22.6.4 Iron metabolism and its disorders 5371 Timo

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22.6.4 Iron metabolism and its disorders 5371 World Health Organization (1968). Nutritional anaemias. Technical Report Series No. 405. World Health Organization, Geneva. World Health Organization (2002). The world health report 2002— reducing risks, promoting healthy life. World Health Organization, Geneva. World Health Organization (2015). The world malaria report. World Health Organization, Geneva. http://apps.who.int/iris/bitstream/10665/200018/1/9789241565158_eng.pdf 22.6.4 Iron metabolism and its disorders Timothy M. Cox and John B. Porter ESSENTIALS Iron deficiency and iron storage disease—the latter principally due to inherited and acquired anaemias such as thalassemia—are dis- orders of massive clinical significance across the globe. Iron de- ficiency is the most common cause of anaemia, affecting about 1 billion people; about 0.75 million people have thalassaemia. Largely neglected by health services in rich and resource-poor countries alike, disorders of iron metabolism, whether inherited, nutritional, or otherwise, represent a long-standing public health challenge. Improved screening methods for detection, diagnosis, and appro- priate supplementation—as well as genetic counselling—can offer a great deal to relieve the burden in stricken communities. Advances in chelation therapy have improved the survival of patients with iron-loading anaemias and transfusion-related haemochromatosis, and better understanding of the molecular pathophysiology of iron homeostasis now offers the prospect of definitive therapies to con- trol pathological erythropoiesis and the inappropriate drive to ac- quire lethal quantities of toxic iron. Iron homeostasis Iron is an essential nutrient, the recommended daily allowance being 10 to 20 mg, depending on the bioavailability of food iron con- stituents. Iron requirements are greater during periods of growth in childhood and adolescence and in pregnancy, also in menstruating women and during lactation. Patients with chronic haemorrhage and intravascular haemolysis need sufficient iron to compensate for in- creased losses. Iron is a critical component of haem proteins and nonhaem en- zyme systems required for oxygen transport, mitochondrial respir- ation, and

essential enzymatic reactions. High-affinity iron-binding proteins have evolved to facilitate iron transport and delivery to sites of storage and utilization, and especially for haem biosynthesis. Most iron in the body is coordinated in protoporphyrin IX as haem (ferroprotohaem). Small amounts of iron circulate in the plasma, bound in the ferric form to the glycoprotein, transferrin. Iron is stored in the mononuclear phagocyte system principally as intracellular fer- ritin and its proteolytic degradation product, haemosiderin. Iron absorption occurs in the duodenum and upper jejunum. The processes are complex, but the following proteins are in- volved: the divalent metal transporter protein (DMT1), ferrireductase, hephaestin, ferroportin 1 and a haem transporter. Capacity for safe storage of iron in intracellular deposits (mainly in tissue macrophages) is limited, and the ability to dispose of ex- cess iron in the body by excretion is negligible. Iron balance is maintained by rigorous control of absorption from the diet, which is principally orchestrated by the actions of hepcidin. The mech- anisms by which hepcidin release is controlled by iron status and in response to activity of the erythroid marrow are only now being understood. Iron deficiency Causes—iron-poor diets alone are a rare cause of iron deficiency an- aemia, except in growing children. It is critical to consider (1) en- hanced loss of iron—due to excess menstruation, pregnancy, and losses from the gastrointestinal tract (hookworms, ulcerating lesions, angiodysplasia); and (2) malabsorption of iron (e.g. after gastrointes- tinal surgery and or coeliac disease). At least one rare inherited dis- ease causes iron-refractory iron deficiency anaemia (IRIDA). Clinical features—apart from symptoms of anaemia and pallor, the most common are restless legs, angular cheilosis, atrophic glossitis, hair loss, and dystrophy of the nails with longitudinal ridging and koilonychia. Investigation and diagnosis—iron deficiency causes microcytic anaemia, usually in association with reduced serum transferrin sat- uration with iron (<16%), a raised total plasma transferrin, and a re- duced serum ferritin concentration (<12 μg/litre). In many cases, the cause will be obvious (e.g. menorrhagia in a young woman), but in others, diligent investigation will be required (e.g. to diag- nose or exclude gastrointestinal cancer and other sources of occult blood loss). Management—apart from dealing with the underlying cause, this involves iron-replacement therapy, which should normally be ad- ministered orally, although parenteral preparations may (rarely) be necessary. Iron storage disease (haemochromatosis, iron overload) Ferrous and ferric ions are chemically reactive so that excess of free iron is toxic; tissues with elevated concentrations of the metal show functional impairment and structural injury leading to ‘iron storage disease’ (haemochromatosis). Causes—(1) genetic, hereditary, or primary haemochromatosis; or (2) secondary haemochromatosis, including (a) diseases character- ized by ‘ineffective’ or disordered erythropoiesis (e.g. β-thalassaemia) which induce inappropriate absorption of iron by the intestine; (b) repeated blood or red cell transfusion; and (c) administration of iron in quantities or formulations that overwhelm natural homeo- static mechanisms. Clinical features—these depend on the rate and distribution of ex- cess iron and include (1) heart disease—cardiomyopathy is a leading cause of death in refractory anaemias such as β-thalassaemia; (2) fibrotic liver disease and cirrhosis, sometimes complicated by hepatocellular cancer; (3) endocrine failure (e.g. hypogonadotropic hypogonadism, diabetes mellitus, and mineralocorticoid failure); (4) skin and joint manifestations; and (5) microbial infection.

 Investigation and diagnosis—iron storage disease is usually sus- pected on the basis of (1) raised serum ferritin measurements, and (2) when the saturation of serum transferrin exceeds 60%. Definitive diagnosis requires confirmation of the genetic mutation (most commonly C282Y for the HFE gene in persons of European descent) and of iron overload, either by noninvasive iron deter- mination by liver or cardiac magnetic resonance

imaging or by liver biopsy with specific elemental analysis and/or histochemical staining for iron. Management—in the early stages, potentially fatal sequelae of iron toxicity can be prevented by prompt institution of measures to deplete iron: (1) where erythropoiesis is normal—by repeated venesection; and (2) for patients with disordered (ineffective) haem- atopoiesis or inadequate venous access—iron chelators. Hitherto, parenteral administration of desferrioxamine has provided the best standard of care, but orally active chelators (deferiprone and deferasirox) have better acceptability and efficacy. Future developments—striking advances in understanding the control of iron balance by the hormone hepcidin, the molecular pathogenesis of iron loading in dyserythropoietic anaemias, and of the role of the transforming growth factor-β superfamily of pro- teins and hypoxia-inducible factors in the regulation of erythropoi- esis, are driving innovative clinical research. These advances offer a good chance of introducing transformative new treatments for life- shortening iron-loading anaemias due to ineffective erythropoiesis. Introduction Disorders of iron metabolism frequently contribute to disease and are of immense significance for global health. Iron deficiency is the dominant cause of anaemia and affects more than 1 billion people; about 0.75 million people have thalassaemia and about 0.33 mil- lion have sickling disorders and other haemoglobinopathies (see also Chapter 22.6.3). These conditions erode working capacity as well as well-being. Moreover, the iron-loading anaemias and sickling disorders are expensive to treat, in some regions affecting the economies of whole societies. Iron deficiency is rife: it affects women in rich and deprived countries alike, but is most common among the poor, in in- fants and the elderly, those who eat little or no meat, and those with hookworm infestation. At the same time, the prevalence of haemoglobinopathies and other anaemias such as myelodysplasia that cause inappropriate absorption of iron and ineffective erythropoiesis, with or without the need for red cell transfusions, mean that iron storage disease is also a massive challenge in global health. In addition, as discussed in Chapter 12.7.1, Hereditary haemochromatosis occurs at a high gene frequency in certain populations, including those of European descent (adult haemo- chromatosis, due to mutations in HFE) and of sub-Saharan African origin (African iron overload), in whom the nature of the predisposing gene is unknown. Other rare genetic forms of haemochromatosis (‘juvenile’) in which endocrine failure and cardiomyopathy due to iron toxicity occur in childhood or early adult life are due to defects in the complex signalling pathways that control iron homeostasis. General aspects of iron metabolism and disease Iron is the fourth most common element in the Earth’s crust; while essential for aerobic life, its electrochemical properties also pose challenges for living organisms. The metal assumes two readily inter- convertible redox states (divalent ferrous iron, Fe^{2+}, and trivalent ferric iron, Fe^{3+}) which are highly reactive. In the environment, iron exists principally in the oxidized ferric state, which, under neutral conditions, is then rapidly hydrolysed to insoluble polyhydroxide complexes, which cannot be assimilated. High-affinity iron-binding proteins, which form stable ferric complexes, have evolved to facili- tate iron transport and delivery to sites of storage and utilization. Iron is essential for human health As a component of metalloenzymes and complexed to form haem, iron participates in the transport of oxygen by haemoglobin and myoglobin and harvesting metabolic energy obtained via the elec- tron transport chain by the agency of cytochromes. In addition, iron-dependent enzymes are required for critical reactions (e.g. xenobiotic metabolism) as well as the biosynthesis of dopamine, catecholamines, and melanin. Iron deficiency, which affects infants, children, young adults, and older people in all populations, is prob- ably the most frequent organic illness worldwide; its high prevalence is evidence of the critical availability of iron as a key nutrient. Iron deficiency is associated with anaemia and nonhaematopoietic manifestations that impair work efficiency and contribute to chronic ill health,

as well as loss of mucosal integrity; iron deficiency an- aemia is associated with pica, in effect an obsessive craving to eat or chew unusual materials which are often of no nutritional value. Pica, which reflects mental changes induced by iron deficiency, has important socioeconomic, environmental, and behavioural associ- ations with poverty and hookworm infection. The causal role of iron deficiency is confirmed by a rapid response to iron supplementation. Iron and infection The study of iron metabolism in animals and plants is a vast and continually expanding field in biology. Since the need for and avail- ability of iron for growth of pathogens is critical for host defence as well as microbial virulence, deeper understanding of iron physi- ology has become an imperative in pathogen research. Strong se- lective pressures throughout evolution have resulted in adaptations to cellular iron deficiency as well as mechanisms to sequestrate bio- available iron—so limiting microbial invasion and growth. In contrast, not only are patients with iron overload susceptible to certain bacterial and fungal infections, but indiscriminate iron sup- plementation may have catastrophic effects and exacerbate infec- tions. The protozoal infection, malaria, provides an example of this effect, despite its frequent occurrence in populations where anaemia is rife. Recent studies in which the ferroportin gene was deleted se- lectively in murine erythroid cells showed accumulation of excess intracellular iron, cellular damage, and haemolysis—as well as a poor outcome from experimental malaria. In humans, a prevalent mutation in ferroportin (glutamate replacing histidine at position 248) impairs the normal turnover of ferroportin when iron is defi- cient and increases abundance of the iron exporter. Not only is there is evidence that inheritance of the variant protein protects against severe malaria, in African populations this mutant allele of human

 ferroportin is over-represented—presumably as a consequence of evolutionary selection. Interrelations between iron and infection are increasingly important in clinical medicine but space does not permit further detailed discussion here.
Iron toxicity ‘Free’ iron that is not coordinated by an iron-binding molecule interchanges between ferric and ferrous oxidation states, thereby promoting the formation of damaging free radicals; these mediate the injury to cells and tissues that characterizes iron storage disease. Hydroxyl radicals, the most damaging reactive oxygen species, are generated by interactions between superoxide and ferric ions; they catalyse the modification of cell membrane lipids—a common fea- ture of iron-induced tissue injury. There are clear clinical parallels between secondary haemochromatosis related to the iron-loading anaemias and the severe genetic forms of this disease, usually termed juvenile haemochromatosis. After many years of detailed study, these subjects are converging also with the expectation of credible therapeutic development for the anaemias, which remain a worldwide challenge in medical practice. When iron accumulates excessively, the iron-binding capacity of plasma transferrin is often exceeded, so that a fraction of the iron present in the blood occurs as low molecular weight species that are only bound loosely to plasma proteins. This nontransferrin-bound iron in human plasma induces a distinct pattern of tissue iron up- take compared with physiological transferrin-mediated delivery: it catalyses the peroxidation of unsaturated lipids and generates re- active complexes which damage DNA. Iron-mediated injury to DNA Iron-mediated DNA modification is potentially mutagenic and, as in the inherited copper toxicity disease, Wilson disease, is likely to contribute to the known association between iron excess and cancer. These disorders, characterized by excess hepatic deposition of multi- valent transition metals, induce oxidative stress and increase the risk of liver cancer. The frequency of p53 mutated alleles in noncancerous tissue may be a biomarker of genomic injury and identify individ- uals at increased cancer risk: a higher frequency of transversions at codon 249

have been reported in liver samples from patients with haemochromatosis compared with tissue from control subjects who do not have excess iron. These findings suggest that the generation of reactive free radical species from iron overload lead to mutations in the p53 tumour suppressor gene that may contribute to the greatly increased risk of hepatocellular cancer. Body iron composition and evaluation of iron status Body iron composition The total amount of iron in the adult body is between 3 and 4 g, most of which is coordinated to protoporphyrin IX as haem (Fig. 22.6.4.1). Haem is found principally as haemoglobin and myoglobin; appre- ciable quantities are found also in the viscera, especially the liver, kidney, and intestine, where it is present in cytochromes and other iron proteins. Cytochromes of the electron transport chain and of the P450 system for the metabolism of xenobiotics are abundant in these organs and, notably in specific regions of the brain. In an adult, about 2.5 g of iron is complexed in haemoglobin, with an additional 0.5 g of iron in myoglobin, principally in muscles. Plasma iron Very small amounts of iron circulate in the plasma: here it is bound in the ferric form to the glycoprotein, transferrin, which is normally only about one-third saturated with iron, so that with a mean con- centration of 3 g/litre for a protein of molecular weight 80 000, the en- tire plasma compartment contains less than 2 mg of elemental iron. In health, the concentration of another iron protein, ferritin, in the plasma does not exceed about 250 µg/litre. Plasma or serum fer- ritin does not itself contain appreciable iron; rather, serum ferritin concentrations usually reflect the stores of iron in the body. Serum ferritin concentrations below the healthy reference range nearly al- ways indicate iron deficiency. In contrast, a high concentration of serum ferritin may reflect high iron stores but also occurs in inflam- matory states, liver disease (e.g. from hepatic steatosis), or certain cancers (e.g. Hodgkin lymphoma). Storage compartment Iron is stored in the mononuclear phagocyte system (previously known as the reticuloendothelial system) principally as intra- cellular ferritin and its cognate proteolytic degradation product, haemosiderin. In healthy men, body iron stores do not exceed 1.5 g and are usually 0.5 g or less in healthy women. Deposits of nonhaem iron that serve as stores in the iron-rich tissues can be revealed by staining with Perls' reagent (acid potassium ferrocyanide), with which they give a strong Prussian blue reaction. Faint staining with Perls' reagent is seen in healthy parenchymal liver cells; but Plasma 4 mg Erythrocytes 2500 mg 5 mg daily 20 mg daily 20 mg daily RBC destruction Absorption 1–2 mg daily Monocyte-macrophage system Loss 1–2 mg daily Bone marrow RBC production Body stores 1000 mg Myoglobin and respiratory enzymes 300 mg Fig. 22.6.4.1 Daily flux of iron through storage and transport compartments.

section 22 Haematological disorders 5374 the principal deposits of storage iron are observed in bone marrow, macrophages of the spleen, and in Kupffer cells, specialized macro- phages of the liver. Evaluation of iron status The manifestations of iron deficiency and overload are discussed in the following sections. Laboratory investigation of iron status includes measurement of transferrin saturation and serum ferritin (Box 22.6.4.1). In health, transferrin is about one-third saturated with iron. In acute (or chromic) inflammation, increased hepcidin biosynthesis brought about by inflammatory mediators such as interleukin (IL)-6, decreases transferrin saturation. However, the total plasma transferrin concentration is decreased or within the healthy reference range. In contrast, in iron deficiency, low serum iron is typically accompanied by increased total transferrin, and hence a low transferrin saturation. The presence of hypochromic microcytic red cells supports the diagnosis of iron deficiency, although it is important to remember that small red cells occur also in thalassaemia carriers. Low serum ferritin concentrations are a useful confirmatory biomarker of iron deficiency. An elevated concentration of serum ferritin implies in- creased body iron but serum ferritin is an acute phase reactant and is increased in acute or chronic

inflammation, so that an elevated serum ferritin is not specific. Serum ferritin is persistently raised in patients with hyperferritinaemia-cataract syndrome (see later); although they do not develop systemic iron excess. If mistakenly treated by phlebotomy, the serum ferritin (principally containing light-chain subunits) does not fall, and may even rise paradoxically. Serum ferritin concentrations are also raised independently of iron overload in malignant disease (including common cancers as well as lymphomas including Hodgkin lymphoma), or released from the liver in hepatitis—including steatohepatitis. Again, it is im- portant to emphasize that while low serum ferritin almost invariably indicates iron deficiency, a raised serum ferritin does not always in- dicate iron overload. Additional diagnostic blood tests It may sometimes be necessary to undertake additional tests to confirm the presence of iron deficiency or iron overload. While in most cases blood film examination is avoided, it is surprising as to how informative this simple preparation and microscopy can be—particularly for detecting the host response to blood loss (polychromasia with modest macrocytosis with a reticulocytosis) and the presence of red cell fragments that indicate intravascular haemolysis. There are advocates for the measurement of free circulating trans- ferrin receptors, which may be determined by immunoassay: ex- pression of soluble transferrin receptor protein is enhanced under conditions of iron deficiency and plasma concentrations are ele- vated in the presence of functionally iron-deficient erythropoiesis. However, serum transferrin receptor concentrations are elevated under conditions of erythroid hyperplasia in the bone marrow and especially when ineffective erythropoiesis occurs (megaloblastic an- aemia, haemoglobinopathies, sideroblastic anaemia). Direct estimation of storage iron Staining of iron stores in the bone marrow with Perls' reagent is a time-honoured, robust, but mildly invasive method for resolving difficulties that arise in the investigation of patients with suspected iron deficiency anaemia. Although an examination of the amount of iron (usually graded semi-quantitatively on a scale from 0 to 4, reflecting the strength of Prussian blue staining) does not provide any information as to the availability of the iron for haemoglobin formation, it does provide useful information as to the appropri- ateness of iron therapy for hypochromic anaemia. Bone marrow examination, moreover, may be diagnostic in patients suffering from hypochromic anaemias due to primary or sideroblastic change in the marrow, since the characteristic ring sideroblasts, with or without other myeloblastic changes, will be apparent. Liver iron concentration obtained from liver biopsies has been used to quantify iron overload and evaluate responses to chela- tion treatment but is invasive. More recently, determination of liver iron concentration by magnetic resonance imaging (MRI) is being used to confirm iron overload in the case of unexplained hyperferritinaemia (raised serum ferritin concentration). This non- invasive approach is slowly replacing liver biopsy for confirming iron overload; it is also used to assess cardiac iron in transfusional iron overload (see 'Transfusional iron overload'). This analysis re- quires a calibrated and externally validated method using T2* or R2 measurements but can be performed on most 1.5-Tesla MRI ma- chines. Low concentrations of hepatic iron ascertained by MRI in the presence of hyperferritinaemia indicate liver disease or an in- flammatory process—thus meriting investigations for these condi- tions rather than iron overload. Human iron physiology Erythropoiesis and recycling of iron Iron is essential for the biosynthesis of haem and haemoglobin formation during the maturation of red cell precursors. The circu- lating iron-binding plasma glycoprotein, transferrin is the principal physiological mediator of iron delivery and transport about the body. For assimilation into haem, iron is transported and taken up from plasma transferrin by the recycling endocytosis of the iron-bound ligand via transferrin receptor type 1; the iron moiety is incorporated Box 22.6.4.1 Laboratory investigations for iron deficiency anaemia • Serum ferritin concentration (low) • Serum iron concentration (low) • Transferrin saturation (low) • Soluble transferrin receptora (elevated) • Serum hepcidin

concentrationb Note: in addition to haemoglobin concentration, check red cell indices (mean cell volume and mean cell haemoglobin content). A reticulocyte count and film examination are desirable to judge the extent of compensatory erythro- poiesis for haemorrhage (or haemolysis) and may support the diagnosis of haemoglobinopathy, sideroblastic anaemia, or coeliac disease (dimorphic picture with hyposplenic features). a More reliable for diagnosis of iron deficiency than transferrin parameters but not universally available. b Highly specialized investigation: if inappropriately elevated, suggests an- aemia of chronic disorders.

22.6.4 Iron metabolism and its disorders 5375 into protoporphyrin IX by mitochondrial ferrochelatase which util- izes ferrous ions that are generated by chemical reduction within the cell. The lifespan of the red cell is up to 120 days and about 1% of the steady-state haemoglobin pool turns over daily. This requires de novo synthesis of approximately 6 g of haemoglobin into which 20 mg of iron is incorporated in the haem moiety. Most of the iron required for haemoglobin synthesis in the basal state is recycled from senescent red cells after their destruction by macrophages (Fig. 22.6.4.1). Iron is delivered to the erythron in the plasma by transferrin that binds to transferrin receptors on eryth- roid precursors The iron-transferrin–receptor complex is internal- ized and, after acidification in endosomes, its iron is released. After recycling to the cell surface, the apotransferrin, which has a low affinity for the receptor at neutral pH, is released and can thus be reutilized. Increased delivery of iron occurs in association with erythroid ex- pansion and disturbed maturation of red cell precursors that express cell surface transferrin receptors under the influence of the renal hormone, erythropoietin (see ‘Erythropoietin’). Erythropoiesis is stimulated in the presence of hypoxia, after bleeding and haemolysis, as well as in dyserythropoietic conditions (such as thalassaemia and megaloblastic anaemia), and net assimilation of iron from the diet is increased. Storage of iron Ferritin, the principal protein for the safe storage of iron, is widely distributed in nature. As a large 24-subunit multimer, ferritin com- prises heavy and light polypeptide chains. Differences in the sub- unit composition influence the rates of iron uptake and release in different tissues. The main function of ferritin is the sequestration of ferric iron in a soluble, accessible, but nontoxic form. Partial pro- teolytic breakdown of iron-loaded ferritin molecules leads to the formation of haemosiderin, which exists as relatively insoluble ag- gregates in storage cells of the macrophage series; both proteins can release stored iron but the turnover of iron in haemosiderin is re- tarded compared with that intracellular ferritin. The heavy-chain component of ferritin has antioxidative prop- erties. Transcription (in contrast to iron-regulated translational expression) of the ferritin heavy-chain gene, FTH1, located on chromosome 11q12.3, is increased by the regulatory factor, NF-κB. This appears to inhibit tumour necrosis factor (TNF)-α-induced apoptosis by suppressing iron-mediated generation of reactive oxygen species. Iron homeostasis Iron, an essential nutrient, is fastidiously conserved by the body and only a fraction of that which is utilized in the bone marrow and other tissues is lost by obligatory processes involving exfoliation of epi- thelia and intercurrent blood loss, such as that incurred by trauma or menstruation. Net iron balance is ultimately controlled at the level of absorption of organic and inorganic iron from the diet by the small intestine. Assimilation of iron Iron is absorbed from dietary sources principally in the duodenum and proximal jejunum. The amount of iron available varies greatly, and even under optimal circumstances only a small fraction is nor- mally absorbed: in adult men, the daily requirement is on average 0.8 mg, whereas adult women of reproductive age need about 2 mg daily. Depending on the bioavailability of the constituent iron, the recommended daily allowance of iron in the diet is 10 to 20 mg. Digestion of food iron Inorganic ferric ions are released by digestion and reduced by lu- minal factors such as

ascorbate, as well as the action of ferrireductase (DCYTB); Fe2+ ions are taken up via the divalent metal transporter (DMT1) in the brush-border membrane. Iron complexed to haem enters enterocytes through a distinct uptake pathway. Within en- terocytes, the membrane-bound copper protein, hephaestin, is im- plicated in reoxidation of ferrous to ferric ions; Fe3+efflux across the basolateral membrane and delivered to unbound plasma apotransferrin is mediated by the transporter, ferroportin (FPN1). Haem iron may be more readily absorbed than inorganic iron in the human intestine and, depending principally on the content in meat, may constitute an important source of iron in nonvegetarians. Dietary phytates and medication including antacids and tetracyc- line antibiotics, as well as proton pump inhibitors, H2 antagon- ists, and prior upper gastrointestinal surgery, strongly influence the intraluminal bioavailability and hence absorption of inorganic food iron. Meeting increased needs for iron The requirement for iron is greater in patients with recurrent bleeding or in those who are blood donors; iron requirements are also greater during periods of growth in childhood, adolescence, and during pregnancy, when the daily requirement may be as much as 5 mg. Given its iron content, loss of 1 ml of blood represents approxi- mately 0.5 mg of iron; this relationship simplifies estimates of iron required to meet that incurred by blood losses, for example, by menorrhagia (>80 ml/month) or from other sources. Depending on peripartum blood loss, each pregnancy represents a maternal invest- ment of up to 1.5 g iron, which greatly exceeds savings due to the cessation of menstruation. Iron absorption and release from tissue stores In health, iron absorption in the duodenum and upper jejunum is a finely regulated process that matches the acquisition of iron from the diet to body requirements for erythropoiesis; this compensates for daily obligatory losses of iron (e.g. from the exfoliation of epithelia or menstruation). Iron uptake Genetic studies of mutant strains of mice with abnormalities of iron metabolism have shed light on the iron-absorption mechanism. The divalent metal transporter protein, DMT1, which is expressed in the upper small intestine and cells of the erythron, is essential for uptake of ferrous ions. The human DMT1 gene maps to the long arm of chromosome 12 and encodes a 12-membrane-spanning protein that is expressed in the apical membrane of the upper intestine. DMT1 is also produced in developing erythroid cells, in which it is respon- sible for the intracellular delivery of iron derived from transferrin after endocytosis for haemoglobin synthesis.

section 22 Haematological disorders 5376 Reduction of inorganic iron A ferrireductase (cytochrome b reductase) is encoded by DCYTB; the cognate protein reduces ferric to ferrous ions and is localized to the intestinal brush-border membrane and present in red cell precursors. Mucosal ferrireductase occurs at the apical microvillous mem- brane of mammalian intestinal mucosa and this activity is increased by nutritional iron deficiency. Dietary iron is found in haem proteins as a component of haemoglobin, myoglobin, and tissue cytochromes. Haem iron Haem iron occurs principally but not exclusively in meat, which is an important facultative source of iron in the diet of many humans. Haem uptake by enterocytes requires a membrane protein, but the identity of this putative carrier remains controversial. After uptake as the intact molecule, the haem moiety is opened up by the action of haem oxygenases in the intestinal mucosa to release iron (and carbon monoxide). Efflux of iron to the portal plasma Ferric ions derived from the dietary sources are exported from en- terocytes by the carrier molecule ferroportin, which is localized to the basolateral membrane of intestinal epithelial cells. Ferroportin is also responsible for the efflux of iron liberated from the iron stores in the macrophage compartment; it is the principal site for the regula- tion of iron flux and distribution and its activity is controlled by the master regulatory hormone, hepcidin. Heterozygous deficiency of ferroportin gives rise to a dominantly inherited

pathological storage of iron in macrophages and macro- phage-rich organs such as the liver and spleen (see Chapter 12.7.1). Homozygosity for such mutations would probably be incompatible with life. Intracellular oxidation of ferrous iron in the epithelium A putative copper-binding protein, hephaestin, which maps to the X chromosome and has sequence similarity to caeruloplasmin, appar- ently mediates oxidation of ferrous iron and cooperates functionally with ferroportin in promoting the transepithelial transport of iron in enterocytes. Inherited defects of hephaestin in experimental mice lead to iron deficiency anaemia; none have yet been identified in humans. Iron release from tissue stores Most storage iron is present in macrophages in the bone marrow, spleen, and Kupffer cells of the liver. Ferric ions released by break- down of ferritin and haemosiderin are exported by the action of ferroportin and bind the plasma protein transferrin. Adaptive responses to iron status In iron deficiency, or even when iron stores are modestly depleted, more of the bioavailable food iron is absorbed. Hypoxia also en- hances the absorptive capacity of the small intestine and induces the ferrous iron carrier, divalent metal transporter, DMT1, as well as the accompanying ferrireductase, encoded by DCYTB. Dysregulation of iron absorption in iron-loading anaemias For reasons that are only now being understood, certain anaemias, particularly those associated with dyserythropoiesis and hence in- effective erythropoiesis, also increase absorption of iron in the intestine. Where the anaemia is long-standing (e.g. congenital or ac- quired sideroblastic anaemia), or in haemoglobinopathies such as β-thalassaemia, inappropriate intestinal absorption of iron accom- panies massive expansion of the erythropoietic marrow; hepcidin biosynthesis in the liver is suppressed and toxic iron overload re- sults. This secondary haemochromatosis can occur in the absence of iron loading that results from multiple red cell transfusions. Oral iron toxicity Maintenance of iron balance by the intestine normally protects the body from the potentially toxic effects of iron-rich diets. Only under exceptional circumstances, such as the ingestion of alcoholic beverages containing abundant iron due to peculiarities of manufacture (e.g. the kaffir beers that are fermented in iron pots by the South African Bantu), does excess dietary iron lead to iron storage disease. It seems probable that those individuals who develop iron storage disease in the context of long-standing excessive ingestion of highly available iron, harbour genetic cofactors such as mutant alleles of the adult haemochromatosis gene, HFE, or because of an underlying haematological disorder, such as α- or β-thalassaemia trait or a cell-intrinsic haemolytic anaemia. There are numerous reports of haemochromatosis associated with red cell disorders, including the congenital dyserythropoietic disorders (see Chapters 22.6.7 and 22.6.8) and red cell enzyme defects, such pyruvate kinase deficiency (see Chapter 22.6.10). Physiological regulation of iron status While iron is critical for health, its essential electrochemical prop- erties also confer great danger in its use, handling, and storage. Elaborate mechanisms exist to maintain the balance of supply (ac- quired by the small intestine from dietary sources) and requirements without incurring tissue injury. Body iron balance is regulated at the level of acquisition from the intestine: in operational terms, net ab- sorption of dietary iron is modulated by the iron status of the tis- sues (‘storage regulator’) and controlled by activity of erythropoiesis in the bone marrow (‘erythroid-regulator’). These aspects are dis- cussed in more detail in following sections. Hepcidin—an iron-regulatory hormone Hepcidin plays a critical role in the control of iron homeostasis. This polypeptide (molecular mass c.2800) is a member of a family of cysteine-rich peptides with antimicrobial activities. Hepcidin serves as an ‘iron regulatory hormone’, which inhibits efflux of iron from macrophages and enterocytes. Hepcidin is present in serum and urine but is generated in, and secreted by the liver. Hepcidin biosynthesis and release by the liver is induced by iron loading and suppressed by anaemia, hypoxia, and by inflammatory cytokines—including IL-6, which also activates the hepcidin promoter through signal transduction and

transcriptional regulation. Mode of action A critical action of hepcidin is attributed to its capacity to bind to ferroportin—the transport protein which mediates export of ferric ions from mammalian cells and which is expressed particularly on the basolateral membrane of enterocytes and the plasma membrane of macrophages. Binding of hepcidin to ferroportin on the plasma mem- brane induces ferroportin internalization and degradation in the lyso- some. Thus, hepcidin inhibits net absorption of dietary iron as well as its release from the macrophage storage compartment (Fig. 22.6.4.2).

22.6.4 Iron metabolism and its disorders 5377 Dominant mutations in the SLC40A1 gene that encodes ferroportin lead to an adult form of haemochromatosis (HFE4) in males and females associated with prominent deposition of iron in macrophages present in the marrow, liver, and spleen (see Chapter 12.7.1). Recently, ferroportin was found to be highly abundant in mature red cells and the capacity to export iron is suppressed by iron supplementation. This may in part explain the adverse effects of indiscriminate mass administration of iron in populations with a high level of malaria exposure. Congenital deficiency of hepcidin due to recessively transmitted mutations in the HAMP gene that maps to chromosome 19q23 is also associated with greatly enhanced absorption of iron and ju- venile haemochromatosis (HFE2B—see Chapter 12.7.1). In con- trast, excess synthesis and secretion of hepcidin by the liver inhibits the release of iron from the storage compartment and also decreases net absorption of iron from the diet by the small intestine. The main action of hepcidin has been verified in mice, and humans, in whom there is a hereditary deficiency of the peptide: intestinal absorption of iron proceeds in an unrestrained manner and thus iron circu- lates that is unbound to transferrin. This reactive pool of plasma iron is responsible for unregulated distribution of the iron and is associated with rapid parenchymal injury in many tissues (human hepcidin deficiency is a subtype of juvenile haemochromatosis (see Chapter 12.7.1)). Biosynthesis of hepcidin is transcriptionally regulated in the liver in response to the concentrations of extracellular and intracellular iron. This is achieved by formation of a macromolecular complex of bone morphogenetic protein receptors and their iron-specific ligands as well as modulators and molecules that serve as iron sensors (Fig. 22.6.4.3). Overall, molecular regulation of hepcidin appears to be orches- trated through the bone morphogenetic protein/smooth mothers against decapentaplegic homologue (BMP/SMAD) signalling pathway (see later). BMP6 expression is upregulated in response to iron and induces hepcidin through phosphorylation of the SMAD1/ 5/8 complex (see Chapter 12.7.1). Haemojuvelin (HJV), the hereditary haemochromatosis type 2A associated protein, and the transferrin receptor-2 isoform, which is mutated in another rare adult form of haemochromatosis (HFE3), also interact with this multimolecular complex on the plasma mem- brane. While the details of the molecular interactions of HJV with its partners are yet to be fully worked out, as with hepcidin encoded by HAMP1, mutations in the HJV gene on chromosome 1q21 cause a severe, juvenile form of iron overload—indicating its critical role in regulating net iron balance. HJV interacts with hepcidin and Dietary iron Hepcidin Hepcidin Plasma transferrin Fe Tfn Ferroportin Fe3+ Haem Fe3+ Fe2+ Spleen Macrophage storage compartment Upper small intestine Marrow macrophages Reduction (DCyt B) Uptake (DMT1) Fe Tfn Ferroportin Ferroportin Fe Tfn Inflammation IL6 etc Erythropoiesis Transferrin saturation Liver Kupffer cells + + – Hepcidin HAMP1 gene transcription Fig. 22.6.4.2 Hepcidin regulates iron balance by controlling ferroportin expression. Hepcidin, HAMP, is a regulatory polypeptide hormone synthesized and released by hepatocytes according to systemic iron status and erythropoietic activity; hepcidin release is also increased in the presence of inflammation. Hepcidin reduces the availability and assimilation of iron for erythropoiesis and storage by

regulation of iron acquisition from the diet and release of iron from the storage and recycling compartment. Hepcidin release is controlled negatively by the so-called storage and erythroid regulators. Inflammatory signals, mediated by interleukin-6 and Janus kinase/signal transducers and activators of transcription (JAK-STAT) pathway, enhance release. Hepcidin binds to ferroportin on plasma membranes and promotes internalization and degradation of this transporter: this reduces iron efflux from storage macrophages and across the basolateral membrane of enterocytes in the small intestinal mucosa.

section 22 Haematological disorders 5378 Liver – Receptor Spleen and other storage macrophages Erythroferrone and other red cell factors EPO Kidney Lungs Erythron Red cell mass Upper small intestine mucosa Ferroportin Ferroportin Fe3+ Tfn Fe3+ Tfn O2 Hepcidin Hepcidin Pa O2 JAK2 - STAT HIFS Heart Dietary iron + + (a) Fig. 22.6.4.3 (a) Homeostatic control and the interplay of erythropoiesis and iron supply. Erythroid drive is the dominant influence on iron homeostasis. This comprises several physiological pathways that ensure a balanced supply of iron for haemoglobin formation sufficient to meet tissue requirements for oxygenation. Ineffective erythropoiesis profoundly disrupts this tightly regulated control system and leads to inappropriate iron loading with tissue injury (secondary haemochromatosis). Tissue hypoxia inhibits the action of Fe2+-dependent prolyl hydroxylases which normally initiate proteasomal destruction of hypoxia-inducible factor (HIF)-1α and HIF2α by rendering them susceptible to ubiquitination by the von Hippel–Lindau complex (see text). When tissue Po2 is lowered, as in anaemia or high altitude, HIF-2α induces erythropoietin (EPO) transcription. On binding to its receptor (EPOR), in colony-forming units EPO activates the JAK2/STAT5 pathway for erythroid gene expression in the marrow. As the red cell population expands, erythroferrone and other soluble red cell factors are released: erythroferrone attenuates hepcidin synthesis and coordinates the iron supplied by plasma transferrin with the demands for haemoglobin formation.

Ferroportin Hormone release Ferroportin Iron storage macrophage Fe Fe DMT1 Ferrireductase Fe3+ Iron transferrin TfR1 BMP receptor SMADS Iron transferrin TfR2 BMP6 HFE Fe3+ Hepcidin Hepcidin Proximal intestine Intestinal mucosal epithelium Fe3+ transferrin Fe3+ transferrin Hepcidin mRNA Hepatocyte Tf Tf Haemojuvelin + + + HAMP1 gene + (c) Fig. 22.6.4.3 (b) Hypoxia and iron deficiency increase transcription of TMPRSS6, encoding the transmembrane protease 6 (matriptase-2), which cleaves membrane-bound haemojuvelin (HJV), a bone morphogenetic protein (BMP)-6 coreceptor essential for suppressing hepcidin when iron is in excess; matriptase-2 attenuates the regulation of hepcidin synthesis by erythroferrone. Fig. 22.6.4.3 (c) Molecular control of hepcidin by systemic iron-sensing negative transcriptional control of HAMP1 encoding hepcidin by iron status is mediated by the canonical bone morphogenetic protein (BMP) receptor kinase pathway. This involves principally BMP6, BMP2, and smooth mothers against decapentaplegic (SMAD) protein signalling (SMAD4). Cell-surface BMP ligand interactions are modulated by iron transferrin and multiprotein receptor interactions in hepatocytes which include transferrin receptors 1 and 2 (TFR1 and TFR2), the HFE1 protein, and haemojuvelin, HJV. Mutations in these proteins and ferroportin (FPN1) lead to distinct forms of hereditary haemochromatosis; TMPRSS6 mutations impair BMP/ SMAD signalling and cause iron-refractory iron deficiency anaemia (IRIDA) due to excessive hepcidin action. Fe3+ Fe3+ Fe3+ Fe3+ TFR2 Iron transferrin TFR1 BMP6 BMPR HJV TMPRSS1 HFE Soluble haemojuvelin Ferroportin Ferroportin Hormone release Iron storage macrophage Proximal intestine Intestinal mucosal epithelium ApoTf Fe3+ transferrin Fe3+ transferrin Fe3+ DMTI Ferrireductase Food iron Fe3+ Fe3+ ApoTf Hepcidin Hepcidin DCytB Fe2+

Fe3+ Hepcidin mRNA HAMP1 gene SMAD4 SMAD4 SMADS Pi P Tf Tf Liver parenchymal cell SMADS (b)

section 22 Haematological disorders 5380 thus serves as a coreceptor for BMP signalling in the regulation of hepcidin expression. Activin receptors Hepcidin expression is influenced by BMPs and requires activa- tion of serine/threonine kinases present on the surface of liver parenchymal cells. This control pathway responds to BMP signal- ling that involves activin through the participation of the two type I activin receptor serine kinases and two type II receptor kinases— respectively named ALK2 and ALK3 and the ActRIIA and BMPR2 receptor pairs. Downstream signalling events of the BMP pathway that regu- lates hepcidin expression ultimately induces phosphorylation of intracellular transducing molecules, termed receptor-interacting or R-SMADs—a complicated hybrid term derived from the nomen- clature of conserved homeobox genes that regulate development in Drosophila and vertebrates. Cell-surface binding of the activin- related receptor serine kinases leads to phosphorylation of SMAD4 which is a nuclear transcription factor. SMAD4 forms heteromeric molecular complexes which influence proliferative and/or differ- entiation functions and have been implicated in the regulation of hepcidin. Erythropoietin Erythropoietin is a major determinant of the rate and amount of iron used in the body (see Chapter 22.3.5). It is a secreted glycoprotein that coordinates red cell formation with the availability of oxygen to the tissues.
A negatively regulated feedback system ensures there is sufficient oxygen transported by haemoglobin to meet the demands of aerobic metabolism. Erythropoietin is, in effect, a hormone that controls the production of red cells in the bone marrow in response to changes in tissue oxygenation reflected by the Pao2: it thus affects the demand for iron by the erythron as well as the flux of iron bound to transferrin in and out of the plasma compartment. Understanding the regulation and mode of action of erythropoi- etin is essential to understanding the pathophysiology of secondary haemochromatosis, which is the principal cause of death in patients with the iron-loading anaemias. The stimulus for erythropoietin secretion is reduced capillary par- tial pressure of oxygen in the juxtamedullary renal cortex, which op- erationally reflects the oxygenation status of the entire body. When tissue oxygen tension (Po2) decreases, release of erythropoietin from peritubular interstitial fibroblasts in the adult kidney (or, in the fetus, by hepatic perisinusoidal cells) is increased. Erythropoietin stimulates the differentiation and maturation of red cell precursors in response to hypoxia (and blood loss); the hormone also stimulates expansion of the erythroid progenitor population in the marrow. The effects are coordinated at the level of transcription by the hypoxia-inducible factor-2 (HIF-2α), which induces expression of the erythropoietin gene in the adult kidney (and fetal liver) and is itself regulated by oxygen-sensitive iron enzymes. Erythropoietin represses the default programme of cell death (apoptosis) in erythrocyte progenitors and serves as a driving erythropoietic stimulus which, in cooperation with other growth factors such as IL-3, IL-6, and stem cell factor, ensures erythroid- cell lineage development from stem cells. Under the influence of erythropoietin, erythroid burst-forming units (BFU-Es) and eryth- roid colony-forming units (CFU-Es), proliferate. These rapidly dif- ferentiate to form proerythroblasts, basophilic erythroblasts, and normoblasts; these cells all express erythropoietin receptors until the reticulocyte stage is reached, since reticulocytes lack nuclei and hence all transcriptional activity. Control of erythropoietin expression Erythropoietin expression is driven by binding of the oxygen- labile HIF-α subunits, HIF-1α, HIF-2α, and HIF-3α, to hypoxia-responsive elements in the erythropoietin gene (see Chapter 22.3.5). There is evidence that HIF-2α is the principal factor controlling physiological erythropoietin expression as well as the accompanying adaptations in expression of DMT1 and ferrireductase (DCYTB) in the small

intestine—which support enhanced iron absorption in response to hypoxia and iron deficiency. The preferential use and specificity of HIF-2α for regulation of the principal haematological responses to hypoxia are impaired when the von Hippel–Lindau (VHL) system and other regulatory pathways are activated by disease-related mutations. In the pres- ence of iron, oxygen-dependent degradation domains (containing specific proline residues modified by at least three Fe2+-dependent prolyl-4-hydroxylases that require oxoglutarate) decrease the abundance of the HIF-α subunit. Prolyl hydroxylation of the HIF proteins promotes their binding by the VHL protein complex, which serves as an ubiquitin ligase and promotes their degradation by proteasomes. An additional level of control by molecular oxygen is exerted on HIF-α: its transcriptional activity is modulated by hydroxyl- ation of a specific asparagine residue in the C-terminal transactiva- tion domain. This factor inhibiting HIF (FIH) is also an iron- and 2-oxoglutarate-dependent dioxygenase; its action interferes with recruitment of promiscuous coactivators (CREB-binding protein and p300) to the HIF transcriptional complex. Under conditions of hypoxia, reciprocal inactivation of FIH enhances recruitment of CREB-binding protein and so induces expression of HIF-2α target genes. Expression of the prolyl and asparaginyl hydroxylases is itself con- trolled by the HIFs, so that high abundance of HIF-α under stress is self-limiting and partially declines during sustained periods of hypoxia. These oxygen-sensitive enzymes are Fe2+-dependent and 2- oxoglutarate-dependent oxygenases and they incorporate atomic oxygen into the target protein. Under hypoxic conditions, the hy-droxylation is suppressed, and HIF-α subunits serve only as a basally active transcriptional complex. There is evidence in human cells that HIF-2α is principally responsible for the physiological con- trol of erythropoietin expression—the affinity of the prolyl system for oxygen has been estimated in the healthy arterial Po2 range (c.150 mmHg) but the reciprocal asparaginyl system has a greater oxygen affinity (Po2 c.60 mm). Mode of action of erythropoietin Erythropoietin binds to its homodimeric receptor which is abun- dant on the surface of erythroid progenitors, thereby inducing conformational changes that activate the associated cytoplasmic Janus-activated kinase 2 (JAK2) by autophosphorylation of target

22.6.4 Iron metabolism and its disorders 5381 tyrosine residues. Phosphorylation leads to the engagement of adap- tors and effectors, including the signal transducers and activators of transcription factor-5 (STAT-5). STAT-5 activates diverse molecular targets including the extracellular signal regulated kinases: Jun N- terminal kinases, p38 mitogen-activated protein kinase (MAPK), and phosphoinositide-3 kinase p38. Activation of phosphoinositide- 3 kinase/protein kinase B pathway via JAK2 also leads to phosphor- ylation of the key transcription factor, GATA-1, that is critical for programming erythroid differentiation. Effectors of erythropoietin action STAT5 signalling in the nucleus drives transcriptional expression of several genes, including those encoding: • the antiapoptotic mitochondrial protein, B-cell lymphoma-extra large, which promotes erythroid cell survival by binding free cytochrome C • transferrin receptor 1, which mediates uptake of protein-bound iron in plasma • the iron-responsive element 2 binding protein, that binds to a stem-loop sequence in the 5′-untranslated regions of L-chain fer- ritin (inhibiting translation) and to the 3′-untranslated region of transferrin receptor mRNA (to prevent its degradation). Binding of erythropoietin to its receptor also suppresses the action of death receptors such as the Fas ligand receptor, TNFα, and apop- tosis that is mediated by the TNF-related apoptosis-inducing ligand (TRAIL). Homeostatic control systems in iron metabolism The fastidious relationship between iron supplied from the diet, and that required for erythropoiesis, haem protein biosynthesis, and compensation for insensible losses, depends on the recycling of haemoglobin iron and iron balance physiologically controlled by the regulatory hormone, hepcidin. In health, the magnitude of the

tissue iron stores and rate of red cell formation affect net iron absorption and hepcidin activity is influenced by two principal dy- namic factors: 1. An entity that regulates the pool of stored and circulating iron (the so-called ‘storage’ regulator named by Clement Finch, and representing a hormonal feedback loop). Fundamentally the storage regulator allows the requirements for growth and physiological iron losses to be met. This control system has a limited capacity to influence net acquisition of iron (maximum about 2 mg daily). 2. A further conceptual entity, the ‘erythroid regulator’—a high- capacity control system which serves to support the drive for red cell production in the face of limited supplies of iron irrespective of body iron balance. The erythron accounts for about 80% of plasma iron flux: in iron deficiency related to haemorrhage, daily absorption of dietary iron may rise from less than 1 to 30 to 40 mg but even in iron-overloaded patients with ß-thalassaemia, daily acquisition of 3 to 4 mg iron is often long sustained. Under basal and physiological conditions, both the iron storage and the erythroid regulators influence the expression, secretion, and action of the principal iron hormone, hepcidin. This ensures an adequate supply of iron, while avoiding its gratuitous accumulation of this reactive and potentially toxic metal. Storage regulator The ‘iron-storage regulator’ reflects the overall stimulation hepcidin secretion by hepatocytes in response to adequate concentrations of intracellular iron. A plausible candidate has been postulated to be BMP6, but other members of this protein family may be in-volved. In effect, hepatic expression of hepcidin correlates with nonparenchymal iron stores and iron saturation of transferrin. A feedback loop that incorporates the control of ferroportin func- tion mediated by hepcidin would ensure the retention of intracel- lular iron in storage macrophages. Erythroid regulator The concept of the more elusive ‘erythroid regulator’ (or regulators) reflects the indirect signal that downregulates hepcidin secretion by the liver: this would serve to meet the requirements for iron during erythroid expansion in the bone marrow driven by erythropoietin. When additional iron bound to transferrin for haemoglobin syn- thesis in the marrow is required, immature erythroid cells are postu- lated to use a signal to downregulate hepcidin secretion by the liver, so enhancing entry of iron to the plasma from macrophage storage compartment with supplementation of new incoming iron assimi- lated from the diet in the small intestine. Several molecular candidates for the erythroid regulator have been proposed, including (1) cytokine growth differentiation factor 15 (GDF15), a member and modulator of the TGFβ superfamily; (2) twisted gastrulation (TWSG1), which suppresses hepcidin ex- pression and in human cells has been reported to inhibit secretion of hepcidin indirectly; but perhaps the most plausible candidate is (3) erythroferrone (ERFE, also termed FAM132B), a member of the TNFα family which is released from the spleen and bone marrow of mice within a few hours after experimental removal of blood and immediately precedes the physiological decrease of circulating hepcidin. Administration of erythropoietin induces release of this putative hormonal factor only from bone marrow and spleen. The action of erythroferrone in murine erythroblasts is influ- enced by the systemic erythropoietin and its cognate receptor- mediated signalling through the Jak2/Stat5 pathway. Mice engineered genetically to lack the Fam132b protein, while ap- parently healthy in the steady state, have defects in their red cell maturation. After phlebotomy or administration of erythropoi- etin, Fam132b−/− mice have exaggerated hepcidin expression, so that compensatory acquisition of iron in these conditions of ‘stress erythropoiesis’ is severely impaired. These characteristics strongly support the putative physiological role for this protein as the ‘erythroid regulator’ in mammalian iron homeostasis. If this is, as expected, the case, the protein represents a factor of major biomed-ical significance, especially for therapeutic exploitation. Other factors Erythroblasts also sense the availability of iron by circulating iron bound to plasma transferrin via the second transferrin receptor (TFR2) that is mutated in rare forms of haemochromatosis (HFE3) (Chapter 12.7.1). This

iron signal appears to attenuate the sensi- tivity of the erythroid precursors to erythropoietin. By inhibiting

section 22 Haematological disorders 5382 transcription of hepcidin in the liver, erythroferrone enhances the activity of ferroportin and so promotes release of iron from the macrophage storage compartment and net absorption of iron in the small intestine. Erythroferrone mediates some actions of erythro- poietin, which allows the body to compensate for blood loss as well as failure of adequate oxygen delivery to the peripheral tissues in- duced by hypoxia. This adaptive response is a physiological adaptation to iron defi- ciency, after blood loss and under hypoxic conditions; red cell for- mation in the bone marrow influences iron homeostasis. The release of erythroferrone from erythroblasts would reduce hepcidin release by the liver and so increase acquisition of iron for erythropoiesis. The nature of the erythroid regulator of iron homeostasis has been unclear but the properties of erythroferrone render it an attractive candidate to explain the pathological and inappropriate increase in the acquisition of iron in conditions such as ß-thalassaemia and other iron-loading anaemias. The common factor is ineffective erythropoiesis and erythroid hyperplasia driven by the action of erythropoietin. In operational terms, ineffective erythropoiesis disrupts the regulatory mechanisms that maintain systemic iron balance: the pathological marrow due to erythroid expansion driven in part by erythropoietin and tissue hypoxia overcomes the ‘storage regu- lator’ and causes unregulated net absorption and delivery of iron in the face of adequate stores. Hepcidin is inappropriately and mark- edly suppressed if there is no prior burden of iron from red cell transfusions in dyserythropoietic anaemias such as ß-thalassaemia intermedia, absorption of iron can enhanced up to 10-fold, which is grossly in excess of requirements for erythropoiesis; iron toxicity and secondary haemochromatosis are the result. Finally, in mouse models at least, increasing concentrations of hepcidin or transferrin may alleviate anaemia and dyserythropoiesis and restrict iron up- take by erythroblasts. Iron deficiency General aspects Iron deficiency is a major challenge for global health and the most common cause of anaemia worldwide (see also Chapter 22.6.3). The deficiency reflects requirements for iron that exceed that obtained from the diet (often in a diet that is otherwise adequate), the effects of chronic blood loss, and malabsorption. Contributory causes include the loss of iron by excess exfoliation of cells, as in inflammatory diseases of the gastrointestinal tract, and (more rarely) urinary iron loss due to intravascular haemolysis associ- ated with haemoglobinuria. The availability of iron modulates erythropoiesis: iron restriction reduces the synthesis of haem and α-globin chains in ß-thalassaemia. About 30% of the world’s population (estimated by the World Health Organization to be 7.6 billion at the end of 2017)—more than 2.25 billion people—are anaemic and about 50% will have iron deficiency anaemia. Even in rich countries, such as the United States of America and those in Europe, up to 20% of menstruating women have signs of iron deficiency. In children and young adults, there is a frequency of between 5 and 10% of iron deficiency anaemia, particularly in deprived socioeconomic groups. Iron deficiency anaemia impairs well-being, cognitive and phys- ical performance, as well as working capacity, and to a considerable extent these factors contribute to poverty. Administration of iron during pregnancy improves maternal, neonatal, infant, and even long-term outcomes in children. Moreover, there is evidence sug- gesting that in children, judicious iron supplementation may be able to improve cognitive, psychomotor, and physical development. Many epidemiological studies have been based on the erroneous attribution of microcytic anaemia to iron deficiency. Diverse condi- tions, including the anaemia of chronic disorders and genetic dis- eases with haemoglobinopathies such as β thalassaemia trait, are characterized by hypochromic or microcytic red cell indices, and iron deficiency is but one cause. Population surveys based on the

detection of iron-deficient erythropoiesis, especially those using de- termination of free red cell zinc protoporphyrin concentrations by fluorimetry, enhance the detection of true iron deficiency anaemia; determinations of serum ferritin concentrations can also help to discriminate between the anaemia of chronic disease and true iron deficiency. Causes of iron deficiency Nutritional factors Iron deficiency anaemia is often attributed to an iron-poor diet, but in the absence of significant blood loss or intestinal parasites including hookworm, even the most iron-poor diets rarely cause iron deficiency anaemia, except in growing children. The amount of iron required to repair obligatory losses is very small, so that at least 90% of the iron required for de novo haemoglobin formation in erythropoiesis is retrieved from senescent erythrocytes broken down by the mononuclear phagocyte system. Furthermore, once iron deficiency develops, striking adaptive changes occur in the absorptive mechanism in the upper small in- testine so that assimilation of bioavailable iron in the diet is greatly enhanced. Iron deficiency, and the response to the removal of a unit of blood, may increase the overall absorptive efficiency of the intestine for iron up to 10-fold—thus greatly enhancing the in- corporation of dietary iron. Iron deficiency is associated with the recruitment of a greater length of mucosal surface for participation in the absorption of luminal iron in the upper small intestine. In experimental animals with iron deficiency, mucosal expression of DMT1 on the brush-border membrane of the intestinal epithelium is induced. Iron deficiency is also associated with enhanced intes- tinal expression of mucosal ferrireductase activity, thus increasing absorptive capacity for inorganic ferric iron released by digestion of food. The extent to which the absorptive pathway for organic iron complexed as haem is modified by iron deficiency and/or anaemia is less well studied. Composition of foods Alcoholic beverages may be a significant source of iron, and the absorption of haem iron present in red meat, poultry, and fish is usually between 15 and 35%. Between 2 and 20% of nonhaem iron present in fruit and vegetable sources is absorbed. Natural enhan- cers of iron absorption such as ascorbic acid, which maintains

 ferrous iron in its reduced form in the intestinal lumen, promote direct uptake by DMT1. Fructose and other organic compounds of low molecular weight also form soluble ferrous complexes after release from nonhaem sources in food. Healthy individuals in rich countries, ingest between about 10 and 15 mg of iron in the so- called Western diet, daily. Adult men with normal iron stores ab- sorb approximately 2% of the nonhaem iron ingested, whereas men with iron deficiency can absorb more than 20% of iron from this source in the diet; the comparable figures for haem iron are 26 and 47%, respectively. Components that interfere with the availability of food iron Many compounds present in the diet also inhibit or impede the absorption of iron released by digestion in the lumen. These com- pounds include tannin, especially present in tea; phytates, present in bran and nuts; dietary fibre; and other inhibitory factors including drugs such as tetracycline antimicrobials, proton pump inhibitors, and alkalis. Some vegetarians of Asian origin ingest large amounts of phosphate and phytates, which inhibit the absorption of iron pre- sent in diets that may contain up to 30 mg of assayable total iron each day. Another example is spinach—although very rich in iron, much of this is not readily available. When ingested, spinach induces formation of odiferous black stools: the passage of unabsorbed iron through the small intestine and its delivery to the colon leads to the formation of insoluble ferrous sulphide complexes due to the pres- ence of sulphur-reducing bacteria. Loss of iron Menstrual losses Women in the reproductive age group lose iron regularly as a re- sult of menstrual bleeding. The recommended daily allowance for women is higher than in all other groups: this must supply sufficient bioavailable iron to meet the increased needs. The average require- ment for healthy menstruating women is approximately 1.4 mg of iron daily to replace

losses, compared with men, who lose about 0.8 to 0.9 mg of iron per day. Menorrhagia is a frequent, and a frequently invoked cause of iron deficiency due to excess losses of iron compared with iron intake in women. In the United Kingdom, 5% of women aged 30 to 49 years consult their general practitioners each year with menorrhagia and similar figures apply to other developed countries. Established men- orrhagia (defined as the loss of more than 80 ml of blood each normal cycle) causes anaemia in two-thirds of women. Benign leiomyomas ('fibroids') occur in about 10% of women with menorrhagia overall, but in 40% of those with severe menorrhagia (>200 ml per cycle). Iron deficiency anaemia occurs in two-thirds of women with proven menorrhagia. Quantitative determination of menstrual loss is often imprac- tical in everyday clinical work; to resolve this matter in practice, Duckitt has suggested that menorrhagia may be defined oper- ationally as a complaint of regular excessive menstrual blood loss that interferes with the physical, emotional, social, and ma- terial quality of life. Menorrhagia may occur alone or with other symptoms. It is important to be cautious if other potential causes of iron deficiency anaemia are to be sought since about half of women having a hysterectomy for menorrhagia are found to have an apparently normal uterus; however, this does not necessarily imply that the procedure was not warranted. A prevailing view is that 'idiopathic ovulatory menorrhagia' reflects disordered endometrial synthesis of prostaglandins—this may account for the beneficial effects of nonsteroidal anti-inflammatory drugs in the management of menorrhagia (with or without symptoms of dysmenorrhoea). Although the rates of hysterectomy are decreasing, about 20% of British women, and 35% of those in North America have under- gone the procedure before the age of 60 years; in at least half of these women, menorrhagia is the principal complaint. Pregnancy Pregnancy is associated with iron deficiency, particularly during the mid and last trimester, when growth of the fetus is rapid. Twin preg- nancies and frequent childbirth, especially in women of low socio-economic groups, often cause iron deficiency anaemia. Although anaemia is an important influence on maternal health and resili- ence, a large study has reported no reliable association between maternal anaemia and the complications of pregnancy, including preterm labour. Pregnancy itself is associated with the development of adap- tive responses in the intestine and iron transport proteins that enhance the avidity of the gastrointestinal tract for bioavailable food iron. Clearly, socioeconomic and sociopolitical consider- ations are likely to influence the population occurrence of iron deficiency in women of the reproductive age group, particularly since the investment of about 1 to 1.5 g of iron occurs with each pregnancy carried to term. This estimate includes blood loss asso- ciated with the birth and the investment of iron placed in human milk, which contains up to 0.5 mg/litre of iron bound to a whey protein, lactoferrin. Intestinal parasites—hookworm More than 400 million people are estimated to suffer from hook- worm infestation. The helminth causes ill-health and misery associ- ated with fatigue and inefficiency across the globe. The two common hookworms of humans are Ancylostoma duodenale and Necator americanus. These helminths attach themselves to the lining of the small intestine by their buccal capsules and cause chronic blood loss by sucking blood from the intestinal villi. Hookworm is widely distributed in Southern Europe, Africa, the Middle East, the Indian subcontinent, East Asia, and the New World, especially Brazil and the Southern United States of America. The infestation may be light, so that iron loss is not sufficient to cause frank iron deficiency. In hookworm disease, involving Old World and New World hookworms, heavy infestation occurs as a result of repeated exposure of the skin to soil contaminated by inva- sive hookworm larvae. Mucosal immunity may also be reduced in the susceptible host. Although it is not known exactly what hook- worms remove from human blood, intact red cells transit through the nematode gut: each Anclyostoma worm induces the loss of up to 300 µl of blood daily, whereas each Nectator worm causes the loss of up to 50 µl. Hookworm

anaemia is often regarded as a disease of poor farmers who have only poor sanitation; but hookworm infestation

section 22 Haematological disorders 5384 has a very wide distribution with long-standing industrial conno- tations beyond subsistence agriculture. Hookworm is well known as an occupational disease in miners, particularly deep miners of metal ore rather than colliers. The disease is widely reported in Swiss tunnel workers and North as well as South Europe, California, and Queensland. Notorious as ‘miners’ anaemia’ and with typically florid ‘bunches’ (cutaneous larva migrans) in those who worked in the deep copper/tin mines in Cornwall, the link between occupational skin exposure to hot and humid, mineral-rich soils and wholly inad- equate access to sanitation has been established since the early years of the 20th century. Clearly, induction of frank anaemia in hookworm infestation will be dependent on the iron content of the diet, the extent of tissue iron stores, and the duration and intensity of the mucosal helminth infestation itself. The heaviest infections usually affect rural workers in agricultural communities or miners where re- peated exposure occurs in isolated locations and where crops or minerals are harvested under conditions of poor sanitation. The iron deficiency anaemia of hookworm disease may be difficult to diagnose when the mucosal inflammation that accompanies heavy infestation is associated with reduction in serum proteins such as albumin and transferrin; this, combined with an acute phase re- sponse, may at first lead to a mistaken diagnosis of the anaemia of chronic disorders. Hookworm infestation is an under-recognized cause of maternal anaemia, and this has precluded the use of anthel- mintic treatment in health provision for pregnant women. In sub-Saharan Africa, it has been estimated that nearly 40 mil- lion women of reproductive age are infected with hookworm; of these, about 7 million were pregnant in 2005. As expected, increasing intensity of infestation was associated with worsening anaemia in pregnant women living in poor countries. Given the number of pregnant women at risk of preventable hookworm- related anaemia, there is an urgent unmet need to determine the benefits of anthelmintic treatment and to develop safe preventive methods against this infestation. These measures will then need effective introduction—without the fear of damaging the fetus or mature offspring—to the benefit of the rural poor who are most at risk. Since up to two-thirds of the haemoglobin iron released by the worms can be reabsorbed in the intestine, significant anaemia oc- curs only when there is a heavy parasite load. Patients with hook- worm disease experience fatigue, dyspnoea, palpitations, and mental changes—including pica related to severe iron deficiency. Nonspecific abdominal pain occurs and radiographic examin- ation of the intestine or endoscopy may reveal duodenitis with a punctate inflammation associated with partial villus atrophy of the duodenojejunal mucosa. Oedema may result from cardiac failure in severe cases, but is more frequently due to hypoalbuminaemia caused by parasite-related protein-losing enteropathy. Hookworm disease may be associated with other opportunistic helminth infec- tions such as ascariasis or strongyloidiasis (the latter with a risk of the fatal hyperinfection syndrome). From many aspects, hookworm infestation contributes to a vicious cycle of poverty due to incapacity for work as result of illness and the preferential use of poor labour in rural environments or in deep mining, where the risk of hookworm invasion is greatest. Intrinsic gastrointestinal disease (see Section 15) The gastrointestinal tract is a key and often cryptic source of blood loss which should always be considered in patients with iron defi- ciency anaemia. Ulcerating lesions of the small and large intestine— including cancers—are often responsible for iron deficiency anaemia. Chronic intermittent bleeding can also arise from unusual sources such as Meckel’s diverticula, strictures, angiodysplastic le- sions, hamartomas, and other benign but ulcerating tumours, such as leiomyomas. Gastric ulcers cause chronic intermittent bleeding, but

duodenal ulcers rarely cause chronic gastrointestinal blood loss; these typically cause episodes of acute bleeding. Oesophageal ulceration and inflammatory lesions cause iron deficiency anaemia, but caution is needed in attributing blood loss sufficient to cause iron deficiency to such a source, especially hiatal hernia, unless other potential sites of bleeding have been excluded. Unusual sources of gastrointestinal bleeding include multiple telangiectatic lesions of Osler–Rendu–Weber disease (hereditary haemorrhagic telangiectasia)—in which bleeding may occur any- where from the nasal or oropharynx down to the stomach and upper intestine. The connective tissue disease, pseudoxanthoma elasticum, is also associated with recurrent, often severe, upper gastrointestinal haemorrhage. The blue bleb naevus syndrome, Peutz–Jeghers syndrome, and other hereditary gut polyposes are rare causes of chronic gastrointestinal bleeding. Inflammatory dis- ease of the lower small intestine and colon such as Crohn disease and ulcerative colitis, usually associated with chronic intestinal blood loss, may present with an abdominal history in which iron deficiency anaemia is prominent. Miscellaneous causes of blood loss Very occasionally, iron deficiency anaemia due to self-bleeding may have to be considered: blood may be removed from almost any site but bizarre tactics may be adopted to conceal the process, thus re- quiring considerable ingenuity, and often intensive detective work, to identify the source. Bronchial or pulmonary blood loss The striking appearance of expectorated blood means that the iron deficiency anaemia associ- ated with frank haemoptysis will demand little diagnostic skill, but disease-related haemorrhages sufficient to induce chronic anaemia are very rare. In contrast, recurrent intra-alveolar pulmonary haem- orrhage may be massive but is often cryptic, even though it may cause unexplained illness and severe anaemia—as in Goodpasture syndrome. Urinary tract blood loss With respect to haematuria, it should be remembered that not all red discoloration of urine is due to red cells: where there is doubt, haemoglobinuria and myoglobinuria should be considered. In rare circumstances, the differential diag- nosis should include other pigments such as those derived from beetroot, or the oxidized pyrrole, porphobilin, derived from the porphyrin precursor, porphobilinogen. Occasionally, alkapton, the oxidized product of homogentisic acid in alkaptonuria may take on a red hue rather than black and give rise to confusion—as may the presence of anthocyanins and phenolphthalein (the latter usually in nonfresh, alkaline urine), in individuals who use these substances as purgatives.

22.6.4 Iron metabolism and its disorders 5385 Haemolysis Iron can be lost in the urine through the kidney in conditions where chronic intravascular haemolysis occurs, and this may be sufficient to induce iron deficiency in the absence of overt changes in urine colour. Often the loss of iron is chronic and oc- curs through the exfoliation of haemosiderin-rich tubular epithe- lial cells into the urine; under these circumstances the urine is not discoloured. Patients with haemolysis due to prosthetic cardiac valve malfunc- tion, paraprosthetic leaks, or valvular defects causing shear stress or other mechanical effects may have frank haemoglobinuria and methaemoglobinuria. Testing the urine for free haem and protein by stick urinalysis and examination of the blood film for characteristic red cell fragments, strongly suggests the diagnosis. Haemoglobinuria In paroxysmal nocturnal haemoglobinuria, chronic intravascular haemolysis causes sustained urinary iron loss with or without visible haemoglobinuria. In these circum- stances, free haemoglobin is released, which quickly saturates the capacity of the plasma proteins haptoglobin and haem-binding protein, haemopexin; free haemoglobin thus appears in the glom- erular filtrate from which it is endocytosed by proximal tubular cells and degraded. After degradation to haemosiderin, iron is lost in the urine as the iron-loaded epithelium is exfoliated; consequen- tial haemosiderinuria is readily detected by diagnostic microscopy of the centrifuged urine deposit after reaction with Perls’

reagent (Prussian blue granular staining). While haemosiderinuria is a diag- nostic sign, it does not immediately follow a bout of haemolysis, ap- pearing only after 2 to 3 days, although it may persist for several weeks after a haemolytic attack. In march haemoglobinuria, mechanical injury to erythrocytes in the circulation of the feet may induce similar features with conse- quential iron deficiency, for example, in service recruits and high- performance athletes. In recent years, an often misdiagnosed but closely related syndrome, aptly termed ‘foot-strike haemolysis’ by American physicians, has been identified in marathon runners and regular ‘joggers’. This condition, which occurs in both sexes but par- ticularly in young athletic women, is characterized by signs of iron deficiency with hypochromia, polychromasia, and macrocytosis. These changes are due to accelerated erythropoiesis as well as red cell fragmentation (visible on blood film examination). The true source of iron loss—through the renal glomeruli—often escapes detection, unless haemosiderinuria is specifically sought at times close to the period of exercise. Malabsorption of iron The inability to release and absorb adequate amounts of iron from the diet is an important but frequently overlooked cause or con- tributor to iron deficiency. Diseases of the stomach, duodenum, and upper jejunum may be responsible for the malabsorption of food iron, but simple radioactive tracer measurements may fail to iden- tify the absorptive defect. On the other hand, properly conducted radioactive food labelling studies show that there is malabsorption of nonhaem and haem food iron after gastric bypass surgery or in- testinal resection. Acquired defects of the intestinal mucosa other than inflam- matory disorders may contribute to malabsorption of therapeutic iron. Young children with iron deficiency anaemia refractory to oral therapy that was corrected by parenteral supplementation have been reported. Careful investigation in some has revealed an absorptive defect for iron which was corrected itself by systemic iron supple- mentation, raising the possibility that severe iron deficiency itself prejudices the ability of the mucosal epithelium in the upper small intestine to carry out its normal absorptive function. However, no further investigations to identify the nature of this acquired meta- bolic defect are available. Rarely, iron deficiency may result from inflammatory disease of the upper intestine that causes malabsorption. Coeliac disease in in- fants and adults may be responsible, and the iron deficiency is often combined with deficiency of folic acid. Sometimes large pharma- cological doses of iron with or without folic acid may overcome the anaemia caused by coeliac disease, but unless a strict gluten-free diet is adhered to after the iron supplements cease, the anaemia re- curs. Although malabsorption of food iron contributes to the iron deficiency associated with coeliac disease, loss of iron exacerbates the effects of malabsorption, coexisting iron loss being related to increased epithelial exfoliation with crypt hyperplasia and at times bleeding due to local ulceration. Malabsorption of food iron due to abnormal motility and maldigestion associated with upper gastrointestinal surgery is com- pounded by anacidity caused by gastritis or acid-suppressing agents, which—if administered for long periods—lead to gastric atrophy. Long-term administration of alkalis and certain iron-chelating drugs such as the tetracycline antimicrobials can also impair iron absorption. Dietary factors, such as ingestion of food containing excess phytate compared with bioavailable inorganic iron, can crit- ically reduce gastrointestinal absorption of iron. Bariatric surgery which leads to diminished gastric acid secretion and bypasses the duodenum is complicated by iron deficiency anaemia; prophylactic supplementation is thus recommended after this procedure, par- ticularly in menstruating women. Geophagia Geophagia—the deliberate consumption of non-nutritive earth, soil, chalk, or clay—has an under-recognized association with iron deficiency. The behaviour occurs principally in certain rural or poor urban populations and the relationship with malabsorption of iron and iron deficiency is complex. The condition is an extreme form of pica and has a cultural history extending from ancient times in the records of several

Roman physicians but also in the palaeontology of Africa. Geophagia may be restricted to individuals or be prac- tised by family or community groups. It is a classical feature of iron deficiency with pregnancy in which perverted taste perceptions are often present (pica). In adults, the condition has been associated strongly with poverty, and while it occurs in individuals it has also attracted attention as a cult behaviour most often affecting women, who may as a result regularly ingest large quantities of calcium, so- dium, or potassium salts, often contaminated with lead, together with silicates present in earth and clay. Colonial African doctors in the 19th century noted wide- spread geophagia in slaves. Here, geophagia was linked to poor health and declining work output; the African explorer, David Livingstone, described earth-eating among slaves in Zanzibar but apparently rejected poverty as the explanation. Geophagia

section 22 Haematological disorders 5386 was reported in African slaves transported to Brazil as well as southern parts of North America, where it was known by the term ‘cachexia Africana’. The disorder occurs contemporaneously in Georgia and Louisiana, sub-Saharan as well as urban South Africa, but has been reported in individuals and groups at various times from nearly every part of the world, including India and the Far East. In some individuals, famine or malnutrition is present, but geophagia may accompany group religious practices or reflect a form of ritualistic purification. Geophagia occurs most typically in black women belonging to African American communities in the south-eastern United States of America and in rural as well as urban areas in South Africa and Nigeria. The behaviour is prevalent among pregnant women in Africa, more than half of whom may report the practice: re- cently, a high frequency of the disorder has been noted in pregnant Hispanic women in Mexico and the south-western United States of America. Cultural perspective As a cult behaviour, geophagia is often considered to represent a psychiatric state, but this view is less easy to sustain in whole communities where it resembles a cul- tural practice that occurs within the context of poverty all over the world. The phenomenon noted historically in slaves subjected to cruel and inhuman mistreatment often seems to have persisted in their disadvantaged latter-day descendants. Reports after the 2008 earthquake on Haiti and the consequential socioeconomic disaster, indicated an outbreak of geophagia with widespread consumption of ‘bon bons de terres’—biscuits made from soil, vegetable oil, and crude salt. In summary, whatever the psycho- pathological causes, the association of geophagia with starvation and deprivation—as well as postcolonial failures of international politics—is striking. The complex behaviours and at times frank psychopathology of geophagia is repeatedly emphasized in reports of compulsive pica and earth consumption by disturbed patients residing in long- term psychiatric institutions, often with bizarre consequences. Despite apparent associations with migration, slavery, famine, poverty, other pica-like behaviours, and iron deficiency in many societies, the cultural, psychiatric, and nutritional factors that contribute to geophagia are neither constant by association nor inevitably driven by single psychosocial or pathophysiological mechanisms. Effects of geophagia The main clinical consequences of geophagia relate to an entrained pica behaviour aggravated by the chronic neuropsychiatric effects of iron deficiency; there is symptomatic an- aemia and often weight loss. In South Africa the condition occurs in women who purchase earth which may be contaminated with appre- ciable quantities of lead. Other toxic minerals such as arsenic can be accidentally coingested, especially in China and parts of the Indian subcontinent. Ingestion of large quantities of indigestible material may cause intestinal bloating or, occasionally, life-threatening ob- struction and perforation. A more frequent association is a parasitic helminth infestation such as ascariasis, and the dog tapeworm, toxocara. Demographically and geographically in the New World, the condition may be asso- ciated with endemic hookworm anaemia, typically Necator

ameri- canus but also Strongyloides spp., parasites that are not acquired by oral ingestion but by environmental exposure and skin invasion. In the Old World, Africa, and Europe, geophagia is highly associ- ated with iron deficiency and geohelminth infections, most notably with Ascaris lumbricoides and Trichuris trichiura. Children who pursue geophagia acquire these infections and also the dog parasite, Toxocara canis. A notable feature, revealed by a longitudinal study in more than 800 pregnant women from western Kenya, was the high frequency of geohelminth reinfection in the months after effective anthelmintic treatment at term. Reinfection was strongly associated with the practice of geophagia. Genetic causes of iron deficiency anaemia While illustrative of the importance of molecular components in- volved in iron metabolism, so far as can be determined, mono- genic causes of iron deficiency are exceptionally rare. Mutations affecting serum transferrin, the proteins involved in transferrin receptor cycle, enzymes that bring about the formation of the first committed precursor of haem biosynthesis (5-aminolaevulinic acid), and proteins of the iron sulphur cluster may induce hypochromic anaemia, usually accompanied by iron overload. Hepcidin plays an indirect role in erythropoiesis by controlling the availability of iron in the plasma. Inappropriate elevation of hepcidin concentrations occur in the rare genetic iron-refractory iron deficiency anaemia (IRIDA) and in the anaemia of chronic disease. Iron-refractory iron deficiency anaemia Original studies in members of a Sardinian family with microcytic anaemia due to defective iron absorption and utilization identified the molecular basis of an apparently ultra-rare condition that may in fact account for many instances of hitherto undiagnosed iron deficiency anaemia unresponsive to oral iron and with limited re- sponsiveness to parenteral iron. At first thought to be inherited as a recessive trait, it is now clear that heterozygotes can also be affected. After excluding the involvement of known genes implicated in iron metabolism, a genome-wide search identified a locus encompassing the matriptase-2 gene, TMPRSS6 (also known as transmembrane protease, serine 6), which is located on human chromosome 22q. In the original pedigree, affected patients were found to harbour a homozygous splicing mutation which is pre- dicted partially to inactivate protease function. Plasma and urinary hepcidin concentrations were later shown to be inappropriately ele- vated. The corresponding murine gene (Tmprss6) has been shown to be an essential component of a pathway that is sensitive to iron lack and suppresses the release of hepcidin. The type II transmembrane serine protease, TMPRSS6 or matriptase-2, cleaves HJV present on the plasma membrane, thus attenuating its action on the hepcidin promoter mediated by BMP6 signalling through the transducer, SMAD4 in the liver. BMP6 and related ligands bind to the BMP receptor and coreceptor, membrane HJV, to induce phosphorylation of the SMAD proteins and formation of heteromeric complexes with the common mediator SMAD4. The complexes stimulate transcription of the HAMP gene to induce synthesis of hepcidin. Matriptase- 2 activity further downregulates hepcidin transcription because the release of soluble HJV serves as an antagonist of the bone

 morphogenetic pathway by competing with the membrane form for BMP ligands. In summary, impaired TMPRSS6 (matriptase-2) activity en- hances hepcidin biosynthesis with elevated plasma concentra- tions of this master hormonal regulator: the outcome is refractory microcytic anaemia and persistent functional iron deficiency due to suppressed iron absorption adversely complicated by im- paired release of iron from the exiguous stores in macrophage compartment. This genetic disease is richly informative for under- standing iron physiology and in particular opens up a potential avenue for therapeutic development in secondary iron storage disease (Box 22.6.4.2). Clinical manifestations of iron deficiency Symptoms of iron deficiency include fatigue, pallor, sore tongue, palpitations, irritability, and little-

recognized mental changes, such as pica. Iron deficiency is a notable cause of the restless legs syn- drome (Willis–Ekbom syndrome), sometimes known as delusional parasitosis. Iron deficiency induces behavioural changes in experimental ani- mals, but lethargy in humans is often the only symptom apart from pica, which includes craving for soils and the ingestion of silica- rich earths, sometimes viewed as a cult practice, geophagia. Other variant cravings are sufficiently characteristic to be dignified by spe- cial terms, such as ryzophagia (rice), amylophagia (other starches, including potato), lithophagia (stones or gravel), cautopyreiophagia (burned matches), and trichophagia (hair); but milk, salty and sour foods, sweets, and dates are all recorded from different regions and probably reflect specific cultural familiarities. Pagophagia (craving for ice or cold drinks), noted in Hippocratic times, is strongly linked to iron deficiency, especially in younger subjects. The pain associ- ated with biting into and chewing solid ice, sometimes leading to fractured teeth, only emphasizes the compelling nature of this capricious behavioural fixation. Pagophagia has been reported as a manifestation of iron deficiency 2 or 3 years after Roux-en-Y gastro- intestinal bypass surgery for pathological obesity management; it responds rapidly to parenteral iron replenishment. Iron deficiency combined with the abnormal taste preferences may account for the bizarre food craving that constitutes part of the folklore of preg- nancy in many cultures, and in women from economically deprived, as well as rich nations. There may be a complaint of dysphagia associated with the de- velopment of an oesophageal web (Patterson–Brown–Kelly or Plummer–Vinson syndrome), which usually occurs in elderly or middle-aged women with chronic iron deficiency. Clinical signs of severe iron deficiency include pallor and nonerythropoietic manifestations: angular cheilosis, atrophic glossitis, and dystrophy of the nails with longitudinal ridging or koilonychia (which has a predilection for the nails of elderly women with long-standing iron deficiency). Moderate hair loss may also be a feature of integumental iron deficiency. Severe iron deficiency may occasionally coexist with splenomegaly, which resolves after iron treatment. Signs of underlying disease include peripheral oedema (hypoalbuminaemia associated with massive hookworm infec- tion) and oronasal and palatal telangiectasia associated with Osler–Rendu–Weber disease (hereditary haemorrhagic telangi- ectasia). Papilloedema has also been reported and may reverse after treatment of the anaemia. Finally, care should be taken to search for peripheral signs of systemic diseases such pseudoxanthoma elasticum, hereditary haemorrhagic telangiectasia, and Peutz– Jeghers syndrome: these hereditary causes of bleeding are readily missed but their identification has material significance in clinical management. Diagnosis of iron deficiency Routine haematological parameters will reveal microcytic an- aemia, usually in association with an unequivocal reduction in serum transferrin saturation (<16%) and a reduced serum ferritin concentration (<12 µg/litre) (Box 22.6.4.1). Blood film will con- firm microcytosis but may also show increased variation in red cell size and shape often with atypical forms such as ‘target’ forms and ‘pencil’-shaped cells. The absence of these features and of an acute phase reactive response may suggest dyserythropoietic or sideroblastic anaemia or thalassaemia trait. ß-thalassaemia trait is also associated with a raised HbA2 while α-thalassaemia trait is not. Patients with iron deficiency consequent upon intravas- cular haemolysis (e.g. related to mechanical heart valves or march haemoglobinuria) also show red cell fragmentation and polychromasia with macrocytosis due to compensatory erythro- poiesis. Paroxysmal nocturnal haemoglobinuria similarly induces iron deficiency with net renal excretion of iron also due to urinary shedding of haemosiderin-loaded tubular epithelial cells. Lead poisoning may be associated with iron-deficient indices, with or without full-blown sideroblastic changes. A bone marrow aspirate stained with Perls’ reagent for iron in marrow macrophages will rapidly confirm reduced or absent stainable iron in the storage compartment: the presence of ring sideroblasts, dyserythropoietic

features, and/or megaloblastic change would also guide differen- tial diagnosis. Box 22.6.4.2 Modulators of erythropoiesis to treat iron-loading anaemias • Inhibitors of Janus-activated kinase-2 (JAK2) signalling:

— Targets erythropoietin, the master regulator of erythropoiesis.

— Binding of erythropoietin to its receptor activates the cytoplasmic kinase JAK2 and signal transducers and activators of transcription to effect hypoxic responses.

— Clinical JAK2 inhibitors include ruxolitinib, which is used clinically for myeloproliferative diseases. • Attenuating TGFβ signalling with ligand traps:

— Activin signalling involves the transforming growth factor-β (TGFβ) family and other components of erythropoietin-induced erythroid proliferation, such as GDF11, which prevents differentiation. Excess activin signalling is a feature of dyserythropoiesis.

— Sotatercept and ACE-536 are undergoing trials to reduce activin I and II signalling thereby inducing apoptosis of progenitors and stimulate erythroid differentiation. • Hepcidin augmentation:

— Use of hepcidin mimetics or ‘minihepcidins’ but small peptides have a short half-life.

— Transgenic overexpression of hepcidin—challenging to obtain appropriate regulation.

— Inhibition or repression of TMPRSS6, the upstream regulator of hepcidin expression.

section 22 Haematological disorders 5388 The presence of immunoreactive serum transferrin receptors may provide additional evidence in favour of iron deficiency anaemia, but because an increased concentration of these receptors occurs in several marrow disorders and the enzyme-linked immunosorbent assay tests are relatively expensive, the role of this analyte in the routine diagnosis of iron deficiency is not yet established. Red cell zinc protoporphyrin concentrations greater than 35 µg/dl of whole blood are usually observed in patients with iron deficiency; values greater than 100 µg/dl are generally associated with lead toxicity. Extremely high values may indicate the presence of erythropoi- etic protoporphyria or lead poisoning in the latter, free, rather than zinc protoporphyrin IX accumulates. Modest elevations in erythro- cyte protoporphyrin can be observed in patients with haemolytic anaemias, sideroblastic anaemia, and occasionally, the anaemia of chronic disorders. Investigation of iron deficiency The identification of iron deficiency anaemia must be regarded as an illness description rather than a satisfactory diagnosis for any pa- tient in its own right: management should always include a serious attempt to determine its root cause. Common errors occur when iron deficiency is ascribed to the presence of other facile causes such as ‘poor diet’ or menorrhagia—ostensibly factors that are challen- ging to quantify. All too often, mild oesophagitis or gastritis re- ported at endoscopy placates the incurious investigator when the underlying cause is bleeding from a coincidental source such as a cryptic gastrointestinal cancer for which a diligent search is often required. Malabsorption of iron as a result of, for example, coeliac disease, or chronic urinary loss of iron consequent upon intravas- cular haemolysis, are also important causes that are frequently not even considered until late into the illness. History A full evaluation of the patient with iron deficiency should include a detailed and systematic dietary history, including the consump- tion of drugs, such as aspirin and nonsteroidal

anti-inflammatory agents which may be responsible for gastrointestinal bleeding. Additional gastrointestinal symptoms should be explored (e.g. change in bowel habit), together with other evidence of blood loss (e.g. presence of melaena or rectal bleeding). Enquiry should be made to quantify the extent of menstrual loss, if the bleeding has been ascribed, as is often the case, to menorrhagia in women of reproductive age. Attention should be paid to the family history and a travel history to consider causes such as hereditary haemorrhagic telangiectasia, Peutz–Jeghers syndrome, familial polyposis coli, or hookworm dis- ease. Patients with malabsorption of iron often have accompanying nutritional deficiencies; coeliac disease has several well-known asso- ciations with autoimmune disorders such as type 1 diabetes mellitus and is most common but not at all exclusive to patients with Irish ancestry. Examination Clinical examination should extend from detailed enquiry about previous gastrointestinal disease or surgery to an examination for visceral enlargement, abdominal lymphadenopathy, splenomegaly, masses, and other features suggestive of intra-abdominal pathology such as portal hypertension and abdominal cancer. Hereditary haemorrhagic telangiectasia, pseudoxanthoma elasticum, or Peutz– Jeghers syndrome may be suspected in the presence of subtle or lo- calized cutaneous, oronasal, or palatal lesions. Investigations for source(s) of bleeding The presence of iron deficiency anaemia demands a convincing explanation and a robust causal diagnosis: while malabsorption may be neglected, occult haemorrhage is usually the most im- portant to identify. The gastrointestinal system is the most fre- quent source of bleeding but can present a laborious challenge for diagnostic pursuit. Patients over the age of 60 years should be evaluated promptly, with upper and lower gastrointestinal endos- copy carried out as soon as reasonably possible. Iron deficiency anaemia in premenopausal women is often due to menorrhagia, dietary deficiency, hiatal hernia, or a combination of these factors, but endoscopy should not be neglected in men and women under 60 years in the presence of features such as weight loss or change in bowel habit. With patients in whom the cause of the iron deficiency is not apparent, intensive studies may be needed to confirm the pres- ence and identify the source of gastrointestinal bleeding, including detection of occult blood in several consecutive samples of stool. Sophisticated endoscopic and radiographic studies of the gastro- intestinal tract and serological studies for the presence of coeliac disease may be required, and occasionally there is a need to quan- tify the amount of blood loss daily in the faeces or during menstrual flow by using radiolabelled chromium red cell studies. In difficult cases, percutaneous visceral angiography of the coeliac and mesenteric arteries has proved useful for detecting sites of ac- tive gastrointestinal bleeding, due, for example, to angiodysplasia that are beyond the reach of conventional endoscopic procedures. In those patients who are actively bleeding, such a procedure can iden- tify local sites of blood loss greater than 0.5 to 1.0 ml/min. The recent introductions of fibreoptic double-balloon enteroscopy and wireless capsule endoscopy offer powerful, largely noninvasive means to examine the entire small-intestinal mucosa extensively for the presence of bleeding lesions. Additional procedures dependent on well-resourced radiological facilities include CT enterography and small bowel MRI. Meckel’s diverticulum is a potential cause of obscure gastro- intestinal bleeding in young adults and children. Some Meckel’s diverticula can be diagnosed by scintigraphic studies using technetium-99m labelled pertechnetate, which may be concentrated in the ectopic gastric mucosa. Meckel’s diverticulum and intestinal strictures, particularly in the ileum, may occasionally be revealed by retrograde colonic contrast radiographic studies. Other diagnostic tests include searching for endomysial (transglutaminase) antibodies, with confirmatory duodenojejunal biopsy to detect coeliac disease. Examination of the urine and sometimes sputum may be required to detect occult iron loss in ex- foliated macrophages or proximal tubular cells, respectively, where intrapulmonary

haemorrhage or renal iron loss from glomeruli is suspected. Sometimes extensive diagnostic procedures fail to identify the cause of iron deficiency when occult gastrointestinal bleeding is responsible. Under these circumstances it is sometimes appropriate

22.6.4 Iron metabolism and its disorders 5389 for an experienced surgeon to conduct a diagnostic laparotomy to try to identify the bleeding lesion, although this is rarely required when there is access to the full range of modern imaging techniques. Occasionally, in younger adults and children, diagnostic laparotomy is indicated to identify Meckel's diverticula, intestinal stricture, and congenital abnormalities such as duplications that serve as occult sources of blood loss. The patient with recurrent chronic iron deficiency anaemia often presents a formidable challenge. First and foremost, an un- equivocal diagnosis of iron deficiency is needed, and—so far as possible—blood loss, even from cryptic sources, should be ex- cluded. While losses of iron through bleeding are frequently re- sponsible, iron deficiency due to malabsorption of iron and even urinary losses as a result of chronic compensated intravascular haemolysis may need to be considered. Expert examination of the blood film with a reticulocyte count and review of present and past measures of iron status, as well as dietary review and other measures of nutritional status, are fundamental. There is a need to capture the past travel and surgical history, as well as a compre- hensive list of prescribed and other drugs. Experienced physicians may need to seek interdisciplinary expertise in radiology, nuclear medicine, and surgery before prematurely abandoning the search for the causal lesion. Management of iron deficiency General aspects As a general rule, iron should only be recommended as a treatment for iron deficiency where that diagnosis is established beyond reasonable doubt: the presence of other causes of anaemia (e.g. defi- ciency of folic acid or haemoglobinopathy) can be easily missed to the detriment of the patient, and positive harm can be done by gratuitous iron supplementation. Many microbes require iron and there is more than a hypothetical risk of infection if iron is given unnecessarily. Treatment of causes of anaemia, including bleeding, is clearly a critical aspect of the management of iron deficiency anaemia. Bleeding lesions in the gastrointestinal tract may require specific treatment; coeliac disease should be treated with a gluten-free diet. Occasionally, patients with a chronic bleeding disorder for which surgery is not effective, such as hereditary haemorrhagic telangiectasia, may require long-term iron supplementation at doses less than that required to treat the acute iron deficiency state. In such circumstances, periodic monitoring is required to ensure that the level of iron replacement is adequate to meet the demands of the bone marrow for de novo haem synthesis and that iron overload is not occurring. It should be recognized that relief of iron deficiency will improve many symptoms suffered by a pa- tient even though they may suffer from an incurable underlying disease. Treatment with iron should continue until iron stores are replen- ished: there is no excuse for inadequate therapy, especially in those patients who are likely to suffer recurrent bleeding. Particular atten- tion is needed for iron-deficient patients who have had episodes of acute bleeding treated by blood transfusion and who at the time of therapy are not anaemic. These patients require appropriate iron re- placement to replenish iron stores for their long-term restitution of health. Because iron therapy leads to a reduction in the avidity of the transport system of the intestine for iron, it should be continued for several months after the anaemia has been corrected to re-establish appropriate iron stores, ideally as reflected by a serum ferritin deter- mination within the normal range. Iron should be replaced not only to restore the normal haemo- globin concentration but to replenish body iron stores. It is neces- sary to replace iron depleted in somatic tissues such as the muscles, where it is an essential component of mitochondrial cytochromes and other proteins critical for optimal aerobic metabolism. Occasionally, a therapeutic trial of oral iron

for a defined period is justified to verify a suspected diagnosis of iron deficiency anaemia. The effects of therapeutic iron supplementation should be moni- tored: a reticulocyte response is normally observed in peripheral blood, peaking 7 to 10 days after initiating treatment, and with a significant increase in blood haemoglobin concentration apparent within 2 to 4 weeks. If there is no evidence of continued blood loss, the haemoglobin concentration should come within the healthy - reference range within 2 months. Failure to meet these expectations suggests either that the anaemia is not caused by iron deficiency, or that there is continued depression of bone marrow function, or that there is persistent blood loss—for which further investigation is needed. Malabsorption of dietary iron is rarely severe enough to compromise the haematological response to pharmacological supplementation and an adequate response to oral iron does not preclude the existence of impaired assimilation of physiologically available iron in the small intestine. Oral delivery of iron Iron salts are best administered by mouth unless there are over whelming reasons for using the parenteral route—parenteral prepar- ations of iron are associated with a greatly increased risk of toxicity. The outdated iron—dextran complex as well as newer iron–sucrose preparations are associated with hypersensitivity, including se- vere anaphylactoid reactions. Oral ferrous salts are better absorbed than ferric salts, but in practice show little difference among pre- parations in terms of rate of repair of anaemia at a given dosage of elemental iron. It is usual to treat iron deficiency anaemia with preparations of oral iron that contain 100 to 200 mg of elemental iron daily. For full- blown iron deficiency anaemia, ferrous sulphate 200 mg is typically administered three times daily (equivalent to 3 × 65 mg of elemental iron). Some patients are unable to tolerate such a dose of iron be- cause of constipation, diarrhoea, or abdominal pain and flatulence; the presence of tarry, black stools with a sulphurous odour further impair acceptability and the required persistence with therapy. Under these circumstances, the dose of iron may be reduced and this, rather than a change of iron salt preparation, usually improves tolerability. The frequency of unwanted effects with ferrous sulphate is generally similar to that of other iron salts when comparable quan- tities of elemental iron are ingested. Once established, the optimal therapeutic response to oral iron increases the blood haemoglobin concentration by 1–2 g/litre per day. Replenishment of iron has a slow effect on the epithelial changes of iron deficiency: the atrophic glossitis may take several months to improve as iron stores are replenished. The nonhaematological ef- fects of iron deficiency in skeletal and cardiac muscle are also slow

 to respond. In contrast, the behavioural manifestations, for example, pica syndromes, often respond to iron therapy within a few days. Slow-release oral preparations of iron are available, which the manufacturers often claim release sufficient iron over a 24-h period for optimal haematological responses after once daily dos- ages. However, these preparations are likely to distribute the iron beyond the upper jejunum and thereby bypass those regions of the intestine in which iron absorption is most avid. Compound preparations of iron including B vitamins and folic acid are avail- able, but there is little justification for prescribing these except for prophylactic use in pregnancy (see following sections). In infants and children, sugar-free preparations of iron complexes are available in the form of polysaccharide iron or iron–sodium EDTA (sodium iron edetate) complexes, which can be used safely as recommended by the manufacturer. In premature infants, up to 2.5 ml of syrup containing approximately 5 mg/ml may be used twice daily; up to 5 ml three times daily may be given to children aged 6 to 12 years. Pregnancy Prophylactic iron is recommended in pregnant women who have risk factors for iron deficiency such as a diet that lacks bioavailable iron (often with low or no meat consumption) or prior menorrhagia. Prophylactic iron is also used in the management of infants of low birth weight,

including premature babies, twins, and infants de- livered by Caesarean section. Compound preparations of iron with folic acid are used for the treatment and prevention of iron and folic acid deficiencies in preg- nancy. To prevent neural tube defects in women planning a preg- nancy, the United Kingdom Department of Health advises that a medicinal or food supplement of 400 µg/day of folic acid is taken before conception and during the first 12 weeks of pregnancy. Parenteral delivery of iron Provision of iron by the parenteral route does not normally lead to more rapid repair of anaemia than when adequate oral iron prepar- ations are used. Given its potential toxicity, the only justification for the use of parenteral iron is in patients who are unable to cooperate with or tolerate oral iron therapy; those with severe gastrointestinal disease that causes malabsorption or continuing severe blood loss; or in the management of anaemia in advanced chronic kidney dis- ease where the bone marrow works best (with or without admin- istration of exogenous erythropoiesis-stimulating agents) when serum ferritin is elevated to a supranormal level, which cannot be attained with oral iron supplementation. Iron dextran was withdrawn in 1992. Current preparations (ferric carboxymaltose (Ferrinject), where 1 ml of solution contains 50 mg of iron for injection/infusion, and iron sucrose (Venofer), where 1 ml of solution contains 20 mg of iron (as iron(III)-hydroxide sucrose complex)), are less likely to cause severe ‘anaphylactoid’ reactions than the now obsolete iron dextran (Imferon) preparations. Care should be taken to exclude patients with a history of hypersensitivity reactions and intravenous preparations should only be administered where true iron deficiency has been confirmed. The main difficulty that appears to arise with these and related preparations is that the first pharmaceutical iron products for par- enteral use (e.g. Imferon) were based on high molecular weight iron dextran; this agent was withdrawn from the market due to manufac- turing difficulties. Recent preparations have been lower molecular weight iron dextrans (Infed/Cosmofer), but while safer than the high-molecular forms these still carry an appreciable risk of severe sensitivity reactions, which occur despite prior test dosing that is re- commended before the full-dose administration. Other authorized products for intravenous use such as iron gluconate (Ferrlecit) and iron sucrose (Venofer) apparently con- tain loosely bound iron and hence are administered only in rela- tively low doses of, say, 100 mg total infusion. Thus there remains a need for effective preparations of iron for intravenous infusion which are safe and allow larger corrective and preventive dosing for patients with marked iron deficiency and with no other op- tion for treating it. Latterly, several innovative new formulations of iron preparations have been introduced including ferumoxytol (Feraheme) and ferric carboxymaltose (Ferinject); the most recent is iron isomaltoside 1000 (Monofer). This preparation is composed of iron and chemically modified isomalto-oligosaccharides with a mean molecular weight of 1000 Da and principally 3–5 bound glu- cose units; the drug is finding acceptance and is now authorized in European countries. The drug allows for a high-dose iron infu- sion that will replete iron stores in many patients at a single visit; the formulation contains 100 mg iron/ml but is not entirely free of sensitivity reactions. Unwanted and toxic effects of parenteral iron preparations A history of allergic disorders including asthma, eczema, and prior anaphylaxis are regarded as contraindications to the use of paren- teral iron, as is liver disease and concurrent infection. Moreover, these drugs cannot be recommended for children. Side effects in- clude nausea, vomiting, taste disturbances, hypotension, paraesthe- siae, abdominal disorders, fever, flushing, anaphylactoid reactions, and the reactivation of inflammatory arthropathy. Injection site re- actions, including phlebitis occur. Parenteral iron should probably be avoided in patients with pre- existing cardiac disease including ar- rhythmias or angina. Parenteral iron is contraindicated in children below the age of 14 years. The Committee for Orphan Medicinal Products of the European Medicines Agency continues to emphasize that intravenous iron products should be administered

when staff trained to evaluate and manage anaphylactic/anaphylactoid reactions as well as re- suscitation facilities are immediately available. Patients should be monitored for signs of hypersensitivity during and for at least 30 min after each administration of an intravenous iron product. It is important to note that the committee considers that the risk of hypersensitivity is increased in patients with known allergies (including drug allergies) and in patients with immune or inflam- matory conditions (e.g. systemic lupus erythematosus, rheumatoid arthritis), as well as in patients with a history of severe asthma, eczema, or other atopic allergy. In these patients, intravenous iron products should only be used if the benefit is clearly judged to out- weigh the potential risk and in full knowledge and consultation with the recipient. Having considered the overall regulatory data, the Committee for Medicinal Products for Human Use under the European Medicines Agency has concluded that the benefits of intravenous iron- containing medicinal products continue to outweigh the risks in the

22.6.4 Iron metabolism and its disorders 5391 treatment of iron deficiency situations when the oral route is insuffi- cient or poorly tolerated. A recent review by experienced North American haematologists is relatively sanguine about the rarity of serious adverse events with contemporary parenteral iron products. Of importance, however, the United States Food and Drug Administration note that the agency received 49 reports of death temporally associated with ad- ministration of intravenous iron during the 5 years 2011 to 2016, 30 of which were adjudicated and determined to be anaphylaxis. The development of porphyria cutanea tarda in patients receiving renal replacement therapy is almost invariably an indication and consequence of iatrogenic iron overload. Administration of parenteral iron Iron–sucrose complex is given by slow intravenous infusion. Iron carboxymaltose can be administered undiluted as a slow intra- venous injection (infusion time dependent on dose) or diluted as a slow infusion. The maximum single dose is 20 mg iron/kg body weight, not exceeding 1000 mg of iron. Doses are calculated, ac- cording to the manufacturer’s instructions, from the haemoglobin concentration, which should be reassessed no earlier than 4 weeks after the final administration to allow time for a haematological response. At least 15 min of close observation should elapse after the test dose before the therapeutic dose is administered. Iron isomaltose offers convenient dosing options up to 20 mg iron/kg body weight with, at the time of writing, no test dose recommended by the manu- facturer. Where less than 1 g is required, infusion should be under- taken slowly over at least 15 min; infusion of higher total doses should extend for at least 30 min. However, these recommendations are not always successful and careful studies on the nature of iron- induced hypersensitivity reactions suggest that most are comple- ment mediated and not true anaphylactic reactions. A convincing case for managing these reactions in at-risk patients by reducing the rate of administration has been made by Szebeni and colleagues, to whom the reader is referred. Unusual syndromes with iron- deficient erythropoiesis Congenital deficiency of transferrin There are a few reports of deficiency or virtual absence of serum transferrin in infants with disturbed growth, marked hypochromic anaemia, and disordered iron metabolism associated with systemic iron storage leading to tissue injury. This disease is extremely rare but holds great fascination for those investigators with an interest in the pathophysiology of iron metabolism. Profound deficiency of serum transferrin disturbs the normal ligand–receptor signalling mechanisms indicated in the overall control of body iron balance and absorption in the intestine. Hypo- or atransferrinaemia in hu- mans appears to be inherited as an autosomal recessive trait; the gene encoding human serum transferrin maps to chromosome 3. Infusions of serum transferrin or plasma restore normal growth and improve the abnormalities of iron homeostasis; iron-deficient erythropoiesis is also corrected, with resolution of

the anaemia. The half-life of transferrin in the plasma is 5 to 10 days and so infusions of plasma or purified preparations enriched with transferrin can be administered at intervals. Since most individuals with transferrin deficiency produce limited amounts of the protein antigen, immune reactions to exogenous human transferrin appear to be either mild or rare. Absolute deficiency of transferrin receptors, for example, as oc- curs in mouse embryos generated as a result of gene disruption technology in embryonic stem cells, is incompatible with normal development beyond the late embryo stage. Acquired defects in the transferrin receptor There is at least one well-documented instance of an acquired de- fect of iron delivery associated with signs of iron-deficient erythro- poiesis caused by loss of human transferrin receptor function. This condition was associated with the development of antinuclear factor and other autoantibodies as part of an autoimmune illness in an adult woman with hypochromic anaemia. Autoantibodies directed against the transferrin receptor were identified in the serum of the patient, but the anaemia, with its attendant sideropenia, ultimately responded to a combination of steroids and azathioprine therapy, and the titre of transferrin receptor autoantibodies of peripheral blood cells diminished. The extent to which this phenomenon oc- curs generally during the course of autoimmune disorders associ-ated with anaemia is unknown. Other causes of refractory iron-deficient erythropoiesis Iron-refractory iron deficiency anaemia The IRIDA syndrome was originally reported as a rare autosomal recessive iron metabolism disorder characterized by iron deficiency anaemia (hypochromic, microcytic) that is often unresponsive to oral iron intake and partially responsive to parenteral iron treat- ment. The disorder is due fundamentally to excess action of the iron-regulatory hormone, hepcidin, and is caused by mutations that impair the action of matriptase-2, a transmembrane serine protease encoded by the TMPRSS6 gene. As explained earlier, this protease controls release of hepcidin by the liver and contributes to the main- tenance of iron homeostasis. IRIDA shows a mixed pattern of autosomal inheritance with di- verse expressivity in affected pedigrees; there is usually milder ex- pression in heterozygous individuals who inherit only one copy of a mutant TMPRSS6 allele from a parent. Although it has been only recently recognized, over 50 cases in families of diverse ethnic origin are reported. Although present lifelong, in most patients the manifestations of anaemia are not severe and development is not impaired during childhood. Women harbouring TMPRSS6 mutations are generally more severely affected, as would be expected in conditions associ- ated with iron deficiency. Investigations reveal a hypochromic, microcytic anaemia with very low serum iron and transferrin saturation; if measured, serum hepcidin concentration may be elevated, but given that reliable clin- ical assays are not always available, low hepcidin determinations are not always found—the reference range is broad and serum hepcidin concentrations fluctuate. The ratio between the iron saturation of serum transferrin and immunoreactive hepcidin has been proposed to give better discrimination but has yet to be widely accepted. A fur- ther confounding feature is that serum ferritin concentrations may be within the normal range and are often modestly elevated after treatment with intravenous iron. While the diagnosis is suggested by the history of long-term anaemia and parental consanguinity, it

 is likely that many patients with this condition escape diagnosis and that artefactual anaemia or frank malingering may be considered. The only definitive method for making the diagnosis of IRIDA syn- drome is molecular analysis of the TMPRSS6 gene. Unexplained iron deficiency Despite increasing awareness of the need to determine its cause, un-explained iron deficiency in children and adults is a frequent occur- rence worldwide. In some patients in whom intensive investigation fails, the expected parameters of iron deficiency associated with iron-deficient erythropoiesis are present after a failure to respond to generous oral

supplementation with iron salts; administration of parenteral iron, however, leads to reticulocytosis with resolution of iron-deficient red cell indices. At the time of writing, in such patients no convincing molecular lesions have been identified in DMT1, ferroportin, hephaestin, or uncharacterized moieties involved in the transport of iron across the intestine. However, despite the absence of wide-scale systematic studies, it seems likely that IRIDA, in rare homozygotes and more frequent heterozygotes, may be responsible for numerous undiag- nosed patients with iron-deficient erythropoiesis that responds only to parenteral iron supplementation. With the advent of better diag- nostic facilities in centres where advanced techniques are used in the management of malignant diseases of the blood, molecular diag- nosis of IRIDA may become incorporated into clinical practice and there is a case for it to be provided by diagnostic genetic services. Determining the frequency of pathological mutations in TMPRSS6 in different populations would go far in justifying the provision of such diagnostic tests for patients with unexplained sideropenic anaemia. Secondary iron storage disease (secondary haemochromatosis) Primary iron storage disease (hereditary or genetic haemochroma- tosis) occurs when excess iron accumulates as a result of heredi- tary defects that lead to enhanced net absorption of iron. Usually tissue injury and iron storage occur slowly, so that in most cases damage from excess iron takes over two decades to manifest itself. However, the increasing recognition of patients with genetic forms of juvenile haemochromatosis accompanied by avid net accumu- lation of iron, even during childhood, has strong clinical parallels with aggressive secondary haemochromatosis due to the iron- loading anaemias. In both cases, early parenchymal iron loading, from multiple sources, is associated with injury to the endocrine system and heart. Juvenile and adult haemochromatosis is de- scribed in detail in Chapter 12.7.1. General aspects Iron storage disease results from repeated blood transfusions or sustained increases in iron absorption accompanying a primary disorder of haematopoiesis with anaemia; these two principal causal influences may coexist in the same patient. Each transfused unit of blood contains about to 225 mg of iron as haemoglobin, so patients receiving repeated blood transfusions to support an- aemia typically accumulate iron at about 10 times the rate that occurs from conditions associated with chronically increased iron absorption. While about 0.75 million people have thalassaemia, there are 332 000 conceptions annually estimated by the World Health Organization to be affected by diseases affecting globin chains. About 275 000 have a sickle-cell disorder and need early intervention; 56 000 infants have a major thalassaemia, including at least 30 000 who need regular transfusions and 5500 who die with the complications of thalassaemia major in the first months of life. Iron storage disease occurs in patients who have received oral iron therapy over many years as medicinal tonics or as treatment for re- fractory anaemia. However, it is unknown if this occurs in the con- text of other coexisting disorders or the presence of mutant alleles of the HFE gene, underlying bone marrow disease, or the coinheritance of a thalassaemia trait or red cell disorder such as pyruvate kinase de- ficiency. Conversely, iron excess may develop spontaneously in pa- tients with haemolytic (and especially dyserythropoietic) anaemias alone through the recently identified mechanisms of hepcidin sup- pression by a bone marrow-derived factor (e.g. erythroferrone). However, iron overload most commonly results from repeated blood transfusion with or without underlying expansion of eryth- roid marrow (Box 22.6.4.3). Based on understanding of the molecular cell biology of iron homeostasis and the control of erythropoiesis, several treatments are being evaluated. These potential therapies interrogate respect- ively, the pathological action of hepcidin and the action of members of the transforming growth factor-ß ligand superfamily on activin- like receptors in erythropoiesis: both avenues have reached a prom- ising stage of clinical investigation. Whatever the physiochemical basis, the mechanisms of iron- mediated toxicity are probably shared between

the iron storage syndromes: primary genetic haemochromatosis and the sec- ondary haemochromatosis associated with blood transfusion and the iron-loading anaemias certainly have many clinical features in common. Causes of iron overload Transfusional iron overload Each millilitre of whole donor blood contains the equivalent of 0.47 mg/ml of elemental iron complexed with protoporphyrin, hence as stated previously, a ‘unit’ of transfused red cells derived from an ori- ginal donation of 475 ml thus contains about 225 mg of elemental Box 22.6.4.3 Anaemias associated with iron storage disease from transfusion and/or increased iron absorption • Congenital dyserythropoietic anaemia types I and II • β-Thalassaemia including transfusion-dependent thalassaemia major and the intermediate phenotype (nontransfusion dependent) • Sideroblastic anaemia (congenital or acquired) • Hereditary spherocytosis (when in association with one or more mu- tant alleles of the HFE gene) • Megaloblastic anaemia • α-Thalassaemia (haemoglobin H disease) (rarely) • Pyruvate kinase deficiency (from transfusion and increased iron absorption) • Diamond–Blackfan and aplastic anaemia (from transfusion)

22.6.4 Iron metabolism and its disorders 5393 iron, which is eventually retrieved after red cell breakdown in the macrophage system as a result of the actions of haem oxygenase, which releases bilirubin, carbon monoxide, and one atom of iron per haem molecule linked to each globin subunit, thus each molecule of haemoglobin A yields four iron atoms. There is no physiological mechanism by which excess iron ac- quired from transfusions can be excreted: after phagocytosis of the senescent red cells by macrophages, the iron is initially retained by the mononuclear phagocyte system. Continued delivery of iron de- rived from transfused red cells leads to the excess of iron-loaded ferritin and its breakdown product, haemosiderin, in parenchymal cells throughout the body, with ensuing tissue injury and functional impairment. Initially this occurs preferentially in hepatocytes but subsequently extends to the endocrine system and myocardium. After the transfusion of 15 to 20 units of blood (representing about 5 g of elemental iron), iron toxicity becomes evident. After 100 to 200 transfused units, myocardial iron accumulation and severe toxicity is inevitable. Iron-loading anaemias In dyserythropoietic anaemias not requiring regular blood trans- fusion, such as milder thalassaemia phenotypes (intermedia) and some sideroblastic anaemias, symptoms and signs of iron storage disease are caused by excessive and sustained absorption of iron from the diet by the intestine. Thus, although some patients with β-thalassaemia intermedia are treated by occasional transfusion, much of the excess iron stored in the body originates from ingested rather than transfused iron and is initially deposited in periportal hepatocytes. Net absorption of dietary iron in β-thalassaemia inter- media may be increased more than fivefold above that in healthy age-matched subjects. In regularly transfused patients with β-thalassaemia major, including the prevalent iron-loading condition, haemoglobin E/ ß-thalassaemia, massive expansion of the erythropoietic marrow is suppressed to render absorption of iron normal or near normal. However, in patients with thalassaemia who are intermittently transfused, erythroid hyperplasia persists and excessive absorption of iron from the diet contributes significantly to the iron storage de- rived from transfused cells; several grams of additional iron may be acquired each year by this route. Patients with hypochromic anaemias due to sideroblastic change in the marrow are particularly at risk because they are often wrongly diagnosed as chronic or recurrent iron deficiency anaemia; they thus receive long-term iron supplementation that serves merely to exacerbate the iron-loading state. It is noteworthy, however, that pa- tients with haemolytic anaemia due to sickle cell-haemoglobin C disease do not commonly develop marked iron overload as a result of enhanced iron absorption: full-blown iron storage disease ap- pears mainly in transfused patients with chronic anaemias. Particular difficulties arise in refractory

anaemias and haemo globinopathies in which there is a hyperplastic bone marrow with ineffective erythropoiesis that drives the inappropriate absorption of iron by the intestine. Patients with a rare inherited anaemia, Diamond–Blackfan anaemia, have few or no red cell precursors so that iron bound to transferrin is not cleared by the bone marrow; the plasma concentration of free, nontransferrin iron rises, and in trans- fused patients poses a high risk of systemic iron overload. In the South African Bantu and related sub-Saharan African populations, excess iron is ingested in an unusually bioavailable form in beers and other alcoholic drinks prepared by fermentation in iron pots (e.g. kaffir beers). Soluble complexes of readily bioavail- able iron in these drinks contribute to secondary haemochromatosis with frank scurvy in young- and middle-aged men. Although much of the iron is at first detected in the mononuclear phagocyte system (and is seen particularly in Kupffer cells on liver biopsy), associated hypogonadism and vitamin C deficiency later induce scurvy and osteoporosis. Dietary adjustment and iron chelation therapy may relieve the disorder, which is becoming less common after its rec- ognition in the early 1950s. Family studies point to a genetic com- ponent which predisposes individuals to this secondary iron storage disease within given pedigrees, but the causal gene or genes have not been identified. Pathophysiology of iron loading in ß-thalassaemia In ß-thalassaemia, imbalanced globin-chain biosynthesis leads to anaemia that is characterized by the deposition of aggregated free α-globin chains in the cytoplasm. Excess hemichrome pigment with increased nonhaem iron is also present, and this is implicated in re- active oxygen-mediated oxidative stress and Heinz body formation. Examination of the marrow shows marked expansion but eryth- roid differentiation does not proceed to full maturation and there are signs of extensive programmed death of red cell precursors. Dyserythropoiesis in ß-thalassaemia is thus characterized by several abnormalities: expansion (often massive) of erythroid progenitors, accelerated differentiation of the erythroid precursor cell population (to the point of the development of polychromatophilia), and ar- rested red cell maturation. In operational terms, ineffective erythropoiesis disrupts the homeostatic mechanisms of systemic iron balance: the patho- logical marrow signal overcomes the control exerted by the physio- logical ‘storage regulator’ so that net absorption and delivery of iron proceeds in an unregulated manner, even in the face of adequate or increased iron stores. Hepcidin is inappropriately and pro- foundly suppressed. If there is no prior burden of iron from red cell transfusions in dyserythropoietic anaemias such as ß-thalassaemia intermedia, absorption of iron can be enhanced as much as ten- fold and is greatly in excess of requirements for erythropoiesis. The pathological excess of labile iron readily induces further cytotoxicity and the consequential effects of secondary haemochromatosis. Inherited disorders of ferritin Deficiency of the ferritin heavy chain Studies in members of a large Japanese pedigree with autosomal dominant iron-storage disease resembling adult-onset genetic haemochromatosis, HFE1, found affected patients to harbour a mu- tation (A49U) in the iron-responsive element of H ferritin mRNA. This results in a deficiency of the H-ferritin protein component of the ferritin multimer with impaired ferroxidase activity and capacity for iron storage. Inherited defects in the ferritin light chain Two classes of informative mutations have been reported in the ferritin light-chain gene. Rare frameshift mutations which dis- tort the C-terminus of the L-ferritin subunit cause a dominant

 neurodegenerative disease with adult-onset dementia due to oxi- dative injury and iron deposition in neurons. The casual mutations impede assembly of the 24 mixed subunits of the multimeric ferritin molecule and markedly impair its iron-storage efficiency. In contrast, point mutations that affect only the untranslated 5′ iron-responsive element in the cognate FTL mRNA cause a domin- antly transmitted form of cataract with presentation in

childhood. This condition is known as the hyperferritinaemia-cataract syn- drome, in which excess free light chains precipitate in the lens of the eye without evidence of systemic disease or iron excess, but with markedly elevated serum ferritin concentrations which are often er- roneously ascribed to iron overload or even haemochromatosis. Clinical features of iron overload Many of the clinical features of established secondary iron storage disease are similar to those observed in the hereditary forms of ju- venile haemochromatosis (see Chapter 12.7.1). The consequences of transfusional iron overload and the benefits of chelation treat- ment are probably best documented in patients with ß-thalassaemia major. Initially, iron derived from transfused red cells accumulates as storage iron in the macrophage system; subsequently excess iron appears in hepatocytes and ultimately in the endocrine system and heart. This distribution pattern reflects iron transferred from molecular species other than transferrin: there is a striking susceptibility of par- ticular cell populations within the tissues that show the most prom- inent signs of iron-related injury to this form of iron. For example, in the entire adenohypophysis it is the limited population of but a few hundred gonadotroph cells that accumulate iron, ultimately leading to hypogonadotropic hypogonadism as the first manifestation of an- terior pituitary failure. This endocrinopathy causes arrested sexual development and infantilism. Iron also accumulates preferentially in the β-cells of pancreatic islets, leading to diabetes mellitus; in the zona glomerulosa of the adrenal glands, with adrenal failure due to mineralocorticoid deficiency; and in the chief cells of the parathy- roid glands, ultimately causing hypoparathyroidism. Transfusional iron overload, as in established hereditary forms of haemochromatosis (especially juvenile haemochromatosis), has a striking predilection for the myocardium, which is also attrib- uted to unregulated uptake of nontransferrin-bound iron, with risk increasing with the total number of units transfused. Myocardial disease (cardiomyopathy) can cause sudden death from about 15 years of age in untreated patients caused by tachyarrhythmias and/or heart block due to injury to the cardiac conducting system. Refractory heart failure due to extensive cardiac myocyte injury and fibrosis is also frequent. Modern iron-chelation regimens are able to prevent and to some extent reverse iron-related cardiomyopathy, but the effects of iron toxicity in the endocrine system are largely irreversible. Susceptibility to infection Iron overload is associated with a greatly increased the risk of micro- bial infection as free iron (i.e. that which unbound to transferrin), is readily utilized, and is a competitive trophic and survival factor. Indeed, infection is the second most common cause of death in pa- tients with ß-thalassaemia who receive regular red cell transfusions. The following microbial pathogens have been associated with in- fections in patients with iron overload: Yesinia enterocolitica, Listeria monocytogenes, Plasmodium falciparum, noncholera vibrios (e.g. Vibrio vulnificus), Mycobacterium tuberculosis, and Mycobacterium avium complex. Fungal pathogens include Candida albicans, Aspergillus spp., and the agents of mucormycosis. It is noteworthy that patients receiving desferrioxamine, a nat- ural high-affinity iron-binding molecule obtained from the Gram- positive bacterium, Streptomyces pilosus, remain at risk from severe infections (e.g. Yersina enterocolitica and Vibrio vulnificus). Many iron-chelators, including desferrioxamine, are related to natural siderophores which are produced by microbes and harnessed to scavenge environmental iron in stable complexes that are subse- quently taken up after binding to dedicated microbial receptor complexes; these systems serve as important microbial virulence factors. Pharmacological use of iron-chelating drugs may bypass the requirement for endogenous siderophores to compete for scarce en- vironmental iron and thus render the treated host more susceptible to invasive infections. Diagnosis and monitoring In transfusional iron overload (without chelation therapy), the amount of excess body iron can be estimated quite reliably from the transfusion history. The extent of life-threatening systemic (extrahepatic) iron deposition increases

when the saturation of serum transferrin by iron is greater than 60%. Above 70% sat- uration, significant and potentially damaging concentrations of nontransferrin iron (‘unbound’) are likely to be present, and iron uptake is altered categorically from the physiological distribution mediated by the transferrin receptor. When nontransferrin bound iron is present, ‘free’ iron in the plasma is independently transferred to tissues, which occurs in an unregulated and potentially toxic manner. Serum ferritin The serum glycoprotein form of ferritin is a moderately reliable bio- marker of iron overload. While most ferritin in the serum is unsat- urated and has an infinitesimal capacity to transport iron, secretion of the protein occurs in response to hepatic iron store. Serum ferritin concentration—readily determined by immunoassay—is in wide use for monitoring excess iron storage and its treatment. Serum ferritin may either under- or overestimate the burden of iron in the body and is a poor surrogate biomarker for risk management in relation to haemochromatosis. For example, in nontransfusion-dependent ß-thalassaemia, ferritin provides under- estimates of liver iron concentrations, whereas in the presence of pre-existing liver disease such as hepatitis C, or in the presence of fatty liver, serum ferritin alone may cause the degree of iron overload to be overestimated. Hyperferritinaemia occurs in unrelated dis- eases including the hyperferritinaemia-cataract syndrome, Hodgkin lymphoma, and other malignancies. Vitamin C deficiency tends to depress serum ferritin concentrations. A downward trend in serum ferritin concentration generally indicates negative iron balance (more iron removed than trans- fused) and an upward trend probably indicates inadequate dosing or inadequate adherence to chelator therapy. However, coexisting liver damage or inflammatory conditions can be responsible for increasing serum ferritin concentrations. For these reasons, it is ad- visable to obtain independent evidence of iron overload, its distribu- tion, and its response to treatment.

22.6.4 Iron metabolism and its disorders 5395 Liver iron concentration Liver iron concentration is the most reliable predictor of total body iron, typically by way of the formula of Angelucci (derived from liver biopsy data) in which body storage iron in mg iron/kg body weight = 10.6 × the liver iron concentration expressed as mg/g dry weight of tissue. However, with the advent of validated MRI tech- niques to measure tissue iron, biopsy for tissue iron quantification and histological examination is rarely carried out today, especially in rich ‘developed’ countries. The upper reference limit of liver iron concentration is about 1.8 mg/g dry tissue weight; values above 7 mg/g indicate inadequate chelation and increased risk of tissue injury; and values greater than 15 mg/g (1.5% of dry liver weight) are associated with severe extrahepatic iron deposition and myocardial injury. Although noninvasive radiological techniques usually obviate the need for liver biopsy and direct chemical quantification of tissue iron, in some cases examination of liver biopsy samples may allow staging of the disease, particularly in relation to coincidental viral hepatitis in which fibrosis and cirrhosis combined with iron deposits in the parenchymal cell can confound the clinical pathological findings. In such circumstances, biopsy may contribute information of authentic and valuable diagnostic utility with regard to iron overload. Cardiac iron overload While serial determinations of liver iron concentration enable ab- solute iron overload and the direction of change to be monitored, liver iron concentration is a relatively unreliable predictor of car- diac iron deposition and associated injury with impaired function. Myocardial biopsy is both invasive and unreliable as a means to in- vestigate suspected myocardial iron overload. The use of cardiac MRI, most notably with the T2* technique, is a desirable tool for identifying the presence of increased cardiac iron as well as the direction of change with chelation therapies. Patients with a T2* less than 20 ms have increased cardiac iron while those with T2* values less than 10 ms are at high risk of developing heart failure within the next year if chelation is not intensified. Where possible, it is

recommended that centres monitoring patients with transfusional iron overload should systematically undertake such assessments in patients who are at risk. The frequency of monitoring will depend on the degree of systemic and heart iron overload and the underling haematological condition responsible for the iron storage. Generally it is recommended that patients with transfusion- dependent ß-thalassaemia have cardiac T2* carried out annually unless the signal has been repeatedly found to be within the healthy reference range and iron loading is otherwise well controlled. Management—iron-chelating drugs Until now, the use of iron-selective chelating agents has been the mainstay of management in patients with iron overload. This stratagem has been the subject of intensive clinical research since the introduction of desferrioxamine (dissociation constant for ferric iron, 10−31 M) more than 50 years ago. The life-saving effects of intra- muscular desferrioxamine in ß-thalassemia were shown in 1981, and over the last 30 years the field has expanded with the develop- ment of safe, orally active iron-chelating agents. Chelatable pools of iron in the tissues are derived from red cell breakdown in the macrophage compartment as well as proteolysis of ferritin in the liver. These operationally described pools of iron are dynamic but also finite: they are generated continuously, so that the most effective removal of toxic iron deposits depends on continuous exposure to chelating agents. At any moment, only a fraction of the body iron is accessible to chelating agents and thus the process of depleting the excessive iron is slow. However, given the extraor- dinary affinity of the iron chelators in clinical use, even in the pres- ence of excess systemic iron accumulation, care is needed to reduce the dose of the agent to minimize toxicity as the iron is removed. The primary goal of chelation therapy is to remove body iron to match the iron accumulation rate, or if substantial concentrations of tissue iron have already accumulated, to induce negative iron balance with the prevention or amelioration of iron-induced tissue injury. Chelation therapy is effective in transfusional iron overload and prolongs life expectancy; it prevents heart disease, endocrine failure, and hepatic fibrosis. While the best evidence of the benefit of long-term chela-tion accrued from studies of patients with transfusion-dependent ß-thalassaemia, other anaemias requiring transfusion, such as myelodysplasia, Diamond–Blackfan anaemia, and pyruvate kinase deficiency, benefit from this treatment. Patients with sickle cell dis- ease who have received multiple transfusions may be at less risk of myocardial iron overload compared with those with thalassaemia at apparently similar burdens of excess iron, but there is a risk of liver injury if no chelation is given. Use of chelation for patients with transfusion-dependent myelodysplasia needs to take into account the age and prognosis of the patient. Compelling evidence gathered over 30 years shows that survival in patients with transfusion-dependent β-thalassaemia is improved by treatment with subcutaneous desferrioxamine, which prevents, and can reverse, the cardiac manifestations of iron-storage disease. It must be noted, however, that full compliance with this demanding treatment is required for benefit to accrue, which requires equal commitment from the patient and the medical and nursing per- sonnel who provide care. Three iron chelators are licensed for the treatment of iron over- load: desferrioxamine, which must be administered parenterally due to poor gastrointestinal absorption, and the two orally active chelators, deferiprone and deferasirox. The chemical characteris- tics, pharmacokinetics, routes of iron excretion, and recommended doses of these agents are summarized in Table 22.6.4.1. Guidelines for starting chelation therapy are founded on long clinical experience with desferrioxamine. With this agent, overnight subcutaneous infusions at least 5 nights a week were typically started when the serum ferritin concentration exceeded 1000 µg/litre or after 20 transfusion episodes. While this paradigm has also been used for other iron chelators, it is probably overcautious because the ototoxicity, retinal toxicity, and bone growth abnormalities observed at low serum ferritin concentrations and

at desferrioxamine doses higher than 40 mg/kg are unlikely to occur with other chelators. For example, serum ferritin concentrations as low as 500 µg/litre are often achieved with the orally absorbed iron chelator, deferasirox. Desferrioxamine Desferrioxamine is a hexadentate iron chelator which binds ferric iron (iron(III)) in a 1:1 molar/atomic ratio. Its relatively high mo- lecular weight limits gastrointestinal iron absorption and this, to- gether with a short systemic half-life, means that desferrioxamine

section 22 Haematological disorders 5396 has to be given by continuous infusion. Desferrioxamine promotes urinary excretion of iron that is derived from red cell catabolism; an approximately equal amount of iron is depleted from hepatocellular stores and excreted into the faeces via the biliary system. Administration and dosing The usual route for desferrioxamine administration is by slow sub- cutaneous infusion over 8–12 h, five to seven times per week; this is can be done on an ambulatory basis in adults, but nocturnal admin- istration is more often used, particularly in children. Nocturnal ad- ministration relies on the use of slow clockwork, battery-operated, or balloon infusion devices. Although electrical syringe pumps are in common use, smaller (and conveniently quieter) infusion devices are now available. Light, prefilled balloon pumps, though expensive, are also in use. Patients with a high requirement for transfusion (>0.5 mg/kg day of iron accumulation) generally require higher doses than those with low transfusion requirements. In patients without cardiac dis- ease, the daily administration of oral ascorbic acid at 2 to 3 mg/kg increases iron excretion as ferrioxamine. In patients with heart failure or high myocardial iron, continuous intravenous desferrioxamine (maximum 60 mg/kg), delivered through an indwelling line can often relieve acute heart failure and also slowly decrease the loading of iron in the myocardium. Desferrioxamine has sometimes been given intravenously together with blood transfusion, but the im- pact on iron balance is small; the drug should not be added directly to the blood, but to avoid potentially toxic bolus administration of desferrioxamine should be coadministered through a separate intra- venous route through the same cannula. Side effects and monitoring The most important unwanted effects of desferrioxamine are ototox- icity and retinal toxicity. These are more likely at higher doses and where iron overload is less marked, and children are more suscep- tible to these toxicities than adults. It is prudent to monitor visual acuity and auditory function at intervals during treatment. The daily dose in children is 20 to 40 mg/kg of body weight and doses should not exceed 40 mg/kg because of risks to growth and bone
Table 22.6.4.1 Characteristics of iron-chelating drugs Compound Desferrioxamine Deferasirox Deferiprone Molecular weight (Da) 560 373 139 Route of absorption Subcutaneous, intravenous, intramuscular Oral Oral Half-life of iron-free chelator 20–30 min 12–16 h 3–4 h Maximum plasma concentration of iron-free drug (µM) 7–10 80 90–450 Minimum plasma concentration with daily dosing (µM) 0 20 0 Elimination of iron complex Urine = faeces Iron complex removed more slowly than free drug Mainly faeces Mainly urine Metabolism Mainly in liver to iron-binding metabolites Mainly in liver to iron binding glucuronides

> 90% eliminated in faeces, 60% unmetabolized. Glucuronide formed in liver does not bind iron Recommended dose mg/kg per day titrated for rate and level of iron loading 30–60 5–7 ×/week 20–40 once daily 75–100 in 3 divided doses Main adverse effects Ocular, auditory, bone growth retardation, local reactions, allergy Gastrointestinal, increased creatinine, proteinuria Hepatitis

> Gastrointestinal, arthralgia, agranulocytosis/neutropenia Potential drug interactions Vitamin C (in doses >200 mg) Prochlorperazine —Inducers of uridine diphosphate glucuronyl transferase —Bile acid sequestrants —Substrates of cytochrome P450 (CYP)- 3A4/5, CYP2C8, or CYP1A2 Drugs inducing neutropenia Aluminium-based antacids Vitamin C? Licensed indications <6 yearsa First line for thalassemia major age 2–6 First line—age 2–6 years USA Second line—age 2–6 years Europe Insufficient information for licensing Licensed indication <6 years First line in TDT First-line TDT. First line NTDT Other chelation not tolerated Chelator toxicity monitoring Pure tone audiometry yearly Serum creatinine—before starting, then weekly for first month—thereafter monthly Neutrophil count 1–2 weekly Retinal assessment yearly Urine protein/creatinine ratio monthly Aspartate alanine transaminase monthly Aspartate alanine transaminase monthly NTDT, nontransfusion-dependent thalassaemia; TDT, transfusion-dependent thalassaemia. a Licensing in children differs between USA and Europe.

22.6.4 Iron metabolism and its disorders 5397 development of higher doses. In adults with established iron over- load, the effective dose is 40 to 50 mg/kg five to seven times per week (licensed up to 60 mg/kg in the United States of America). The dose must also be reduced when serum ferritin concentration decreases, because the risk of retinal or auditory toxicity increases when the dose, relative to serum ferritin, is high. Rarely, minor gastroenterological disturbances, myalgia, and very rarely anaphylaxis may occur. Desferrioxamine interacts unfavour- ably with phenothiazines and coma may result, especially in patients with modest iron overload. Apart from minor localized skin reac- tions, desferrioxamine is usually otherwise well tolerated. These re- actions can usually be controlled by reducing the concentration of the drug in the infusion (and always <10% weight/volume) and by alternating the infusion sites. Hydrocortisone in doses of up to 100 mg has been reported to reduce severe cutaneous reactions. Some patients receiving desferrioxamine develop infections with microorganisms such as yersinia and fungi, including Candida and Mucor spp., that have fastidious requirements for iron. Iron- overloaded patients may also develop other systemic microbial in- fections and are particularly susceptible to fulminating sepsis caused by the marine vibrio, V. vulnificus. It seems likely that under these circumstances the ferrioxamine complex may serve as nature in- tended, that is, as an available iron ligand for uptake by microbial siderophore systems. Despite the inconvenience of its use, long-term studies of patients receiving desferrioxamine for iron storage disease in homozygous ß-thalassaemia syndromes show that it is largely safe; moreover, desferrioxamine improves cardiac function and life expectancy and arrests hepatic fibrosis in secondary haemochromatosis. The intro- duction of desferrioxamine has also been associated with decreasing rates of endocrine failure such as diabetes, hypothyroidism, and hypoparathyroidism. Should these complications develop, prompt replacement of deficient hormones (or vitamin D analogues in hypoparathyroidism) should be introduced. Sex-steroid hormone replacement, for patients developing hypogonadotropic hypo- gonadism, should improve growth, sexual development, bone density, and self-esteem. Deferasirox For many patients, the orally active tridentate ferric iron che- lator, deferasirox, offers an attractive option for once-daily therapy without the discomfort and limitations of continuous subcutaneous infusions. Once-daily administration is effective as a consequence of the long plasma half-life of the

chelator. Deferasirox is able to attain sufficient trough concentrations to complex labile iron species that are present in the plasma. Deferasirox chelates the same pools of iron as desferrioxamine, but—unlike the latter—the iron complex is excreted almost entirely in the faeces. In Europe, the drug is indicated for the treatment of chronic iron overload in adults and children over the age of 6 years with thalassaemia major who receive frequent blood transfusions (equivalent to >7 ml packed red cells/kg per month). Deferasirox is also licensed for other forms of transfusional iron overload in which desferrioxamine is either contraindicated or inadequate. Administration and dosing As with desferrioxamine, the effective dose depends on the rate of iron accumulation derived from the breakdown of transfused red cells. For a patient receiving between 0.3 and 0.5 mg/kg per day of iron loading, a dose of 20 to 30 mg/kg once daily is sufficient to balance input and excretion. For patients in whom a negative iron balance is needed as a result of pre-existing marked iron overload, or who have a transfusional iron loading rate estimated to be greater than 0.5 mg/kg per day, a dose of up to 40 mg/kg per day may be given. Dose increments up to this value can also be given in patients whose serum ferritin concentrations fail to decrease, according to the extent of iron overload (as judged by transfusion history and serum ferritin concentration). Dose adjustments should be made at intervals of 3–6 months. At 20 to 30 mg/kg deferasirox per day, liver iron concentrations and serum ferritin concentrations decrease without impaired safety over a follow-up period as long as 5 years in ß-thalassaemia major and sickle cell disease. Progressive removal of cardiac iron over 3 years of follow-up has been shown: normalization of cardiac iron over this period occurs in patients with moderate myocardial iron loading (myocardial T2* of 10–20 ms), and significant improvement has been shown in patients with more severe myocardial T2* of be- tween 6 and 10 ms. Improvement or stabilization in liver fibrosis has been demonstrated in a 3-year prospective study. Deferasirox has recently been licensed for the treatment of nontransfusional iron overload in ß-thalassaemia intermedia pa- tients where low doses of 5 to 10 mg, carefully titrated against serum ferritin and liver iron, are effective and well tolerated. A newer formulation of deferasirox, in a film-coated tablet, is now available; the correct dose of this formulation is 0.7 times the effective dose of the deferasirox dispersible tablet because of its increased ab- sorption. This formulation appears to be well-tolerated and gener- ally more acceptable to patients than the dispersible preparation. Side effects and monitoring Monitoring of baseline and monthly hepatic and renal function (including tests for proteinuria) is required weekly for the first month of treatment with deferasirox, or after dose increments, and monthly thereafter. Modest increases in serum creatinine concen- tration (about 30%) occur in about one-third of patients, but these rarely progress. Since acute kidney injury has been occasionally re- ported, in patients whose serum creatinine concentrations rise, or where the serum creatinine exceeds the upper healthy reference limit, dose reduction, or temporary interruption is recommended. Annual ear and eye examinations, as well growth parameters and sexual development in children, should be carefully monitored, but inner ear and retinal toxicities are very rare. Gastrointestinal effects are common but usually mild to moderate and readily controlled. About 10% of patients experience a skin eruption soon after starting treatment: mild to moderate skin eruptions can initially controlled by dose reduction, but with a severe skin reaction the treatment must be stopped and—after healing—carefully reintroduced at a low dose followed by cautious increments. Deferiprone Deferiprone, a bidentate oral iron chelator, has been licensed in Europe and, more recently, the United States of America for treat- ment of iron overload in patients with thalassaemia over 6 years of age unable to tolerate desferrioxamine or where desferrioxamine is contraindicated. This drug, of the hydroxypyridone class, has a short plasma half-life due to its rapid glucuronidation in the

section 22 Haematological disorders 5398 liver; it is used at a dose of 75 to 100 mg/kg per day of body weight daily in three divided doses. Iron is excreted almost entirely in the urine. Direct, truly randomized ‘head-to-head’ comparisons between deferiprone and deferasirox monotherapy have yet to be undertaken. Administration and dosing Deferiprone monotherapy induces negative body iron balance in about one-third of patients with severe homozygous β-thalassaemia at 75 mg/kg per day, with attendant reductions in serum ferritin concentrations. In the remaining patients, where negative iron balance is not achieved or maintained, desferrioxamine is often added at conventional doses between twice and five times a week to achieve this goal—a stratagem referred to as combination therapy (see ‘Combinations of iron-chelating agents’). High doses (100 mg/ kg per day) of deferiprone may be more effective at improving ab- normal myocardial T2* than desferrioxamine alone when given at standard doses five times a week. Side effects and monitoring Deferiprone may cause serious toxicity, including neutropenia and the occasional incidence of agranulocytosis; weekly monitoring of the neutrophil count is recommended. Before the drug was ap- proved, there was controversy about the progression or lack of pro- gression of liver fibrosis on this treatment. Combinations of iron-chelating agents Combinations of iron-chelating drugs, although not specifically li- censed, have been widely used when the desired therapeutic effect with monotherapy has not been not achieved, or where the patient finds it difficult to adhere to chelation monotherapy with sufficient frequency. In principle, combinations may work either by increasing total exposure to chelating molecules or by true synergism, where one chelator with rapid kinetic access to iron (e.g. deferiprone) shut- tles iron onto a ‘sink’ chelator with higher iron affinity but slower kinetic access (e.g. desferrioxamine). The most commonly used combination has been deferiprone with subcutaneous desferrioxamine, but using diverse regimens and dosing, especially where iron balance was not achieved with deferiprone monotherapy or when use of desferrioxamine was insuf- ficient and thus ineffective. Subcutaneous desferrioxamine, given at night, combined with daily deferiprone renders synergy improbable, as with such a regimen the chelators are unlikely to coexist usefully in the tissues. However, near 24-h exposure to chelating molecules can be achieved with this combination if desferrioxamine is given every night. In a randomized prospective trial, this stratagem has also been shown to be more effective at decreasing myocardial iron deposits (estimated by MRI (T2*)), than standard doses of desferrioxamine as a monotherapy. Deferasirox has been combined with desferrioxamine in patients with severe myocardial siderosis (T2* 5–10 ms): it brought about rapid removal of iron from the liver as well as the heart, and deteri- oration of left ventricular function was arrested. In one randomized clinical study, deferiprone combined with deferasirox was reported to be effective at removing heart and liver iron. No new toxicities have so far been reported with the combined therapies. Management—other aspects of care The single most important aspect of care is adherence to iron che- lation therapy and monitoring, especially for infants and other young patients with iron-loading anaemias such as ß-thalassaemia. Regular attendance of special clinics is advisable so that wide- ranging professional support from familiar personnel can reinforce medical care delivered with attention to continuity and nurturing independence. In ß-thalassaemia major, the pre transfusion blood haemoglobin concentration should be maintained between 95 and 105 g/litre with a mean interval concentration of 120 g/litre. If transfusion require- ments increase in the context of an enlarging spleen, intensification of the transfusion regimen may decrease spleen size, and this ultim- ately will reduce the transfusion requirement. In patients suffering from ß-thalassaemia, except as a last resort, the high risk of throm- bosis and infection normally should preclude splenectomy. Patients with secondary iron overload should be monitored not only for the progression of their iron storage as determined by parameters of iron metabolism, but also

clinically for the presence of iron-mediated tissue injury. Monitoring the effectiveness of che- lation treatment is most frequently undertaken by serial determin- ation of serum ferritin concentrations. Trends in ferritin identify under- or overtreatment and also identify poor adherence to therapy at an early point. The relative value of liver iron determinations and cardiac T2* MRI were discussed earlier in this chapter. Regular clin- ical monitoring and assessment of cardiac, hepatic, and endocrine function assist in the assessment of iron storage disease and thera- peutic efficacy of iron chelation therapy. Regular echocardiography and electrocardiography are also essential aspects of management and therapeutic monitoring. Endocrine disease Infantilism with hypogonadotropic hypogonadism are frequent manifestations in patients with secondary iron storage disease and contribute greatly to psychosocial difficulties and social exclusion, as well as depression, in adolescents and children. Prompt diagnosis and institution of appropriate sex hormone replacement is a crit- ical component of holistic care. Consideration of puberty induction using recombinant gonadotropins and advice about later fertility management are key to the well-being of these patients, particularly since long-term survival is increasingly a reality of contemporary haematological care. Hormone determinations and careful clinical monitoring are essential to search for the presence of other aspects of endocrine failure, including hypoparathyroidism and adrenocortical failure, which may be very difficult to detect but critically important to treat. Vigilance should be maintained for the development of diabetes mellitus. Cardiac disease Decreasing ejection fraction of the left ventricle usually precedes the functional complications of myocardial iron overload; it thus carries a poor prognosis and mandates urgent intensification of the iron- chelating regimen, often to include use of continuous infusion of desferrioxamine. Desferrioxamine given continuously through a permanent indwelling portable catheter safely sited in the superior vena cava,

 with careful attention to preventing sepsis, is a satisfactory method for securing reversal of cardiac disease in high-risk patients with serum ferritin concentrations that persist at greater than 2500 µg/ litre or in whom hepatic iron concentrations exceed 1.5% of dry liver weight. Histological studies in postmortem cardiac tissue obtained from patients with severe cardiac iron deposition and heart failure leading to death or transplantation showed limited interstitial fi- brosis and no evidence of the extensive replacement cardiac fibrosis that characterized the disease before chelation therapy; this accords with the view that heart failure associated with cardiac haemo- chromatosis is potentially reversible. Continuous intravenous infusions of desferrioxamine not ex- ceeding 50 to 60 mg/kg daily are now recommended for patients in whom the left ventricular function has deteriorated below reference values or where there is evidence of very severe myocardial iron loading (T2* ≤6 ms). This regimen is often administered with sup- plemental deferiprone at standard doses to accelerate the rate of iron removal. High-dose intravenous infusions may cause unacceptable toxic injury, especially in the retina and inner ear. Improved out- comes occur with the use of anticoagulation induced by warfarin, and scrupulous attention to cutaneous needle resiting and skin care is necessary if the risk of thrombosis and complicating infections is to be kept to a minimum. Skeletal disease Patients with stunted growth and infantilism frequently develop skeletal disease beyond that related to their expanded bone marrow, and investigations should be carried out to search for osteopenia and osteoporosis for which additional therapy will be needed. Bone dis- ease and growth arrest may be caused by the overenthusiastic use of desferrioxamine in young infants: the daily dose of desferrioxamine should be reduced to below 40 mg/kg, which usually restores normal growth velocity. Pregnancy Desferrioxamine is not recommended for use in during pregnancy, but despite

this many successful pregnancies have been reported without fetal injury. Where possible, the drug should be avoided during the middle trimester and should almost certainly be avoided, because of unknown teratogenicity, in early pregnancy or at the time of any planned conception. Nonetheless, it may be reasonable to re- start desferrioxamine therapy in the final trimester of pregnancy if the risks to the mother from iron storage disease are high. No information is available on the use of deferasirox or deferiprone and these drugs cannot be recommended in pregnancy until more experience is forthcoming. Psychological matters Psychological difficulties, sometimes accompanied by disturbed behaviour, are prevalent in children and adolescents receiving iron-chelation therapy. Transfusion for chronic anaemias and ap- propriate counselling is needed over long periods to build trust with the patients and their families, thereby to support engagement with, and adherence to, this essential treatment. Prognosis The principal causes of death in secondary iron storage disease are car- diac failure and arrhythmia, endocrine failure, and the consequences of diabetes mellitus, infection, and hepatocellular carcinoma. When associated with transfusion therapy and intestinal hyperabsorption of iron in the chronic anaemias with dyserythropoiesis, secondary haemochromatosis is rapidly fatal unless it is treated. However, the outcome of iron storage disease in patients with chronic anaemia is now greatly improving, with enhanced life quality and duration. Of patients with β-thalassaemia major who are unable to comply with iron chelation therapy, less than one-third survive to 25 years of age. One study has indicated that 95% of patients with β-thalassaemia who administer desferrioxamine subcutaneously more than 250 times each year will survive to 30 years, whereas only 12% of those who do not achieve this dosing will survive to that age. In the United Kingdom, the overall survival is 50% at 35 years; but the actuarial survival at 40 years was 80% in more than 100 patients treated at one specialist centre. Continuous intravenous desferrioxamine can reverse life-threatening arrhythmias in cardiac iron overload and also improve or reverse left ventricular or biventricular heart failure in most pa- tients. The actuarial survival of patients with β-thalassaemia and life-threatening iron-storage disease has been reported to be greater than 60% at 13 years when so treated, emphasizing the benefits of care administered at a dedicated treatment centre. From 1980 to 1999, there were 12.7 deaths from all causes per 1000 patient-years. In 2000 to 2003, the death rate from all causes fell significantly to 4.3 per 1000 patient-years and mortality attributable to iron overload decreased from 7.9 to 2.3 deaths per 1000 patient-years. Moreover, effective iron chelation reduces the frequency of hypogonadism, diabetes, and growth retardation. As cardiac disease is becoming less frequent in patients with thal- assaemia major, many are now reaching their sixth decade of life. The risks of liver disease, including hepatocellular carcinoma, re-main to be determined. Delayed consequences on health of other potential sequelae, such as endocrine failure, should also benefit from better treatment, but the consequences of delayed puberty or infantilism are hard to shake off in the surviving, late-treated adult. Chelation therapy improves the quality of life as well as survival in β-thalassaemia and has salutary effects that are comparable in other forms of transfusional iron overload. An important aspect of the new chelation regimens, either as monotherapy or in combination, will be to determine whether it will be possible safely to achieve lower serum ferritin concentrations than those typically obtained with desferrioxamine monotherapy. Since this may further reduce the adverse consequences of iron overload, the best means to opti- mize contemporary therapy still requires intensive clinical explor-ation and comprehensive evaluation of the effects on quality of life measures. Future perspectives Chelation is an effective and proven therapy but the optimal solution for iron overload would be its prevention. While regular transfusion and iron chelation are the mainstay of treatment for severe forms of thalassemia, this is in effect a therapeutic supportive stratagem which does not

definitively address the underlying pathophysiology of the diseased marrow and persistent drive to toxic iron loading. Patients with this disease still have large unmet needs, which im- pair their capacity for physical fulfilment and an enriching quality of life that is free of the need for intensive medication and clinical monitoring.

section 22 Haematological disorders 5400 Cell and genetic therapies Haematopoietic stem cell transplantation and lentiviral-mediated haematopoietic stem cell gene therapy are attractive developments for definitive correction of some forms of marrow disease (e.g. the thalassaemia syndromes and sickling disorders). However, these emerging treatments are not available worldwide and are often com- plicated by the pre-existing effects of ineffective erythropoiesis, es- tablished iron overload, and transfusion-related immunization. In particular, these prior conditions increase the risk of infection and in particular susceptibility to cardiotoxic marrow conditioning drugs. Several trials of gene transfer using third-generation lentiviral vec- tors to correct haemoglobinopathies, including sickle cell anaemia and ß-thalassaemia syndromes, are underway. These developments reflect ambitious desires to introduce a single curative step which would be superior to haematopoietic stem cell (bone marrow) transplantation. In June 2019, the EMA conditional approved Zynteglo, a therapy based on re-infusing endogenous CD34+ stem-cells transduced ex-vivo with the wild type beta-globin gene in transfusion-dependent patients who are 12 years or older. The main limit to gene therapy seems to be the con- ditioning regimen, and at present, ex vivo gene transfer to endogenous stem cells does not avoid this requirement. Experimental gene editing in animal models is encouraging, but routine use in human recipients has yet to be proven safe, specific, and effective. Other potential treatments Identification of pathological disturbances in dyserythropoiesis that drive iron overload immediately point to alternative targets for the treatment of this disease. The experimental agents under investiga- tion are based on contemporary molecular understanding of iron homeostasis and its relationship to erythropoiesis. Specific treat- ments in late-phase clinical exploration include (1) the clinical use of selective Janus kinase 1/2 inhibitors, (2) parenteral administration of long-acting minihepcidins, (3) activin receptor type IIA and/or IIB ligand trap biological agents that are predicted to attenuate patho- logical SMAD2/3 activation in erythroid precursors, and (4) puta- tive inhibitors of erythroferrone or its release. In a cognate field, the anaemia of renal failure due to erythropoietin deficiency, a novel HIF prolyl hydroxylase inhibitor (roxadustat) effectively treats the anaemia, with improved iron utilization, hepcidin concentrations and erythropoeitin—without the increased risk of stroke or death associated with exogenous erythropoetin therapy. JAK2 inhibitors in β-thalassemia Preclinical studies using convincing disease models in living rodents have shown that a JAK2 inhibitor markedly decreased spleen size and attenuated ineffective erythropoiesis, thus clinical use of JAK2 inhibi- tors may correct splenomegaly and obviate the need for splenectomy and frequent blood transfusion, and with these effects management of the iron-loading anaemia is likely to be greatly improved. Ruxolitinib, a JAK2 inhibitor, has shown modest effects on spleen size in small phase 2 studies in patients with transfusion-dependent thalassaemia. Activins, TGFβ signalling, and hepcidin regulation Increased haemoglobin concentrations were a chance finding in ex- ploratory clinical studies of ACE-536, an activin receptor II ligand trap, conducted in healthy volunteers and women with postmenopausal osteoporosis. The observation led to a careful review of the role of activins in the development of the bone marrow and stimulated an examination of the haematological effects of these agents in disordered erythropoiesis. Two activin receptor II ligand traps (sotatercept and luspatercept) that block GDF-11, and ACE-536 (a recombinant pro- tein containing a modified activin receptor type IIB), are being inves- tigated for the treatment of

ineffective erythropoiesis in thalassaemia and myelodysplasia. ACE-536 promotes late-stage erythroid differen- tiation by binding to TGFβ superfamily ligands, thereby inhibiting sig- nalling through the SMAD2/3 transcription factors. The challenge Credible scientific advancement in this field is long overdue, but with continuing refinement of practice, the availability and experience of oral iron chelators, the outlook for patients with otherwise fatal iron- loading anaemias is improving. Most patients are located in resource- poor regions, but even where access to emerging molecular therapies is restricted, patients with limited capacity to pay for novel agents can look forward to increasingly competitive pricing of the second- generation oral iron-chelating drugs. The ultimate challenge will be to match longer survival with improvement in quality of life. FURTHER READING Anderson GJ, et al. (2005). Mechanisms of haem and non-haem iron absorption: lessons from inherited disorders of iron metabolism. Biometals, 18, 339–48. Anderson LJ, et al. (2004). Myocardial iron clearance during reversal of siderotic cardiomyopathy with intravenous desferrioxamine: a pro- spective study using T2* cardiovascular magnetic resonance. Br J Haematol, 127, 348–55. Andrews NC, Schmidt PJ (2007). Iron homeostasis. Annu Rev Physiol, 69, 69–85. Auerback M, Adamson JW (2016). How we diagnose and treat iron defi- ciency anemia. Am J Hematol, 91, 31–8. Babitt JL, et al. (2006). Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. Nat Genet, 38, 531–9. Bain BJ, Bates IM, Laffan MA (2016). Dacie and Lewis, Practical Haematology, 12th edition. Elsevier Health Sciences, London. Baksi AJ, Pennell DJ (2014). Randomized controlled trials of iron che- lators for the treatment of cardiac siderosis in thalassaemia major. Front Pharmacol, 5, 217. Benoist B, et al. (2008). Worldwide prevalence of anaemia 1993–2005. World Health Organization, Geneva. Borgna-Pignatti C, et al. (2004). Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica, 89, 1187–93. Bothwell T, et al. (1989). Nutritional iron requirements and good iron absorption. J Intern Med, 226, 357–65. Bowes O, et al. (2014). Hereditary hyperferritinaemia cataract syn- drome. Lancet, 383, 1520. Breda L, Rivella S (2014). Modulators of erythropoiesis: emerging therapies for hemoglobinopathies and disorders of red cell produc- tion. Hemato Oncol Clin North Am, 28, 375–86. Bryant BJ, et al. (2013). Ascertainment of iron deficiency and depletion in blood donors through screening questions for pica and restless legs syndrome. Transfusion, 53, 1637–44. Camaschella C, Nai A (2016). Ineffective erythropoiesis and regula- tion of iron status in iron loading anaemias. Br J Haematol, 172, 512–23. Cappellini MD, Motta I (2017). New therapeutic targets in transfusion- dependent and -independent thalassemia. Hematology, 2017, 278–3.

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