromia and target cells; (d) irregularly contracted cells, a 'hemi-ghost', a target cell and a stomatocyte.

haemoglobin C (as occurs in the less common cells containing haemoglobin C crystals) [111]. Deoxygenation favours S-like polymerization, whereas oxygenation favours C-like crystallization [110,112]. It seems likely that the formation of SC poikilocytes is consequent on both processes occurring simultaneously in the one cell. On scanning electron microscopy, the forms seen include folded cells (compared to a taco), triconcave cells and stomatocytes [98].

Features of hyposplenism, such as Howell–Jolly bodies and Pappenheimer bodies, are less common than in sickle cell anaemia. Polychromasia and nucleated red blood cells are likewise less common, whereas target cells (Fig. 4.24c) show a similarly high frequency and irregularly contracted cells (Fig. 4.24d) are much more common. 'Hemi-ghosts' may be present (Fig. 4.24d). An assessment of the blood count and film usually permits the distinction between sickle cell/haemoglobin C disease and sickle cell anaemia. However, those cases that lack sickle cells, boat-shaped cells and SC poikilocytes (Fig. 4.24d) can be difficult to distinguish from haemoglobin C disease.

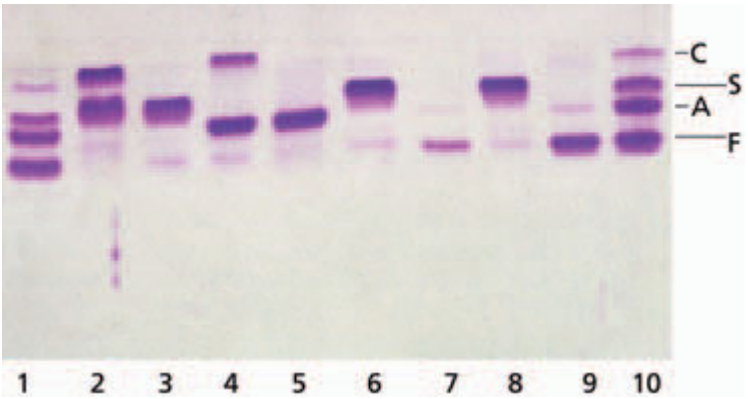


Fig. 4.25 Haemoglobin electrophoresis on agarose gel at pH 6.2 showing a patient with sickle cell/haemoglobin C disease (lane 2); lanes 1 and 10, control sample containing haemoglobins F, A, S and C.

Other investigations

Haemoglobins S and C are present in similar proportions (Fig. 4.25). The haemoglobin F percentage ranges from normal to slightly elevated, with mean values of 1.1–3.3% having been reported in different studies. The haemoglobin F percentage is significantly higher in females than in males [105]. As in sickle cell anaemia, the haemoglobin F percentage is affected by the β gene haplotype, averaging 3.2% with the Senegal haplotype and 1.5% and 1.4% with the Benin and Bantu haplotypes, respectively [105]. In 98 UK subjects, the mean haemoglobin F was 1.46% (standard deviation 1.81%), with adult levels being reached by 9 years of age [50]. The percentage of F cells is increased; in one study, the mean level was 27% (range 5–73%), in comparison with normal levels of 0.5–7% [92]. Little information is available on the haemoglobin A_2 percentage in sickle cell/haemoglobin C disease as, on cellulose acetate electrophoresis, haemoglobin A_2 comigrates with haemoglobin C.

The sickle solubility test is positive and immunoassays demonstrate the presence of haemoglobins S and C with no haemoglobin A.

Bilirubin is normal or mildly elevated. LDH is elevated in comparison with control subjects, but is less elevated than in sickle cell anaemia. The red cell life span ranges from moderately shortened to slightly less than normal. The oxygen dissociation curve shows reduced oxygen affinity, i.e. a right-shifted curve and a higher P_{50}. The reduction in oxygen affinity is less than that seen in sickle cell anaemia [87].

Diagnosis

The diagnosis rests on the demonstration of the presence of haemoglobin S and haemoglobin C with haemoglobin A being absent. The identity of the two variant haemoglobins must be confirmed by at least two independent techniques. It is important not to confuse compound heterozygous states for haemoglobin S and haemoglobin C-Harlem, O-Arab or E (see pp. 175, 176 and 178) with sickle cell/haemoglobin C disease, as all have two bands in the same positions on cellulose acetate electrophoresis at alkaline pH. In compound heterozygosity for haemoglobins S and E, the band in the C position constitutes a lower percentage than the S band. Homozygous haemoglobin S with coexisting haemoglobin G-Philadelphia will also have bands in the positions of S and C, but the band in the C position, which represents the hybrid $\alpha^{G\text{-Philadelphia}}\beta^{S}$ haemoglobin, constitutes an appreciably lower percentage than the band representing S plus G-Philadelphia.

Interactions with other haemoglobinopathies and other haematological diseases

There is conflicting evidence as to the effect of coexisting α thalassaemia trait. In one series of patients, α thalassaemia trait was associated with a lower risk of osteonecrosis and retinopathy, but an earlier series did not show this [101].

Individuals with sickle cell/haemoglobin C disease who are also heterozygous for the α chain

variant, haemoglobin G-Philadelphia, have disease of variable severity. One reported case was more severe than is usual in sickle cell/haemoglobin C disease [113], while another had a mild clinical course with abundant crystals in circulating cells and numerous folded cells [114]. The latter is considered to be the more typical clinical picture, attributable to the presence of the G-Philadelphia α chain both increasing the likelihood of crystallization of haemoglobin C and decreasing the likelihood of polymerization of haemoglobin S [98]. Haemoglobin electrophoresis is complex. At alkaline pH, there are bands with the mobility of haemoglobins S (about 35%), C (about 47%) and a slow G–C hybrid (about 15%) [113]. The 'S' band represents haemoglobins S and G-Philadelphia. The 'C' band represents haemoglobin C and the S–G hybrid. At acid pH, there are two bands with the mobility of haemoglobins S and C.

A severe phenotype has been observed with coincidental hereditary spherocytosis [81].

Sickle cell/β thalassaemia

Sickle cell/β thalassaemia is a compound heterozygous state for β^S and either β^+ thalassaemia or β^0 thalassaemia [115,116]. In sickle cell/β^0 thalassaemia, there is no haemoglobin A, whereas, in sickle cell/β^+ thalassaemia, a variable amount of haemoglobin A is present. The reduced concentration of haemoglobin S within red cells, together with the greater or lesser increase in the percentages of haemoglobins A_2 and F, lessens the likelihood of sickling and lessens the haemolysis (in comparison with sickle cell anaemia); however, this is counterbalanced by the higher haemoglobin concentration and increased blood viscosity.

Clinical features

Patients with sickle cell/β^0 thalassaemia have less evidence of haemolysis than patients with sickle cell anaemia but, despite this, the frequency of painful crises is, if anything, greater [61]. The explanation may lie in the higher haemoglobin concentration. Patients with sickle cell/β^+ thalassaemia may have both less haemolysis and a reduced incidence of painful crises in comparison with individuals with sickle cell anaemia. The amelioration of the disease is proportional to the percentage of haemoglobin A present. They may, however, have a higher incidence of proliferative retinopathy, as a consequence of the higher haemoglobin concentration [81]. Splenomegaly persists longer than in sickle cell anaemia, particularly in those with sickle cell/β^+ thalassaemia. Patients with sickle cell/β^+ thalassaemia and persisting splenomegaly remain susceptible to splenic infarction during aeroplane flights, whereas those with sickle cell/β^0 thalassaemia resemble patients with sickle cell anaemia as they are likely to have suffered recurrent splenic infarction and consequent atrophy and therefore do not remain susceptible [102]. Sometimes massive splenomegaly leads to hypersplenism. Overexpansion of the bone marrow cavity in the skull may cause frontal bossing. Sickle cell/β thalassaemia is generally more severe in Mediterranean populations than in those of African descent because of the greater prevalence of β^0 thalassaemia in the former group.

Laboratory features

Blood count

Anaemia is milder than in sickle cell anaemia, the haemoglobin concentration varying from about 5 g/dl to within the normal range. The distribution of haemoglobin concentration is bimodal, being higher in those with sickle cell/β^+ thalassaemia than in those with sickle cell/β^0 thalassaemia; mean values in one study were 10.7 and 8.1 g/dl, respectively [9]. The MCV, MCH and MCHC are reduced, again showing a bimodal distribution. Mean values observed were 72 and 69.8 fl for MCV, 22.6 and 20.1 pg for MCH and 31.5 and 28.8 g/dl for MCHC [116]. For both groups, sickle cell/β^0 thalassaemia and sickle cell/β^+ thalassaemia, the mean values for MCV, MCH and MCHC are lower than those in sickle cell anaemia, but overlap occurs. The RDW is markedly increased in sickle cell/β^0 thalassaemia and moderately increased in sickle cell/β^+ thalassaemia [70]. It should be noted that, in patients with sickle cell/β thalassaemia who develop megaloblastic anaemia, the MCV and MCH, although elevated in comparison with baseline values, may be within the normal range.

The reticulocyte count is elevated, in sickle cell/β^0 thalassaemia to around 8–9% on average and in sickle cell/β^+ thalassaemia to around 3% on average [104]. During complicating bacterial or parvovirus infection or megaloblastic anaemia, the usual elevation of the reticulocyte count is lacking.

Coexisting α thalassaemia increases the haemoglobin concentration, MCV and MCH and reduces the reticulocyte count [9].

Blood film

The blood film abnormalities are more severe in sickle cell/β^0 thalassaemia (Fig. 4.26) than in sickle cell/β^+ thalassaemia (Fig. 4.27). Classical sickle cells are quite uncommon, particularly in sickle cell/β^+ thalassaemia. There are some boat-shaped cells. There is hypochromia and microcytosis, and circulating nucleated red blood cells show defective haemoglobinization. Target cells are prominent and basophilic stippling may be apparent. Features of hyposplenism may be present, particularly in sickle cell/β^0 thalassaemia. In patients with hyposplenism, Pappenheimer bodies are often very prominent. Polychromasia is present unless there is associated erythropoietic failure caused by infection or megaloblastosis.

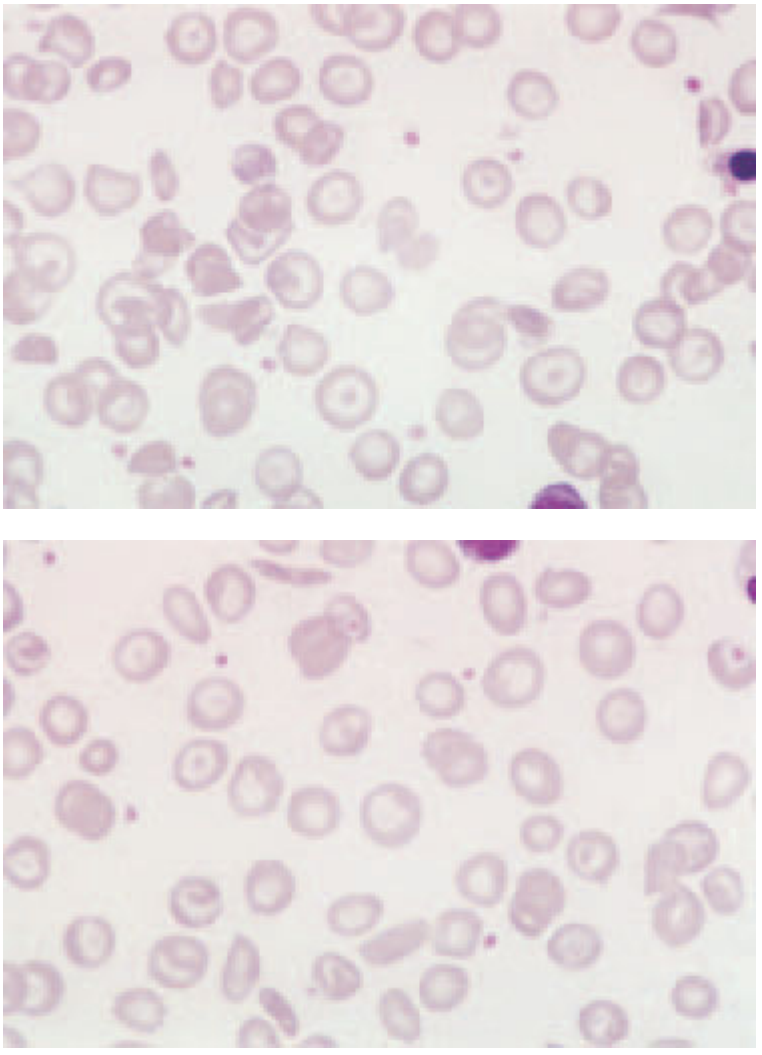


Fig. 4.26 Blood films in two patients with sickle cell/β^0 thalassaemia showing: (a) target cells, a nucleated red blood cell and a number of partly sickled cells; (b) hypochromia, microcytosis, target cells and a number of partly sickled cells.

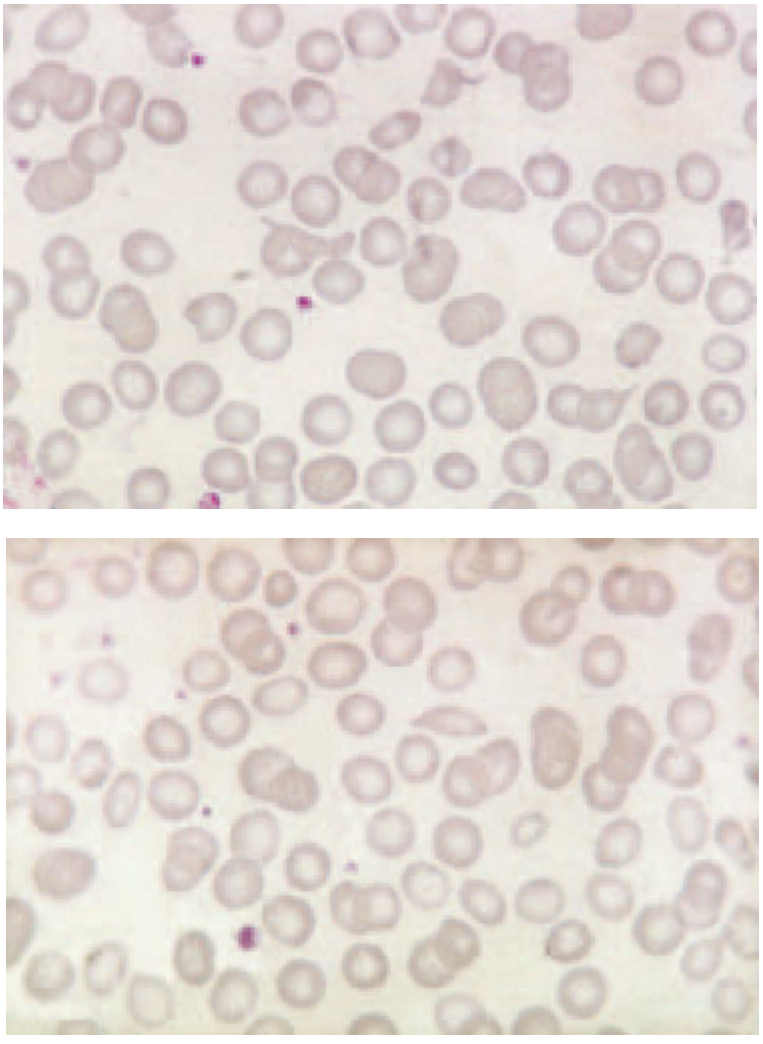


Fig. 4.27 Blood films in two patients with sickle cell/β^+ thalassaemia showing: (a) hypochromia, poikilocytosis and a probable sickle cell; (b) hypochromia, microcytosis and one partly sickled cell. The first patient had 70% haemoglobin S, 24% haemoglobin A, 6% haemoglobin A_2, red blood cell count (RBC) 5.07×10^{12}/l, haemoglobin concentration (Hb) 10.6 g/dl, mean cell volume (MCV) 64 fl, mean cell haemoglobin (MCH) 21 pg and mean cell haemoglobin concentration (MCHC) 32.9 g/dl. The second patient had 59% haemoglobin S, 25% haemoglobin A and A_2, 13% haemoglobin F, RBC 4.71×10^{12}/l, Hb 10.6 g/dl, haematocrit (Hct) 0.32, MCV 68 fl, MCH 22.5 pg and MCHC 33 g/dl.

Other investigations

Haemoglobin S comprises more than 50% of total haemoglobin, in contrast with sickle cell trait when it is less than 50%. In patients with sickle cell/β^0 thalassaemia, there is no haemoglobin A, whereas, in those with sickle cell/β^+ thalassaemia (Fig. 4.28), the amount of haemoglobin A varies from almost undetectable to, rarely, as high as 45% (Table 4.9) [117–120]. Haemoglobin F is usually 5–15% and the percentage of F cells is considerably increased. As for sickle cell anaemia, the haemoglobin F concentration is influenced by the β gene haplotype associated with the β^S mutation, being higher with the Senegal and Arab–Indian haplotypes. Because of the overlap in values, the haemoglobin F percentage is not very useful in separating sickle cell/β^0 thalassaemia from sickle cell anaemia; in one series of Jamaican patients, the F percentage tended to be higher in compound heterozygotes, but the difference was not significant [116]. Haemoglobin A_2 tends to be somewhat elevated, usually 3.5–5.5%, with the level being higher when the β thalassaemia gene is β^0 rather than β^+ [121]. Higher levels of haemoglobins F and A_2 (and a milder clinical course) have been observed when the β thalassaemia mutation is a large (290-bp) deletion [122]. The higher level of haemoglobin A_2 in sickle cell/β^0 thalassaemia can be useful in helping to make

a distinction between the compound heterozygous state and sickle cell anaemia with microcytosis consequent on coexisting α thalassaemia trait, in which haemoglobin A_2 is usually in the range of 2–4%. Although there is some overlap in haemoglobin A_2 percentages, this is the most useful variable for making the distinction; the haemoglobin concentration, reticulocyte count and haemoglobin F percentage show more overlap (Table 4.7).

The red cell life span is reduced, particularly in sickle cell/β^0 thalassaemia, but not to the same extent as in sickle cell anaemia. The α:β chain synthesis ratio in peripheral blood reticulocytes is increased in sickle cell/β thalassaemia, whereas it is normal in sickle cell anaemia.

In the neonatal period, the diagnosis of sickle cell/β^+ thalassaemia can be difficult [85]. Confusion with sickle cell trait can occur if almost all the haemoglobin present is haemoglobin F and the proportions of haemoglobins S and A are so low that it is not clear which is present in the greater amount. Neonates with sickle cell/β^+ thalassaemia may also have only haemoglobins S and F, so that confusion with sickle cell anaemia and sickle cell/β^0 thalassaemia is possible. Only a provisional diagnosis can be made in this circumstance. Family studies and follow-up are needed for a definitive diagnosis.

The bone marrow aspirate (Fig. 4.29) shows erythroid hyperplasia, sickle cells and a variable degree of iron overload.

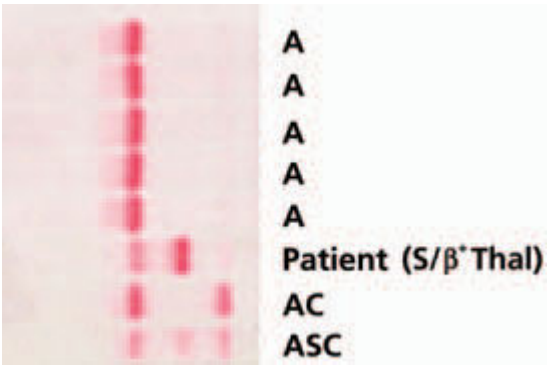


Fig. 4.28 Haemoglobin electrophoresis on cellulose acetate at alkaline pH in a patient with sickle cell/β^+ thalassaemia compound heterozygosity; ASC, control sample containing haemoglobins A, S and C.

Diagnosis

The diagnosis of compound heterozygosity for haemoglobin S and β^+ thalassaemia is straightforward, merely requiring the demonstration of both haemoglobin A and haemoglobin S by two independent techniques and the confirmation that haemoglobin S is present as a larger proportion than haemoglobin A. The diagnosis of compound heterozygosity for haemoglobin S and β^0 thalassaemia is more difficult as a distinction must be made from sickle cell anaemia with microcytosis (e.g. due to coexisting α thalassaemia) (see above). When a precise diagnosis is important, e.g. for genetic counselling, and is not clear from family studies and from a consideration of the proportions of various haemoglobins, DNA analysis should be carried out. A distinction also needs to be made from compound heterozygosity for haemoglobin S and deletional hereditary persistence of fetal haemoglobin, particularly with coexisting α thalassaemia trait; in this instance, clinical features, haemoglobin concentration, MCV and haemoglobin F percentage are useful (Table 4.7).

Table 4.9 Percentage of haemoglobin A in compound heterozygosity for haemoglobin S and β^+ thalassaemia. (Derived from references [115–120].)

Mutation and ethnic group	Haemoglobin A (%)
C→G at IVS2, position 745 (Greek/Turkish)	3–5
G→C at IVS1, position 5 (Indian)	3–5
G→A at IVS1, position 110 (Greek/Turkish)	8–14
C→T at –88 (black)	18–25
A→G at –29 (black)	18–25
G→T at IVS1, position 5 (Greek/Turkish)	18–25

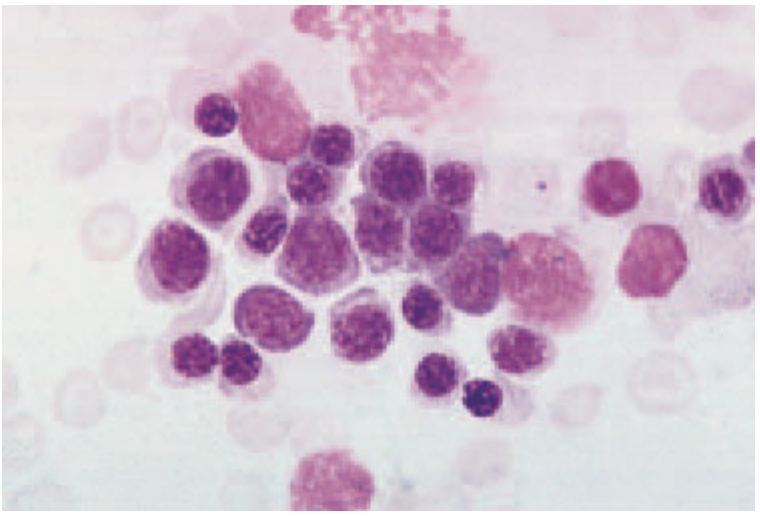


Fig. 4.29 Bone marrow aspirate in sickle cell/β^0 thalassaemia compound heterozygosity showing erythroid hyperplasia and one sickle cell.

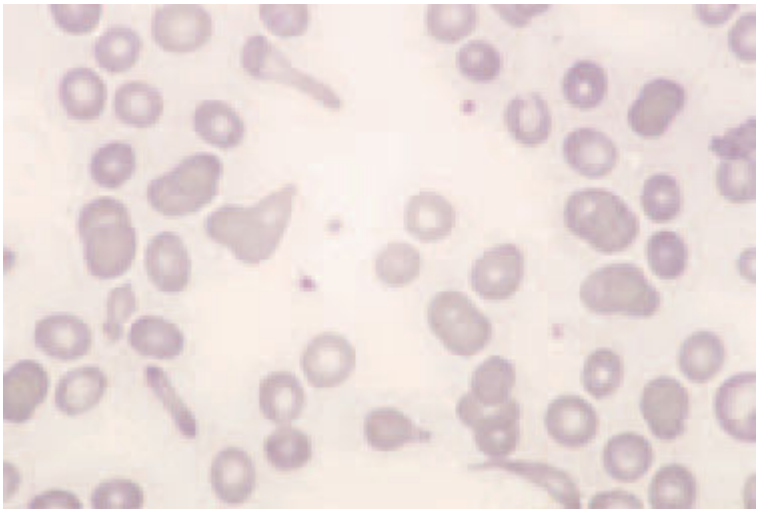


Fig. 4.30 Blood film in sickle cell/haemoglobin D-Punjab compound heterozygosity.

Other causes of sickle cell disease

Sickle cell/haemoglobin D-Punjab/D-Los Angeles disease

Compound heterozygosity for sickle cell haemoglobin and haemoglobin D-Punjab (D-Los Angeles) leads to sickle cell disease which is, on average, slightly milder than sickle cell anaemia [9,96,123–127]. This compound heterozygous state has been observed in Afro-Americans, Afro-Caribbeans, Central and South Americans (Mexicans and Venezuelans) and Turks and, in addition, in a number of individuals of mixed ancestry (Northern European/American Indian, English/African, English/Afro-Caribbean) including several individuals who appeared to have only Mediterranean or Northern European ancestry. The clinical features are a mild or moderate haemolytic anaemia with sickling crises. Persisting splenomegaly is more common than in sickle cell anaemia. The haemoglobin concentration is usually between 5 and 10 g/dl and the reticulocyte count between 5% and 20% (occasionally higher). The MCV is very variable, but macrocytosis is quite common with some individuals having an MCV of 110–120 fl. The blood film (Fig. 4.30) shows anisocytosis, poikilocyto-

sis, target cells, sickle cells, boat-shaped cells, nucleated red cells and sometimes macrocytes. The bone marrow shows erythroid hyperplasia and sickle cells (Fig. 4.31). On cellulose acetate electrophoresis at alkaline pH, haemoglobins S and D-Punjab show the same electrophoretic mobility, but HPLC and electrophoresis at acid pH separate these two haemoglobins from each other. Haemoglobin D forms a somewhat higher proportion of total haemoglobin than does haemoglobin S [127]. In a few cases, haemoglobin F is significantly elevated, e.g. 13–20% [126], but usually is present in only small amounts. Haemoglobin A_2 may be slightly elevated [127].

It should be noted that coinheritance of haemoglobin S and haemoglobin D variants other than haemoglobin D-Punjab/haemoglobin D-Los Angeles does not have the same adverse effects as sickle cell/haemoglobin D-Punjab compound heterozygosity. For example, two Nigerians with haemoglobin S/haemoglobin D-Ibadan were asymptomatic [125]. Similarly, haemoglobin D-Iran does not interact adversely with haemoglobin S.

Sickle cell/haemoglobin O-Arab disease

Compound heterozygosity for sickle cell haemoglobin and haemoglobin O-Arab ($\alpha_2\beta_2^{121Glu\rightarrow Lys}$) leads to sickle cell disease that is generally severe [9,96,128–130]. Sickle cell/haemoglobin O-Arab has been observed in Arabs, Africans (Sudanese and Kenyans), Afro-Caribbeans, Afro-Americans and Americans who appeared to be of Caucasian ancestry. The haemoglobin concentration in adults varies between 6.1 and 9.9 g/dl. The reticulocyte count is usually between 8% and 10% (1–15% reported). The reported MCVs have been quite variable, from normal to moderately macrocytic levels (82–110 fl in adults). The blood film (Fig. 4.32) is similar to that in sickle cell anaemia. The oxygen affinity is reduced, comparable to that seen in sickle cell anaemia. On electrophoresis on cellulose acetate at alkaline pH, haemoglobin O-Arab has a similar mobility to haemoglobin C (Fig. 4.33), but, at acid pH, the mobility depends on the electrophoresis medium. On agarose gel, it is slightly slower than haemoglobin S (Fig. 4.34). On HPLC, there are two abnormal peaks, one in the position of haemoglobin S and the other between haemoglobins S and C (Fig. 4.35). Haemoglobin O-Arab and haemoglobin C-Harlem can easily be confused with each other when present in the compound heterozygous state with haemoglobin S. The difference in mobility on citrate agar at acid pH is most useful in making the distinction (Table 4.10). Haemoglobin S forms a somewhat higher proportion of total haemoglobin than does haemoglobin O-Arab [127].

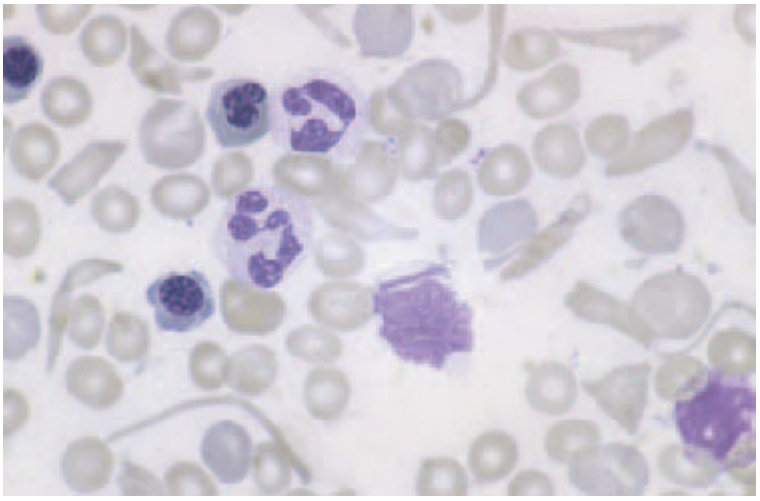


Fig. 4.31 Bone marrow aspirate in sickle cell/haemoglobin D-Punjab compound heterozygosity showing prominent sickle cell formation.

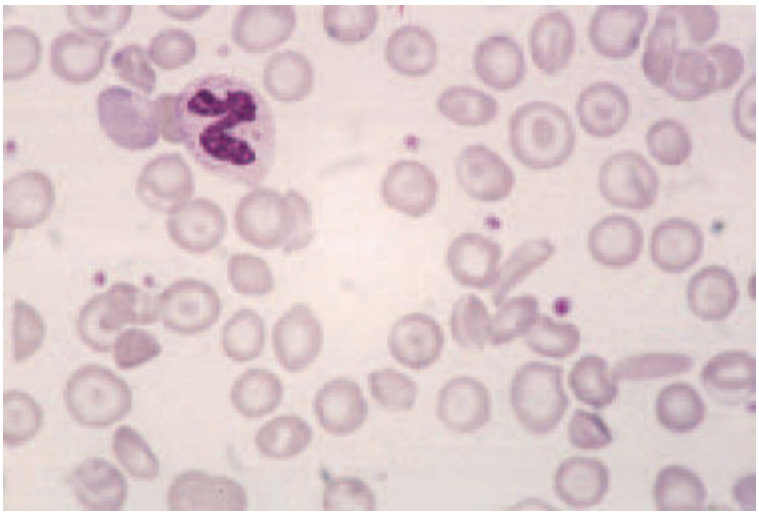


Fig. 4.32 Blood film in sickle cell/haemoglobin O-Arab compound heterozygosity showing hypochromia, target cells and partially sickled cells. (Note: O-Arab in this patient was misidentified as C-Harlem in the previous edition of this book; the correct identity has been confirmed by family studies, citrate agar electrophoresis and mass spectrometry.)

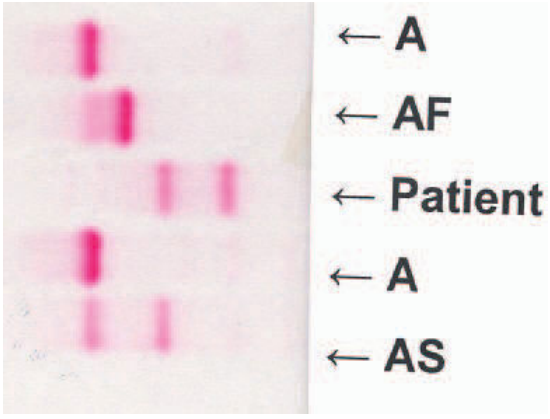


Fig. 4.33 Haemoglobin electrophoresis on cellulose acetate at alkaline pH in sickle cell/haemoglobin O-Arab compound heterozygosity (Patient). By this technique, the pattern cannot be distinguished from that of sickle cell/haemoglobin C compound heterozygosity; other samples contain the haemoglobins indicated.

Sickle cell/haemoglobin C-Harlem compound heterozygosity

This condition is slightly milder than sickle cell anaemia. The blood film shows similar features. Haemoglobin electrophoresis at alkaline pH resembles that of sickle cell/haemoglobin C disease, whereas, at acid pH on citrate agar (but not agarose gel), there is a single band with the mobility of haemoglobin S (Table 4.10).

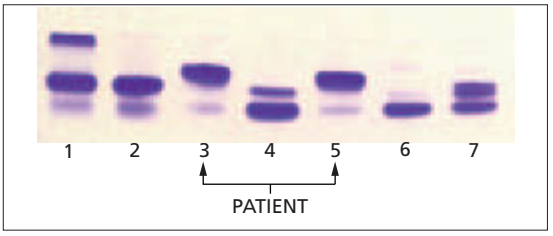


Fig. 4.34 Haemoglobin electrophoresis on agarose gel at acid pH in sickle cell/haemoglobin O-Arab compound heterozygosity (lanes 3 and 5) showing a faint F band and broadening of the S band in the direction of C by the presence of O-Arab. From left to right, lanes are: (1) F, A and C; (2) F and A; (3) S plus O-Arab; (4) F and A; (5) S plus O-Arab; (6) F; (7) F, A and S. The mobility of O-Arab on this medium is more readily apparent in the absence of haemoglobin S (see Fig. 5.24).

Sickle cell/haemoglobin Lepore

Compound heterozygosity for sickle cell haemoglobin and haemoglobin Lepore-Boston [131,132] has been reported in Mediterranean (Greek and Italian), Afro-Caribbean and Afro-American populations. Sickle cell/haemoglobin Lepore leads to sickle cell disease of variable severity, but resembling sickle cell/β thalassaemia more closely than sickle cell anaemia. Of the 10 cases reported up to 1997, three were severe and seven were mild [132].

The haematological variables reported in adults [9,96,132] have included a haemoglobin concentra-

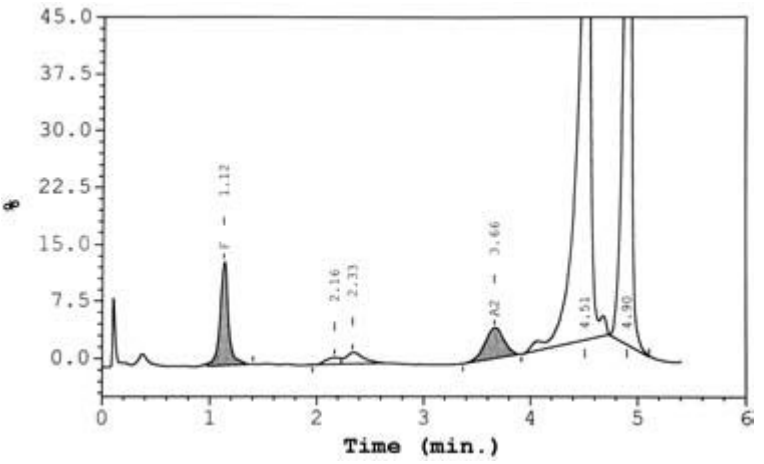


Fig. 4.35 HPLC chromatogram in a patient with haemoglobin S/O-Arab compound heterozygosity; the haemoglobin O-Arab in this and another patient had retention times on a Bio-Rad Variant II of 4.89 and 4.90 min respectively; from left to right, the peaks are altered F, haemoglobin F, altered S and haemoglobins A_2, S and O-Arab.

Table 4.10 Making a distinction between haemoglobin O-Arab and haemoglobin C-Harlem.

	Haemoglobin O-Arab	Haemoglobin C-Harlem
Frequency	Uncommon	Rare
Clinical severity of compound heterozygous state with haemoglobin S	As severe as sickle cell anaemia	Somewhat milder than sickle cell anaemia
Sickle solubility test	Negative	Positive
Mobility on cellulose acetate electrophoresis at alkaline pH	Mobility of C	Mobility of C
Mobility on agarose gel electrophoresis at acid pH	Slightly slower than S (i.e. slightly towards C)	With S
Mobility on citrate agar electrophoresis at acid pH	Somewhat faster than S (i.e. slightly on the A side of S)	With S
High performance liquid chromatography	Between S and C	Between S and C
Isoelectric focusing	With E	With E

tion of 8–13.3 g/dl, MCV of 66.5–83 fl, MCH of 24.3–27.6 pg and reticulocyte count of 3–13% (33% in one case). The blood film shows anisocytosis, hypochromia, microcytosis and some sickle cells.

As haemoglobin Lepore has the same mobility as haemoglobin S on electrophoresis at alkaline pH, the only bands apparently present are haemoglobins F, S and A_2, and diagnostic confusion with sickle cell anaemia and sickle cell/β^0 thalassaemia can therefore occur. However, other techniques, such as HPLC, show that haemoglobin Lepore is usually around 10–12% of total haemoglobin (20% in one case), while haemoglobin S is 63–90% and haemoglobin F is 5–25%. Electrophoresis at acid pH shows two bands, one with the mobility of haemoglobin A, which represents haemoglobin Lepore. The proportion of haemoglobin A_2 is variable, having been reported to be reduced, normal or slightly elevated in different cases (0.9–4%) [9,132].

Sickle cell/$\delta\beta^0$ thalassaemia

Sickle cell/$\delta\beta^0$ thalassaemia has been observed in Mediterranean populations (Greek, Sicilian, other

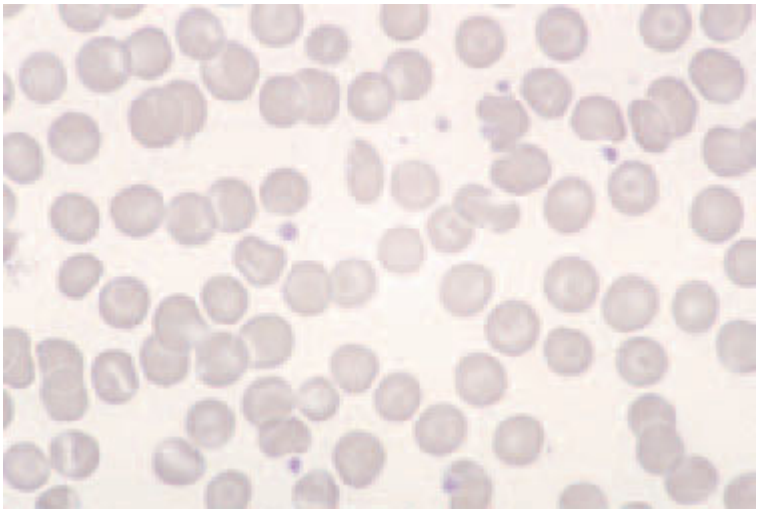


Fig. 4.36 Blood film in sickle cell/hereditary persistence of fetal haemoglobin compound heterozygosity showing mild poikilocytosis and target cell formation.

Italian), Arabs and Afro-Americans [9,96]. This compound heterozygous state is generally much milder than sickle cell anaemia because the high percentage of haemoglobin F protects against sickling. There is mild anaemia and splenomegaly.

The blood count shows a haemoglobin concentration of around 10–12 g/dl and the MCV is slightly reduced (76–83 fl). The reticulocyte count is slightly elevated, usually 2–4%. The blood film shows anisocytosis, poikilocytosis and hypochromia. Haemoglobin S is the major haemoglobin component, with haemoglobin F being 15–37% of total haemoglobin. The proportion of haemoglobin A_2 is normal or low (1.5–3.1%). Sickle cell/$\delta\beta^0$ thalassaemia differs from microcytic cases of sickle cell anaemia, having a higher haemoglobin concentration, lower reticulocyte count and lower haemoglobin A_2 percentage (Table 4.7). However, definitive diagnosis requires DNA analysis.

Sickle cell/hereditary persistence of fetal haemoglobin

Compound heterozygosity for haemoglobin S and deletional or pancellular hereditary persistence of fetal haemoglobin (HPFH) is either asymptomatic or produces quite mild sickle cell disease [96]. Haemoglobin S/HPFH has been reported in Africans, Afro-Caribbeans and Afro-Americans. There may be mild haemolytic anaemia and splenomegaly or minor clinical features consequent on sickling. The haemoglobin and reticulocyte count are usually normal, but microcytosis is common and occasionally there is mild anaemia and reticulocytosis of 2–4%. The blood film (Fig. 4.36) may show anisocytosis, microcytosis and target cells. Haemoglobin electrophoresis or HPLC shows haemoglobin F of 15–35% (usually 20–30%), low-normal or slightly reduced haemoglobin A_2 and haemoglobin S comprising around 60–80% of total haemoglobin. Virtually all cells are F cells [92]. Haemoglobin A is absent. The proportions of various haemoglobins in haemoglobin S/HPFH are similar to those in haemoglobin S/β^0 thalassaemia. Distinction between the two is aided by the usual lack of symptoms and by the fact that the haemoglobin concentration and reticulocyte count are often normal in haemoglobin S/HPFH (Table 4.7). Definitive diagnosis requires family studies or DNA analysis.

Sickle cell/haemoglobin E compound heterozygosity

Compound heterozygosity for haemoglobin S and haemoglobin E produces a condition that is either asymptomatic or clinically mild [9,96,133–136]. Sickle cell/haemoglobin E has been observed in Turks, Afro-Americans, Afro-Caribbeans, Saudi Arabians

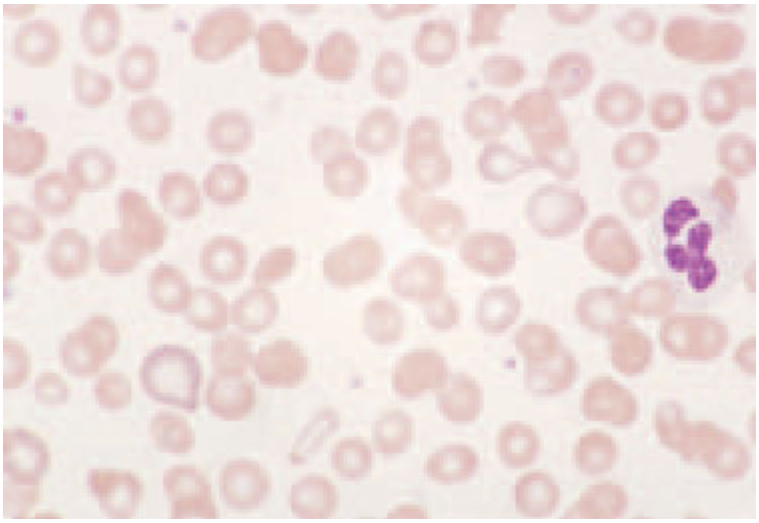


Fig. 4.37 Blood film in sickle cell/haemoglobin E compound heterozygosity showing microcytosis and poikilocytosis. (By courtesy of Dr R. Gupta.)

and a Pakistani. There are sometimes minor symptoms that are probably related to sickling. There may be mild haemolysis, often compensated, and splenomegaly. Recurrent splenic infarction during aeroplane flights has been reported [134]. The haemoglobin concentration may be normal or reduced (8–14.6 g/dl) with sometimes a slight increase in the reticulocyte count (1.5–4%). The MCV may be normal or reduced (71–97 fl in adults). The blood film (Fig. 4.37) may show target cells which are sometimes numerous. Sickle cells have been observed [133], but this is not usual. Haemoglobin S is a larger proportion of total haemoglobin than haemoglobin E, e.g. around 60% [127,134,136] (Fig. 4.38). The haemoglobin E percentage tends to be higher than in individuals with haemoglobin E trait [127]. Haemoglobin F may be normal or slightly elevated [127,135].

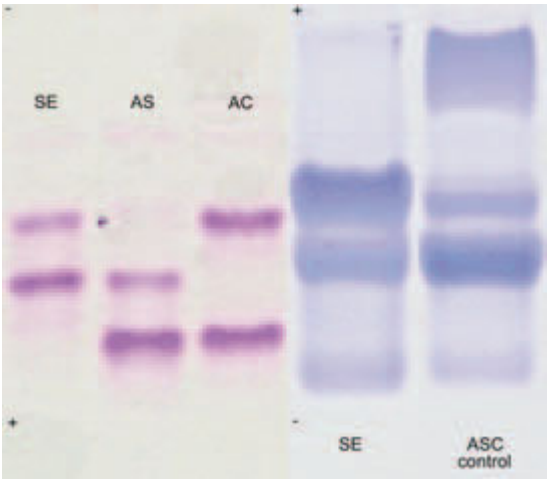


Fig. 4.38 Haemoglobin electrophoresis at acid and alkaline pH in sickle cell/haemoglobin E compound heterozygosity; the first three lanes from the left are cellulose acetate electrophoresis at alkaline pH; the two lanes on the right are agarose gel electrophoresis at acid pH; ASC, control sample containing haemoglobins A, S and C. (By courtesy of Dr R. Gupta, Mr M. Jarvis and Dr A. Yardumian.)

Sickle cell/$\delta^0\beta^+$ thalassaemia

Sickle cell/$\delta^0\beta^+$ thalassaemia, as the result of the formation of a $\delta\beta$ fusion gene, has been reported in four brothers in a Senegalese family. The haemoglobin concentration varied from 10.9 to 13.4 g/dl, MCV from 76 to 85 fl, haemoglobin S from 58% to 70%, haemoglobin A from 12% to 16% and haemoglobin F from 12% to 30% [137]. The propositus was asymptomatic.

Other rare compound heterozygous states

Compound heterozygosity for haemoglobin C and haemoglobin C-Harlem appears to produce a much milder disease than sickle cell/haemoglobin C disease. One reported patient who presented with

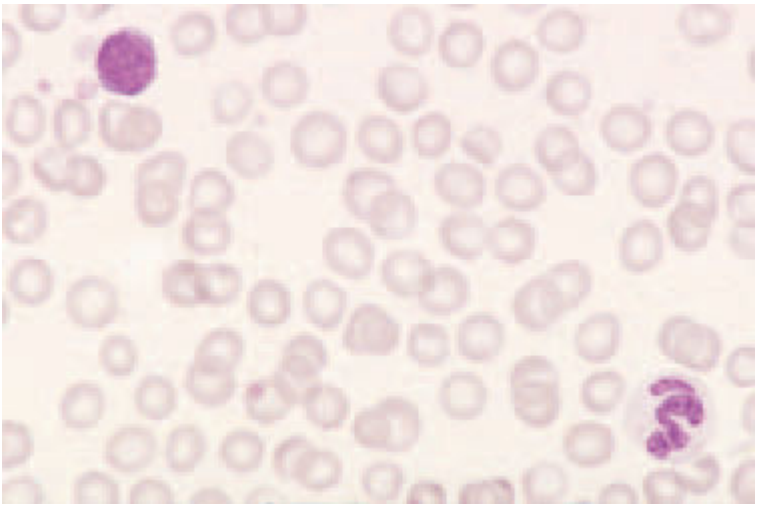


Fig. 4.39 Blood film from a 1-year-old child with haemoglobin S/haemoglobin Siriraj compound heterozygosity showing anisocytosis and hypochromia. The red cell indices were RBC 4.87×10^{12}/l, Hb 11.2 g/dl, MCV 67 fl, MCH 23.3 pg and MCHC 34.4 g/dl.

haematuria had anaemia and splenomegaly, but no symptoms suggestive of sickling [7]. The blood film showed many target cells and occasional sickle cells. Compound heterozygosity for haemoglobin S and haemoglobin S-Antilles produces a very severe form of sickle cell disease [19]. Compound heterozygosity for haemoglobin S and haemoglobin S-Oman has been described, presenting at the age of 1 year [138]; it is likely that the phenotype will be severe as this double substitution haemoglobin can cause disease in heterozygotes. Compound heterozygosity for haemoglobin S and the electrophoretically silent variant, haemoglobin Quebec-Chori, causes sickle cell disease [24]. Compound heterozygosity for haemoglobin S and mildly unstable haemoglobins, such as haemoglobin Hope and haemoglobin Siriraj (Fig. 4.39), can cause mild haemolysis [96]. A compound heterozygote for haemoglobin S and haemoglobin Hofu (a fast-moving haemoglobin) had significant anaemia (haemoglobin concentration 9.6 g/dl), 73% haemoglobin S and apparently clinical features of sickling [127]. Compound heterozygosity for haemoglobin S and the γβ fusion haemoglobin, haemoglobin Kenya, has an interestingly mild phenotype, given that haemoglobin S is 60–70% of total haemoglobin [139]. Haemoglobin Kenya is about 18% and haemoglobin F about 8%; haemoglobins F and A_2 will inhibit sickling and one might be tempted to postulate that this could also be true of haemoglobin Kenya. Compound heterozygosity for haemoglobin S and various other variant haemoglobins can cause haematological abnormalities as a result of the characteristics of the second variant, rather than as a result of any interaction between the two variant haemoglobins; this appears to be true of haemoglobins I-Toulouse (unstable), San Diego (high oxygen affinity), Shelby (mildly unstable), Hope (unstable and low oxygen affinity) and North Shore ('thalassaemic') [52]. Compound heterozygosity for haemoglobin S and haemoglobin Monroe leads to a clinical syndrome resembling haemoglobin S/β^0 thalassaemia, as haemoglobin Monroe is unstable and constitutes only about 2% of total haemoglobin [140].

There are many β chain variants that do not interact with haemoglobin S, so that compound heterozygotes have clinical and haematological features resembling those of sickle cell trait. These include haemoglobins Camden, Caribbean, D-Ouled Rabah, D-Ibadan, Detroit, G-Galveston, G-San Jose, G-Szuhu, J-Amiens, J-Baltimore, J-Bangkok, K-Ibadan, K-Matupo, Korle Bu, K-Woolwich, Mobile, N-Baltimore, Ocho-Rios, Osu-Christiansborg, Pyrgos and Richmond [54,69].

Sickle cell disease in heterozygotes

Three variant haemoglobins in which the $\beta^{6Glu \rightarrow Val}$ substitution is one of two substitutions are capable of

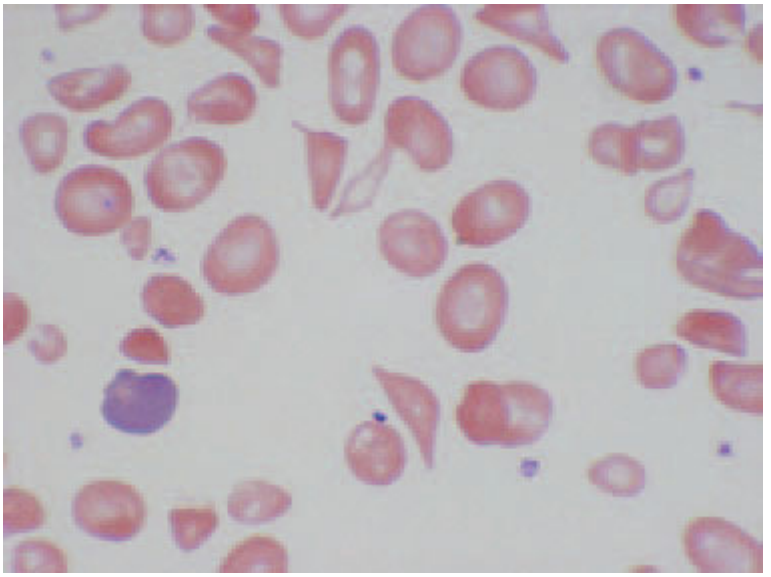


Fig. 4.40 Blood film from a patient with compound heterozygosity for haemoglobin S and haemoglobin S-Oman showing the 'Napoleon hat' red cells that are characteristic of haemoglobin S-Oman. (With thanks to Dr Samir Al Azzawi, Muscat, Sultanate of Oman.)

producing sickle cell disease in heterozygotes. They are haemoglobin S-Antilles, haemoglobin S-Oman and haemoglobin Jamaica Plain (Table 4.2). In the case of haemoglobin S-Oman, severe disease occurs in heterozygotes with coinheritance of $-\alpha/\alpha\alpha$, who have 20–27% of the variant haemoglobin, whereas those with coinheritance of $-\alpha/-\alpha$, who have 13–15% of haemoglobin S-Oman, have no significant clinical disease. The morphology of sickle cells in patients who are simple or compound heterozygotes for haemoglobin S-Oman differs from the morphology of classical sickle cells. There are cells that are pointed at both ends but fat in the middle; they have been compared to a yarn/knitting needle or to 'Napoleon hats' [138] (Fig. 4.40). Affected heterozygotes also differ clinically from individuals with sickle cell anaemia in that splenomegaly can persist into adult life.

Sickle cell disease can also occur in heterozygotes if there is coinheritance of another condition leading to a high concentration of 2,3-diphosphoglycerate (2,3-DPG) and reduced oxygen affinity. For example, a patient who had coinherited a severe pyruvate kinase deficiency had a two-fold increase in 2,3-DPG leading to reduced oxygen affinity and symptomatic sickling crises [26].

Check your knowledge

One to five answers may be correct. Answers to almost all questions can be found in this chapter or can be deduced from the information given. The correct answers are given on p. 189.

4.1 The coinheritance of haemoglobin S and the following haemoglobins usually produces a clinically significant sickling disorder
(a) haemoglobin C
(b) haemoglobin G-Philadelphia
(c) haemoglobin D-Punjab
(d) haemoglobin Lepore
(e) haemoglobin A

4.2 Haemoglobin S occurs in a significant proportion of individuals from the following ethnic groups
(a) Australian aboriginals
(b) Greeks
(c) southern Italians and Sicilians
(d) Saudi Arabs
(e) Nigerians

4.3 Recognized features of sickle cell trait include
(a) a defect in urine concentrating ability
(b) an increased incidence of gallstones
(c) an increased reticulocyte count
(d) leg ulcers
(e) susceptibility to clinically significant sickling in conditions of severe hypoxia

4.4 The following variant haemoglobins have the same mobility as haemoglobin S on cellulose acetate electrophoresis at alkaline pH
(a) haemoglobin C
(b) haemoglobin D
(c) haemoglobin E
(d) haemoglobin F
(e) haemoglobin G

4.5 The likelihood of red cell sickling occurring is increased by
(a) acidosis
(b) a lower partial pressure of oxygen
(c) an increased percentage of haemoglobin F
(d) reduced blood flow through tissues
(e) a lower mean cell haemoglobin concentration

4.6 In comparison with individuals with sickle cell anaemia, patients with compound heterozygosity for haemoglobin S and haemoglobin C usually have
(a) a higher percentage of haemoglobin A
(b) more severe anaemia
(c) a higher incidence of proliferative retinopathy
(d) a higher incidence of ischaemic necrosis of the femoral head
(e) earlier onset of blood film features of hyposplenism

4.7 Significant disease would be predicted in 25% of offspring if the partner of a pregnant woman with sickle cell trait had
(a) α thalassaemia trait
(b) β thalassaemia trait
(c) δ thalassaemia trait
(d) $\delta\beta$ thalassaemia trait
(e) non-deletional hereditary persistence of fetal haemoglobin

4.8 The disease phenotype is usually appreciably less severe than that of homozygosity for haemoglobin S in
(a) sickle cell/haemoglobin C disease
(b) sickle cell/β^+ thalassaemia
(c) sickle cell/deletional hereditary persistence of fetal haemoglobin
(d) sickle cell/$\delta\beta^0$ thalassaemia
(e) sickle cell/haemoglobin E

4.9 On haemoglobin electrophoresis at alkaline pH, homozygosity for haemoglobin S cannot be distinguished from
(a) sickle cell/haemoglobin C disease
(b) sickle cell/β^0 thalassaemia
(c) sickle cell/haemoglobin D-Punjab
(d) sickle cell/haemoglobin Lepore
(e) heterozygosity for both haemoglobin S and haemoglobin G-Philadelphia

4.10 A higher mortality rate in sickle cell anaemia correlates with
(a) a higher white cell count
(b) coexisting α thalassaemia trait
(c) a lower percentage of haemoglobin F
(d) male gender
(e) previous cerebrovascular accident

4.11 The blood count in sickle cell/haemoglobin C disease is characterized by
(a) generally mild anaemia
(b) reticulocytosis
(c) increased mean cell volume
(d) increased mean cell haemoglobin concentration
(e) increased red cell distribution width and haemoglobin distribution width

4.12 The haemoglobin F percentage in sickle cell anaemia is affected by
(a) age
(b) gender
(c) haplotype of the β globin gene cluster
(d) the F-cell locus on the X chromosome
(e) hydroxycarbamide (hydroxyurea) therapy

Further reading

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Answers to questions

4.1	(a) T	4.3	(a) T	4.5	(a) T	4.7	(a) F	4.9	(a) F	4.11	(a) T
	(b) F		(b) F		(b) T		(b) T		(b) T		(b) T
	(c) T		(c) F		(c) F		(c) F		(c) T		(c) F
	(d) T		(d) F		(d) T		(d) T		(d) T		(d) T
	(e) F		(e) T		(e) F		(e) F		(e) F		(e) T
4.2	(a) F	4.4	(a) F	4.6	(a) F	4.8	(a) T	4.10	(a) T	4.12	(a) T
	(b) T		(b) T		(b) F		(b) T		(b) F		(b) T
	(c) T		(c) F		(c) T		(c) T		(c) T		(c) T
	(d) T		(d) F		(d) T		(d) T		(d) T		(d) T
	(e) T		(e) T		(e) F		(e) T		(e) T		(e) T