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22.6.7 Disorders of the synthesis or function of haemoglobin 5427 Disorders of haemoglobin can be genetic or acquired and may be caused by disordered production of one or more globin chains or structural change in the globin chain. The most important disorders are the genetic conditions thalassaemia and sickle cell disease. Thalassaemias A heterogeneous group of genetic disorders, all resulting from a re- duced rate of production of one or more of the globin chains of haemoglobin and inherited in a simple Mendelian fashion. They are clinically classified according to their severity into major (a se- vere transfusion-dependent disorder), intermediate (characterized by anaemia and splenomegaly), and minor (a symptomless carrier state) forms. The β thalassaemias, which occur in patients with ethnic origin from a broad belt ranging from the Mediterranean and parts of North and West Africa through the Middle East and Indian sub- continent to South-East Asia, are the

most important types of thalassaemia because they are very common and produce severe anaemia in their homozygous and compound heterozygous states. Most countries in which the disease is common are putting a major effort into programmes for its prevention (population screening and prenatal diagnosis). Symptomatic management of severe dis- ease requires regular blood transfusion, judicious use of splenec- tomy if hypersplenism develops, and chelating agents to reduce iron overload. Sickle cell disease Haemoglobin S differs from haemoglobin A by the substitution of valine for glutamic acid at position 6 in the β globin chain, and homozygosity for haemoglobin S produces the state of sickle cell disease. This occurs very frequently in African populations and sporadically throughout the Mediterranean region and the Middle East, with extensive pockets in India. Typical presentation is in in- fancy with symptoms related to anaemia or infection, but clinical manifestations are very variable, ranging from an almost incidental finding on routine haematological examination to severe haemolytic anaemia interspersed with frequent exacerbations or crises, which can take various forms and may be life-threatening. Management of both acute and chronic complications remains largely supportive, with hydroxycarbamide being the only clinically proven effective treatment to date in routine clinical use. However, investigational agents targeting the complex pathophysiology of sickle cell anaemia are in clinical trials and promise to improve outcomes for patients with this disease. Introduction Disorders of the synthesis or structure of haemoglobin may be either inherited or acquired. The inherited disorders of haemo- globin are the commonest single-gene disorders with hundreds of millions of carriers worldwide, and at least 300 000 severely af- fected homozygotes or compound heterozygotes born annually. The greatest prevalence is in low and middle income countries of the tropical belt. The main reason for the extremely high frequency of these conditions in these regions is that heterozygotes show a vari- able degree of resistance to infection with Plasmodium falciparum malaria. There has therefore been intense selection for these muta- tions in countries where malaria is common. As economic condi- tions improve in these countries, and infant and childhood death rates fall, the genetic disorders of haemoglobin place a major burden on health services. As a result of migration of populations from high-incidence areas for the haemoglobin disorders, these conditions are now seen with increasing frequency in all parts of the world. Some of them, particularly sickle cell anaemia and the more severe forms of thal- assaemia, can produce life-threatening medical emergencies. It is important for all clinicians to have a working knowledge of their clinical features, management, and prevention. Haemoglobin disorders are also of particular interest because they were the first group of diseases to be analysed genetically. More is known about their molecular pathology than any other genetic disorders and their study has given us insight into the wide reper- toire of mutations that underlie inherited diseases in humans. Before describing the haemoglobin disorders, it is necessary to discuss briefly the structure, function, and synthesis of haemoglobin and the way that it is genetically determined. The structure, function, genetic control, and synthesis of haemoglobin Structure Human haemoglobin is heterogeneous at all stages of develop- ment; different haemoglobins are synthesized in the embryo, fetus, and adult, each adapted to the particular oxygen requirements. Each human haemoglobin has a tetrameric structure made up of two different pairs of globin chains, each attached to one haem mol- ecule (Fig. 22.6.7.1). At each stage of development, the tetramer is made from two alpha (α)-like chains and two beta (β)-like chains. The α-like chains are α and zeta (ζ) globins, encoded by adjacent genes on the telomeric tip of chromosome 16. Of these, ζ globin is transcribed in embryonic life, and α globin in fetal and adult life. The β globin locus on chromosome 11 encodes β-like chains expressed at different stages of maturation—epsilon (ε) globin expressed em- bryonically, gamma (γ) globin in the fetus, and delta (δ) globin and β globin in the adult. Thus, adult and fetal haemoglobins have α chains

combined with β chains (Hb A, α2β2), δ chains (Hb A2, α2δ2), or γ chains (Hb F, α2γ2). In embryos, ζ chains combine with γ chains to produce Hb Portland (ζ2γ2), or with ε chains to make Hb Gower 1 (ζ2ε2), and α and ε chains combine to form Hb Gower 2 (α2ε2). Fetal haemoglobin (Hb F α2γ2) is itself heterogeneous; there are two kinds of γ chains which differ in their amino acid composition at position 136, where they have either glycine (Gγ) or alanine (Aγ). The Gγ and Aγ chains are the products of separate (Gγ and Aγ) loci in the β globin cluster. Function The sigmoid shape of the oxygen dissociation curve, which reflects the allosteric properties of haemoglobin, ensures that oxygen is rap- idly taken up at high oxygen tensions in the lungs, and that it is re- leased readily at the lower tensions encountered in the tissues. The shape of the curve is due to cooperativity between the four haem molecules. When one takes on oxygen, the affinity for oxygen of the

section 22 Haematological disorders 5428 remaining haems of the tetramer increases dramatically. This is be- cause haemoglobin can exist in two configurations, deoxy (T) and oxy (R) (T and R stand for tight and relaxed states, respectively). The T form has a lower affinity than the R form for ligands such as oxygen. During the sequential addition of oxygen to the four haems, transition from the T to R configuration occurs and the oxygen af- finity of the partially liganded molecule increases rapidly. The position of the oxygen dissociation curve can be modi- fied in many ways. First, oxygen affinity decreases as CO_2 tension rises, the Bohr effect. This facilitates oxygen delivery to the tissues, where the pH falls due to CO_2 generation. The opposite effect oc- curs in the lungs. Oxygen affinity is also modified by the level of 2,3-bisphosphoglycerate (2,3-BPG) in the red cell. Increasing con- centrations move the curve to the right, reducing oxygen affinity. Diminishing concentrations have the opposite effect. The 2,3-BPG mechanism plays an important role in the response to hypoxia (see Chapter 22.6.2). Genetic control The arrangement of the two main families of globin genes is illus- trated in Fig. 22.6.7.2. The β-like globin genes form a linked cluster on chromosome 11 that spans about 60 kb; they are arranged in the order 5′-ε-Gγ-Aγ-ψβ-δ-β-3′. The α-like globin genes form a linked cluster on chromosome 16, in the order 5′-ζ-ψζ-ψα-α2-α1-3′. The ψβ, ψζ, and ψα genes are pseudogenes; their sequences resemble the β, ζ, or α genes but contain mutations which prevent them from functioning as structural genes. They may be ‘burnt out’ remnants of genes which were functional at an earlier stage of evolution. The molecular machinery required for gene expression has been defined comprehensively for the globin genes, in part through the study of patients with unusual forms of thalassaemia. The promoters, regulatory elements, 5′ and 3′ untranslated regions and splice sites are all well defined for both α globin and β globin. Individual globin chains combine with haem, which is synthesized through a separate pathway to form definitive tetrameric haemoglobin molecules. Classification of the disorders of haemoglobin The main groups of disorders of haemoglobin are shown in Box 22.6.7.1. The genetic disorders are divided into those in which there is a reduced rate of production of one or more of the globin chains, the thalassaemias, and those in which a structural change in a globin chain leads to instability or to abnormal oxygen transport. In addition, there is a harmless group of mutations, known collect- ively as hereditary persistence of fetal haemoglobin, that interfere with the normal switching of fetal to adult haemoglobin production. Tyr HC2 Val E11 FG2 F9 G1 F1 G5 V M F8 C3 C7 M CD1 CD2 CD E7 C P D1 E1 D7 B5 A16 B1 AB1 G19 GH4 A1 H5 E20 EF1 G15 NA2 EF3 H16 V M NA1 NH3 + HI C1 E5 Fig. 22.6.7.1 The α chain subunit of human haemoglobin showing the position of the haem molecule in a cleft formed by the globin chain. The helical parts of the chain are given letters of the alphabet and each amino acid residue in each helical region has a specific number, for example, Val E11 is the 11th amino acid in the E helical region. The nonhelical regions of the N- and C-terminal ends of the chains are labelled NA and HC respectively. Reproduced by

permission of Dr M F Perutz and the editors of the Cold Spring Harbor Symposia for Quantitative Biology. 1 Kb 11 Embryo Fetus Hb Portland Hb Gower 1 Hb Gower 2 HbF HbA HbA2 16 105 104 31 31 30 32 99 100 Adult ζ2 ψζ1 ψα 2 ψβ α2 α1 ε Gγ Aγ β δ Fig. 22.6.7.2 The genetic control of human haemoglobin. Two of the genes are enlarged to show the introns (unshaded) and exons (purple) plus 3′ and 5′ untranslated regions (lilac). 1 kb = 1000 nucleotide bases.

22.6.7 Disorders of the synthesis or function of haemoglobin 5429 The acquired disorders of haemoglobin can also be subdivided into those characterized by defective synthesis of the globin chains and those in which the structure of the haem molecules is altered, leading to inefficient oxygen transport. Like all biological classifications, this way of classifying the haemoglobin disorders is not entirely satisfactory. For example, some structural variants are synthesized in reduced amounts and hence produce the clinical picture of thalassaemia. The thalassaemias Historical introduction The thalassaemias are the commonest of the inherited haemato- logical disorders and, indeed, are the commonest single-gene dis- orders in the world population. The condition was first recognized in 1925 by Thomas B. Cooley, who described infants who became profoundly anaemic and developed splenomegaly over the first year of life. A milder form was described independently in the same year by Fernando Rietti. As further cases were identified the dis- order was variously called von Jaksch's anaemia, splenic anaemia, erythroblastosis, Mediterranean anaemia, or Cooley's anaemia. In 1936, George Whipple and Lesley Bradford recognized that many of their patients came from the Mediterranean region and hence they invented the word 'thalassaemia' from the Greek word meaning 'the sea'. Although it was realized later that the disorder occurs throughout the world and is not localized to the Mediterranean re- gion, the name has stuck. Thalassaemia is extremely heterogeneous. Its clinical picture can result from the interaction of many different genetic defects. This chapter concentrates mainly on the clinical and haematological aspects; readers who wish to learn more about the molecular pathology and population genetics of thalassaemia are referred to reviews and monographs listed at the end of this chapter. Definition and classification The thalassaemias are a heterogeneous group of genetic disorders of haemoglobin synthesis, all of which result from a reduced rate of production of one or more of the globin chains of haemoglobin. They are divided into the α, β, δβ, or εγδβ thalassaemias, according to which globin chain is produced in reduced amounts (Box 22.6.7.2). In some thalassaemias, no globin chain is synthesized at all; these are called α° or β° thalassaemias. In others, the α+ or β+ thalassaemias, globin chain is produced but at a reduced rate. Thalassaemia occurs in populations in which structural haemoglobin variants are also common and an individual may inherit a thalassaemia gene from one parent and a gene for a structural haemoglobin variant from the other. Both α and β thalassaemia occur commonly in some countries and hence individuals may carry genetic changes causing both types. These different interactions produce an extremely complex and clin- ically diverse series of genetic disorders which range in severity from death in utero to extremely mild, symptomless hypochromic anaemias. The thalassaemias are inherited in a simple Mendelian fashion. Heterozygotes are usually symptomless, although they can be easily recognized haematologically. More severely affected patients are either homozygotes for α or β thalassaemia, compound het- erozygotes for different molecular forms of α or β thalassaemia, or compound heterozygotes for thalassaemia and a structural haemoglobin variant. Clinically, the thalassaemias are classified according to their severity into major, intermediate, and minor forms. Thalassaemia major is a severe transfusion-dependent dis- order. Thalassaemia intermedia is characterized by anaemia and splenomegaly though not of such severity as to require regular transfusion. Thalassaemia minor is the symptomless carrier state.

While these descriptive terms do not have a precise genetic meaning, they remain useful in clinical practice and may be sim- plified further into transfusion-dependent and non transfusion-dependent thalassaemia. β Thalassaemias The β thalassaemias are the most important types of thalassaemia because they are very common and produce severe anaemia in their homozygous and compound heterozygous states (Table 22.6.7.1). Distribution Patients with the β thalassaemias have an ethnic origin that re- lates to a broad belt ranging from the Mediterranean and parts of North and West Africa through the Middle East and Indian subcon- tinent to South-East Asia (Fig. 22.6.7.3). The high incidence zone stretches north through the Balkans and the southern parts of Russia and includes the southern regions of China. The disease is particu- larly common in South-East Asia where it occurs from southern Box 22.6.7.1 Disorders of haemoglobin Genetic • Thalassaemia • Structural variants • Hereditary persistence of fetal haemoglobin • α Thalassaemia/mental retardation syndromes Acquired • Methaemoglobin • Carbonmonoxyhaemoglobin • Sulphaemoglobin • Glycosylated haemoglobin • Acquired HbH disease • Disorders associated with raised levels of haemoglobin F Box 22.6.7.2 The thalassaemias α Thalassaemia • α° • α+ β Thalassaemia • β° • β+ δβ Thalassaemia • (δβ)° • Haemoglobin Lepore (δβ)+ • (εγδβ)° Thalassaemia • δ Thalassaemia

section 22 Haematological disorders 5430 China, through Thailand, the Malay peninsula and Indonesia, to some of the Pacific islands. In these populations, and in some of the Mediterranean islands and mainland countries, gene frequencies for the various forms of β thalassaemia range between 2 and 20%. It should be remembered that β thalassaemia is not entirely con- fined to these high-incidence regions; it occurs sporadically in every racial group. Molecular pathology The precise molecular lesions responsible for the defective synthesis of the β globin chains have been determined for many patients with β thalassaemia. The disease is extremely heterogeneous with nearly 400 different mutations found to date which result in the clinical phenotype of β thalassaemia. With the exception of a deletion of about 600 bases at the 3´ end of the β globin gene, which is only found in certain popula- tions of northern India, deletions are an uncommon cause of β thalassaemia. Most of the mutations are single base changes or small deletions and insertions of one or two bases. These occur in both introns and exons, and also outside the coding regions. Nonsense, frameshift, and splice site mutations have all been de- scribed. Mutations activating cryptic splice sites have been ob- served, causing a β+ thalassaemia with severity dependent on the relative usage of the normal and abnormal splice sites. Many single base substitutions have also been found in the flanking re- gions of the β globin genes. They alter either the proximal pro- moter regions or adjacent transcriptional regulatory machinery (e.g. enhancers). Because there are so many different β thalassaemia mutations it follows that many patients who are apparently homozygous for β thalassaemia are, in fact, compound heterozygotes for two different molecular lesions. Pathophysiology The mutations that cause β thalassaemia result in absent or re- duced β chain production. The synthesis of α chains proceeds at a normal rate and hence there is imbalanced globin chain synthesis (Fig. 22.6.7.4). In the absence of their partner chains the excess α chains are unstable and precipitate in the red cell precursors, forming large intracellular inclusions. These interfere with red cell maturation, and hence there is a variable degree of intramedullary destruction of red cell precursors, (ineffective erythropoiesis). Those red cells which mature and enter the circulation contain α chain inclusions which interfere with their passage through the microcirculation, particularly in the spleen. These cells are prema- turely destroyed. However, the mechanisms of the destruction of red cell precursors and their progeny are extremely complex and are not simply a reflection of mechanical damage to the red cells. Free

α chains and their degradation products, particularly haem and iron, cause severe oxidative damage to the red cell membrane proteins. The end result is a dehydrated, rigid erythrocyte with a markedly shortened survival. Table 22.6.7.1 The β, δβ, and γδβ thalassaemias Type of thalassaemia Findings in homozygote Findings in heterozygote β° Thalassaemia majora, b Thalassaemia minor Hbs F and A2 Raised Hb A2 β+ Thalassaemia majora, b Thalassaemia minor Hbs F, A, and A2 Raised Hb A2 δβ Thalassaemia intermedia Thalassaemia minor Hb F only Hb F 5–15%; Hb A2 normal (δβ)+ Thalassaemia major or intermedia Thalassaemia minor (Lepore) Hbs F and Lepore Hb Lepore 5–15%; Hb A2 normal εγδβ Not viable Neonatal haemolysis Thalassaemia minor in adults, with normal Hbs F and A2 a Occasionally have thalassaemia intermedia phenotype. b Many patients with thalassaemia are compound heterozygotes for different molecular forms of β° or β+ thalassaemia. CODON 6 – 1 bp IVS 1 – 1G A IVS 2 – 1G A IVS 2 – 745 C G CODON 39 CAG TAG IVS 1 – 6T C IVS 1 – 110 G A IVS 1 – 5 G C IVS 1 – 1 G T CODONS 41– 42.bp DEL. CODONS 26 GAG AAG (HbE) IVS 1 – 5 G C IVS 2 – 654 C T CODONS 41 – 42.4bp DEL. CODON 17 AAG TAG CODON 26 GAG AAG(HbE) –28A G –29A G –29 A G –88 C T CODON 24 T A POLY-A T C IVS 1 – 5 G C 619 bp DELETION CODON 8/9 + G IVS 1 – 1 G T CODONS 41– 42.4 bp DEL. IVS 1 – 110 G A IVS 1 – 5 G C IVS 1 – 6 T C CODON 39 CAG TAG CODON 8 2bp DEL Fig. 22.6.7.3 World map showing the distribution of the different β thalassaemia mutations.

22.6.7 Disorders of the synthesis or function of haemoglobin 5431 If untreated, the anaemia acts as a stimulus to increase erythro- poietin production, causing massive expansion of the bone marrow which may lead to serious deformities of the skull and long bones. Because the spleen is being constantly bombarded with abnormal red cells, it hypertrophies. The resulting spleno-megaly and bone marrow expansion gives rise to an increase in the plasma volume which, together with pooling of the red cells in the enlarged spleen, causes an exacerbation of an already severe degree of anaemia. As mentioned previously, fetal haemoglobin production largely ceases after birth. However, some adult red cell precursors (F cells) retain the ability to produce a small number of γ chains. Because the latter can combine with excess α chains to form haemoglobin F, cells which make relatively more γ chains in the bone marrow of β thalassaemics are partly protected against the deleterious effect of α chain precipitation. Red cell precursors which produce haemo-globin F are selected in the marrow and peripheral blood of these patients. Thus, they have relatively large amounts of haemoglobin F in their red cells. Furthermore, because δ-chain synthesis is un- affected, the disorder is characterized by a relative or absolute in- crease in haemoglobin A2 (α2δ2) production. If the anaemia is corrected with blood transfusion the erythro-poietic drive is reduced, growth and development are improved, and bone deformities do not occur. However, as each unit of blood contains 200 mg of iron, regular transfusion results in the steady accumulation of iron in the liver, endocrine glands, and myocardium. Even though well-transfused thalassaemic chil- dren grow and develop normally, they die of iron overload unless steps are taken to remove iron (see iron chelation, in ‘Symptomatic treatment’). The severe homozygous or compound heterozygous forms of β thalassaemia These are the commonest and most important forms of thalas- saemia and give rise to a major public health problem in many parts of the world. Clinical features Most severe forms of β thalassaemia present within the first year of life, as fetal haemoglobin production declines, with failure to thrive, poor feeding, intermittent bouts of fever, or failure to improve after an intercurrent infection. At this stage, the af- fected infant is pale and splenomegaly may already be present. Diagnosis depends on the haematological changes outlined in the following paragraphs. The clinical manifestations of the severe forms of β thalassaemia have to be described in two contexts: (1) the well-transfused child

and (2) the child with chronic anaemia throughout early life. In the well-transfused thalassaemic child, early growth and de- velopment is normal. Splenomegaly is minimal. However, there is a gradual accumulation of iron and the effects of tissue siderosis start to appear by the end of the first decade in the unchelated patient. The normal adolescent growth spurt fails to occur. Hepatic, endocrine, and cardiac complications of iron overloading produce a variety of problems including diabetes, hypopara- thyroidism, adrenal insufficiency, and progressive liver failure. Secondary sexual development is delayed or does not occur at all. Short stature and lack of sexual development may lead to serious psychological problems. By far the commonest cause of death, which usually occurs toward the end of the second or early in the third decade in patients who do not receive iron chelation, is pro- gressive cardiac damage. Ultimately these patients die either as a result of protracted cardiac failure or suddenly, as the result of an acute arrhythmia. Children who have been both adequately transfused and che- lated may grow and develop normally, pass through a normal puberty, and survive to adult life in good health. However, even children who have been well managed in this way may still suffer from complications as they get older, particularly delayed sexual maturation, growth disturbances, and osteoporosis. It seems likely that many of these problems are due to subtle damage to the hypothalamic–pituitary axis with secondary hypogonadism. The clinical picture in children who are inadequately trans- fused is quite different. Rates of growth and development are Excess Precipitation Haemolysis Anaemia Transfusion Tissue hypoxia Erythropoietin Marrow expansion Iron loading Destruction of red blood cell precursors Splenomegaly (pooling, plasma volume expansion) Ineffective erythropoiesis High oxygen affinity of red cells Bone deformity Increased metabolic rate Wasting Gout Folate deficiency Endocrine deficiencies Cirrhosis Cardiac failure Death Selective survival of HbF-containing cells HbF Increased iron absorption α α2γ2 β γ Fig. 22.6.7.4 The pathophysiology of β thalassaemia.

section 22 Haematological disorders 5432 markedly slowed. There is progressive splenomegaly; hyper splenism may cause a worsening of the anaemia. Because of the bone marrow expansion there may be deformities of the skull with marked bossing and overgrowth of the zygomata giving rise to the classical facial appearance of β thalassaemia (Fig. 22.6.7.5). These findings are reflected by radiological changes which include a lacy, trabecular pattern of the long bones and phalanges and a typical ‘hair-on-end’ appearance of the skull (Fig. 22.6.7.7). These bone changes may be associated with recurrent fractures. There is increased susceptibility to in- fection which may cause a catastrophic drop in the haemoglobin level. Because of the massive marrow expansion, these chil- dren are hypermetabolic, run intermittent fevers, lose weight (Fig. 22.6.7.6), have increased requirements for folic acid, and may become acutely folate depleted with worsening of their anaemia. Increased turnover of red cell precursors occasion- ally gives rise to hyperuricaemia and secondary gout. There is a bleeding tendency which, partly due to thrombocyto- penia secondary to hypersplenism, may be exacerbated by liver damage associated with iron loading and extramedullary haemopoiesis. There is also an increased risk of thrombotic complications, reflecting procoagulant properties of the ab- normal red cell membranes. The bone deformities of the skull can cause distressing dental complications with poorly formed teeth and malocclusion, and inadequate drainage of the sinuses and middle ear which may lead to chronic sinus infection and deafness. If these children survive to puberty, they develop the same complications of iron loading as the well-transfused patients. In this case, some of the iron accu- mulation results from an increased rate of gastrointestinal absorption (due to decreased hepcidin production—see also Chapter 22.6.4) as well as that derived from the inadequate transfusion regimen.
Laboratory features There is always a severe anaemia. The haemoglobin values on presentation

range from 20 to 80 g/litre. The appearance of the stained peripheral blood film is grossly abnormal (Fig. 22.6.7.8). The red cells show marked hypochromia and variation in shape Fig. 22.6.7.5 Homozygous β thalassaemia: skull and facial deformity due to bone marrow expansion.
Fig. 22.6.7.6 Gross wasting of the limbs and hepatomegaly in an undertransfused child.
Fig. 22.6.7.7 Radiological changes of the skull in homozygous β thalassaemia.

22.6.7 Disorders of the synthesis or function of haemoglobin 5433 and size. There are many hypochromic macrocytes and misshapen microcytes, some of which are mere fragments of cells. There is anisochromia, basophilic stippling, and some nucleated red cells in the peripheral blood. After splenectomy, nucleated cells are found in large numbers. In the postsplenectomy film, many of the nucle- ated cells and mature erythrocytes show ragged inclusions after incubation of the blood with methyl violet, representing free ex- cess α globin. There is usually a slight elevation in the reticulocyte count. The white cell and platelet counts are normal unless there is hypersplenism in which case they are reduced. The bone marrow shows marked erythroid hyperplasia. The haemoglobin F level is always elevated. In β° thalassaemia there is no haemoglobin A and the haemoglobin consists of F and A2 only. In β+ thalassaemia the level of haemoglobin F ranges from 30 to 90% of the total haemoglobin. The haemoglobin A2 level is usually normal and is of no diagnostic value. There are biochemical changes of increased haemolysis and progressive iron loading. The bilirubin level is usually elevated and haptoglobins are absent. The serum iron and serum ferritin rises progressively. Most transfusion-dependent children have a totally saturated iron-binding capacity. Liver biopsies show a marked increase in hepatic iron, which may be distributed both in the reticuloendothelial and parenchymal cells, and magnetic res- onance imaging (MRI) of the heart and liver manifest markedly shortened relaxation times, consistent with iron loading (see later in this chapter: Fig. 22.6.7.18). As well as folic acid deficiency, vitamin E and ascorbate defi- ciency is common in thalassaemic children. The endocrine com- plications of iron loading include diabetes, and parathyroid or adrenal insufficiency. Growth hormone levels are usually normal. Heterozygous β thalassaemia Carriers for β thalassaemia, apart from symptoms of mild an- aemia, are usually well except in periods of stress such as preg- nancy, when they may become more anaemic. Splenomegaly is rarely present. There is a mild degree of anaemia with haemoglobin values of 90 to 110 g/litre. The red cells are hypochromic and microcytic. The reticulocyte count is usually normal. The bone marrow shows moderate erythroid hyperplasia. Haemoglobin analysis shows an elevated haemoglobin A2 level of 4 to 6%, and there may also be a slight elevation of haemoglobin F to approximately 1 to 3%. A less common form occurs in which the haemoglobin A2 is not elevated (see ‘Other β thalassaemia variants’). β Thalassaemia in association with haemoglobin variants Although numerous interactions between thalassaemia and struc- tural haemoglobin variants have been described, in clinical practice only three are of importance: sickle cell β thalassaemia, haemo- globin C β thalassaemia, and haemoglobin E β thalassaemia. Sickle cell β thalassaemia The clinical manifestations which result from the interaction of the β thalassaemia and sickle cell genes vary considerably from race to race, and depend on the severity of the thalassaemia de- terminant. In African populations, there are mild forms of β+
thalassaemia which, when they interact with the sickle cell gene, produce a condition characterized by mild anaemia and few sickling crises. By contrast, in Mediterranean populations, the combination of a β° or severe β+ thalassaemia determinant from one parent with a sickle cell gene from the other may give a clin- ical picture which is indistinguishable from sickle cell anaemia (see later). The diagnosis of sickle cell thalassaemia rests on the clinical features of a sickling disorder

found in association with a per- ipheral blood picture with typical thalassaemic red cell changes, that is, a low mean cell haemoglobin and mean cell volume. In the more severe forms of sickle cell β° thalassaemia, there may be an elevated reticulocyte count and sickled red cells are found on the peripheral blood film. The diagnosis can be confirmed by high-performance liquid chromatography (HPLC) or haemo- globin electrophoresis, which in sickle cell β+ thalassaemia shows haemoglobin S together with 10 to 30% haemoglobin A and an elevated haemoglobin A2. In sickle cell β° thalassaemia, the haemoglobin consists mainly of haemoglobin S with an elevated level of haemoglobins F and A2 and is therefore indistinguishable from homozygous sickle cell disease. To confirm the diagnosis, DNA analysis is required. Haemoglobin C thalassaemia This disorder is restricted to patients of West African ethni- city and to some North African and southern Mediterranean populations. It is characterized by a mild haemolytic anaemia associated with splenomegaly. The peripheral blood film shows numerous target cells and thalassaemic red cell changes with a moderately elevated reticulocyte count. Haemoglobin HPLC shows a preponderance of haemoglobin C. The diagnosis is con- firmed by finding the haemoglobin C trait in one parent and the β thalassaemia trait in the other, or again by recourse to DNA sequencing. Haemoglobin E β thalassaemia This is the commonest form of severe thalassaemia in many Asian countries where it causes a serious public health burden. The Fig. 22.6.7.8 Peripheral blood film in homozygous β thalassaemia (×630, Leishman stain).

section 22 Haematological disorders 5434 mutation that underlies haemoglobin E produces an alternative splice site in the β globin gene. This mutation results in the pro- duction of a β globin variant which is produced at a much lower rate than normal β globin. Thus, when a haemoglobin E gene is inherited together with a severe β thalassaemia mutation there is a marked inefficiency of β chain production. However, one of the major characteristics of haemoglobin E β thalassaemia, which causes particular difficulties for its management, is its extraor- dinary clinical heterogeneity. At one end of the spectrum it is indistinguishable from β thalassaemia major, while at the other end there are patients who grow and develop normally without the need for transfusion. While some of this phenotypic vari- ability can be ascribed to the inheritance of β thalassaemia alleles of varying severity, some is also due to coinheritance of various modifier genes, including those for α thalassaemia or increased haemoglobin F production. The explanation for much of this phenotypic variation remains unclear. In the more severe forms of this condition the findings are very similar to those in severe β thalassaemia (Fig. 22.6.7.9), while in the milder forms they resemble those of β thalassaemia intermedia, as described later in this chapter. Complications in- clude susceptibility to infection, hypersplenism, iron loading, neurological lesions due to extramedullary erythropoietic masses extending inwards from the inner tables of the skull or vertebrae, folate deficiency, and recurrent pathological frac- tures. From the limited data that are available, it seems that patients at the milder end of the clinical spectrum, though often quite anaemic, survive in good health well into adult life. They do not appear to develop cardiac complications unless they have become particularly iron loaded from increased intestinal absorption. The diagnosis of haemoglobin E thalassaemia is confirmed by finding haemoglobins E and F and little or no haemoglobin A on HPLC and by demonstrating the haemoglobin E trait in one parent and the β thalassaemia trait in the other. Other β thalassaemia variants It is not uncommon to encounter patients with the clinical and haematological features of heterozygous β thalassaemia who do not have an elevated haemoglobin A2 level. Many of these in- dividuals are heterozygotes for both β and δ thalassaemia. It is important to recognize this interaction because, if it is inherited together with a typical β thalassaemia gene, it can produce a severe transfusion-dependent disorder. Hence this variant is

important in antenatal screening programmes. It can only be identified for certain by genetic analysis of the affected locus. Families are oc- casionally encountered in which there is a more severe form of heterozygous β thalassaemia associated with anaemia, jaundice, and splenomegaly. In some of these families it is apparent that the affected individuals are in fact compound heterozygotes for β thalassaemia and the so-called silent β thalassaemia gene, that is, a determinant which cannot be identified haematologically in heterozygotes. In other families, a severe form of β thalassaemia behaves as a single gene disorder with full expression in heterozy- gotes, that is, it follows a dominant form of inheritance. In most of these families, the disorder results from the synthesis of a highly unstable β globin chain. The δβ thalassaemias See Table 22.6.7.1. Molecular genetics and classification Disorders due to reduced β and δ chain synthesis are much less common than those due to defective β chain production alone. They are remarkably heterogeneous at the molecular level and may result from deletions of the β and δ globin genes (the (δβ)° thalassaemias). Unequal crossing over between the δ and β globin gene loci may also occur, with the production of δβ fusion genes. These produce δβ fusion chains which combine with α chains to form haemoglobin variants called the Lepore haemoglobins (Lepore was the family name of the first patient to be recognized with this disorder). Clinical and haematological changes The (δβ)° thalassaemias have been reported in many populations, although there are no high- frequency areas. In the homozygous state there is a mild degree of anaemia with haemoglobin values of 80 to 100 g/litre. There is often a moderate degree of splenomegaly but these patients are usually symptomless except during periods of stress such as infection or pregnancy. Haemoglobin analysis shows 100% haemoglobin F. Heterozygous carriers have thalassaemic blood pictures, elevated levels of haemoglobin F of 5 to 20%, and normal levels of haemoglobin A2. The homozygous state for haemoglobin Lepore is character- ized by a clinical picture which is usually similar to that of homo- zygous β thalassaemia although in some cases it may be milder and nontransfusion dependent. The haematological findings are similar to those of β thalassaemia. The haemoglobin consists of F and Lepore only. Heterozygous carriers have thalassaemic Fig. 22.6.7.9 Bossing of the skull in haemoglobin E thalassaemia.

22.6.7 Disorders of the synthesis or function of haemoglobin 5435 blood pictures associated with about 5 to 15% haemoglobin Lepore. The (εγδβ)0 thalassaemias There are several rare forms of thalassaemia which result from long deletions of the β globin gene cluster which, as well as re- moving or inactivating the β genes, involve the δ, γ, and embryonic ε genes. They also involve the main regulatory sequence upstream of the β globin gene cluster, the locus control region. This means that there is no output of globin chains from this gene cluster at all. Clearly, the homozygous state for these disorders would not be compatible with survival. Heterozygotes often have severe haemo- lytic disease as neonates with anaemia and hyperbilirubinaemia. If they survive the neonatal period they grow and develop nor- mally; in adult life they have the haematological picture of het- erozygous β thalassaemia with mild anaemia, hypochromic microcytic red cells, and a haemoglobin pattern consisting of haemoglobin A, no elevation of haemoglobin F, and a normal level of haemoglobin A2. Hereditary persistence of fetal haemoglobin There is a complex family of conditions characterized by per- sistent fetal haemoglobin synthesis into adult life associated with no major haematological abnormalities. In some cases they result from long deletions of the β globin gene cluster, similar to those that cause δβ thalassaemia. Indeed, they form a continuum with this condition; homozygotes have 100% fetal haemoglobin, elevated haemoglobin levels, and no clinical find- ings. Other forms result from point mutations in

the promoter regions of the γ globin genes. In this case there is increased γ chain production together with reduced β chain production on the affected chromosome. Hence, homozygotes have markedly elevated levels of haemoglobin F but also produce some haemo- globin A. Finally, there is a group in which persistent low levels of haemoglobin F, in the 3 to 10% range, are observed. They re- sult from mutations either within the β globin gene cluster or on other chromosomes. The only clinical importance of this complex group of condi- tions is that they may interact with the thalassaemias or struc- tural haemoglobin variants and reduce the severity of different phenotypes by increasing the amount of haemoglobin F that is produced. The α thalassaemias Although the α thalassaemias are commoner on a global basis than the β thalassaemias, they pose less of a public health problem be- cause their severe forms only occur in a few regions. Distribution The α thalassaemias occur in patients whose ethnic origin re- lates to the Mediterranean region, parts of West Africa, the Middle East, parts of the Indian subcontinent, and throughout South-East Asia from southern China through Thailand, the Malay peninsula, and Indonesia to the Pacific island popula- tions (Fig. 22.6.7.10). The serious forms of α thalassaemia are restricted mainly to patients of Mediterranean and South-East Asian ethnicity. Inheritance and molecular pathology As both haemoglobins A and F have α chains, genetic disorders of α chain synthesis result in defective fetal and adult haemoglobin production. In the fetus, deficiency of α chains means there is a relative excess of γ chains which form γ4 tetramers also known as haemoglobin Bart's (Fig. 22.6.7.11). In adults, a deficiency of α chains leads to a relative excess of β chains which form β4 tetramers, or haemoglobin H, the adult counterpart of haemoglobin Bart's. However, a critical level of globin chain imbalance is required be- fore detectable amounts of haemoglobins Bart's or H appear in the red cells, and in individuals with mild forms of α thalassaemia this level is not reached; significant amounts of these haemoglobin vari- ants occur only in the red cells of patients who have a severe degree of α chain deficiency. As normal individuals receive two α globin genes from each of their parents, αα/αα, the genetic basis of the α thalassaemias is 5–40% 1–15% 60% 40–80% 5–80% 5–15% α + Thalassaemia α ° Thalassaemia Fig. 22.6.7.10 World map showing the distribution of the α thalassaemias. Adult Fetus Normal Excess Excess HbF HbA Hb Bart's [High oxygen affinity] HbH High oxygen affinity Unstable Inclusions Hypochromia Haemolysis Hypoxia α Thalassaemia γ2 α2 α2 α2γ2 α2β2 β4 γ4 β2 Fig. 22.6.7.11 The pathophysiology of α thalassaemia.

 more complicated than that of the β thalassaemias. It is useful to define these conditions in heterozygotes. First, there is a

more se- vere form, α° thalassaemia, which results from loss of both of the linked α globin genes, − −/αα. The second type, α+ thalassaemia, arises due to the deletion –α/αα, so there is still some output of α globin from the affected chromosome. This is almost completely silent in carriers; their red cells are normal or are only slightly hypochromic. In clinical practice we encounter two symptomatic types of α thal- assaemia, the haemoglobin Bart's hydrops syndrome and haemo- globin H disease (Table 22.6.7.2).

The former results from the homozygous inheritance of α° thalassaemia. Haemoglobin H disease by contrast usually results from the coinheritance of both α° and α+ thalassaemia. These genetic interactions are summarized in Fig. 22.6.7.12. Like the β thalassaemias, the α thalassaemias are extremely heterogeneous at the molecular level. Various deletions can remove either both the α globin genes or the main regulatory regions of the α globin gene cluster and cause α° thalassaemia, but there are only

two that are common. One is found in patients of South-East Asian ethnicity. The other occurs mainly in Mediterranean popu-lations. Similarly, there are several different-sized deletions that re-move a single α globin gene to produce the deletion forms of α+ thalassaemia; the commonest are those that remove either 3.7 or 4.2 kb of the α gene cluster (Fig. 22.6.7.13). Nondeletion forms of α+ thalassaemia are also seen, and many of them are similar to those that produce β thalassaemia. A particularly

common form of nondeletion α+ thalassaemia, found in up to 5% or more of some South-East Asian populations, results from a single base change in the α globin chain termination codon UAA, which changes to CAA. The latter is the code for the amino acid glutamine. When the ribo- somes reach this point, instead of the chain terminating, they read through mRNA that is not normally translated until another stop codon is reached. An elongated α chain variant is synthesized, but the mRNA is destabilized by read-

through of sequences which are not normally translated and so the variant is also produced at a reduced rate. It is called haemoglobin Constant Spring after the name of the town in Jamaica in which it was discovered.

Genotype–phenotype relationships

Molecular studies explain much of the clinical variability of α thalassaemia in different populations. Since the haemoglobin Bart's hydrops syndrome requires the homozygous inheritance of α° thalassaemia (– –/– –), this condition only occurs in

populations in which α° thalassaemia is common. Most forms of haemoglobin H disease are due to the inheritance of α° thalassaemia from one parent and α+ thalassaemia from the other (–α/– – or –αT/– –). Thus,

Table 22.6.7.2 The α thalassaemias Type Homozygotes Heterozygotes α° Hb Bart’s hydrops Thalassaemia minor α+ (deletion) Thalassaemia minor Thalassaemia minorb αT (nondeletion) Hb H diseasea Thalassaemia minoret a Haemoglobin H disease more

commonly results from the compound heterozygous inheritance of α° and either variety of α+ thalassaemia. b Heterozygotes for the α+ determinant typically have reduced mean cell volume and mean cell haemoglobin; in a minority of cases, the red cells indices fall within the normal range. Normal Hb Bart’s hydrops Hb H disease Normal α° Thal. trait α° Thal. trait α° Thal. trait α° Thal. trait α+ Thal. trait α+ Thal. trait α° Thal. trait α° Thal. trait Fig. 22.6.7.12 The genetics of

α thalassaemia. The purple α genes represent gene deletions or otherwise inactivated genes. The open α genes represent normal genes. α° Thalassaemia and α+ thalassaemia are defined in the text. – – – 3′HVR 30 20 10 0 −10 −50

--MC --CAL --THAI --FIL --CL --BRIT --SA –(α)20.5 --MED --SEA --SPAN –(α)5.2 θ1 ψζ1 inter- ζHVR ζ2 ψα2 α2 α1 α3.7 α3.5 α4.2 yα1 Fig. 22.6.7.13 The different-sized deletions responsible for some forms of α° or α+ thalassaemia. The α globin gene cluster is shown at the top of the figure. Two highly variable regions (HVR) are shown. The abbreviations on the right-hand side indicate the source of origin of patients with the deletions: MED, Mediterranean; SEA, South-East Asia. The three smaller deletions at the bottom of the figure show some of the main classes of α+ thalassaemia. The superscripts 3.7, 4.2, and 3.5 indicate the size of the deletions in kb.

22.6.7 Disorders of the synthesis or function of haemoglobin 5437 haemoglobin H disease is also restricted mainly to Mediterranean and Asian populations. On the other hand, α+ thalassaemia occurs very commonly throughout the whole of the tropical belt; α° thalas- saemia does not occur commonly in many of these regions, so that the haemoglobin Bart's hydrops syndrome and haemoglobin H dis- ease are not seen. The homozygous state for α+ thalassaemia (–α/ –α) is characterized by a mild hypochromic anaemia, very similar to the heterozygous state for α° thalassaemia. To complicate matters, sometimes the homozygous state for the nondeletion forms of α+ thalassaemia, αTα/αTα, are more severe and cause haemoglobin H disease. Pathophysiology The pathophysiology of α thalassaemia is different from that of β thalassaemia. A deficiency of α chains leads to a relative excess of γ chains or β chains which form haemoglobins Bart's and H respect- ively. These more soluble tetramers do not precipitate significantly in the bone marrow, and erythropoiesis is thus more effective than in β thalassaemia. However, haemoglobin H is unstable and precipi- tates in red cells as they age. The large inclusion bodies produced in this way are trapped in the spleen and other parts of the microcir- culation leading to a shortened red cell

survival. Both haemoglobins Bart's and H have a very high oxygen affinity; because they have no α chains there is no haem–haem interaction and their oxygen dis- sociation curves resemble that of myoglobin, making them physio- logically useless. Haemoglobin Bart's hydrops syndrome This condition is a cause of fetal loss throughout South-East Asia and in Greece and Cyprus. Affected infants produce no α chains and hence can make neither fetal nor adult haemoglobin. The clinical picture is very characteristic (Fig. 22.6.7.14). Infants are usually stillborn between 28 and 40 weeks. Live-born infants take a few gasping respirations and then expire within the first hour after birth. They show the typical picture of hydrops fetalis with ex- treme pallor, generalized oedema, and massive hepatosplenomegaly. There is a high frequency of other congenital abnormalities, and a very large, friable placenta, all due to severe intrauterine anaemia. The haemoglobin values are in the 60 to 80 g/litre range and there are gross thalassaemic changes of the peripheral blood film. The haemoglobin consists of approximately 80% haemoglobin Bart's and 20% of the embryonic haemoglobin Portland (ζ2γ2). It is believed that these infants survive to term because they continue to produce embryonic haemoglobin at this level; haemoglobin Bart's is, as men- tioned previously, useless as an oxygen carrier. This syndrome is also characterized by a high incidence of ma- ternal pre-eclampsia and considerable obstetric difficulties due to the presence of the large, abnormal placenta. Haemoglobin H disease Haemoglobin H disease usually results from the inheritance of α° thalassaemia from one parent and α+ from the other. It may also result from the inheritance of α° thalassaemia and haemo- globin Constant Spring or from the homozygous state for a severe, nondeletion form of α thalassaemia. The latter form of inheritance is particularly common in Saudi Arabia. Recent evidence suggests that, overall, this condition is more severe in those who have in- herited α° thalassaemia together with haemoglobin Constant Spring or other nondeletion forms of the disease compared with those who have inherited three α gene deletions. There is a variable degree of anaemia and splenomegaly but it is most unusual to see severe thalassaemic bone changes or the growth retardation characteristic of homozygous β thalassaemia. Patients usually survive into adult life although the course may be inter- spersed with severe episodes of haemolysis associated with infec- tion, or worsening of the anaemia due to progressive hypersplenism. Oxidant drugs such as sulphonamides may increase the rate of pre- cipitation of haemoglobin H and therefore exacerbate the anaemia. (a) (b) Fig. 22.6.7.14 The haemoglobin Bart's hydrops syndrome: (a) a hydropic infant with massively enlarged placenta; (b) autopsy findings with an enlarged liver. By permission of Professor P. Wasi.

 Haemoglobin values range from 70 to 100 g/litre. The blood film shows typical thalassaemic changes. There is a moderate reticulocytosis. Incubation of the red cells with brilliant cresyl blue generates numerous inclusion bodies by precipitation of the haemoglobin H under the redox action of the dye (Fig. 22.6.7.15) The haemoglobin comprises 5 to 40% haemoglobin H together with haemoglobin A and a normal or reduced level of haemoglobin A2. The haematological findings in the α° and α+ thalassaemia traits are summarized in Table 22.6.7.2. They can only be identified with certainty by analysis of the α globin genes. α Thalassaemia and intellectual disability or myelodysplasia There is an increasingly important and heterogeneous group of α thalassaemias which are not restricted to individuals from tropical backgrounds. They are observed in all racial groups and have been best characterized in those of northern European origin. These con- ditions are characterized by variable degrees of intellectual disability, dysmorphic features, and α thalassaemic blood pictures. They follow a completely different form of inheritance from the commoner gen- etic forms of α thalassaemia. There are two major varieties of this condition. The first is due to lesions that involve the α globin gene cluster on

chromosome 16, ATR-16. There is another group re- sulting from mutations on the X chromosome, ATR-X. The ATR-16 disorders are characterized by a variable degree of intellectual disability and dysmorphic features. The blood film shows mild α thalassaemic changes and some cells which contain typical haemoglobin H inclusion bodies. In some cases the condition re- sults from long deletions which remove the end of the short arm of chromosome 16 and extend for 1 to 2 Mb. In other cases, the loss of the end of the short arm of chromosome 16 is the result of an in- herited cytogenetic abnormality, including translocations and other rearrangements. The ATR-X syndrome is characterized by a much more consistent series of dysmorphic features including typical facial features and genital abnormalities, and more severe intellectual disability. This is accompanied by a very mild form of haemoglobin H disease. This condition is inherited as a typical sex-linked disorder affecting males and results from mutations of the ATR-X gene which regulates tran- scription via an effect on chromatin structure. Female carriers may show a very small proportion of red cells containing haemoglobin H bodies. Acquired mutations of ATR-X are sometimes found in older patients who have a mild form of haemoglobin H disease associated with myelodysplasia. The relationship between the mutations and the disease of the bone marrow is still not clear. Thalassaemia intermedia Definition and pathogenesis The term ‘thalassaemia intermedia’ is used to describe patients with the clinical picture of thalassaemia which, although not transfusion dependent, is associated with a much more severe degree of anaemia than that found in carriers for α or β thalassaemia. Many of the con- ditions which have been described previously in this section follow this clinical course, for example, haemoglobin C or E thalassaemia, the various δβ thalassaemias and haemoglobin Lepore disorders, haemoglobin H disease, and the wide variety of conditions which can result from the interactions of the different β and δβ thalassaemia determinants. However, some children with this condition have parents with typical heterozygous β thalassaemia blood pictures and elevated haemoglobin A2 levels. These patients appear to be homo- zygous for β thalassaemia, yet they run a much milder course than is usually the case with this condition. Some of them have inherited an α thalassaemia determinant as well as being homozygous for β thalassaemia. This reduces the overall degree of globin chain imbal- ance and consequently the severity of the dyserythropoiesis which usually accompanies homozygous β thalassaemia; hence these chil- dren run a milder clinical course. In other cases, particularly in indi- viduals of African ethnicity, relatively mild forms of homozygous β thalassaemia seem to reflect the action of less severe β thalassaemia mutations. Finally, some intermediate forms of β thalassaemia seem to result from the coinheritance of a gene for unusually effective haemoglobin F production. Clinical and haematological changes The clinical features of the intermediate forms of thalassaemia are extremely variable. At one end of the spectrum are patients who are virtually symptom free except for moderate anaemia. At the other end there are patients who have haemoglobin values of 50 to 70 g/ litre and who develop marked splenomegaly, skeletal deformities due to expansion of bone marrow, and, as they get older, become iron loaded because of increased intestinal iron absorption. Recurrent leg ulceration, folate deficiency, symptoms due to extramedullary haemopoietic tumour masses in the chest and skull (Figs. 22.6.7.16 and 22.6.7.17), gallstones, and a tendency to infection are character- istic of this group of thalassaemias. Due to the heterogeneity of these disorders, it is only possible to determine the course that is likely to evolve in any individual patient by following the disorder very carefully from early childhood. Differential diagnosis of the thalassaemias There are few conditions that are likely to be confused with the more severe forms of homozygous β thalassaemia or haemoglobin H dis- ease. The ethnic background of the patient, the presence of anaemia from early life, and the characteristic haematological changes make the diagnosis relatively easy. Once thalassaemia is

suspected, the parents and near relatives should be examined for the carrier states for α or β thalassaemia. Both disorders can be distinguished from simple iron deficiency by the finding of a normal ferritin level and by the associated changes in the haemoglobin pattern on HPLC.
Fig. 22.6.7.15 Supravital staining with brilliant cresyl blue highlights prominent red cell inclusion bodies in Hb H disease.

22.6.7 Disorders of the synthesis or function of haemoglobin 5439 It should be remembered, however, that in some groups iron defi- ciency and heterozygous thalassaemia frequently occur together in the same person, particularly during pregnancy. The sideroblastic anaemias can be easily distinguished from thalassaemia by the mor- phological appearances of the red cells and the presence of ring sideroblasts in the bone marrow. It should be remembered that there are some rare forms of acquired haemoglobin H disease in elderly patients with myelodysplasia. Laboratory diagnosis of thalassaemia The homozygous states for the severe forms of β thalassaemia are easily recognized by the haematological changes associated with very high levels of haemoglobin F; haemoglobin A2 values vary so much that they are of no diagnostic help. The heterozygous states are recognized by microcytic hypochromic red cells, a high red cell count and an elevated level of haemoglobin A2. The δβ thalassaemias are characterized by the finding of 100% haemoglobin F in homo- zygotes and 5 to 15% haemoglobin F together with a normal level of haemoglobin A2 in heterozygotes (Table 22.6.7.1). When β thalassaemia is diagnosed, quantitative HPLC or haemo- globin electrophoresis will exclude the presence of an abnormal haemoglobin variant such as haemoglobin E or Lepore. The precise nature of the genetic lesion may need be determined by sequencing the affected loci, or by multiplex-ligation dependent probe analysis for deletions. The haemoglobin Bart’s hydrops syndrome is recognized by the finding of a hydropic infant with a severe anaemia, a thalassaemic blood picture, and 80% or more haemoglobin Bart’s on HPLC. Haemoglobin H disease is identified by the finding of a typical thalassaemic blood picture with an elevated reticulocyte count, and variable amounts of haemoglobin H on HPLC. There are no really useful, simple diagnostic tests for the different α thalassaemic carrier states although α° thalassaemia heterozygotes usually have typical thalassaemic red cell changes with a normal haemoglobin A2 value. It is essential for counselling purposes to diagnose the different car- rier states for α thalassaemia; blood samples should be referred to a laboratory for DNA analysis of the globin genes. Prevention and treatment Thalassaemia produces a severe public health problem and a serious challenge for medical resources in many populations. Since there is no definitive treatment, most countries in which the disease is common are putting a major effort into programmes for its prevention. Prevention Since the carrier states for the β thalassaemias can be easily rec- ognized, it is possible to screen populations and provide ante- natal genetic counselling. When heterozygous carrier mothers are found, their partners are tested; if they are also carriers, the couple are offered the possibility of prenatal diagnosis and the option to discuss termination of pregnancies where fetuses are affected by severe forms of thalassaemia. Fig. 22.6.7.16 (a) Chest radiograph with a right paravertebral mass of extramedullary haemopoietic tissue in β thalassaemia intermedia. (b) Transverse thoracic image from computed tomography scan of the same patient, showing the extent of the extramedullary haemopoietic mass complicated by haemothorax. Fig. 22.6.7.17 Cranial MRI of a patient with thalassaemia intermedia, showing significantly thickened skull vault consistent with extramedullary haematopoiesis.

 Prenatal diagnosis Prenatal diagnosis can be offered to couples at risk for having chil- dren with severe forms of β thalassaemia and haemoglobin Bart’s

hydrops. Prenatal diagnosis of thalassaemia is typically carried out by genetic analysis of fetal tissue obtained by chorionic villus sam- pling between the 11th and 14th week of gestation. As prenatal diag- nosis of thalassaemia is now well established in many countries, it is important to discuss the genetic implications of the condition when carriers are detected by chance, even in low-prevalence areas. Symptomatic treatment The symptomatic management of severe β thalassaemia requires regular blood transfusion, the judicious use of splenectomy if hypersplenism develops, and the administration of chelating agents to prevent iron overload. When the diagnosis of severe β thalassaemia is suspected during the first year of life, the infant should be followed for several weeks to make sure that the haemoglobin has fallen to a level at which regular transfusion will be necessary. It is difficult to be dogmatic about exactly when transfusions should be started. A severely anaemic infant who is feeding poorly, inactive, or other- wise failing to thrive, will almost certainly need to be transfused. The object is to maintain the pretransfusion haemoglobin level at about 95 g/litre. This usually requires transfusion of 10 to 15 mg/kg red cells every 4 weeks, with extended red cell phenotyping to reduce the risk of alloimmunization. The rate of transfusion should not exceed 4 to 5 ml/kg per h. In patients who are profoundly anaemic or show evi- dence of cardiac insufficiency, the rate should be no more than 2 ml/ kg per h. It is important to calculate the annual blood consumption by dividing the total volume of blood transfused over 12 months by the patient’s weight in the middle of the year. If it is higher than 200 ml/kg body weight, splenectomy may be considered. Hypersplenism is becoming much less common where children are maintained on an adequate transfusion regimen. Increasing blood requirements, or other evidence of hypersplenism, such as pancytopenia, should prompt one to consider splenectomy. It should be avoided before the age of 6 years because of the particularly high incidence of infection in asplenic children. Two to three weeks before splenectomy the child should be given (1) pneumococcal vaccine, (2) Haemophilus influenzae type B vaccine, and (3) menin- gococcal A and C vaccine. After the operation the children should be maintained on oral penicillin V, 125 mg twice daily, increasing to 250 mg twice daily for older children. For those who are allergic to penicillin, erythromycin should be given. For patients given adequate transfusion support, iron overload be- comes a critical factor in the determining the morbidity and mortality of thalassaemia. Meticulous attention to chelation is required if pa- tients are to avoid significant iron loading in the heart, liver, and endo- crine organs, with clinical manifestations including diabetes mellitus, hypogonadotropic hypogonadism, hypothyroidism, and hypopara- thyroidism. The anterior pituitary appears to be especially sensitive to the effects of iron overload, and delayed sexual maturation and subfertility may be observed even in the context of good iron chelation. Regular assessment of endocrine function therefore forms a key part of the long-term management of transfusion-dependent thalassaemic patients. The secondary effects of endocrine dys- function, such as osteoporosis (which may have contributions from reduced growth hormone and sex hormone secretion, hypo- parathyroidism and vitamin D deficiency, as well as collagen gene polymorphisms), must also be sought and treated where possible. Assessment of iron loading has historically relied on serum fer- ritin assays, which often reflect total body iron stores only imper- fectly, and on formal measurement of the iron concentration in tissue obtained at liver biopsy. Liver iron concentration can now be accur- ately determined using MRI (e.g. Ferriscan®—Fig. 22.6.7.18) which is a favoured modality for monitoring iron overload and the response to chelation. All patients with transfusion-dependent thalassaemia should undergo regular MRI assessment of liver iron concentration, with a target of 3–7 mg/g dry weight. Cardiac T2* MRI should also be undertaken in patients with evidence of iron loading, since this will give a reproducible estimate of the cardiac iron burden. Relaxation times of greater than 20 ms suggest effective iron chelation, while a T2* MRI less than 15 ms

suggests a need for intensified chelation. Three iron chelating agents are available for clinical use: desferri oxamine (deferoxamine), deferasirox, and deferiprone. Randomized clinical trials comparing all three agents are still needed, and the choice of first-line agent is therefore based on consideration of its side effect profile and tolerability, along with patient preference. 0 50 100 150 200 250 317 R2(/s) Voxels Transverse Relaxation Rate (R2) Image Transverse Relaxation Rate (R2) Distribution Transverse Relaxation Rate R2 (/s) Distribution Mean ± SD: 204.1 ± 43.2 264 211 158 105 52 0 0 80 160 240 320 400 Fig. 22.6.7.18 MRI assessment of hepatic iron loading. The increased transverse relaxation rate is consistent with significant iron deposition.

22.6.7 Disorders of the synthesis or function of haemoglobin 5441 There is greatest experience with desferrioxamine, which has been used for iron chelation in patients with thalassaemia for over 40 years. Although an effective chelator, its route of administra- tion remains a significant disadvantage: desferrioxamine must be delivered parenterally, typically by subcutaneous infusion over 12 hours for five nights out of seven. Difficulties with compliance are therefore the main limitation to its usefulness in the clinic. The initial dose of desferrioxamine should not exceed 25 to 35 mg/ kg body weight per 24 h, and iron excretion may be potentiated if patients also receive 100 mg vitamin C by mouth on the days of the infusion. A careful titration of ferritin level against dosage prevents over-treatment, and monitoring must include regular audiometry and ophthalmic assessment to assess for the known complications of this treatment. Randomized phase III trials comparing desferrioxamine with deferasirox have shown that both agents can effect similar reductions in liver iron concentrations. However, its oral bioavailability makes deferasirox an increasingly popular first-line agent. Complications include transient gastrointestinal upset, typically reversible renal dysfunction with proteinuria, and rashes. It is unsuitable for use in patients with renal dysfunction. Although deferiprone may be less effective in reducing total body iron in some thalassaemic patients, preliminary data suggest it may have a specific role in reducing myocardial iron deposition, particu- larly in conjunction with desferrioxamine. This, plus the recognized risk of marrow suppression and agranulocytosis with deferiprone (such that patients are advised to have a weekly full blood count once starting this agent) has meant that deferiprone has been less widely adopted as a first-line choice for chelation. Decompensated cardiac failure as a consequence of cardiac siderosis remains a major cause of mortality in patients with transfusion-dependent thalassaemia. The development of features suggestive of cardiac failure in the context of significant cardiac iron loading should prompt immediate treatment with a continuous in- fusion of desferrioxamine pending stabilization. The addition of deferiprone may also be of use in this setting. Management of patients with thalassaemia intermedia The intermediate forms of thalassaemia should be managed by careful observation, folic acid supplementation, and, in the face of a falling haemoglobin and increasing spleen size, the judicious use of splenectomy. The increased risk of venous thrombosis in patients with thalassaemia intermedia may be exacerbated be splenectomy, and the risks and benefits of the procedure must be considered on an individual patient basis. It is important to monitor the iron status regularly because some of these patients become iron loaded as a consequence of increased intestinal absorption and chelation therapy may be necessary later in life. Toward a cure for thalassaemia major Currently, haematopoietic stem cell transplantation (see Chapter 22.8.2) is the only cure for thalassaemia major. To min- imize transplant related mortality, the procedure should ideally be undertaken before patients develop end-organ damage due to iron deposition—typically in childhood. Although few prospective studies and fewer controlled trials have been performed in this field, disease-free survival is now approximately 80%, with a transplant- related mortality of approximately 5% in young patients

with a matched sibling donor. The transplant-related mortality in adult patients is significantly higher, and haemopoietic stem cell trans- plantation is therefore limited to patients who have had excellent iron chelation with limited end-organ damage. There has been process toward the development of gene therapy for the thalassaemias. This process aims to genetically modify the patient's own haematopoietic stem cells, whether by lentiviral gene transfer of intact β globin genes, or by CRISPR-mediated gene editing of the globin genes themselves or other loci implicated in the silencing of the fetal γ globin expression. The modified haem- atopoietic cells are then returned to the patient in an autologous transplant. In July 2019 Bluebird, a gene therapy company, has an- nounced positive clinical results from the use of third-generation lentiviral vectors in transfusion-dependent beta-thalassaemia— about 80% of recipients able to be free of blood transfusions. Similar studies are underway using CRISPR-Cas9 editing technology to correct the sickle haemoglobin defect in human autologous haem- atopoietic stem cells. The long-awaited hope of a definitive therapy by gene correction for these important inherited disorders of haemoglobin synthesis appears to becoming a reality. However, ensuring realistic access to these costly and labour-intensive stra- tagems for the innumerable patients who are affected, will be a formidabe challenge. Structural haemoglobin variants Over 400 structural haemoglobin variants have been described, most of which result from single amino acid substitutions. Many of them are harmless and have been discovered during surveys of the electrophoretic patterns of human haemoglobin. Of course, this approach underestimates the number of variants because it only identifies those in which the amino acid substitution alters the charge of the haemoglobin molecule. Single amino acid substitutions cause clinical disorders only if they alter the stability or functional properties of the haemo- globin molecule. A classification of these diseases is shown in Table 22.6.7.3. They include the sickling disorders, chronic or drug- induced haemolytic anaemia associated with unstable haemo- globins, and polycythaemia or congenital cyanosis, associated with high- and low oxygen affinity haemoglobin variants, respect- ively. There is a rare group of haemoglobin variants that produce methaemoglobinaemia. Table 22.6.7.3 Clinical disorders due to structural haemoglobin variants Disorder Variants Haemolysis and tissue damage Haemoglobin S Drug-induced haemolysis Haemoglobin Zürich and other unstable haemoglobins Chronic haemolysis Unstable haemoglobin variants Haemoglobin C Congenital polycythaemia High-affinity variants Congenital cyanosis Haemoglobin(s) M Low-affinity variants Hypochromia: thalassaemic phenotype Haemoglobin E Haemoglobin Constant Spring

 Nomenclature The structural haemoglobin variants are named by letters of the al- phabet or by the place of origin of the first patient in whom they were characterized. The heterozygous carrier state is termed the 'trait' and the homozygous condition the 'disease'. The sickling disorders Sickling disorders (Table 22.6.7.4) consist of the homozygous state of sickle cell disease (SS), and the compound heterozygous state for haemoglobin S together with haemoglobins C, D, E, or other structural variants. Several disorders result from the inher- itance of the sickle cell mutation together with different forms of thalassaemia (described previously). Pathogenesis Haemoglobin S differs from haemoglobin A by the substitution of valine for glutamic acid at position 6 in the β globin chain. Although this has been known for well over half a century, it is still not absolutely clear how it gives rise to the sickling phenomenon. The latter appears to be due to the unusual solubility characteristics of haemoglobin S which undergoes liquid crystal (tactoid) forma- tion as it becomes deoxygenated. In this state, aggregates of sickled haemoglobin molecules arrange themselves in parallel, rod-like fibres, made up of a complex solid core about 21 nm in diameter, composed of 14 filaments arranged as 7 pairs of double filaments.

Much is now known about the complex interactions whereby the β6 valine substitution stabilizes the molecular stacks in the deoxy configuration of haemoglobin. There is considerable variation in the extent to which different haemoglobins are able to participate with haemoglobin S in the sickling process. This accounts for some of the clinical variability of the different sickling conditions. For example, haemoglobin F is almost completely excluded from the sickling process; increasing concentrations in the red cell reduce the rate of sickling. The pathophysiology of sickling is a dynamic process. Red cells containing sickle haemoglobin at a high concentration endure a series of cycles of sickling (prompted by deoxygenation or inflam- matory stimuli) and desickling, with progressive membrane damage and loss of plasticity. Finally these dry, rigid cells become irrevers- ibly sickled (Fig. 22.6.7.19). Sickling of this type has two main ef- fects. First, sickled erythrocytes have a shortened survival, leading to a chronic haemolytic anaemia. This in turn results in anaemia, cholelithiasis, and free haemoglobin mediated changes in nitric oxide availability with endothelial dysfunction and increased resting vascular tone. Second, the abnormal red cells tend to adhere to vas- cular and intercellular adhesion molecules on endothelial cells, with the production of aggregates, blockage of the vessels, vascular stasis, subsequent reperfusion damage and, ultimately, oxidant and inflam- matory damage to tissues. Distribution The sickling disorders occur very frequently in African popula- tions and, sporadically, throughout the Mediterranean region and the Middle East There are extensive pockets in India. The high fre- quency of the sickle cell gene occurs because carriers are more re- sistant than normal individuals to P. falciparum malaria. Clinical features Except in conditions of extreme hypoxia, such as flying in an un- pressurized aircraft, the sickle cell trait causes no clinical disability. However, it is possible for individuals to suffer vaso-occlusive epi- sodes if they become oxygen deprived under anaesthesia. Therefore all individuals of the appropriate racial background should have a sickling test (see ‘Laboratory diagnosis’ under ‘Haemoglobin SC dis- ease’) before receiving an anaesthetic. If the test is positive, homozy- gous sickle cell disease should be excluded first; but even in patients with sickle trait alone anaesthetics should be given with special attention to oxygenation and care should be taken to avoid post- operative dehydration. Sickle cell anaemia runs an extremely variable clinical course. At one end of the spectrum it is characterized by a severe haemo- lytic anaemia interspersed with frequent exacerbations, or crises. Other cases may be extremely mild and only found by chance on routine haematological examination. The reason for these remark- able differences in phenotypic expression, which are only partly understood, include the level of haemoglobin F, coinheritance of α thalassaemia, climate, and socioeconomic factors. Since reactivation of haemoglobin F would be a potential therapeutic intervention in the β globin disorders (both sickling diseases and β thalassaemia), the genetic control of γ globin expression has been subject to intense investigation. Polymorphisms in the γ G promoter, at the HMIP Table 22.6.7.4 The major sickling disorders Disorder Genotype (Normal = αα/αα β/β) SS disease αα/αα βS/βS SC disease αα/αα βS/βC SD disease αα/αα βS/βD S–β thalassaemia αα/αα βS/β° or βS/β+ S–hereditary persistence of fetal Hb αα/αα βS/–a S–α thalassaemia α–/αα or α–/α– βS/βS SS, sickle cell anaemia. See text for details of other conditions. a Indicates β gene deletion. Fig. 22.6.7.19 Sickled red cells in homozygous HbS.

 5443 locus and BCL11a locus (see ‘Towards a cure for the sickling dis- orders’) have been shown to account for much of the variation in fetal haemoglobin expression, but many more modifiers are likely to be found. Typically, sickle cell anaemia presents in infancy with symptoms related to anaemia or infection. Dactylitis is also commonly seen. Infants begin to develop anaemia from about the third month of life. During early development they often have significant spleno- megaly that gradually resolves due to repeated

infarction resulting in functional hyposplenism, though splenic sequestration crises (see following ‘Complications’ section) can result in significant splenic enlargement. The haemoglobin is typically between 60 to 80 g/litre with a reticulocyte count of 10 to 20%. There is chronic, mild icterus with an elevated bilirubin level. Examination of the peripheral blood film shows anisochromia and poikilocytosis with a variable number of sickled erythrocytes. As the children grow older, the haemato- logical changes of hyposplenism develop with the appearance of pits on the surface of the red cells, Howell–Jolly bodies, and distorted red cells. The white cell and platelet counts are usually normal or slightly elevated. Complications The chronic haemolysis of sickle cell disease is interspersed with acute exacerbations of the illness called sickling crises. Furthermore, there are a series of serious and life-threatening long-term complications which develop in many patients with sickle cell anaemia. The different forms of sickle cell crises are summarized in Box 22.6.7.3. The commonest is the painful crisis. This is some- times precipitated by infection, dehydration, or exposure to cold, although quite often no underlying cause can be found. The epi- sode starts with vague pain, often in the back or bones of the limbs, which worsens gradually. The pain is almost certainly due to blockage of small vessels with sickled erythrocytes; aspiration over areas of bone tenderness has shown infarction of marrow tissue. Occasionally, abdominal pain is the major symptom and this may be associated with distension and rigidity, a picture very similar to an acute abdominal emergency. The diagnostic difficul- ties in distinguishing between an abdominal crisis and a surgical abdomen are compounded by the fact that the bowel sounds are often diminished during abdominal crises. The acute chest syndrome is the second commonest cause of hos- pitalization for patients with sickle cell disease. In this particularly serious form of crisis, sickling within the pulmonary vasculature ini- tiates a vicious circle of hypoxia, microvascular occlusion, and fur- ther downstream hypoxia, manifest as acute dyspnoea and pleuritic pain together with infiltrates on the chest radiograph. It is some- times accompanied by a fall in the packed cell volume and platelet count which also may reflect sequestration of sickled cells in the pulmonary vessels. More than 1 in 10 patients will need ventila- tory assistance, and there is a mortality rate of approximately 3%. Patients are treated with supportive therapy including high-flow oxygen, top-up transfusions where possible, and broad-spectrum antibiotics. However, many patients will need an exchange transfu- sion to lower the haemoglobin S percentage in order to break the downward spiral of sickling and hypoxia. Neurological complications may present in a variety of ways. Stroke is particularly common and 11% of patients with sickle cell disease will have had a stroke by the age of 20. Although the exact mechanism by which stroke arises in sickle cell disease is unclear, it is likely to be multifactorial with contributions from endothelial dysfunction, leucocytosis, anaemia, and nitric oxide dysregulation. Transcranial Doppler to identify children with increased middle cerebral arterial blood velocity has been shown to identify those children who will benefit from prophylactic transfusion to minimize the risk of stroke. Haemorrhagic strokes are also seen, typically in older patients, and are thought to be caused by rupture of aneurysms or collaterals akin to those seen in moyamoya disease. MRI studies also show a high frequency of silent infarcts, even within the first few years of life and neurocognitive impairment is not unusual as a result. Sequestration crises occur mainly in babies and young children, and are characterized by a rapid enlargement of the spleen which becomes engorged with sickled erythrocytes. As the crisis pro- gresses a large proportion of the total red cell mass may be trapped in the spleen. Untreated, death may occur due to profound an- aemia, while caution must be exercised with transfusion to ensure that a reversal of the sequestration does not result in an excessively high haemoglobin level. Parents of children with sickling dis- orders should be taught how to detect splenic enlargement in their children, and immediate medical attention should be sought for this serious

complication. Hepatic sequestration may also occur, including in adults, and is easily overlooked if the liver size is not monitored carefully. Priapism is another common and distressing acute complication, which, if recurrent, can result in fibrosis of the corpus cavernosa and subsequent sexual dysfunction. During painful crises, there may be a marked increase in the rate of haemolysis with a fall in the haemoglobin level. Such acute haemolytic episodes are uncommon. More serious are periods of transient red cell aplasia called aplastic crises, which result from intercurrent infection with parvovirus (erythrovirus) B19. Infection with this erythrovirus temporarily blocks the maturation of red cell precursors and results in a sharp drop in haemoglobin in the context of haemolytic anaemias. The combination of worsening anaemia with a reticulocytopenia suggests this diagnosis, and transfusional support is needed until the virus is cleared. Pregnancy in women with sickling disorders may be uneventful, but there is an increased risk of fetal loss, intrauterine growth retard- ation, premature labour, and an increased incidence of painful crises for the expectant mother. Box 22.6.7.3 Acute exacerbations (‘crises’) in sickle cell disease • Thrombotic:

— Generalized or localized bone pain

— Abdominal

— Pulmonary

— Neurological • Aplastic • Haemolytic • Sequestration:

— Spleen

— Liver

— ?Lung • Various combinations of above

section 22 Haematological disorders 5444 Chronic complications Many of the chronic complications of sickle cell anaemia result from infarcts following repeated episodes of vascular occlusion. Almost any organ can be involved. Those at particular risk are areas which rely largely on small vessels for their blood supply. The bones are particularly prone to infarction, and avascular necrosis of the humeral or femoral heads may lead to deformity of the shoulder and hip joints (Fig. 22.6.7.20). Bone infarcts may result in chronic se- questra formation which may become secondarily infected with the production of osteomyelitis. Chronic leg ulcers are also commonly seen, and may prove very difficult to treat effectively. Another organ at particular risk is the kidney. During early childhood, renal function may be impaired but this can be corrected by blood trans- fusion, suggesting that it is due to reversible changes in the renal vascula- ture. However, alterations in renal function are not reversible in later life. Chronic renal failure is one of the commonest causes of death in adults with sickle cell anaemia, and nephrotic syndrome may be seen. Pulmonary disease is seen, with repeated episodes leading to se- vere pulmonary hypertension and right heart failure. Irrespective of the presence of pulmonary hypertension, there is usually some degree of cardiomegaly.
A variety of flow murmurs may be heard, many of which are the result of chronic anaemia. Myocardial infarc- tion or fibrosis is not a typical feature of the disease. Ocular manifestations are also relatively common in sickle cell anaemia although they tend to be more serious in haemoglobin SC disease; they will be considered in this context below. Other im- portant chronic complications include a greatly increased suscepti- bility to pigment gallstone formation and

gallbladder disease. Course and prognosis There are still large gaps in our knowledge about the natural history of sickle cell anaemia, with socioeconomic and ill-defined genetic factors being important factors in determining prognosis. In the developing world, the disease still has a high mortality in the first year or two of life with infection being a major cause of death. Data from the United States Cooperative Study of Sickle Cell Disease (1994) sug- gest that the median age at death for males is 42 years and for females 48 years; more recent data are lacking. In Saudi Arabia and India, a particularly mild form of the condition occurs; mortality is extremely low in childhood and a normal survival seems to be common. Other sickling disorders The other sickling disorders include the interaction of haemoglobin S with haemoglobins C, D, and some of the rarer haemoglobin vari- ants. The interactions with the different forms of β thalassaemia were described earlier. In many of these conditions, the clinical manifest- ations are little different from the sickle cell trait, but haemoglobin SC disease and SD disease more closely resemble sickle cell anaemia. Haemoglobin SC disease This disease is found in West Africa and less frequently in North Africa. Characterized by a milder anaemia than sickle cell disease, it may go unrecognized until adult life. It may present with a com- plication resulting from damage to the microvasculature, probably because of the relatively high haemoglobin level and the combined effects of sickling and red cell rigidity caused by haemoglobin C (see ‘Haemolysis due to common haemoglobin variants other than haemoglobin S’). Aseptic necrosis of the femoral or humeral heads and unexplained haematuria are common complications, and re- peated blockage of the retinal vessels may lead to retinitis proliferans, retinal detachment, and vitreous haemorrhage. Haemoglobin SC disease is diagnosed by finding a mild anaemia, sometimes with splenomegaly, and characteristic morphological changes of the red cells including many target forms, intracellular crystals, and sickle cells. The sickling test is positive and haemo- globin HPLC shows haemoglobins S and C in about equal propor- tions (Fig. 22.6.7.21). Laboratory diagnosis The presence of haemoglobin S can be determined by the sickle solu- bility test. A variety of such tests are available but each is based on the insolubility of reduced sickle haemoglobin in a phosphate buffer. If red cells containing haemoglobin S are lysed in a phosphate buffer, the addition of a reducing agent such as hydrosulphite will result in the formation of a turbid suspension—a positive sickle solubility test. Sickle cell trait causes no haematological changes and is diagnosed by the finding of a positive sickling test together with haemoglobins A and S on electrophoresis or HPLC (Fig. 22.6.7.22). Sickle cell an- aemia is diagnosed by the finding of a variable degree of anaemia, an elevated reticulocyte count, sickled erythrocytes on the peripheral blood film, a positive sickling test, and a haemoglobin electrophor- esis or HPLC pattern characterized by the absence of haemoglobin A and a preponderance of haemoglobin S with a variable amount of haemoglobin F (Figs. 22.6.7.21 and 22.6.7.22). Management Prospective genetic counselling for couples with sickling disorders and sickle trait is available in developed countries. Although prenatal diagnosis of sickle cell disease can be carried out by DNA analysis following chorionic villus sampling, it has not been taken up as ex- tensively as it has for the thalassaemias, not least because the pheno- type of affected children cannot be so accurately predicted. Universal screening programmes for all neonates help identify affected infants Fig. 22.6.7.20 Aseptic necrosis of the left femoral head in sickle cell disease.

 0.96 1.07 F 1.33 1.24 1.73 2.40 A2 3.62 0 0.0 7.5 % 15.0 22.5 30.0 37.5 45.0 (a) 1 2 Time (min.) 3 4 5 6 45.0 (b) 37.5 30.0 22.5 15.0 % 7.5 0.0 0 1 2 3 Time (min.) F 1.09 1.25 2.14 2.28 A2 3.62 4.31 4 5 6 45.0 (c) 37.5 30.0 22.5 15.0 F 1.10 1.27 1.80 2.34 A2 3.64 4.49 5.17 % 7.5 0.0 0 1 2 3 Time (min.) 4 5 6 1 Fig. 22.6.7.21 (a) Normal HPLC trace showing dominant peak for HbA, with a smaller peak for HbA2, and no

variant haemoglobins. (b) HPLC trace for homozygous sickle cell disease (HbSS). Note the increased HbF peak. (c) HPLC trace showing HbSC disease; the rightmost peak corresponds to HbC.

section 22 Haematological disorders 5446 at the earliest opportunity; this helps to minimize the risk of early deaths due to infection through the administration of prophylactic antibiotics and immunization. Affected infants should be given oral penicillin at a dosage of 62.5 mg three times a day, up to 1 year of age, 125 mg twice a day from the age of 1 to 3 years, and 250 mg twice a day thereafter. It is also standard practice for these babies to receive pneumococcal vaccine, and vaccines against meningococcus and H. influenzae. While it used to be believed that the high death rate among infants with sickle cell disease in sub-Saharan Africa and similar environments was due to malaria infection, studies have dem- onstrated that many of these deaths are due to infection with the same organisms that occur in nonmalarious parts of the world. Appropriate prophylactic programmes are therefore critically important. Patients with sickle cell anaemia adapt well to their low haemo- globin levels and regular blood transfusion is not required. Regular folate supplements should be given. Patients should be given access to a centre that has expertise in the management of this disorder and advised to present at the first sign of a painful crisis. They should also be given a card to carry which states their haemoglobin genotype. Painful crises not responding to simple analgesia, oral hydration and rest should be managed in hospital. Patients should be examined for evidence of underlying infection and given adequate rehydration, oxygen, antibiotics where appropriate, and, in particular, analgesia. The use of patient-controlled analgesia pumps can result in the rapid resolution of pain, but must be accompanied by careful monitoring of respiratory function to avoid oversedation. The haemoglobin level and reticulocyte count should be estimated at frequent intervals to antici- pate an aplastic crisis or sequestration episode. It is important to be alert to the possibility of developing acute chest syndrome, the majority of which arise in the context of a pre-existing painful crisis. The acute chest syndrome may be managed initially with oxygen and top-up transfusion (with extended phenotyped, sickle-free blood) where the baseline haemoglobin is low enough to permit it; any deteri- oration warrants red cell exchange transfusion and a low threshold for involvement of the intensive care team. Similarly, cerebral complica- tions should be treated by exchange or top-up transfusion. Exchange transfusion should also be used to cover major surgical interventions, such as total hip replacement for avascular necrosis of the femoral head, or for patients who are having recurrent crises. Ocular manifestations, particularly proliferative retinopathy, re- quire expert ophthalmological treatment, likely to involve laser photocoagulation. Pre-emptive ophthalmic assessment is advised annually for patients with sickling disorders to detect proliferative retinopathy prior to complications such as vitreous haemorrhage. Haematuria is common, and usually resolves without treatment, but it is important to be aware of the possibility of renal medullary carcinoma which is seen almost exclusively in this patient popula- tion. Proteinuria is also a common manifestation of sickle nephrop- athy, and treatment with angiotensin-converting enzyme inhibitors may slow the rate of development of renal impairment. Endstage renal failure should be managed as for any other form of renal in- sufficiency; renal transplantation has been shown to be successful in several studies though regular exchange transfusions are subse- quently needed to maintain the health of the graft. Recurrent priapism may be a problem. Nearly two-thirds of major episodes are preceded by stuttering attacks and therefore it has been suggested that effective therapy at this stage may reduce the risk of sustaining a major attack, with danger of permanent deformity of the penis. Several forms of management have been suggested although none has been studied in sufficient detail. One approach has been to commence etilefrine, an α-adrenergic agonist during the stuttering phase. Acute or fulminant cases

may require intracavernosal irriga- tion with epinephrine. Centres with experience of this complication suggest that conservative treatment should be restricted to 24 h at the most. If there is no improvement, surgical correction is recom- mended, with a cavernosum–spongiosum shunt. The management of leg ulcers is unsatisfactory. They may heal with bed rest and debridement but often relapse. Skin grafting does not always give good results and controlled trials have shown that transfusion does not appear to increase the rate of healing. Increasingly, efforts are being made to emphasize a preventa- tive rather than reactive approach to sickle crises, with the recog- nition that long-term, subclinical sickling will result in end-organ damage however effective the treatment of acute crises. For patients with more than three painful crises per year, treatment with oral hydroxycarbamide has been shown to improve quality of life and reduce the overall mortality of sickle cell disease. Variable increases in fetal haemoglobin production are seen in patients treated with hydroxycarbamide, and this may underlie its beneficial effect. Initial concerns about the possible leukaemogenicity of hydroxycarbamide have not been borne out by long-term studies of its safety. If hydroxycarbamide is not tolerated or ineffective, long-term elective red cell exchange programmes may be used, with good effect. Although this may be a significant burden for the patient and pose a risk of red cell alloimmunization, it may free patients with especially severe clinical phenotypes from frequent and disabling crises. Regular top-up transfusions are avoided where possible to avoid iron overload (with the exception of transfusion in the light of Doppler studies suggesting an increased risk of stroke in children—mentioned previ- ously). The increased viscosity of the blood in patients with sickle cell dis- ease means that over-transfusion (>100 g/litre) should also be avoided. Hb A Hb S 5 4 3 2 1 Origin − + Fig. 22.6.7.22 Haemoglobin electrophoresis showing the haemoglobin pattern in the sickling disorders (starch gel electrophoresis, protein stain, pH 8.5). The following are shown (left to right): (1 and 2) the sickle cell trait; (3) normal; (4) sickle cell anaemia; (5) normal.

22.6.7 Disorders of the synthesis or function of haemoglobin 5447 There has been significant progress in recent years in developing new treatments for sickle cell disease which target the underlying pathophysiology of this condition. Crizanlizumab, monoconal anti- body targeted against the adhesion molecule P-selectin, has been shown in randomized studies to reduce the rate of sickle-related painful crises relative to placebo, by disrupting the cell-cell inter- actions which are thought to be central to development of such ex- acerbations. An alternative strategy of reducing sickle haemoglobin polymerization has also shown some benefit in phase III random- ized trials; voxelotor, a drug which reversibly binds to, and stabilizes, the oxygenated form of haemoglobin has been shown to improve parameters associated with haemolysis. L-glutamine has been ap- proved as a new agent for the management of patients with sickle cell anaemia, again reducing the frequency of painful crises in a phase III randomized controlled study, presumably through anti- oxidative mechanisms. Whether these agents will have an impact on the long term outcomes of patients with sickling disorders remains to be seen. Towards a cure for the sickling disorders The management of sickle cell anaemia remains largely supportive, with hydroxycarbamide being the only widely available effective treat- ment to date. As with thalassaemia major, allogeneic bone marrow transplant programmes exist which offer the possibility of cure to patients who have sustained only minimal end-organ damage and who can therefore tolerate the procedure. Currently this means that bone marrow transplantation is undertaken mostly in children, and requires a careful discussion of the risks and benefits of such a major procedure if the patient’s true clinical phenotype is still unclear. Efforts to understand the normal control of γ globin transcription, and thus to reverse its silencing in adult life, remain the focus of many transla- tional research groups.

The discovery of the critical role of the tran- scription factor BCL11a in the silencing of fetal haemoglobin has been an important step forward in this process. Haemolysis due to common haemoglobin variants other than haemoglobin S After haemoglobin S, the second commonest variant in West Africa is haemoglobin C. Because of its relatively low solubility haemoglobin C appears to exist in a precrystalline state in red cells, causing their rigidity and premature destruction in the microcirculation. The homo- zygous state, haemoglobin C disease, is characterized by a mild haemo- lytic anaemia with splenomegaly, and 100% target cells on the blood film. Haemoglobin analysis shows haemoglobin C with small amounts of haemoglobin F. By contrast with haemoglobin SC, homozygous haemoglobin C is a mild disorder and no specific treatment is required. The commonest haemoglobin variant throughout South-East Asia and the Indian subcontinent is haemoglobin E. The homozy- gous state for this variant, haemoglobin E disease, is characterized by a very mild degree of anaemia with a slight reticulocytosis. The blood film shows mild morphological changes of the red cells which are hypochromic and microcytic, resembling the changes seen in β thalassaemia. No treatment is required. Haemoglobin variants which migrate in the position of haemoglobin S on electrophoresis but which do not sickle have been given the gen- eral title of haemoglobin D. There are several different molecular var- ieties of this variant; the commonest is haemoglobin D Los Angeles. The homozygous state is associated with moderate anaemia, splenomegaly, and a mild degree of haemolysis. The compound heterozygous state with haemoglobin S produces a disorder very similar to sickle cell anaemia. The unstable haemoglobin disorders The unstable haemoglobin disorders are a rare group of inherited haemolytic anaemias which result from structural changes in the haemoglobin molecule that cause intracellular precipitation with the formation of Heinz bodies. Their true incidence is not known. There have been several well-documented families in which patients with one of these haemoglobin variants have had no affected rela- tives, suggesting that the condition has arisen by a new mutation. Aetiology and pathogenesis Most of the unstable haemoglobin variants result from single amino acid substitutions at critical areas of the molecule. For example, substitutions in or around the haem pocket can disrupt the normal structure and allow in water, with subsequent oxidative damage to haem which leads to precipitation of the haemoglobin. Some sub- stitutions, such as those involving proline residues, cause a marked disruption of the secondary structure of a globin chain. A few of these variants result from deletions of either single or several amino acid residues. For example, in haemoglobin Gun Hill, five amino acids are missing including the haem binding site. As the unstable haemoglobins precipitate in the red cells or their precursors, they produce intracellular inclusions, or Heinz bodies, which make the cells more rigid causing their premature destruction in the microcir- culation (Fig. 22.6.7.23). The degradation products of the precipi- tated haemoglobin, notably haem and iron, cause oxidative damage to the red cell membrane proteins in much the same way as the ex- cess α and β chains produced in the thalassaemias. Clinical features All these conditions are characterized by a haemolytic anaemia of varying severity and splenomegaly. There may be a history of the pas- sage of dark urine, particularly during episodes of infection. As in all chronic haemolytic anaemias, there is an increased incidence of pigment gallstones. The condition may become worse during periods of intercurrent infection. In the more severe forms, such episodes are associated with life-threatening anaemia. Patients with unstable haemoglobins are at particular risk of haemolytic episodes following Fig. 22.6.7.23 The peripheral blood film of a patient with an unstable haemoglobin disorder, haemoglobin Hammersmith. This is a postsplenectomy film, which shows small inclusions in many of the red cells (×1000, Leishman stain).

section 22 Haematological disorders 5448 the administration of oxidant drugs. Apart from intermittent icterus and splenomegaly there are no characteristic physical findings. Laboratory diagnosis This condition should be considered in any familial haemolytic an- aemia, particularly if a red cell enzyme deficiency cannot be demon- strated. The peripheral blood film shows the features of haemolysis but the red cell morphology may be relatively normal. Occasionally there is a mild degree of hypochromia and microcytosis. Unless splenectomy has been carried out, Heinz bodies are not seen in the peripheral blood. The most characteristic feature of the unstable haemoglobins is their heat instability. If a dilute haemoglobin solution is heated at 50°C for 15 min, most of the unstable haemoglobins precipitate as a dense cloud. A similar phenomenon can be induced by isopropanol. Sequencing of the globin genes allows a precise molecular diagnosis, and over 140 unstable variants have been identified to date. Treatment Because these conditions are so rare, there has been very little experience of the effects of splenectomy. From the information that is available, and from the senior author's personal experience, it appears that if a child has had several life-threatening episodes of anaemia or is running a steady- state haemoglobin level which is impairing development or well-being, splenectomy should be undertaken. It is interesting to note that some of these haemoglobin variants produce a 'right shift' in the oxygen dissoci- ation curve, and a measurement of the P50 as part of the pre-splenectomy assessment may help to decide whether to proceed to surgery; a marked right shift, that is, an increased P50, indicates that the anaemia should be more easily tolerated than if the oxygen dissociation curve is moved in the opposite direction with a low P50. An accurate history from the child or its parents is probably more helpful, however. Haemoglobin variants which cause abnormal oxygen binding The first high-affinity haemoglobin identified was haemoglobin Chesapeake, detected as an abnormal haemoglobin band in a pa- tient with otherwise unexplained polycythaemia. Since then, over 90 haemoglobin variants of this type have been defined, all associated with familial polycythaemia. Aetiology The high oxygen affinity haemoglobin variant may result from single amino acid substitutions in either the α or β globin chains, in critical parts of the haemoglobin molecule which are involved in the config- uration changes that underlie haem–haem interaction and the pro- duction of a sigmoid oxygen dissociation curve. Many occur at the junctions between the α and β subunits. Others involve the amino acids which are involved with the binding of 2,3-bisphosphoglycerate (2,3-BPG) to haemoglobin. As mentioned earlier, increasing con- centrations of 2,3-BPG tend to push the oxygen dissociation curve to the right; fetal haemoglobin has a high oxygen affinity (left-shifted curve) because it cannot interact with 2,3-BPG; mutations of the BPG binding sites have a similar effect. Pathophysiology The high oxygen affinity variants have a left-shifted oxygen dis- sociation curve with a reduced P50, which may be detected using a standard blood gas analyser. The variant haemoglobin holds on to oxygen more avidly than normal haemoglobin. This leads to tissue hypoxia. This in turn causes an increased output of erythropoietin and an elevated red cell mass. Clinical features Many patients with high oxygen affinity variants are completely healthy and are only found to carry the variant when a routine haem- atological examination shows an unusually high haemoglobin level or packed cell volume. There have been one or two reports of arterial or venous occlusive disease in these patients. However, this is un- common. Most patients are asymptomatic. There is no splenomegaly and no other associated haematological findings. Although it might be expected that a high oxygen affinity haemoglobin would cause de- fective oxygenation of the fetus, this has not been observed clinically. Diagnosis The condition should be suspected in any patient with polycy- thaemia associated with a left-shifted oxygen dissociation curve. A raised or inappropriately normal serum erythropoietin will be seen. The diagnosis can be confirmed by haemoglobin analysis. Treatment In

asymptomatic patients with high oxygen affinity haemoglobin variants no treatment is necessary. The difficulty arises if the patient has associated vascular disease with symptoms of coronary or cere- bral artery insufficiency. These patients require a high haemoglobin level for oxygen transport; half their haemoglobin is physiologically useless. Venesection is therefore not usually recommended for these patients, though there is insufficient evidence to support categorical statements how these patients should be managed. Low oxygen affinity variants At least 60 haemoglobin variants with reduced oxygen affinity have been reported. The first to be described, haemoglobin Kansas, was found in a mother and son with unexplained cyanosis. The subjects were asymptomatic and had normal haemoglobin levels without any evidence of haemolysis. Like many of the high affinity vari- ants, the amino acid substitution in this variant was at the interface between the α and β globin chains. This condition should be thought of in any patient with an unexplained congenital cyanosis; the differ- ential diagnosis is considered later in this chapter. Methaemoglobinaemia, carboxyhaemoglobinaemia, and sulphaemoglobinaemia Methaemoglobinaemia is a condition characterized by in- creased quantities of haemoglobin in which the iron of haem is oxidized to the ferric (Fe^{3+}) form. Carboxyhaemoglobinaemia (carbonmonoxyhaemoglobinaemia) results from the binding of carbon monoxide to the haem molecules. Sulphaemoglobinaemia is a rare condition in which there is a mixture of haemoglobin de- rivatives whose structure is poorly characterized but which can be defined by their specific spectral characteristics. Pathogenesis As mentioned earlier, each haemoglobin molecule has four haem moi- eties. At first sight it is not clear why the oxidation of a proportion of the iron atoms, or the fact that they are liganded to carbon monoxide,

 should cause such profound changes in oxygen transport. However, oxidation of 30% of the haem molecules has a much more serious ef- fect on tissue oxygenation than a reduction of the haemoglobin level by the same amount. This is because, if a single haem is oxidized, it so alters the conformation of the haemoglobin molecule that the oxygen affinity of the other three haems is increased. Thus methaemoglobin, carboxyhaemoglobin, and cyanmethaemoglobin all have very high oxygen affinities with left-shifted oxygen dissociation curves, and hence are associated with impaired unloading of oxygen to the tissues. Methaemoglobinaemia Methaemoglobin causes a variable degree of cyanosis. It should be suspected in any patient with significant central cyanosis in whom there is no evidence of cardiorespiratory disease. The de- gree of cyanosis produced by 50 g/litre of deoxygenated haemo- globin can be produced by 15 g/litre methaemoglobin and 5 g/litre of sulphaemoglobin. Methaemoglobin concentrations of 10 to 20% are tolerated quite well. It is useless as an oxygen carrier; levels above this are thus often associated with dyspnoea and headache. Much depends on the rapidity at which it is formed. Many patients with lifelong methaemoglobinaemia are asymptomatic, while individuals who have accumulated a similar level of methaemoglobin acutely may be acutely dyspnoeic. For reasons that are not clear, it is un- usual for patients with chronic methaemoglobinaemia to have an increased haemoglobin level or red cell count. Methaemoglobinaemia may arise as a result of a genetic defect in red cell metabolism or haemoglobin structure, or may be acquired following the ingestion of various oxidant drugs and toxic agents. Genetic methaemoglobinaemia There are two forms of inherited methaemoglobinaemia. The first, and less common, results from a deficiency of red cell NADH-cytochrome b5 reductase, the second from a structural alteration in either the α or β globin chains of haemoglobin. NADH-diaphorase (NADH methaemoglobin reductase) cata- lyses a step in the major pathway for methaemoglobin reduction. The enzyme reduces cytochrome b5 using NADH as a hydrogen donor. The reduced cytochrome b5, in turn, reduces methaemoglobin to haemoglobin.

There are several different molecular forms of NADH-cytochrome b5 reductase deficiency which have been iden- tified by electrophoretic analysis of NADH-cytochrome b5 reductase in the red cells of affected patients. The condition is inherited in an autosomal recessive manner. Homozygotes have elevated levels of methaemoglobin and are cyanotic from birth. Heterozygotes do not have elevated levels of methaemoglobin but seem to be unusually susceptible to the oxidant action of drugs. For example, severe cyan- osis has been precipitated by the use of antimalarial drugs. There are several abnormal haemoglobin variants which are associated with genetic methaemoglobinaemia, all of which are designated haemoglobin M, and further identified by their place of discovery (e.g. haemoglobin M Boston, haemoglobin M Milwaukee). These variants may affect either the α or β chain, but usually result from amino acid substitutions near the haem pocket. Normally, haem lies between two histidine residues, one called the proximal histidine to which it is attached, and the other called the distal histidine. Oxygen is bound to haem at a site opposite to the distal histidine. If the latter is substituted by tyrosine, as occurs in the α chain variant haemoglobin M Boston and in the β chain variant M Saskatoon, a stable bond is formed between the haem iron and the phenolic ring of the tyrosine. The iron atom is ‘fixed’ in the Fe^{3+} state. These variants are associated with cyanosis which is present from early life. In the case of the α chain variants it is present from birth, while the β chain haemoglobin variants only produce cyanosis after the first few months of life as adult haemo- globin synthesis becomes established. Unlike NADH diaphorase deficiency, which is inherited as a recessive trait, the haemoglobin Ms have a dominant form of inheritance. Thus the diagnosis of gen- etic methaemoglobinaemia and even the affected globin chain can be ascertained by a good clinical history. The diagnosis is confirmed by spectroscopic examination of the blood and by determination of methaemoglobin levels. The precise cause can be established by combination of HPLC and globin gene sequencing, or by an assay of NADH-diaphorase. Genetic methaemoglobinaemia due to NADH-diaphorase defi- ciency is readily treated by the administration of ascorbic acid, 300 to 600 mg daily by mouth in divided doses, or by the administration of methylene blue, either intravenously (1 mg/kg body weight) or by mouth 60 mg three to four times daily. On the other hand, the gen- etic methaemoglobinaemias due to structural haemoglobin variants do not respond to ascorbic acid, methylene blue, or any other treat- ment. Most affected individuals go through life asymptomatic and require no treatment. Acquired methaemoglobinaemia Acquired methaemoglobinaemia usually results from the adminis- tration of drugs or exposure to chemicals which cause oxidation of haemoglobin. There are many agents which are capable of exceeding the red cells’ ability to reduce methaemoglobin. They include ferri- cyanide, bivalent copper, chromate, chlorate, quinones, and certain dyes with a high oxidation–reduction potential. Nitrite, often used as a preservative, is one of the most common methaemoglobin- forming agents. Nitrates, after conversion to nitrites in the gut, may cause serious methaemoglobinaemia in infants. Other agents which commonly cause methaemoglobinaemia include phenacetin, primaquine, sulfonamides, and various analine dye derivatives. If any of the agents listed previously is given in low dose over a long period of time it may lead to chronic methaemoglobinaemia with or without a haemolytic anaemia. However, after exposure to a large amount of these agents, and the development of in excess of 50 to 60% methaemoglobin, the symptoms of acute anaemia develop because methaemoglobin lacks the capacity to transport oxygen. Thus the clinical picture may be characterized by vascular collapse, coma, and death. Methaemoglobinaemia with haemolytic anaemia The haemolytic action of oxidant drugs is described elsewhere (see also Chapter 22.6.11). Chronic methaemoglobinaemia with haemo- lytic anaemia, characterized by Heinz body formation and frag- mented red cells, occurs commonly in patients receiving dapsone, salazopyrine, or phenacetin. This condition is usually innocuous and can be modified by adjusting

the dose of the drug. Occasionally, acute intravascular haemolysis associated with methaemoglobinaemia and disseminated intravascular coagulation occurs. It usually follows the ingestion or infusion of a strong oxi- dizing agent such as chlorate or arsine. There is gross intravascular haemolysis and methaemoglobinaemia together with evidence of disseminated intravascular coagulation. The haemoglobin level may fall very rapidly and may be complicated by renal failure.

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