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912 • HAEMATOLOGY AND TRANSFUSION MEDICINE Clinical examination in blood disease Insets (Glossitis) From Hoffbrand VA, John E, Pettit JE, Vyas P. Color atlas of clinical hematology, 4th edn. Philadelphia: Mosby, Elsevier Inc.; 2010; (Petechiae) Young NS, Gerson SL, High KA (eds). Clinical hematology. St Louis: Mosby, Elsevier Inc.; 2006. Observation Hands Perfusion Telangiectasia Skin crease pallor Koilonychia Pulse Rate Mouth Lips: angular stomatitis, telangiectasia Gum hypertrophy Tongue: colour, smoothness Buccal mucosa: petechiae Tonsils: size Conjunctivae Pallor Jaundice Lymph nodes (see opposite) Abdomen Masses Ascites Hepatomegaly Splenomegaly Inguinal and femoral lymph nodes Feet Peripheral circulation Toes: gangrene Joints Deformity Swelling Restricted movement • General well-being • Colour: pallor, plethora • Breathlessness Urinalysis Blood Urobilinogen Hereditary haemorrhagic telangiectasia Fundi Hyperviscosity

Engorged veins Papilloedema Haemorrhage Fundal haemorrhage in thrombocytopenia
Purpura/petechiae in thrombocytopenia Gangrenous toe in thrombocytosis Swollen joint in
haemophilia Koilonychia in iron deficiency Skin Purpura Bruising Gum hypertrophy in acute myeloid
leukaemia Glossitis and angular stomatitis in iron deficiency

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Bleeding Bleeding can be due to congenital or acquired abnormalities in the clotting system. History and examination help to clarify the severity and the underlying cause of the bleeding problem. Abnormalities detected in the blood are caused not only by primary diseases of the blood and lymphoreticular systems but also by diseases affecting other systems of the body. The clinical assessment of patients with haematological abnormalities must include a general history and examination, as well as a search for symptoms and signs of abnormalities of red cells, white cells, platelets, haemostatic systems, lymph nodes and lymphoreticular tissues. Anaemia Symptoms and signs help to indicate the clinical severity of anaemia. A full history and examination is needed to identify the underlying cause. 6 Lymphadenopathy Lymphadenopathy can be caused by benign or malignant disease. The clinical points to clarify are shown in the box. Pre-auricular Parotid Submandibular Submental Posterior cervical Supraclavicular Anterior cervical Supraclavicular Axillary Epitrochlear Inguinal Femoral Popliteal fossa Lymphadenopathy History • Speed of onset, rate of enlargement • Painful or painless • Associated symptoms: weight loss, night sweats, itch Examination • Sites: localised, generalised • Size (cm) • Character: hard, soft, rubbery • Fixed, mobile • Search area that node drains for abnormalities (e.g. dental abscess) • Other general examination (e.g. joints, rashes, finger clubbing) Anaemia Non-specific symptoms • Tiredness • Lightheadedness • Breathlessness • Development/worsening of ischaemic symptoms, e.g. angina or claudication Non-specific signs • Mucous membrane pallor • Tachypnoea • Raised jugular venous pressure • Tachycardia • Flow murmurs • Ankle oedema • Postural hypotension Bleeding History • Site of bleed • Duration of bleed • Precipitating causes, including previous surgery or trauma • Family history • Drug history • Age at presentation • Other medical conditions, e.g. liver disease Examination There are two main patterns of bleeding:

1. Mucosal bleeding Reduced number or function of platelets (e.g. bone marrow failure or aspirin) or von Willebrand factor (e.g. von Willebrand disease) Skin: petechiae, bruises Gum and mucous membrane bleeding Fundal haemorrhage Post-surgical bleeding
2. Coagulation factor deficiency (e.g. haemophilia or warfarin/ anticoagulant) Bleeding into joints (haemarthrosis) or muscles Bleeding into soft tissues Retroperitoneal haemorrhage Intracranial haemorrhage Post-surgical bleeding 8 Examination of the spleen Characteristics of the spleen • Notch • Superficial • Dull to percussion • Cannot get examining hand between ribs and spleen • Moves well with respiration • Move your hand up from the right iliac fossa, towards the left upper quadrant on expiration. • Keep your hand still and ask the patient to take a deep breath through the mouth to feel the spleen edge being displaced downwards. • Place your left hand around the patient's lower ribs and approach the costal margin to pull the spleen forwards. • To help palpate small spleens, roll the patient on to the right side and examine as before.

914 • HAEMATOLOGY AND TRANSFUSION MEDICINE at times of increased demand. Haematopoietic cells interact closely with surrounding connective tissue stroma, made up of reticular cells,

macrophages, fat cells, blood vessels and nerve fibres (Fig. 23.1). In normal marrow, nests of red cell precursors cluster around a central macrophage, which provides iron and also phagocytoses nuclei from red cells prior to their release into the circulation. Megakaryocytes are large cells that produce and release platelets into vascular sinuses. White cell precursors are clustered next to the bone trabeculae; maturing cells migrate into the marrow spaces towards the vascular sinuses. Plasma cells are antibody-secreting mature B cells that normally represent less than 5% of the marrow population and are scattered throughout the intertrabecular spaces. Stem cells All blood cells are derived from pluripotent haematopoietic stem cells. These comprise only 0.01% of the total marrow cells, but they can self-renew (i.e. make more stem cells) or differentiate to produce a hierarchy of lineage-committed progenitor cells. The resulting primitive progenitor cells cannot be identified morphologically, so they are named according to the types of cell (or colony) they form during cell culture experiments. CFU-GM (colony-forming unit – granulocyte, monocyte) is a progenitor cell that produces granulocytic and monocytic lines, CFU-E produce erythroid cells, and CFU-Meg produce megakaryocytes and ultimately platelets (Fig. 23.2). Growth factors, produced in bone marrow stromal cells and elsewhere, control the survival, proliferation, differentiation and function of stem cells and their progeny. Some, such as, interleukin-3 (IL-3), stem cell factor (SCF) and granulocyte, macrophage-colony-stimulating factor (GM-CSF), act on a wide number of cell types at various stages of differentiation. Others, such as erythropoietin, granulocyte-colony-stimulating factor (G-CSF) and thrombopoietin (Tpo), are lineage-specific. Many of these growth factors are now synthesised by recombinant DNA technology and used as treatments: for example, erythropoietin to correct renal anaemia and G-CSF to hasten neutrophil recovery after chemotherapy. The bone marrow also contains stem cells that can differentiate into non-haematological cells. Mesenchymal stem cells differentiate Disorders of the blood cover a wide spectrum of illnesses, ranging from some of the most common disorders affecting humans (anaemias) to relatively rare conditions such as leukaemias and congenital coagulation disorders. Although the latter are uncommon, advances in cellular and molecular biology have had major impacts on their diagnosis, treatment and prognosis. Haematological changes occur as a consequence of diseases affecting any system and give important information in the diagnosis and monitoring of many conditions. Functional anatomy and physiology Blood flows throughout the body in the vascular system, and consists of: • red cells, which transport oxygen from the lungs to the tissues • white cells, which defend against infection • platelets, which interact with blood vessels and clotting factors to maintain vascular integrity and prevent bleeding • plasma, which contains proteins with many functions, including antibodies and coagulation factors. Haematopoiesis Haematopoiesis describes the formation of blood cells, an active process that must maintain normal numbers of circulating cells and be able to respond rapidly to increased demands such as bleeding or infection. During development, haematopoiesis occurs in the yolk sac, liver and spleen, and subsequently in red bone marrow in the medullary cavity of all bones. In childhood, red marrow is progressively replaced by fat (yellow marrow) so that, in adults, normal haematopoiesis is restricted to the vertebrae, pelvis, sternum, ribs, clavicles, skull, upper humeri and proximal femora. However, red marrow can expand in response to increased demands for blood cells. Bone marrow contains a range of immature haematopoietic precursor cells and a storage pool of mature cells for release Fig. 23.1 Structural organisation of normal bone marrow. Megakaryocyte Bony trabecula Neutrophil Erythroid 'nest' Vascular sinusoid Fat cell Myelocyte Blast cells and progenitor cells Lymphocyte

deformable, with a lipid bilayer to which a 'skeleton' of filamentous proteins is attached via special linkage proteins (Fig. 23.4). Inherited abnormalities of any of these proteins result in loss of membrane as cells pass through the spleen, and the formation of abnormally shaped red cells called spherocytes or elliptocytes (see Fig. 23.8D). Red cells are exposed to osmotic stress in the pulmonary and renal circulation; in order to maintain homeostasis, the membrane contains ion pumps, which control intracellular levels of sodium, potassium, chloride and bicarbonate. In the absence of mitochondria, the energy for these functions is provided by anaerobic glycolysis and the pentose phosphate pathway in the cytosol. Membrane glycoproteins inserted into the lipid bilayer also form the antigens recognised by blood grouping (see Fig. 23.4). The ABO and Rhesus systems are the most commonly recognised (p. 931) but over 400 blood group antigens have been described. Haemoglobin Haemoglobin is a protein specially adapted for oxygen transport. It is composed of four globin chains, each surrounding an iron-containing porphyrin pigment molecule termed haem. Globin chains are a combination of two alpha and two nonalpha chains; haemoglobin A ($\alpha\alpha/\beta\beta$) represents over 90% of adult haemoglobin, whereas haemoglobin F ($\alpha\alpha/\gamma\gamma$) is the predominant type in the fetus. Each haem molecule contains a ferrous ion (Fe^{2+}), to which oxygen reversibly binds; the affinity for oxygen increases as successive oxygen molecules bind. When oxygen is bound, the beta chains 'swing' closer together; they move apart as oxygen is lost. In the 'open' deoxygenated state, 2,3-bisphosphoglycerate (2,3-BPG), a product of red cell metabolism, binds to the haem molecule, reducing its affinity for oxygen. This is termed the Bohr effect and may have exciting clinical applications in the future (Ch. 3). Blood cells and their functions Red cells Red cell precursors formed in the bone marrow from the erythroid (CFU-E) progenitor cells are called erythroblasts or normoblasts (Fig. 23.3). These divide and acquire haemoglobin, which turns the cytoplasm pink; the nucleus condenses and is extruded from the cell. The first non-nucleated red cell is a reticulocyte, which still contains ribosomal material in the cytoplasm, giving these large cells a faint blue tinge ('polychromasia'). Reticulocytes lose their ribosomal material and mature over 3 days, during which time they are released into the circulation. Increased numbers of circulating reticulocytes (reticulocytosis) reflect increased erythropoiesis. Proliferation and differentiation of red cell precursors is stimulated by erythropoietin, a polypeptide hormone produced by renal interstitial peritubular cells in response to hypoxia. Failure of erythropoietin production in patients with renal failure (p. 384) causes anaemia, which can be treated with exogenous recombinant erythropoietin or similar pharmacological agents called erythropoiesis-stimulating agents, e.g. darbepoetin. Normal mature red cells circulate for about 120 days. They are 8 μm biconcave discs lacking a nucleus but filled with haemoglobin, which delivers oxygen to the tissues. In order to pass through the smallest capillaries, the red cell membrane is Fig. 23.2 Stem cells and growth factors in haematopoietic cell development. (BFU-E = burst-forming unit - erythroid; CFU-E = colony-forming unit - erythroid; CFU-GM = colony-forming unit - granulocyte, monocyte; CFU-Meg = colony-forming unit - megakaryocyte; Epo = erythropoietin; G-CSF = granulocyte-colony-stimulating factor; GM-CSF = granulocyte, macrophage-colony-stimulating factor; IL = interleukin; M-CSF = macrophage-colony-stimulating factor; SCF = stem cell factor; Tpo = thrombopoietin) GM - CSF, M - CSF Pluripotent stem cell IL-3, GM - CSF, SCF, IL-12 SCF IL-6 IL-11 Myeloid progenitor cell Lymphoid progenitor cell SCF IL-3 SCF IL-7 T cells B cells Monocytes Eosinophils Basophils Neutrophils Platelets Red cells IL-3 IL-3, GM - CSF, IL-6 IL-3, GM - CSF IL-3 Thymus Thymocyte Pre-B stem cell CFU - GM CFU - Meg BFU - E Epo Tpo CFU - E Megakaryoblast G - CSF IL-3, SCF GM - CSF, IL-5 IL-4, IL-7 IL-2, IL-4, IL-7

916 • HAEMATOLOGY AND TRANSFUSION MEDICINE Fig. 23.3 Maturation pathway of red cells, granulocytes and platelets. The image on the right is a normal blood film. Myeloblast Promyelocyte Myelocyte Metamyelocyte Neutrophil Pronormoblast Early normoblast Late normoblast Megakaryoblast Megakaryocyte Platelet Reticulocyte Red blood cell Fig. 23.4 Normal structure of red cell membrane. Red cell membrane flexibility is conferred by attachment of cytoskeletal proteins. Important transmembrane proteins include band 3 (an ion transport channel) and glycophorin C (involved in cytoskeletal attachment and gas exchange, and a receptor for *Plasmodium falciparum* in malaria). Antigens on the red blood cell determine an individual's blood group. There are about 22 blood group systems (groups of carbohydrate or protein antigens controlled by a single gene or by multiple closely linked loci); the most important clinically are the ABO and Rhesus (Rh) systems (p. 931). The ABO genetic locus has three main allelic forms: A, B and O. The A and B alleles encode glycosyltransferases that introduce N-acetylgalactosamine (open circle) and D-galactose (blue circle), respectively, on to antigenic carbohydrate molecules on the membrane surface. People with the O allele produce an O antigen, which lacks either of these added sugar groups. Rh antigens are transmembrane proteins. RhD antigen Blood group O antigen Blood group A antigen Blood group B antigen Alpha spectrin Beta spectrin Ankyrin Band 3 Protein 4.1 Adducin Glycophorin C Membrane 40% lipid 50% protein 10% carbohydrate Cytoskeleton metabolism, binds to the haemoglobin molecule and lowers its oxygen affinity. These complex interactions produce the sigmoid shape of the oxygen dissociation curve (Fig. 23.5). The position of this curve depends on the concentrations of 2,3-BPG, H⁺ ions and CO₂; increased levels shift the curve to the right and cause oxygen to be released more readily, e.g. when red cells reach hypoxic tissues. Haemoglobin F is unable to bind 2,3-BPG and has a left-shifted oxygen dissociation curve, which, together with the low pH of fetal blood, ensures fetal oxygenation. Strong oxidising agents, such as dapsone, can convert ferrous iron in haemoglobin to its ferric state (Fe³⁺). The resultant methaemoglobin also has a left-shifted oxygen dissociation curve, which can result in tissue hypoxia (p. 135). Genetic mutations affecting the haem-binding pockets of globin chains or the 'hinge' interactions between globin chains result in haemoglobinopathies or unstable haemoglobins. Alpha globin chains are produced by two genes on chromosome 16, and beta globin chains by a single gene on chromosome 11; imbalance in the production of globin chains results in the thalassaemias (p. 951). Defects in haem synthesis cause the porphyrias (p. 378).

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containing 2–5 segments and granules in their cytoplasm. Their main function is to recognise, ingest and destroy foreign particles and microorganisms (p. 64). A large storage pool of mature neutrophils exists in the bone marrow. Every day, some 10¹¹ neutrophils enter the circulation, where cells may be circulating freely or attached to endothelium in the marginating pool. These two pools are equal in size; factors such as exercise or catecholamines increase the number of cells flowing in the blood. Neutrophils spend 6–10 hours in the circulation before being removed, principally by the spleen. Alternatively, they pass into the tissues and either are consumed in the inflammatory process or undergo apoptotic cell death and phagocytosis by macrophages. Eosinophils Eosinophils represent 1–6% of the circulating white cells. They are a similar size to neutrophils but have a bilobed nucleus and prominent orange granules on Romanowsky staining. Eosinophils are phagocytic and their granules contain a peroxidase capable of generating reactive oxygen species and proteins involved in the intracellular killing of protozoa and helminths (p. 233). They are also involved in allergic reactions (e.g. atopic asthma, p. 567; see also p. 84). Basophils

These cells are less common than eosinophils, representing less than 1% of circulating white cells. They contain dense black granules that obscure the nucleus. Mast cells resemble basophils but are found only in the tissues. These cells are involved in hypersensitivity reactions (p. 66).

Monocytes Monocytes are the largest of the white cells, with a diameter of 12–20 μm and an irregular nucleus in abundant pale blue cytoplasm containing occasional cytoplasmic vacuoles. These cells circulate for a few hours and then migrate into tissue, where they become macrophages, Kupffer cells or antigen-presenting dendritic cells. The former phagocytose debris, apoptotic cells and microorganisms (see Box 4.1, p. 64).

Lymphocytes Lymphocytes are derived from pluripotent haematopoietic stem cells in the bone marrow. There are two main types: T cells (which mediate cellular immunity) and B cells (which mediate humoral immunity) (p. 68). Lymphoid cells that migrate to the thymus develop into T cells, whereas B cells develop in the bone marrow. The majority (about 80%) of lymphocytes in the circulation are T cells. Lymphocytes are heterogeneous, the smallest being the size of red cells and the largest the size of neutrophils. Small lymphocytes are circular with scanty cytoplasm but the larger cells are more irregular with abundant blue cytoplasm. Lymphocyte subpopulations have specific functions and lifespan can vary from a few days to many years. Cell surface antigens ('cluster of differentiation' (CD) antigens), which appear at different points of lymphocyte maturation and indicate the lineage and maturity of the cell, are used to classify lymphomas and lymphoid leukaemias.

Haemostasis Blood must be maintained in a fluid state in order to function as a transport system, but must be able to solidify to form a clot following vascular injury in order to prevent excessive bleeding, a process known as haemostasis. Successful haemostasis

Destruction Red cells at the end of their lifespan of approximately 120 days are phagocytosed by the reticulo-endothelial system. Amino acids from globin chains are recycled and iron is removed from haem for reuse in haemoglobin synthesis. The remnant haem structure is degraded to bilirubin and conjugated with glucuronic acid before being excreted in bile. In the small bowel, bilirubin is converted to stercobilin; most of this is excreted, but a small amount is reabsorbed and excreted by the kidney as urobilinogen. Increased red cell destruction due to haemolysis or ineffective haematopoiesis results in jaundice and increased urinary urobilinogen. Free intravascular haemoglobin is toxic and is normally bound by haptoglobins, which are plasma proteins produced by the liver.

White cells White cells or leucocytes in the blood consist of granulocytes (neutrophils, eosinophils and basophils), monocytes and lymphocytes (see Fig. 23.12). Granulocytes and monocytes are formed from bone marrow CFU-GM progenitor cells during myelopoiesis. The first recognisable granulocyte in the marrow is the myeloblast, a large cell with a small amount of basophilic cytoplasm and a primitive nucleus with open chromatin and nucleoli. As the cells divide and mature, the nucleus segments and the cytoplasm acquires specific neutrophilic, eosinophilic or basophilic granules (see Fig. 23.3). This takes about 14 days. The cytokines G-CSF, GM-CSF and M-CSF are involved in the production of myeloid cells, and G-CSF can be used clinically to hasten recovery of blood neutrophil counts after chemotherapy. Myelocytes or metamyelocytes are normally found only in the marrow but may appear in the circulation in infection or toxic states. The appearance of more primitive myeloid precursors in the blood is often associated with the presence of nucleated red cells and is termed a 'leucoerythroblastic' picture; this indicates a serious disturbance of marrow function.

Neutrophils Neutrophils, the most common white blood cells in the blood of adults, are 10–14 μm in diameter, with a multilobular nucleus Fig. 23.5

The haemoglobin-oxygen dissociation curve. Factors are listed that shift the curve to the right (more oxygen released from blood) and to the left (less oxygen released) at given PO_2 . To convert kPa to mmHg, multiply by 7.5. (2,3-BPG = 2,3-bisphosphoglycerate)

PO_2 (kPa or mmHg)	Haemoglobin saturation (SO_2) %
10.7 (80)	100
9.3 (70)	90
8.0 (60)	75
6.7 (50)	50
5.3 (40)	30
4.0 (30)	15
3.3 (25)	10
2.7 (20)	5
2.0 (15)	0

Normal arterial PO₂ Normal venous PO₂ 2,3-BPG H⁺ CO₂ Temperature Shift to left 2,3-BPG H⁺ CO₂ Temperature Shift to right

918 • HAEMATOLOGY AND TRANSFUSION MEDICINE Fig. 23.6 The stages of normal haemostasis. A Stage 1. Pre-injury conditions encourage flow. The vascular endothelium produces substances (including nitric oxide, prostacyclin and heparans) to prevent adhesion of platelets and white cells to the vessel wall. Platelets and coagulation factors circulate in a non-activated state. B Stage 2. Early haemostatic response: platelets adhere; coagulation is activated. At the site of injury, the endothelium is breached, exposing subendothelial collagen. Small amounts of tissue factor (TF) are released. Platelets bind to collagen via a specific receptor, glycoprotein Ia (GPIa), causing a change in platelet shape and its adhesion to the area of damage by the binding of other receptors (GPIb and GPIIb/IIIa) to von Willebrand factor and fibrinogen, respectively. Coagulation is activated by the tissue factor (extrinsic) pathway, generating small amounts of thrombin. C and D Stage 3. Fibrin clot formation: platelets become activated and aggregate; fibrin formation is supported by the platelet membrane; stable fibrin clot forms. The adherent platelets are activated by many pathways, including binding of adenosine diphosphate (ADP), collagen, thrombin and adrenaline (epinephrine) to surface receptors. The cyclo-oxygenase pathway converts arachidonic acid from the platelet membrane into thromboxane A₂, which causes aggregation of platelets. Activation of the platelets results in release of the platelet granule contents, enhancing coagulation further (see Fig. 23.7). Thrombin plays a key role in the control of coagulation: the small amount generated via the TF pathway massively amplifies its own production; the 'intrinsic' pathway becomes activated and large amounts of thrombin are generated. Thrombin directly causes clot formation by cleaving fibrinopeptides (FPs) from A B Thrombin Vascular endothelium Heparans Red cell Nitric oxide Prostacyclin Platelet Activated platelet Tissue factor GPIIb/IIIa binds fibrinogen GPIa binds collagen GPIb binds von Willebrand factor Coagulation activation by tissue factor pathway Subendothelial collagen A B C A B A B Thrombin Thrombin receptor Platelet activation Inhibition of fibrinolysis Clot stabilisation Cleavage of fibrinogen TAFI XIII FPs XIIIa TAFIa Intrinsic pathway Activation of protein C pathway Activation of tissue factor pathway Tissue factor is localised to the area of tissue damage and is followed by removal of the clot and tissue repair. This is achieved by complex interactions between the vascular endothelium, platelets, von Willebrand factor, coagulation factors, natural anticoagulants and fibrinolytic enzymes (Fig. 23.6). Dysfunction of any of these components may result in haemorrhage or thrombosis. Platelets Platelets are formed in the bone marrow from megakaryocytes. Megakaryocytic progenitor cells (CFU-Meg) divide to form megakaryoblasts, which undergo a process called 'endomitotic reduplication', in which there is division of the nucleus but not the cell. This creates mature megakaryocytes, large cells with several nuclei and cytoplasm containing platelet granules. Large numbers of platelets then fragment off from each megakaryocyte into the circulation. The formation and maturation of megakaryocytes is stimulated by thrombopoietin produced in the liver. Platelets circulate for 8–10 days before they are destroyed in the reticuloendothelial system. Some 30% of peripheral platelets are normally pooled in the spleen and do not circulate. Under normal conditions, platelets are discoid, with a diameter of 2–4 µm (Fig. 23.7). The surface membrane invaginates to form a tubular network, the canalicular system, which provides a conduit for the discharge of the granule content following platelet activation. Drugs that inhibit platelet function and thrombosis include aspirin (cyclo-oxygenase inhibitor), clopidogrel, prasugrel and ticagrelor (adenosine diphosphate (ADP)-mediated activation

inhibitors), dipyridamole (phosphodiesterase inhibitor), and the glycoprotein IIb/IIIa inhibitors abciximab, tirofiban and eptifibatide (which prevent fibrinogen binding; p. 500). Clotting factors The coagulation system consists of a cascade of soluble inactive zymogen proteins designated by Roman numerals.

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Investigation of diseases of the blood The full blood count To obtain a full blood count (FBC), anticoagulated blood is processed through automated blood analysers that use a variety of technologies (particle-sizing, radiofrequency and laser instrumentation) to measure the haematological parameters. These include numbers of circulating cells, the proportion of whole blood volume occupied by red cells (the haematocrit, Hct), and the red cell indices that give information about the size of red cells (mean cell volume, MCV) and the amount of haemoglobin present in the red cells (mean cell haemoglobin, MCH). Blood analysers can differentiate types of white blood cell and give automated counts of neutrophils, lymphocytes, monocytes, eosinophils and basophils. It is important to appreciate, however, that a When proteolytically cleaved and activated, each is capable of activating one or more components of the cascade. Activated factors are designated by the suffix 'a'. Some of these reactions require phospholipid and calcium. Coagulation occurs by two pathways: it is initiated by the extrinsic (or tissue factor) pathway and amplified by the 'intrinsic pathway' (see Fig. 23.6D). Clotting factors are synthesised by the liver, although factor V is also produced by platelets and endothelial cells. Factors II, VII, IX and X require post-translational carboxylation to allow them to participate in coagulation. The carboxylase enzyme responsible for this in the liver is vitamin K-dependent. Vitamin K is converted to an epoxide in this reaction and must be reduced to its active form by a reductase enzyme. This reductase is inhibited by warfarin, and this is the basis of the anticoagulant effect of coumarins (p. 939). Congenital (e.g. haemophilia) and acquired (e.g. liver failure) causes of coagulation factor deficiency are associated with bleeding. Tissue factor X Xa Va VIIIa –ve –ve –ve –ve –ve –ve Thrombin Antithrombin Actions of thrombin Intrinsic pathway Activated protein C, protein S Plasmin Inhibitors of plasmin Inhibitors of plasminogen activators Activators of plasminogen Plasminogen t-PA Urokinase Fibrin degradation products (FDP) PAI-1, PAI-2 Tissue factor pathway Natural anticoagulant actions Tissue factor pathway inhibitor (TFPI) Tissue factor E D F Tissue factor (extrinsic) pathway Common pathway Tissue injury Tissue factor VII VIIa X Xa Va V Prothrombin Prothrombin Thrombin Amplification of coagulation by thrombin Intrinsic pathway XI XIa IXa IX VIIIa VIII –ve –ve –ve TAFI α 2-antiplasmin α 2-macroglobulin fibrinogen to produce fibrin. Fibrin monomers are cross-linked by factor XIII, which is also activated by thrombin. Having had a key role in clot formation and stabilisation, thrombin then starts to regulate clot formation in two main ways: (a) activation of the protein C (PC) pathway (a natural anticoagulant), which reduces further coagulation; (b) activation of thrombin-activatable fibrinolysis inhibitor (TAFI), which inhibits fibrinolysis (see E and F). E Stage 4. Limiting clot formation: natural anticoagulants reverse activation of coagulation factors. Once haemostasis has been secured, the propagation of clot is curtailed by anticoagulants. Antithrombin is a serine protease inhibitor synthesised by the liver, which destroys activated factors such as XIa, Xa and thrombin (IIa). Its major activity against thrombin and Xa is enhanced by heparin and fondaparinux, explaining their anticoagulant effect. Tissue factor pathway inhibitor (TFPI) binds to and inactivates VIIa and Xa. Activation of PC occurs following binding of thrombin to membranebound thrombomodulin; activated protein C (aPC) binds to its co-factor, protein S (PS), and cleaves Va and VIIIa. PC and PS are vitamin K-dependent and

are depleted by coumarin anticoagulants such as warfarin. F Stage 5. Fibrinolysis: plasmin degrades fibrin to allow vessel recanalisation and tissue repair. The insoluble clot needs to be broken down for vessel recanalisation. Plasmin, the main fibrinolytic enzyme, is produced when plasminogen is activated, e.g. by tissue plasminogen activator (t-PA) or urokinase in the clot. Plasmin hydrolyses the fibrin clot, producing fibrin degradation products, including the D-dimer. This process is highly regulated; the plasminogen activators are controlled by an inhibitor called plasminogen activator inhibitor (PAI), the activity of plasmin is inhibited by α 2-antiplasmin and α 2-macroglobulin, and fibrinolysis is further inhibited by the thrombin-activated TAFI. Fig. 23.6, cont'd

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23.1 Spurious full blood count results from autoanalysers

Result	Explanation
Increased haemoglobin	Lipaemia, jaundice, very high white cell count
Reduced haemoglobin	Improper sample mixing, blood taken from vein into which an infusion is flowing
Increased red cell volume (mean cell volume, MCV)	Cold agglutinins, non-ketotic hyperosmolarity
Increased white cell count	Nucleated red cells present
Reduced platelet count	Clot in sample, platelet clumping

Fig. 23.7 Normal platelet structure. The platelet surface is populated by glycoproteins, which bind to key structures including fibrinogen, collagen and von Willebrand factor and cell surface receptors for thrombin, ADP and adrenaline (epinephrine). Through internal signalling pathways, platelet activation causes degranulation of alpha and dense granules, which ultimately results in platelet aggregation. Blockade of these pathways by drugs such as aspirin, clopidogrel, ticagrelor, tirofiban and abciximab forms the basis of antiplatelet therapy. (ADP = adenosine diphosphate; GP = glycoprotein)

Cell surface receptors

Platelet glycoproteins	ADP
Mitochondrion	Dense tubule
Alpha granule	Dense granule
Lysosome	Actin and myosin filaments
Glycocalyx membrane	Open canalicular system
GPIIb/IIIa	GPIb/V/IX
GPIa/IIa	GPVI
Adrenaline (epinephrine)	Thrombin

number of conditions can lead to spurious results (Box 23.1). The reference ranges for a number of common haematological parameters in adults are given in Chapter 35.

Blood film examination Although technical advances in full blood count analysers have resulted in fewer blood samples requiring manual examination, scrutiny of blood components prepared on a microscope slide (the 'blood film') can often yield valuable information (Box 23.2 and Fig. 23.8). Analysers cannot identify abnormalities of red cell shape and content (e.g. Howell-Jolly bodies, basophilic stippling, malaria parasites) or fully define abnormal white cells such as blasts.

Bone marrow examination In adults, bone marrow for examination is usually obtained from the posterior iliac crest. After a local anaesthetic, marrow can be sucked out from the medullary space, stained and examined under the microscope (bone marrow aspirate). In addition, a core of bone may be removed (trephine biopsy), fixed and decalcified before sections are cut for staining (Fig. 23.9). A bone marrow aspirate is used to assess the composition and morphology of haematopoietic cells or abnormal infiltrates. Further investigations may be performed, such as cell surface marker analysis (immunophenotyping), chromosome and molecular studies to assess malignant disease, or marrow culture for suspected tuberculosis. A trephine biopsy is superior for assessing marrow cellularity, marrow fibrosis, and infiltration by abnormal cells such as metastatic carcinoma.

Investigation of coagulation

Bleeding disorders In patients with clinical evidence of a bleeding disorder (p. 913), there are recommended screening tests (Box 23.3). Physiological activation of coagulation is predominantly by tissue factor, with amplification of the process by the small amounts of thrombin formed as a result. For ease of description, the terms extrinsic, intrinsic and common pathways are still used (see Fig. 23.6D). Coagulation tests measure the time to clot formation in vitro in a plasma sample after the clotting process is initiated by activators and calcium. The result of the test sample is compared with normal controls. The tissue factor ('extrinsic') pathway (see Fig. 23.6D) is

assessed by the prothrombin time (PT), and the 'intrinsic' pathway by the activated partial thromboplastin time (APTT), sometimes known as the partial thromboplastin time with kaolin (PTTK). Coagulation is delayed by deficiencies of coagulation factors and by the presence of inhibitors of coagulation, such as heparin. The approximate reference ranges and causes of abnormalities are shown in Box 23.3. If both the PT and APTT are prolonged, this indicates either deficiency or inhibition of the

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Platelet function has historically been assessed by the bleeding time, measured as the time to stop bleeding after a standardised incision. However, most centres have abandoned the use of this test. Platelet function can be assessed in vitro by measuring aggregation in response to various agonists, such as adrenaline (epinephrine), collagen, thrombin, arachidonic acid and ADP, agglutination in response to ristocetin or by measuring the constituents of the intracellular granules, e.g. adenosine triphosphate, adenosine diphosphate and their ratio to each other (ATP/ADP). Coagulation screening tests are also performed in patients with suspected DIC, when clotting factors and platelets are consumed, resulting in thrombocytopenia and prolonged PT and APTT. In addition, there is evidence of active coagulation with final common pathway (which includes factors X, V, prothrombin and fibrinogen) or global coagulation factor deficiency involving more than one factor, as occurs in disseminated intravascular coagulation (DIC, pp. 196 and 978). Further specific tests may be performed based on interpretation of the clinical scenario and results of these screening tests. A mixing test with normal plasma allows differentiation between a coagulation factor deficiency (the prolonged time corrects) and the presence of an inhibitor of coagulation (the prolonged time does not correct); the latter may be a chemical (heparins) or an antibody (most often a lupus anticoagulant but occasionally a specific inhibitor of one of the coagulation factors, typically factor VIII). Von Willebrand disease may present with a normal APTT; further investigation of suspected cases is detailed on page 974. Microcytosis (reduced average cell size, MCV < 76 fL) A • Iron deficiency • Thalassaemia • Sideroblastic anaemia Macrocytosis (increased average cell size, MCV > 100 fL) B • Vitamin B12 or folate deficiency • Liver disease, alcohol • Hypothyroidism • Myelodysplastic syndromes • Drugs (e.g. zidovudine, trimethoprim, phenytoin, methotrexate, hydroxycarbamide) Target cells (central area of haemoglobinisation) C • Liver disease • Thalassaemia • Post-splenectomy • Haemoglobin C disease Spherocytes (dense cells, no area of central pallor) D • Autoimmune haemolytic anaemia • Post-splenectomy • Hereditary spherocytosis Red cell fragments (intravascular haemolysis) E • Microangiopathic haemolysis, e.g. haemolytic uraemic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP) • Disseminated intravascular coagulation (DIC) Nucleated red blood cells (normoblasts) F • Marrow infiltration • Severe haemolysis • Myelofibrosis • Acute haemorrhage Howell-Jolly bodies (small round nuclear remnants) G • Hyposplenism • Post-splenectomy • Dyshaematopoiesis Polychromasia (young red cells – reticulocytes present) H • Haemolysis, acute haemorrhage • Increased red cell turnover Basophilic stippling (abnormal ribosomal RNA appears as blue dots) I • Dyshaematopoiesis • Lead poisoning Fig. 23.8 Appearance of red blood cells. A Microcytosis. B Macrocytosis. C Target cells. D Spherocytes. E Red cell fragments. F Nucleated red blood cells. G Howell-Jolly bodies. H Polychromasia. I Basophilic stippling. A B C D E F G H I 23.2 How to interpret red cell appearances

922 • HAEMATOLOGY AND TRANSFUSION MEDICINE warfarin. INR is the ratio of the patient's PT to that of a normal control, raised to the power of the international sensitivity index of the thromboplastin used in the test (ISI, derived by comparison with an international reference standard material). Concentrations of the direct oral anticoagulants (DOACs) cannot be accurately assessed from the PT or the APTT, with which they have a variable and generally poor correlation. Monitoring of heparin therapy is, on the whole, required only with unfractionated heparins. Therapeutic anticoagulation prolongs the APTT relative to a control sample by a ratio of approximately 1.5–2.5. Low-molecular-weight heparins have such a predictable dose response that monitoring of the anticoagulant effect is not required, except in patients with renal impairment (glomerular filtration rate less than 30 mL/min). When monitoring is indicated, an anti-Xa activity assay rather than APTT should be used. Thrombotic disorders Measurement of plasma levels of D-dimers derived from fibrin degradation is useful in excluding the diagnosis of active venous thrombosis in some patients (see Fig. 10.6, p. 187). A variety of tests exist that may help to explain an underlying propensity to thrombosis, especially venous thromboembolism (thrombophilia) (Box 23.4). Examples of possible indications for testing are given in Box 23.5. In most patients, the results do not affect clinical management (p. 975) but they may influence the duration of anticoagulation (e.g. antiphospholipid antibodies, p. 977), justify family screening in inherited thrombophilias (p. 975), or suggest additional management strategies to reduce thrombosis risk (e.g. in myeloproliferative disease and paroxysmal nocturnal haemoglobinuria; p. 950). Anticoagulants can interfere with some of these assays; for example, warfarin reduces protein C and S levels and affects measurement of lupus anticoagulant, while heparin interferes with antithrombin and lupus anticoagulant Fig. 23.9 Bone marrow aspirate and trephine. A Trephine biopsy needle. B Macroscopic appearance of a trephine biopsy. C Microscopic appearance of stained section of trephine. D Bone marrow aspirate needle. E Stained macroscopic appearance of marrow aspirate: smear (left) and squash (right). F Microscopic appearance of stained marrow particles and trails of haematopoietic cells. C B D E F A 23.3 Coagulation screening tests¹

Investigation	Reference range ²	Situations in which tests may be abnormal
Platelet count	150–400 × 10 ⁹ /L	Thrombocytopenia
Prothrombin time (PT)	9–12 secs	Deficiencies of factors II, V, VII or X
Activated partial thromboplastin time (APTT)	26–36 secs	Deficiencies of factors II, V, VIII, IX, X, XI, XII Severe fibrinogen deficiency
Unfractionated heparin therapy		Antibodies against clotting factors
Lupus anticoagulant		Multiple factor deficiency (e.g. DIC)
Fibrinogen concentration	1.5–4.0 g/L	Hypofibrinogenaemia, e.g. liver failure, DIC

1N.B. International normalised ratio (INR) is used only to monitor coumarin therapy and is not a coagulation screening test. 2Ranges are approximate and may vary between laboratories. (DIC = disseminated intravascular coagulation) consumption of fibrinogen and generation of fibrin degradation products (D-dimers). Note, however, that fibrinogen is an acute phase protein that may also be elevated in inflammatory disease (p. 70). Monitoring anticoagulant therapy The international normalised ratio (INR) is validated only to assess the therapeutic effect of coumarin anticoagulants, including

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and sex. Other factors, including pregnancy and altitude, also affect haemoglobin levels and must be taken into account when considering whether an individual is anaemic. The clinical features of anaemia reflect diminished oxygen supply to the tissues (p. 912). A rapid onset of anaemia (e.g. due to blood loss) causes more profound symptoms than a gradually developing anaemia. Individuals with cardiorespiratory disease are more susceptible to symptoms of anaemia. The

clinical assessment and investigation of anaemia should gauge its severity and define the underlying cause (Box 23.7). Clinical assessment • Iron deficiency anaemia (p. 940) is the most common type of anaemia worldwide. A thorough gastrointestinal history is important, looking in particular for symptoms of blood loss. Menorrhagia is a common cause of anaemia in pre-menopausal females, so women should always be asked about their periods. • A dietary history should assess the intake of iron and folate, which may become deficient in comparison to needs (e.g. in pregnancy or during periods of rapid growth; pp. 712, 945 and 1284). • Past medical history may reveal a disease that is known to be associated with anaemia, such as rheumatoid arthritis (anaemia of chronic disease), or previous surgery (e.g. resection of the stomach or small bowel, which may lead to malabsorption of iron and/or vitamin B12). • Family history and ethnic background may raise suspicion of haemolytic anaemias, such as the haemoglobinopathies and hereditary spherocytosis. Pernicious anaemia may also run in families but is not associated with a clear Mendelian pattern of inheritance. • A drug history may reveal the ingestion of drugs that cause blood loss (e.g. aspirin and anti-inflammatory drugs), haemolysis (e.g. sulphonamides) or aplasia (e.g. chloramphenicol). On examination, as well as the general physical findings of anaemia shown on page 912, there may be specific findings related to the aetiology of the anaemia; for example, a patient may be found to have a right iliac fossa mass due to an underlying caecal carcinoma. Haemolytic anaemias can cause jaundice. Vitamin B12 deficiency may be associated with neurological signs, including peripheral neuropathy, dementia and signs of subacute combined degeneration of the cord (p. 1138). Sickle-cell anaemia (p. 951) may result in leg ulcers, stroke or features of pulmonary hypertension. Anaemia may be multifactorial and the lack of specific symptoms and signs does not rule out silent pathology. Investigations Schemes for the investigation of anaemias are often based on the size of the red cells, which is most accurately indicated by the MCV in the FBC. Commonly, in the presence of anaemia: assays. Therefore these tests, when required, should be performed when the patient is not taking anticoagulants.

Presenting problems in blood disease Anaemia Anaemia refers to a state in which the level of haemoglobin in the blood is below the reference range appropriate for age 23.4 Investigation of possible thrombophilia Full blood count Plasma levels • Antithrombin • Protein C • Protein S (free) • Antiphospholipid antibodies, lupus anticoagulant, anticardiolipin antibody/anti-β2GP1 Thrombin/reptilase time (for dysfibrinogenaemia) Genetic testing • Factor V Leiden • Prothrombin G20210A • JAK-2 V617F mutation • CALR mutations Flow cytometry • Screen for GPI-linked cell surface proteins (CD14, 16, 55, 59), deficient in paroxysmal nocturnal haemoglobinuria (CD = cluster of differentiation; GP1 = glycoprotein 1; GPI = glycerol phosphatidyl inositol) 23.5 Possible indications for thrombophilia testing* • Venous thrombosis < 45 years • Recurrent venous thrombosis • Family history of unprovoked or recurrent thrombosis • Combined arterial and venous thrombosis • Venous thrombosis at an unusual site: Cerebral venous thrombosis Hepatic vein (Budd–Chiari syndrome) Portal vein, mesenteric vein *Antiphospholipid antibodies should be sought where clinical criteria for antiphospholipid syndrome (APS) are fulfilled (p. 977). Thrombophilia testing may explain the diagnosis without necessarily affecting management and this limits the clinical value of such an approach. 23.6 Haematological investigations in old age • Blood cell counts and film components: not altered in general by ageing alone, although haemoglobin concentrations fall with increasing age. • Ratio of bone marrow cells to marrow fat: falls. • Neutrophils: maintained throughout life, although leucocytes may be less readily mobilised by bacterial invasion in old age. • Lymphocytes: functionally compromised by age due to a T-cell-related defect in cell-mediated immunity. • Clotting factors: no major changes, although mild congenital deficiencies may be first noticed in old age. • Erythrocyte sedimentation rate (ESR):

raised above the reference range but usually in association with chronic or subacute disease. In truly healthy older people, the ESR range is very similar to that in younger people.

23.7 Causes of anaemia

- Decreased or ineffective marrow production
- Lack of iron, vitamin B12 or folate
- Hypoplasia/myelodysplasia
- Invasion by malignant cells
- Renal failure
- Anaemia of chronic disease
- Normal marrow production but increased removal of cells
- Blood loss
- Haemolysis
- Hypersplenism

924 • HAEMATOLOGY AND TRANSFUSION MEDICINE Fig. 23.10 Investigation of anaemia with normal or low mean cell volume (MCV). (Hb = haemoglobin; MCH = mean cell haemoglobin) MCV normal (76–100 fL) or low (< 76 fL) Blood film and reticulocyte count High reticulocyte count Normal or low reticulocyte count Hypochromia (low MCH) Target cells basophilic stippling Hb electrophoresis Increased HbA2 Normal HbA2 Dimorphic Bone marrow Ferritin Consider ferritin Non-specific If Hb < 80 g/L consider bone marrow to establish diagnosis ? Anaemia of chronic disease No obvious cause ? Bleeding ? Haemolysis Normal or high ? Sideroblastic Low Fe deficient Investigate Check family Betathalassaemia trait Alphathalassaemia trait Fig. 23.11 Investigation of anaemia with high mean cell volume (MCV). (LDH = lactate dehydrogenase) ? Bleeding ? Haemolysis Polychromasia/high reticulocyte count MCV high (> 100 fL) Blood film ± reticulocyte count Clinical clues Alcohol, liver disease, family history of pernicious anaemia, hypothyroidism, drugs, previous abdominal surgery etc. Drugs/cytotoxic agents Investigate cause Liver function tests Hypersegmented neutrophils Target cells, stomatocytes Dysplasia/ cytopenia Dimorphic Marrow Marrow Folate, B12 ? Myelodysplasia ? Sideroblastic anaemia Bilirubin ↑ LDH ↑ Spherocytes Fragments +ve Coombs test Low

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also have aquagenic pruritus (itching after exposure to water), hepatosplenomegaly and gout (due to high red cell turnover). If the JAK-2 mutation is absent and there is no obvious secondary cause, a measurement of red cell mass is required to confirm an absolute erythrocytosis, followed by further investigations to exclude hypoxia, and causes of inappropriate erythropoietin secretion.

Leucopenia (low white cell count) A reduction in the total numbers of circulating white cells is called leucopenia. This may be due to a reduction in all types of white cell or in individual cell types (usually neutrophils or lymphocytes). Leucopenia may occur in isolation or as part of a reduction in all three haematological lineages (pancytopenia; p. 930).

Neutropenia A reduction in neutrophil count (usually < $1.5 \times 10^9/L$ but dependent on age and race) is called neutropenia. The main causes are listed in Box 23.9 and Figure 23.12. Drug-induced neutropenia is not uncommon (Box 23.10). Clinical manifestations range from no symptoms to overwhelming sepsis. The risk of bacterial infection is related to the degree of neutropenia, with counts lower than $0.5 \times 10^9/L$ considered to be critically low. Fever is the first and often only manifestation of infection. A sore throat, perianal pain or skin inflammation may be present. The lack of neutrophils allows the patient to become septicaemic and shocked within hours if immediate antibiotic therapy is not commenced. Management is discussed on page 224.

Lymphopenia This is an absolute lymphocyte count of less than $1 \times 10^9/L$. The causes are shown in Box 23.9. Although minor reductions may be asymptomatic, deficiencies in cell-mediated immunity may result in infections (with organisms such as fungi, viruses and mycobacteria) and a propensity to lymphoid and other malignancies (particularly those associated with viral infections such as Epstein-Barr virus (EBV), human papillomavirus (HPV))

23.8 Classification and causes of erythrocytosis Absolute erythrocytosis

Relative (low-volume) erythrocytosis Haematocrit High High Red cell mass High Normal Plasma volume Normal Low Causes Primary Myeloproliferative disorder Polycythaemia rubra vera (primary proliferative polycythaemia) Secondary High erythropoietin due to tissue hypoxia: High altitude Cardiorespiratory disease High-affinity haemoglobins Inappropriately increased erythropoietin: Renal disease (hydronephrosis, cysts, carcinoma) Other tumours (hepatoma, bronchogenic carcinoma, uterine fibroids, phaeochromocytoma, cerebellar haemangioblastoma) Exogenous testosterone therapy Exogenous erythropoietin administration: Performance-enhancing drug-taking in athletes Diuretics Smoking Obesity Alcohol excess Gaisböck's syndrome • A normal MCV (normocytic anaemia) suggests either acute blood loss or the anaemia of chronic disease, also known as the anaemia of inflammation (ACD/AI) (Fig. 23.10). • A low MCV (microcytic anaemia) suggests iron deficiency or thalassaemia or sometimes ACD/AI (Fig. 23.10). • A high MCV (macrocytic anaemia) suggests vitamin B12 or folate deficiency or myelodysplasia (Fig. 23.11). Specific types of anaemia and their management are described later in this chapter (p. 940). High haemoglobin Patients with a persistently raised haematocrit (Hct) (> 0.52 males,

0.48 females) for more than 2 months should be investigated. 'True' polycythaemia (or absolute erythrocytosis) indicates an excess of red cells, while 'relative', 'apparent' or 'low-volume' polycythaemia is due to a decreased plasma volume. Causes of polycythaemia are shown in Box 23.8. These involve increased erythropoiesis in the bone marrow, either due to a primary increase in marrow activity, or in response to increased erythropoietin (Epo) levels in chronic hypoxaemia, or due to inappropriate secretion of Epo. Athletes who seek to benefit from increased oxygen-carrying capacity have been known to use Epo to achieve this. Apparent erythrocytosis with a raised Hct, normal red cell mass (RCM) and reduced plasma volume may be associated with hypertension, smoking, alcohol and diuretic use (Gaisböck's syndrome). Clinical assessment and investigations Males and females with Hct values of over 0.60 and over 0.56, respectively, can be assumed to have an absolute erythrocytosis. A clinical history and examination will identify most patients with polycythaemia secondary to hypoxia. The presence of hypertension, smoking, excess alcohol consumption and/or diuretic use is consistent with low-volume polycythaemia (Gaisböck's syndrome). In polycythaemia rubra vera (PRV), a mutation in a kinase, JAK-2 V617F, is found in over 90% of cases (p. 970). Patients with PRV have an increased risk of arterial thromboses, particularly stroke, and venous thromboembolism. They may

926 • HAEMATOLOGY AND TRANSFUSION MEDICINE and human herpesvirus 8 (HHV-8)).

Lymphopenia without any obvious cause is common with advancing age. Leucocytosis (high white cell count) An increase in the total numbers of circulating white cells is called leucocytosis. This is usually due to an increase in a specific type of cell (see Box 23.9). It is important to realise that an increase in a single type of white cell (e.g. eosinophils or monocytes) may not increase the total white cell count (WCC) above the upper limit of normal and will be apparent only if the 'differential' of the white count is examined. Neutrophilia An increase in the number of circulating neutrophils is called a neutrophilia or a neutrophil leucocytosis. It can result from an increased production of cells

from the bone marrow or redistribution from the marginated pool. The normal neutrophil count depends on age, race and certain physiological parameters. During pregnancy, not only is there an increase in neutrophils but also earlier forms, such as metamyelocytes, can be found in the blood. The causes of a neutrophilia are shown in Box 23.9. *Many drugs can induce cytopenias. In suspected cases check drug summary of product characteristics.*

23.10 Drugs that can induce neutropenia

Group Examples

Analgesics/antiinflammatory agents Gold, penicillamine, naproxen

Antithyroid drugs Carbimazole, propylthiouracil

Anti-arrhythmics Quinidine, procainamide

Antihypertensives Captopril, enalapril, nifedipine

Antidepressants/ psychotropics Amitriptyline, dosulepin, mianserin

Antimalarials Pyrimethamine, dapson, sulfadoxine, chloroquine

Anticonvulsants Phenytoin, sodium valproate, carbamazepine

Antibiotics Sulphonamides, penicillins, cephalosporins

Miscellaneous Cimetidine, ranitidine, chlorpropamide, zidovudine

Fig. 23.12 Appearance of white blood cells. A Neutrophil. B Eosinophil. C Basophil. D Monocyte. E Lymphocyte.

A B C D E Neutrophils

A Neutrophilia

- Infection: bacterial, fungal
- Trauma: surgery, burns
- Infarction: myocardial infarct, pulmonary embolus, sickle-cell crisis
- Inflammation: gout, rheumatoid arthritis, ulcerative colitis, Crohn's disease
- Malignancy: solid tumours, Hodgkin lymphoma
- Myeloproliferative disease: polycythaemia, chronic myeloid leukaemia
- Physiological: exercise, pregnancy

Neutropenia

- Infection: viral, bacterial (e.g. Salmonella), protozoal (e.g. malaria)
- Drugs: see Box 23.10
- Autoimmune: connective tissue disease
- Alcohol
- Bone marrow infiltration: leukaemia, myelodysplasia
- Congenital: Kostmann's syndrome
- Constitutional: Afro-Caribbean and Middle Eastern descent

Eosinophils

B Eosinophilia

- Allergy: hay fever, asthma, eczema
- Infection: parasitic
- Drug hypersensitivity: e.g. gold, sulphonamides
- Vasculitis: e.g. eosinophilic granulomatosis with polyangiitis (Churg–Strauss), granulomatosis with polyangiitis (Wegener's)
- Connective tissue disease: polyarteritis nodosa
- Malignancy: solid tumours, lymphomas
- Primary bone marrow disorders: myeloproliferative disorders, hypereosinophilic syndrome (HES), acute myeloid leukaemia

Basophils

C Basophilia

- Myeloproliferative disease: polycythaemia, chronic myeloid leukaemia
- Inflammation: acute hypersensitivity, ulcerative colitis, Crohn's disease
- Iron deficiency

Monocytes

D Monocytosis

- Infection: bacterial (e.g. tuberculosis)
- Inflammation: connective tissue disease, ulcerative colitis, Crohn's disease
- Malignancy: solid tumours, chronic myelomonocytic leukaemia

Lymphocytes

E Lymphocytosis

- Infection: viral, bacterial (e.g. Bordetella pertussis)
- Lymphoproliferative disease: chronic lymphocytic leukaemia, lymphoma
- Post-splenectomy
- Lymphopenia
- Inflammation: connective tissue disease
- Lymphoma
- Renal failure
- Sarcoidosis
- Drugs: glucocorticoids, cytotoxics
- Congenital: severe combined immunodeficiency
- HIV infection

23.9 How to interpret white blood cell results

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source of inflammation or primary malignancy in the appropriate drainage area:

- the scalp, ear, mouth and throat, face, teeth or thyroid for neck nodes
- the breast for axillary nodes
- the perineum or external genitalia for inguinal nodes.

Generalised lymphadenopathy may be secondary to infection, often viral, connective tissue disease or extensive skin disease (dermatopathic lymphadenopathy) but is more likely to signify underlying haematological malignancy. Weight loss and drenching night sweats that may require a change of nightclothes are associated with haematological malignancies, particularly lymphoma. Initial investigations in lymphadenopathy include an FBC (to detect neutrophilia in infection or evidence of haematological disease), measurement of erythrocyte sedimentation rate (ESR) and a chest X-ray (to detect mediastinal

lymphadenopathy). If the findings suggest malignancy, a formal cutting needle or excision biopsy of a representative node is indicated to obtain a histological diagnosis.

Splenomegaly The spleen may be enlarged due to involvement by lymphoproliferative disease, the resumption of extramedullary haematopoiesis in myeloproliferative disease, enhanced reticuloendothelial activity in autoimmune haemolysis, expansion of the lymphoid tissue in response to infections, or vascular congestion as a result of portal hypertension (Box 23.12). Hepatosplenomegaly is suggestive of lympho- or myeloproliferative disease, liver disease or infiltration (e.g. with amyloid). Associated lymphadenopathy is suggestive of lymphoproliferative disease. An enlarged spleen may cause abdominal discomfort, accompanied by back pain and abdominal bloating and early satiety due to stomach compression. Splenic infarction produces severe abdominal pain radiating to the left shoulder tip, associated with a splenic rub on auscultation. Rarely, spontaneous or traumatic rupture and bleeding may occur. Investigation should focus on the suspected cause. Imaging of the spleen by ultrasound or computed tomography (CT) will detect variations in density in the spleen, which may be a feature of lymphoproliferative disease; it also allows imaging of the liver and abdominal lymph nodes. Biopsy of enlarged abdominal or superficial lymph nodes may provide the diagnosis, as might a bone marrow biopsy in splenic lymphomas. A chest X-ray or CT of the thorax will detect mediastinal lymphadenopathy. An FBC may show pancytopenia secondary to hypersplenism, when the enlarged spleen has become overactive, destroying blood cells prematurely. If other abnormalities are present, such as abnormal lymphocytes or a leucoerythroblastic blood film, a bone marrow examination is indicated. Screening for infectious or liver disease (p. 852) may be appropriate. If all investigations are unhelpful, splenectomy may be diagnostic but is rarely carried out in these circumstances.

Bleeding Normal bleeding is seen following surgery and trauma. Pathological bleeding occurs when structurally abnormal vessels rupture or when a vessel is breached in the presence of a defect in haemostasis. This may be due to a deficiency or dysfunction of platelets, the coagulation factors or von Willebrand factor, or Eosinophilia A high eosinophil count of more than $0.5 \times 10^9/L$ is usually secondary to infection (especially parasites; p. 233), allergy (e.g. eczema, asthma, reactions to drugs; p. 84), immunological disorders (e.g. polyarteritis, sarcoidosis) or malignancy (e.g. lymphomas) (see Box 23.9). Usually, such eosinophilia is short-lived. In the rarer primary disorders, there is a persistently raised, often clonal, eosinophilia, e.g. in myeloproliferative disorders, subtypes of acute myeloid leukaemia and idiopathic hypereosinophilic syndrome (HES). Recently, specific mutations in receptor tyrosine kinase genes have been found in some primary eosinophilias (e.g. causing rearrangements of platelet-derived growth factor receptors α and β or c-kit), which allow diagnosis and, in some cases, specific therapy with tyrosine kinase inhibitors such as imatinib. Eosinophil infiltration can damage many organs (e.g. heart, lungs, gastrointestinal tract, skin, musculoskeletal system); evaluation of eosinophilia therefore includes not only the identification of any underlying cause and its appropriate treatment but also assessment of any related organ damage.

Lymphocytosis A lymphocytosis is an increase in circulating lymphocytes above that expected for the patient's age. In adults, this is greater than $3.5 \times 10^9/L$. Infants and children have higher counts; age-related reference ranges should be consulted. Causes are shown in Box 23.9; the most common is viral infection.

Lymphadenopathy Enlarged lymph glands may be an important indicator of haematological disease but they are not uncommon in reaction to infection or inflammation (Box 23.11). The sites of lymph node groups, and symptoms and signs that may help elucidate the underlying cause are shown on page 913. Nodes that enlarge in response to local infection or inflammation ('reactive nodes') usually expand rapidly and are painful, whereas those due to haematological disease are more frequently painless. Localised lymphadenopathy should elicit a

search for a 23.11 Causes of lymphadenopathy Infective • Bacterial: streptococcal, tuberculosis, brucellosis • Viral: Epstein-Barr virus (EBV), human immunodeficiency virus (HIV) • Protozoal: toxoplasmosis • Fungal: histoplasmosis, coccidioidomycosis Neoplastic • Primary: lymphomas, leukaemias • Secondary: lung, breast, thyroid, stomach, melanoma Connective tissue disorders • Rheumatoid arthritis • Systemic lupus erythematosus (SLE) Sarcoidosis Amyloidosis Drugs • Phenytoin

928 • HAEMATOLOGY AND TRANSFUSION MEDICINE bleeding from superficial cuts, epistaxis, gastrointestinal haemorrhage or menorrhagia is more likely to be due to thrombocytopenia, a platelet function disorder or von Willebrand disease. Recurrent bleeds at a single site suggest a local structural abnormality rather than coagulopathic bleeding. • Duration of history. It may be possible to assess whether the disorder is congenital or acquired. • Precipitating causes. Bleeding arising spontaneously indicates a more severe defect than bleeding that occurs only after trauma. • Surgery. Ask about operations. Dental extractions, tonsillectomy and circumcision are stressful tests of the haemostatic system. Immediate post-surgical bleeding suggests defective platelet plug formation and primary haemostasis; delayed haemorrhage is more suggestive of a coagulation defect. However, in post-surgical patients, persistent bleeding from a single site is more likely to indicate surgical bleeding than a bleeding disorder. • Family history. While a positive family history may be present in patients with inherited disorders, the absence of affected relatives does not exclude a hereditary bleeding diathesis; about one-third of cases of haemophilia arise in individuals without a family history, and deficiencies of factor VII, X and XIII are recessively inherited. Recessive disorders are more common in cultures where there is consanguineous marriage. • Drugs. Use of antithrombotic, anticoagulant and fibrinolytic drugs must be elicited. Drug interactions with warfarin and drug-induced thrombocytopenia should be considered. Some 'herbal' remedies may result in a bleeding diathesis. Clinical examination may reveal different patterns of skin bleeding. Petechial purpura is minor bleeding into the dermis that is flat and non-blanching (Fig. 23.13). Petechiae are typically found in patients with thrombocytopenia or platelet dysfunction. occasionally to excessive fibrinolysis, which is most commonly observed following therapeutic thrombolysis (p. 500). Clinical assessment 'Screening' blood tests (see Box 23.3) do not reliably detect all causes of pathological bleeding (e.g. von Willebrand disease, scurvy, certain anticoagulant drugs and the causes of purpura listed in Box 23.13) and should not be used indiscriminately. A careful clinical evaluation is the key to diagnosis of bleeding disorders (p. 970). It is important to consider the following: • Site of bleeding. Bleeding into muscle and joints, along with retroperitoneal and intracranial haemorrhage, indicates a likely defect in coagulation factors. Purpura, prolonged 23.13 Causes of non-thrombocytopenic purpura • Senile purpura • Factitious purpura • Henoch-Schönlein purpura (p. 1043) • Vasculitis (p. 1040) • Paraproteinaemias • Purpura fulminans, e.g. in disseminated intravascular coagulation secondary to sepsis 23.12 Causes of splenomegaly Congestive Portal hypertension • Cirrhosis • Hepatic vein occlusion • Portal vein thrombosis • Stenosis or malformation of portal or splenic vein Cardiac • Chronic congestive cardiac failure • Constrictive pericarditis Infective Bacterial • Endocarditis • Sepsis • Tuberculosis • Brucellosis • Salmonella Viral • Hepatitis • Epstein-Barr • Cytomegalovirus Protozoal • Malaria* • Leishmaniasis (kala-azar)* • Trypanosomiasis Fungal • Histoplasmosis Inflammatory/granulomatous disorders • Felty's syndrome in rheumatoid arthritis • Sarcoidosis • Systemic lupus erythematosus Haematological Red cell disorders • Megaloblastic anaemia • Haemoglobinopathies • Hereditary spherocytosis Autoimmune haemolytic anaemias Myeloproliferative disorders • Chronic myeloid leukaemia* • Myelofibrosis* • Polycythaemia rubra

vera • Essential thrombocythaemia Neoplastic • Leukaemias, including chronic myeloid leukaemia*
• Lymphomas Other malignancies • Metastatic cancer – rare Lysosomal storage diseases •
Gaucher's disease • Niemann–Pick disease Miscellaneous • Cysts, amyloid, thyrotoxicosis,
haemophagocytic syndromes *Causes of massive splenomegaly. Fig. 23.13 Petechial purpura.

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Palpable purpura occurs in vasculitis. Ecchymosis, or bruising, is more extensive bleeding into deeper layers of the skin. The lesions are initially dark red or purple but become yellow as haemoglobin is degraded. Retroperitoneal bleeding presents with a flank or peri-umbilical haematoma. Telangiectasia of lips and tongue points to hereditary haemorrhagic telangiectasia (p. 970). Joints should be examined for evidence of haemarthroses. A full examination is important, as it may give clues to an underlying associated systemic illness such as a haematological or other malignancy, liver disease, renal failure, connective tissue disease and possible causes of splenomegaly. Investigations Screening investigations and their interpretation are described on page 920. If the patient has a history that is strongly suggestive of a bleeding disorder and all the preliminary screening tests give normal results, further investigations, such as measurement of von Willebrand factor and assessment of platelet function, should be performed (p. 921).

Thrombocytopenia (low platelet count) A reduced platelet count may arise by one of two mechanisms: • decreased or abnormal production (bone marrow failure and hereditary thrombocytopathies) • increased consumption following release into the circulation (immune-mediated, DIC or sequestration). Spontaneous bleeding does not usually occur until the platelet count falls below $20 \times 10^9/L$, unless their function is also compromised. Purpura and spontaneous bruising are characteristic but there may also be oral, nasal, gastrointestinal or genitourinary bleeding. Severe thrombocytopenia ($< 10 \times 10^9/L$) may result in retinal haemorrhage and potentially fatal intracranial bleeding, but this is rare. Investigations are directed at the possible causes listed in Box 23.14. A blood film is the single most useful initial investigation. Examination of the bone marrow may reveal increased megakaryocytes in consumptive causes of thrombocytopenia, or the underlying cause of bone marrow failure in leukaemia, hypoplastic anaemia or myelodysplasia. Treatment (if required) depends on the underlying cause. Platelet transfusion is rarely required and is usually confined to patients with bone marrow failure and platelet counts below $10 \times 10^9/L$, or to clinical situations with actual or predicted serious haemorrhage.

Thrombocytosis (high platelet count) The most common reason for a raised platelet count is that it is reactive to another process, such as infection, inflammation, connective tissue disease, malignancy, iron deficiency, acute haemolysis or gastrointestinal bleeding (Box 23.15). The presenting clinical features are usually those of the underlying disorder and haemostasis is rarely affected. Reactive thrombocytosis is distinguished from the myeloproliferative disorders by the presence of uniform small platelets, lack of splenomegaly, and the presence of an associated disorder. The key to diagnosis is the clinical history and examination, combined with observation of the platelet count over time (reactive thrombocytosis gets better with resolution of the underlying cause). The platelets are a product of an abnormally expanding clone of cells in the myeloproliferative disorders, chronic myeloid leukaemia 23.14 Causes of thrombocytopenia

Decreased production Marrow hypoplasia • Childhood bone marrow failure syndromes, e.g. Fanconi's anaemia, dyskeratosis congenita, amegakaryocytic thrombocytopenia • Idiopathic aplastic anaemia • Drug-induced: cytotoxics, antimetabolites • Transfusion-associated graft-versus-host disease Marrow infiltration • Leukaemia • Myeloma • Carcinoma (rare) • Myelofibrosis

- Osteopetrosis • Lysosomal storage disorders, e.g. Gaucher's disease Haematinic deficiency • Vitamin B12 and/or folate deficiency Familial (macro-)thrombocytopathies • Myosin heavy chain abnormalities, e.g. Alport's syndrome, Fechtner's syndrome, May-Hegglin anomaly • Bernard-Soulier syndrome • Montreal platelet syndrome • Wiskott-Aldrich syndrome (small platelets) • Mediterranean macrothrombocytopathy Increased consumption Immune mechanisms • Idiopathic thrombocytopenic purpura* • Neonatal alloimmune thrombocytopenia • Post-transfusion purpura • Drug-associated, especially quinine, vancomycin and heparin Coagulation activation • Disseminated intravascular coagulation (see Box 23.68, p. 978) Mechanical pooling • Hypersplenism Thrombotic microangiopathies • Haemolytic uraemic syndrome (HUS) and atypical HUS • Liver disease • Thrombotic thrombocytopenic purpura • Pre-eclampsia Others • Gestational thrombocytopenia • Type 2B von Willebrand disease *Associated conditions include collagen vascular diseases (particularly systemic lupus erythematosus), B-cell malignancy, HIV infection and antiphospholipid syndrome.

23.15 Causes of a raised platelet count Reactive thrombocytosis • Acute and chronic inflammatory disorders • Infection • Malignant disease • Tissue damage • Haemolytic anaemias • Post-splenectomy • Post-haemorrhage Clonal thrombocytosis • Primary thrombocythaemia • Polycythaemia rubra vera • Chronic myeloid leukaemia • Myelofibrosis • Myelodysplastic syndromes (MDSs; refractory anaemia with ring sideroblasts and thrombocytosis (RARS-T), MDS with isolated deletion of 5q)

930 • HAEMATOLOGY AND TRANSFUSION MEDICINE 23.16 Causes of pancytopenia Bone marrow failure • Hypoplastic/aplastic anaemia (p. 968): inherited, idiopathic, viral, drugs Bone marrow infiltration • Acute leukaemia • Myeloma • Lymphoma • Carcinoma • Haemophagocytic syndrome • Myelodysplastic syndromes Ineffective haematopoiesis • Megaloblastic anaemia • Acquired immunodeficiency syndrome (AIDS) Peripheral pooling/destruction • Hypersplenism: portal hypertension, Felty's syndrome, malaria, myelofibrosis • Systemic lupus erythematosus and some forms of myelodysplasia. As with PRV, patients with essential thrombocythaemia may present with thrombosis or, rarely, bleeding. Stroke, transient ischaemic attacks, amaurosis fugax, digital ischaemia or gangrene, aquagenic pruritus, splenomegaly and systemic upset are also features. Patients with myeloproliferative disorders may also present with features such as aquagenic pruritus, splenomegaly and systemic upset. Pancytopenia Pancytopenia refers to the combination of anaemia, leucopenia and thrombocytopenia. It may be due to reduced production of blood cells as a consequence of bone marrow suppression or infiltration, or there may be peripheral destruction or splenic pooling of mature cells. Causes are shown in Box 23.16. A bone marrow aspirate and trephine are usually required to establish the diagnosis. allogeneic transfusion may be avoided by following protocols that recommend the use of low haemoglobin thresholds for red cell transfusion, perioperative blood salvage and antifibrinolytic drugs. Blood products Blood components are prepared from whole blood or specific blood constituents collected from individual donors and include red cells, platelets, plasma and cryoprecipitate (Box 23.17). Plasma derivatives are licensed pharmaceutical products produced on a factory scale from large volumes of human plasma obtained from many people and treated to remove transmissible infection. Examples include: • Coagulation factors. Concentrates of factors VIII and IX are used for the treatment of conditions such as haemophilia A, haemophilia B and von Willebrand disease. Coagulation factors made by recombinant DNA technology are now preferred due to perceived lack of infection risk but plasma-derived products are still used in many countries. • Immunoglobulins. Intravenous immunoglobulin G (IVIgG) is administered as regular replacement therapy to reduce infective complications in patients with primary and secondary immunodeficiency. A short, high-dose course

of IVIgG may also be effective in some immunological disorders, including immune thrombocytopenia (p. 971) and Guillain-Barré syndrome (p. 1140). IVIgG can cause acute reactions and must be infused strictly according to the manufacturer's product information. There is a risk of renal dysfunction in susceptible patients and, in these circumstances, immunoglobulin products containing low or no sucrose are preferred. Anti-zoster immunoglobulin has a role in the prophylaxis of varicella zoster (p. 239). Anti-Rhesus D immunoglobulin is used in pregnancy to prevent haemolytic disease of the newborn (see Box 23.19 below).

- Human albumin. This is available in two strengths. The 5% solution can be used as a colloid resuscitation fluid but it is no more effective and is more expensive than crystalloid solutions. Human albumin 20% solution is used in the management of hypoproteinaemic oedema in nephrotic syndrome (p. 395) and ascites in chronic liver disease (p. 864). It is hyperoncotic and expands plasma volume by more than the amount infused. Blood components and their use are summarised in Box 23.17. Blood donation A safe supply of blood components depends on a well-organised system with regular donation by healthy individuals who have no excess risk of infections transmissible in blood (Fig. 23.14). Blood donations are obtained by either venesection of a unit of whole blood or collection of a specific component, such as platelets, by apheresis. During apheresis, the donor's blood is drawn via a closed system into a machine that separates the components by centrifugation and collects the desired fraction into a bag, returning the rest of the blood to the donor. Each donation must be tested for hepatitis B virus (HBV), hepatitis C virus (HCV), HIV and human T-cell lymphotropic virus (HTLV) nucleic acid and/ or antibodies. Platelet concentrates may be tested for bacterial contamination. The need for other microbiological tests depends on local epidemiology. For example, testing for *Trypanosoma cruzi* (Chagas' disease; p. 279) is necessary in areas of South America and the USA where infection is prevalent. Tests for West Nile virus have been required in the USA since this agent became an infection is a major complication of haematological disorders. It relates to the immunological deficit caused by the disease itself, or its treatment with chemotherapy and/or immunotherapy (pp. 224 and 925).

Principles of management of haematological disease Blood products and transfusion Blood transfusion from an unrelated donor to a recipient inevitably carries some risk, including adverse immunological interactions between the host and infused blood (p. 931), and transmission of infectious agents. Although there are many compelling clinical indications for blood component transfusion, there are also many clinical circumstances in which transfusion is conventional but the evidence for its effectiveness is limited. In these settings,

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Red cell incompatibility Red blood cell membranes contain numerous cell surface molecules that are potentially antigenic (see Fig. 23.4). The ABO and Rhesus D antigens are the most important in routine transfusion and antenatal practice.

ABO blood groups The frequency of the ABO antigens varies among different populations. The ABO blood group antigens are oligosaccharide chains that project from the red cell surface. These chains are attached to proteins and lipids that lie in the red cell membrane. The ABO gene encodes a glycosyltransferase that catalyses the final step in the synthesis of the chain, which has three common alleles: A, B and O. The O allele encodes an inactive enzyme, leaving the ABO antigen precursor (called the H antigen) unmodified. The A and B alleles encode enzymes that differ by a single amino acid. Components for use in specific patient groups are prepared from hepatitis E virus-negative donors in the UK, and plasma donated in the UK is not used at present for producing pooled plasma derivatives in view of concerns about transmission of

variant Creutzfeldt-Jakob disease (vCJD; p. 1127). Adverse effects of transfusion Death directly attributable to transfusion is rare, at less than 0.3 per 100 000 transfusions. Relatively minor symptoms of transfusion reactions (fever, itch or urticaria) occur in up to 3% of transfusions, and usually in patients who have had repeated transfusions. Any symptoms or signs that arise during a transfusion must be taken seriously, as they may be the first warnings of a serious reaction. Figure 23.16 below outlines the symptoms and signs, management and investigation of acute reactions to blood components.

23.17 Blood components and their use

Component Major haemorrhage Other indications

Red cell concentrate¹ Most of the plasma is removed and replaced with a solution of glucose and adenine in saline to maintain viability of red cells ABO compatibility with recipient essential Replace acute blood loss: increase circulating red cell mass to relieve clinical features caused by insufficient oxygen delivery. Order 4–6 U initially to allow high red cell to FFP transfusion ratios of (at least) 2 : 1 Severe anaemia If no cardiovascular disease, transfuse to maintain Hb at 70 g/L If known or likely to have cardiovascular disease, maintain Hb at 90 g/L

Platelet concentrate One adult dose is made from four donations of whole blood, or from a single platelet apheresis donation ABO compatibility with recipient preferable Maintain platelet count > 50 × 10⁹/L, or in multiple or central nervous system trauma

“ 100 × 10⁹/L If ongoing bleeding, order when platelets < 100 × 10⁹/L to allow for delivery time Each adult dose has a minimum of 2.4 × 10¹¹ platelets, which raises platelet count by 40 × 10⁹/L unless there is consumptive coagulopathy, e.g. disseminated intravascular coagulation Thrombocytopenia, e.g. in acute leukaemia Maintain platelet count > 10 × 10⁹/L if not bleeding Maintain platelet count > 20 × 10⁹/L if minor bleeding or at risk (sepsis, concurrent use of antibiotics, abnormal coagulation) Increase platelet count > 50 × 10⁹/L for minor invasive procedure (e.g. lumbar puncture, gastroscopy and biopsy, insertion of indwelling lines, liver biopsy, laparotomy) or in acute, major blood loss Increase platelet count > 100 × 10⁹/L for operations in critical sites such as brain or eyes

Fresh frozen plasma² 150–300 mL plasma from one donation of whole blood ABO compatibility with recipient recommended Dilutional coagulopathy with a PT prolonged 50% is likely after replacement of 1–1.5 blood volumes with red cell concentrate Give initially in (at least) a ratio of 1 FFP:2 red cell concentrate; order 15–20 mL/kg and allow for thawing time. Further doses only if bleeding continues and guided by PT and APTT Replacement of coagulation factor deficiency If no virally inactivated or recombinant product is available Thrombotic thrombocytopenic purpura Plasma exchange (using virus-inactivated plasma if available) is frequently effective Cryoprecipitate²

Fibrinogen and coagulation factor concentrated from plasma by controlled thawing 10–20 mL pack contains: Fibrinogen 150–300 mg Factor VIII 80–120 U von Willebrand factor 80–120 U In UK supplied as pools of 5 U Aim to keep fibrinogen > 1.5 g/L. Pooled units (of 10 donations) will raise fibrinogen by 1 g/L

von Willebrand disease and haemophilia If virus-inactivated or recombinant products are not available ¹Whole blood is an alternative to red cell concentrate. ABO compatibility with recipient essential. ²Pooled plasma can be treated with solvent and detergent or single units treated with methylene blue as an

additional viral inactivation step. Virus-inactivated plasma is indicated for large-volume exposure, as in treatment of thrombotic thrombocytopenic purpura, and for treatment of children in the UK born after 1995. (APTT = activated partial thromboplastin time; FFP = fresh frozen plasma; Hb = haemoglobin; PT = prothrombin time)

932 • HAEMATOLOGY AND TRANSFUSION MEDICINE ABO-incompatible red cell transfusion If red cells of an incompatible ABO group are transfused (especially if a group O recipient is transfused with group A, B or AB red cells), the recipient's IgM anti-A, anti-B or anti-AB binds to the transfused red cells. This activates the full complement pathway (p. 66), creating pores in the red cell membrane and destroying four amino acids and hence attach different sugars to the end of the chain. Individuals are tolerant to their own ABO antigens, but do not suppress B-cell clones producing antibodies against ABO antigens that they do not carry themselves (Box 23.18). They are, therefore, capable of mounting a humoral immune response to these 'foreign' antigens. Fig. 23.14 Blood donation, processing and storage. 1Platelet apheresis involves circulating the donor's blood through a cell separator to remove platelets before returning other blood components to the donor. 2In the UK, plasma for fractionation is imported as a precautionary measure against vCJD. (vCJD = variant Creutzfeldt-Jakob disease; HIV = human immunodeficiency virus; HTLV = human T-cell lymphotropic virus) A A A Donor Education Recruitment Selection Donation Process into blood components Filter to remove leucocytes Test for: HIV HTLV Hepatitis B Hepatitis C Hepatitis E Syphilis ABO + RhD Other blood groups Red cell antibodies Platelet apheresis1 450 mL whole blood collected into 63 mL anticoagulant/preservative Pooled/apheresis platelets Red cells Fresh frozen plasma Plasma2 4°C 22°C -30°C Storage Fractionation Plasma derivatives, e.g. albumin, immunoglobulin Patient 35 days 5 days (agitate) 36 months Confirm compatibility Thaw

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(TA GVHD). The latter occurs when there is sharing of a human leucocyte antigen (HLA) haplotype between donor and recipient, which allows transfused lymphocytes to engraft, proliferate and recognise the recipient as foreign, resulting in acute GVHD (p. 937). Prevention is by gamma- or X-ray irradiation of blood components before their administration to prevent lymphocyte proliferation. Those at risk of TA GVHD, who must receive irradiated blood components, include patients with congenital T-cell immunodeficiencies or Hodgkin lymphoma, patients with aplastic anaemia receiving immunosuppressive therapy with antithymocyte globulin (ATG), recipients of haematopoietic stem cell transplants or of blood from a family member, neonates who have received an intrauterine transfusion, and patients taking T-lymphocyte-suppressing drugs, such as fludarabine and other purine analogues. Transfusion-transmitted infection Over the past 30 years, HBV, HIV-1 and HCV have been identified and effective tests introduced to detect and exclude infected donations. Where blood is from 'safe' donors and correctly tested, the current risk of a donated unit being infectious is very small. By 2013 in the UK, the estimated chance that a unit of blood from a 'safe' donor might transmit one of the viruses for which blood is tested was 1 in 6.6 million units for HIV-1, 1 in 51.5 million for HCV and 1 in 2.6 million for HBV. However, some patients who received transfusions before these tests were available suffered serious consequences from infection; this serves as a reminder to avoid non-essential transfusion, since it is impossible to exclude the emergence of new or currently unrecognised transfusion-transmissible

infection. Licensed plasma derivatives that have been virus-inactivated do not transmit HIV, HTLV, HBV, HCV, cytomegalovirus or other lipid-enveloped viruses. Variant CJD is a human prion disease linked to bovine spongiform encephalitis (BSE; p. 1127). The risk of a recipient acquiring the agent of vCJD from a transfusion is uncertain, but of 16 recipients of blood from donors who later developed the disease, 3 have died with clinical vCJD and 1 other had postmortem immunohistological features of infection. Bacterial contamination of a blood component – usually platelets – is extremely rare (1 proven case in the UK in 2015) but can result in severe bacteraemia/sepsis in the recipient. The transfused red cells in the circulation (intravascular haemolysis). The anaphylatoxins C3a and C5a, released by complement activation, liberate cytokines such as tumour necrosis factor (TNF), interleukin 1 (IL-1) and IL-8, and stimulate degranulation of mast cells with release of vasoactive mediators. All these substances may lead to inflammation, increased vascular permeability and hypotension, which may, in turn, cause shock and renal failure. Inflammatory mediators can also cause platelet aggregation, lung peribronchial oedema and smooth muscle contraction. About 20–30% of ABO-incompatible transfusions cause some degree of morbidity, and 5–10% cause or contribute to a patient's death. The main reason for this relatively low morbidity is the lack of potency of ABO antibodies in group A or B subjects; even if the recipient is group O, those who are very young or very old usually have weaker antibodies that do not lead to the activation of large amounts of complement. The Rhesus D blood group and haemolytic disease of the newborn About 15% of Caucasians are Rhesus-negative: that is, they lack the Rhesus D (RhD) red cell surface antigen (see Fig. 23.4). In other populations (e.g. in Chinese and Bengalis), only 1–5% are Rhesus-negative. RhD-negative individuals do not normally produce substantial amounts of anti-RhD antibodies. However, if RhD-positive red cells enter the circulation of an RhD-negative individual, IgG antibodies are produced. This can occur during pregnancy if the mother is exposed to fetal cells via fetomaternal haemorrhage, or following transfusion. If a woman is so sensitised, during a subsequent pregnancy anti-RhD antibodies can cross the placenta; if the fetus is RhD-positive, haemolysis with severe fetal anaemia and hyperbilirubinaemia can result. This can cause severe neurological damage or death due to haemolytic disease of the newborn (HDN). Therefore, an RhD-negative female who may subsequently become pregnant should never be transfused with RhD-positive blood. In RhD-negative women, administration of anti-RhD immunoglobulin (anti-D) perinatally can block the immune response to RhD antigen on fetal cells and is the only effective product for preventing the development of Rhesus antibodies (Box 23.19). HDN can also be caused by other alloantibodies against red cell antigens, usually after previous pregnancies or transfusions. These antigens include Rhc, RhC, RhE, Rhe, and the Kell, Kidd and Duffy antigen systems. HDN can also occur if there is fetomaternal ABO incompatibility, most commonly seen in a group O mother with a group A fetus. The fetus is generally less severely affected by ABO incompatibility than by RhD, Rhc or Kell antigen mismatch, and the incompatibility is often picked up coincidentally after birth. Other immunological complications of transfusion Rare but serious complications include transfusion-associated lung injury (TRALI) and transfusion-associated graft-versus-host disease 23.19 Rhesus D blood groups in pregnancy • Haemolytic disease of the newborn (HDN): occurs when the mother has anti-red cell immunoglobulin G (IgG) antibodies that cross the placenta and haemolyse fetal red cells. • Screening for HDN in pregnancy: at the time of booking (12–16 weeks) and again at 28–34 weeks' gestation, every pregnant woman should have a blood sample sent for determination of ABO and Rhesus D (RhD) group and testing for red cell alloantibodies that may be directed against paternal blood group antigens present on fetal red cells. • Anti-D immunoglobulin prophylaxis in a pregnant woman who is RhD-negative: antenatal anti-D prophylaxis is offered at

28–34 weeks to RhD-negative pregnant women who have no evidence of immune anti-D. This prevents the formation of antibodies that could cause HDN. Following delivery of an RhD-positive baby, the mother is given further anti-D within 72 hours; a maternal sample is checked for remaining fetal red cells and additional anti-D is given if indicated. Additional anti-D is also given after potential sensitising events antenatally (e.g. early bleeding). Doses vary according to national recommendations.

23.18 ABO blood group antigens and antibodies

ABO blood group	Red cell antigens	Antibodies in plasma	UK frequency (%)
O	None	Anti-A and anti-B	35
A	A	Anti-B	35
B	B	Anti-A	10
AB	A and B	None	6

A A Anti-B

B B Anti-A

AB A and B None

934 • HAEMATOLOGY AND TRANSFUSION MEDICINE antibody screen is negative. This allows group-specific units to be issued quickly and safely, for elective and emergency transfusion. Bedside procedures for safe transfusion

Errors leading to patients receiving the wrong blood are an important avoidable cause of mortality and morbidity. Most incompatible transfusions result from failure to adhere to standard procedures for taking correctly labelled blood samples from the patient and ensuring that the correct pack of blood component is transfused into the intended patient. In the UK in 2015, there were 280 reports of transfusion of an incorrect blood component (11 per 100 000 units transfused). Every hospital where blood is transfused should have a written transfusion policy used by all staff who order, check or administer blood products (Fig. 23.15). Management of suspected transfusion reactions is shown in Figure 23.16.

Transfusion in major haemorrhage The successful management of a patient with major haemorrhage requires frontline clinical staff to be trained to recognise significant blood loss early and to intervene before shock is established. Hospitals should have local major haemorrhage protocols and all clinical staff must be familiar with their content. Good team working and communication are essential to prevent poor clinical outcome, suboptimal or inappropriate transfusion practice and component wastage. Fresh frozen plasma (FFP) should be given as part of initial resuscitation in (at least) a 1 : 2 ratio with red cell concentrate (RCC) until coagulation results are available. If the patient is bleeding, a ratio of FFP to RCC of 1 : 1 should be given until laboratory results are available and use of cryoprecipitate should be considered. Once the bleeding is under control, further Safe transfusion procedures

The proposed transfusion and any alternatives should be discussed with the patient or, if that is not possible, with a relative, and this should be documented in the case record. Some patients, e.g. Jehovah's Witnesses, may refuse transfusion and require specialised management to survive profound anaemia following blood loss.

Pre-transfusion testing To ensure that red cells supplied for transfusion are compatible with the intended recipient, the transfusion laboratory will perform either a 'group and screen' procedure or a 'cross-match'. In the group and screen procedure, the red cells from the patient's blood sample are tested to determine the ABO and RhD type, and the patient's serum is also tested against an array of red cells expressing the most important antigens to detect any red cell antibodies. Any antibody detected can be identified by further testing, so that red cell units that lack the corresponding antigen can be selected. The patient's sample can be held in the laboratory for up to a week, so that the hospital blood bank can quickly prepare compatible blood without the need for a further patient sample. Conventional cross-matching consists of the group and antibody screen, followed by direct confirmation of the compatibility of

individual units of red cells with the patient's serum. Full cross-matching takes about 45 minutes if no red cell antibodies are present, but may require hours if a patient has multiple antibodies. Blood can be supplied by 'electronic issue', without the need for compatibility cross-matching, if the laboratory's computer system shows that the patient's ABO and RhD groups have been identified and confirmed on two separate occasions and their Fig. 23.15 Bedside procedures for safe blood transfusion. The patient's safety depends on adherence to standard procedures for taking samples for compatibility testing, administering blood, record-keeping and observations. MORAG MACDONALD HOSPITAL No. 100198E DOB: 11/07/1956 SEX: Female • Positively identify the patient at the bedside • Label the sample tube and complete the request form clearly and accurately after identifying the patient • Do not write forms and labels in advance Taking blood for pre-transfusion testing Administering blood • Positively identify the patient at the bedside • Ensure that the identification of each blood pack matches the patient's identification • Check that the ABO and RhD groups of each pack are compatible with the patient's • Check each pack for evidence of damage • If in doubt, do not use and return to the blood bank • Complete the forms that document the transfusion of each pack • Check the compatibility label on the pack against the patient's wristband • Always involve the patient by asking them to state their name and date of birth, where possible Surname Forename Date of birth Unique identifier/ hospital number Patient's wristband Blood pack Observations • Transfusions should only be given when the patient can be observed • Blood pressure, pulse and temperature should be monitored before and 15 minutes after starting each pack • In conscious patients, further observations are only needed if the patient has symptoms or signs of a reaction • In unconscious patients, check pulse and temperature at intervals during transfusion • Signs of abnormal bleeding during the transfusion could be due to disseminated intravascular coagulation resulting from an acute haemolytic reaction Record-keeping • Record in the patient's notes, the reason for transfusion, the product given, dose, any adverse effects and the clinical responses

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Fig. 23.16 Investigation and management of acute transfusion reactions. *Use size-appropriate dose in children. (ARDS = acute respiratory distress syndrome; BP = blood pressure; CVP = central venous pressure; DIC = disseminated intravascular coagulation; FBC = full blood count; IV = intravenous)* Bacterial infection of unit • Take down unit and giving set/return intact to blood bank with all other used/unused units • Take blood cultures, repeat blood group/cross-match/ FBC, coagulation screen, biochemistry, urinalysis • Monitor urine output • Commence broad-spectrum antibiotics if suspected bacterial infection (Ch. 6) • Commence oxygen and fluid support • Seek advice Severe allergic reaction • Discontinue transfusion • Give chlorphenamine 10 mg slowly IV • Commence O₂ and fluid support • Give salbutamol nebuliser • If severe hypotension or bronchospasm, give adrenaline (epinephrine) 0.5 mg IM* • Send clotted blood sample to transfusion laboratory • Take down unit and giving set, and return intact to blood bank with all other used/unused units Bacterial contamination? • Blood pack discoloured or damaged • Rapid onset of hyper- or hypotension, rigors or collapse • Temperature $\geq 39^{\circ}\text{C}$ or rise of $\geq 2^{\circ}\text{C}$ Fluid overload • Give oxygen and furosemide 40–80 mg IV* Transfusion-related acute lung injury (TRALI) • Typically within 6–24 hrs of transfusion • Breathlessness, non-productive cough • Chest X-ray bilateral nodular infiltration • Discontinue transfusion • Give 100% oxygen • Treat as ARDS – ventilate if severely hypoxaemic If acute dyspnoea/hypotension • Monitor blood gases • Perform chest X-ray • Measure central venous/pulmonary capillary pressure No Raised CVP Yes Normal CVP

Severe allergic reaction? • Bronchospasm, angioedema, abdominal pain, hypotension Yes No
 Suspected ABO incompatibility? • Wrong blood pack infused • Haemoglobinuria Yes ABO
 incompatibility • Take down unit and giving set; return intact to blood bank • Commence IV saline
 infusion • Monitor urine output/catheterise Maintain urine output at > 100 mL/hr Give furosemide if
 urine output falls* • Treat DIC with appropriate blood components • Inform hospital transfusion
 department immediately No Reaction involves mild fever or urticarial rash only? Fever No Febrile
 non-haemolytic transfusion reaction If isolated temperature $\geq 38^{\circ}\text{C}$, or rise of $1\text{--}2^{\circ}\text{C}$, observations
 are stable and patient is otherwise well • Give paracetamol* • Restart infusion at a slower rate and
 observe more frequently Urticaria Mild pruritus/rash • Give chlorphenamine 10 mg slowly IV* •
 Restart the transfusion at a slower rate and observe more frequently Stop the transfusion •
 Undertake rapid clinical assessment, including temperature, pulse, BP, respiratory rate and O₂
 saturation • Check the identity of recipient details on the unit and compatibility form
 Symptoms/signs of possible acute transfusion reaction • Fever, chills, tachycardia, hyper- or
 hypotension, collapse, rigors, flushing, urticaria, bone, muscle, chest and/or abdominal pain,
 shortness of breath, nausea, generally feeling unwell, respiratory distress

936 • HAEMATOLOGY AND TRANSFUSION MEDICINE targeting of the chemotherapy drug to the
 specific cancer cell. Examples of such antibody-drug conjugates (ADCs) include the linking of the
 intercalating antibiotic calicheamicin to anti-CD33 (gemtuzumab ozogamicin) to treat acute
 myeloid leukaemia, and to anti-CD22 (inotuzumab ozogamicin) to treat acute lymphoblastic
 leukaemia. Small molecules targeted at the mechanisms causing cancer are replacing
 chemotherapy in some disease situations, such as tyrosine kinase inhibitors in chronic myeloid
 leukaemia and inhibitors of B-cell signalling in relapsed chronic lymphocytic leukaemia and
 lymphomas. More details of specific chemotherapies are given later in the chapter. Haematopoietic
 stem cell transplantation Transplantation of haematopoietic stem cells (HSCT) has offered the only
 hope of 'cure' in a variety of haematological and non-haematological disorders (Box 23.22). As
 standard treatment improves, the indications for HSCT are being refined and extended, although
 its use remains most common in haematological malignancies. The type of HSCT is defined
 according to the donor and source of stem cells: • In allogeneic HSCT, the stem cells come from a
 donor – either a related donor (usually an HLA-identical sibling) or a closely HLA-matched volunteer
 unrelated donor (VUD). • In an autologous transplant, the stem cells are harvested from the patient
 and stored in the vapour phase of liquid nitrogen until required. Stem cells can be harvested from
 the bone marrow or from the blood. FFP transfusion should be guided by laboratory results with
 transfusion triggers of PT and/or APTT above 1.5 times normal for a standard dose of FFP (15–20
 mL/kg). Cryoprecipitate should be given if the fibrinogen level falls below 1.5 g/L. Platelets should
 be kept above $50 \times 10^9/\text{L}$; to allow for delivery time, platelets should be requested if there is
 ongoing bleeding and the platelet count has fallen below $100 \times 10^9/\text{L}$. Blood component use in
 major haemorrhage is summarised in Box 23.17 and key points in transfusion medicine in Box
 23.20. Chemotherapy Chemotherapy refers to the use of drugs to treat cancer (Box 23.21; see also
 Fig. 33.2, p. 1317). Many haematological malignancies are sensitive to the effects of chemotherapy
 drugs and, as such, chemotherapy is the mainstay of treatment for most haematological cancers.
 There is a wide range of drugs available that work by damaging DNA or disrupting cellular
 metabolism, in such a way that natural apoptosis mechanisms, such as TP53, are activated and the
 cell dies. Despite cancer cells being more sensitive, chemotherapy is largely non-specific and kills
 some normal cells as well as cancer cells. This leads to common side-effects of treatment, such as
 transient bone marrow failure, mucositis and infertility. The supportive care of patients undergoing

chemotherapy is critical in overcoming these side-effects. It is this supportive care, including blood product support, antibiotics, antifungal drugs, growth factors and antiemetics, that has allowed specialist haematology units to achieve the best possible results from intensive chemotherapy: for example, when treating acute leukaemia. The basic principles of chemotherapy include combining several non-cross-reacting drugs in a regimen that kills a fixed proportion of cancer cells with a given dose. Several cycles of the combination are given to achieve gradual reduction of the tumour burden, to induce remission and, in some instances, to produce a cure (p. 1330). In recent years, chemotherapy has been improved by the addition of treatments that are more targeted to the cancer cell, particularly monoclonal antibodies; for example, rituximab (anti-CD20) has been added to CHOP (cyclophosphamide doxorubicin, vincristine, prednisolone) and other regimens, significantly improving the outcome in a range of CD20-positive B-cell lymphomas, including diffuse large B-cell lymphoma, follicular lymphoma and mantle cell lymphoma. Chemotherapy drugs can also be linked to a monoclonal antibody to allow

23.21 Examples of commonly used groups of cancer drugs in haematology

- Alkylating agents • Cross-link double-stranded DNA by adding an alkyl group, e.g. cyclophosphamide, melphalan, chlorambucil
- Anthracyclines • Intercalate between base pairs in the DNA molecule, e.g. daunorubicin, doxorubicin, idarubicin
- Antimetabolites • Inhibit DNA and RNA synthesis, e.g. cytosine arabinoside, fludarabine, methotrexate
- Vinca alkaloids • Cause disruption of tubulin, e.g. vincristine, vinblastine
- Topoisomerase II inhibitors • Prevent DNA repair, e.g. etoposide, daunorubicin, mitoxantrone

An example of a common combination regimen is CHOP, used in lymphoma: cyclophosphamide, hydroxydaunorubicin (doxorubicin), oncovin (vincristine) and prednisolone, given every 21 days for six cycles

23.20 Key points in transfusion medicine

- A restrictive strategy for red cell transfusion (Hb < 70 g/L) is at least as effective as a liberal strategy (< 100 g/L).
- The majority of reports in haemovigilance schemes such as SHOT relate to errors in the process of transfusion.
- Although transfusion-transmitted infection is a major concern for patients receiving transfusion, it is rare.
- In patients with trauma or burns or those who have had surgery, there is no evidence that resuscitation with albumin or other colloid solutions reduces the risk of death compared to resuscitation with crystalloid solutions.
- It is recommended that transfusion should be carried out at night time only in unavoidable circumstances. (SHOT = Serious Hazards of Transfusion)

23.22 Indications for allogeneic haematopoietic stem cell transplantation

- Neoplastic disorders affecting stem cell compartments (e.g. leukaemias)
- Failure of haematopoiesis (e.g. aplastic anaemia)
- Major inherited defects in blood cell production (e.g. thalassaemia, immunodeficiency diseases)
- Inborn errors of metabolism with missing enzymes or cell lines

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remains a significant risk of the haematological malignancy relapsing. The long-term survival for patients undergoing allogeneic HSCT in acute leukaemia is around 50%. Graft-versus-host disease GVHD is caused by the cytotoxic activity of donor T lymphocytes that become sensitised to their new host, regarding it as foreign. This may cause either an acute or a chronic form of GVHD. Acute GVHD occurs in the first 100 days after transplant in about one-third of patients. It can affect the skin, causing rashes, the liver, causing jaundice, and the gut, causing diarrhoea, and may vary from mild to lethal. Prevention includes HLA-matching of the donor, immunosuppressant drugs, including methotrexate, ciclosporin, alemtuzumab or ATG. Severe presentations are very difficult to control and, despite high-dose glucocorticoids, may result in death. Chronic GVHD may follow acute GVHD or arise independently; it occurs later than acute GVHD. It often resembles a connective

tissue disorder, although in mild cases a rash may be the only manifestation. Chronic GVHD is usually treated with glucocorticoids and prolonged immunosuppression with, for example, ciclosporin. Chronic GVHD results in an increased infection risk. However, associated with chronic GVHD are the graft-versus-disease effect and a lower relapse rate of the underlying malignancy.

Autologous HSCT This procedure can also be used in haematological malignancies. The patient's own stem cells from blood or marrow are first harvested and frozen. After conditioning myeloablative therapy, the autologous stem cells are reinfused into the blood stream in order to rescue the patient from the marrow damage and aplasia caused by chemotherapy. Autologous HSCT may be used for disorders that do not primarily involve the haematopoietic tissues, or for patients in whom very good remissions have been achieved. The most common indications are lymphomas and myeloma. The preferred source of stem cells for autologous transplants is peripheral blood (PBSCT). These stem cells engraft more

Allogeneic HSCT Healthy bone marrow or blood stem cells from a donor are infused intravenously into the recipient, who has been suitably 'conditioned'. The conditioning treatment (chemotherapy with or without radiotherapy) is 'myeloablative' or, increasingly, 'nonmyeloablative'. Myeloablative conditioning destroys malignant cells and immunosuppresses the recipient, as well as ablating the recipient's haematopoietic tissues. Reduced intensity conditioning (non-myeloablative) relies on intense immunosuppression to provide 'immunological space' for transplanted stem cells. The infused donor cells 'home' to the marrow, engraft and produce enough erythrocytes, granulocytes and platelets for the patient's needs after about 3–4 weeks. During this period of aplasia, patients are at risk of infection and bleeding, and require intensive supportive care as described on page 957. It may take several years to regain normal immunological function and patients remain at risk from opportunistic infections, particularly in the first year. An advantage of receiving allogeneic donor stem cells is that the donor's immune system can recognise residual recipient malignant cells and destroy them. This immunological 'graft-versus-disease' effect is a powerful tool against many haematological tumours and can be boosted post-transplantation by the infusion of T cells taken from the donor: so-called donor lymphocyte infusion (DLI). Considerable morbidity and mortality are associated with HSCT. The best results are obtained in patients with minimal residual disease, and in those under 20 years of age who have an HLA-identical sibling donor. Reduced-intensity conditioning has enabled treatment of older or less fit patients. In this form of transplantation, rather than using very intensive myeloablative conditioning, which causes morbidity from organ damage, relatively low doses of chemotherapy drugs, such as fludarabine and cyclophosphamide or busulfan, are used in combination with antibodies such as alemtuzumab (which targets CD52 on mature lymphoid cells) or anti-thymocyte globulin (ATG) to immunosuppress the recipient and allow donor stem cells to engraft. The emerging donor immune system then eliminates malignant cells via the 'graft-versus-disease' effect, which may be boosted by the elective use of donor T-cell infusions posttransplant. Such transplants have produced long-term remissions in some patients with acute leukaemia and myelodysplastic syndromes aged 40–65 years, who would not previously have been considered for a myeloablative allograft.

Complications These are outlined in Boxes 23.23 and 23.24. The risks and outcomes of transplantation depend upon several patient- and disease-related factors. In general, 25% die from procedure-related complications, such as infection and GVHD, and there

23.23 Complications of allogeneic haematopoietic stem cell transplantation

Early • Anaemia • Infections • Bleeding • Acute GVHD • Mucositis – pain, nausea, diarrhoea • Liver veno-occlusive disease

Late • Chronic GVHD • Infertility • Cataracts • Second malignancy (GVHD = graft-versus-host disease)

23.24 Infections during recovery from haematopoietic stem cell transplantation (HSCT)

Infection Time after HSCT Management Herpes simplex (p. 247) 0–4 weeks

(aplastic phase) Aciclovir prophylaxis and therapy Bacterial, fungal 0–4 weeks (aplastic phase) As for acute leukaemia (p. 956) – antibiotic and antifungal prophylaxis and therapy Cytomegalovirus (p. 242) 5–21 weeks (cell-mediated immune deficiency) Antigen screening in blood (PCR) and pre-emptive therapy (e.g. ganciclovir) Varicella zoster (p. 238) After 13 weeks Aciclovir prophylaxis and therapy Pneumocystis jirovecii (p. 318) 8–26 weeks Co-trimoxazole Encapsulated bacteria 8 weeks to years (immunoglobulin deficiency, prolonged with GVHD) Prophylaxis and revaccination (GVHD = graft-versus-host disease; PCR = polymerase chain reaction)

938 • HAEMATOLOGY AND TRANSFUSION MEDICINE disease, while warfarin and other anticoagulants are favoured in VTE (p. 975) and management of atrial fibrillation (p. 471). In some extremely prothrombotic situations, such as coronary artery stenting, a combination of anticoagulant and antiplatelet drugs is used (p. 491). A wide range of anticoagulant and antithrombotic drugs is used in clinical practice. These drugs and their modes of action are given in Box 23.26. Newer agents allow predictable anticoagulation without the need for frequent monitoring and dose titration. Although warfarin remains the mainstay for oral anticoagulation, newer oral anticoagulants (dabigatran, rivaroxaban, edoxaban and apixaban), which can be given at fixed doses with predictable effects and no need for monitoring, have now been approved for the prevention of perioperative VTE, the treatment of established VTE and the prevention of cardioembolic stroke in patients with atrial fibrillation. Heparins Unfractionated heparin (UFH) and low-molecular-weight heparins (LMWHs) act by binding via a specific pentasaccharide in the heparin molecule to antithrombin. Fondaparinux is a synthetic pentasaccharide, which also binds antithrombin and has similar properties to LMWH. These agents enhance the natural anticoagulant activity of antithrombin (see Fig. 23.6E). Increased cleavage of activated proteases, particularly factor Xa and thrombin (IIa), accounts for the anticoagulant effect. LMWHs preferentially augment antithrombin activity against factor Xa. For the licensed indications, LMWHs are at least as efficacious as UFH but have several advantages: • LMWHs are nearly 100% bioavailable and so produce reliable dose-dependent anticoagulation. • LMWHs do not require monitoring of their anticoagulant effect (except possibly in patients with very low body quickly, marrow recovery occurring within 2–3 weeks. There is no risk of GVHD and no immunosuppression is required. Thus autologous stem cell transplantation carries a lower procedurerelated mortality rate than allogeneic HSCT at around 5%, but there is a higher rate of recurrence of malignancy because the anti-malignancy effect is solely dependent on the conditioning chemotherapy with no ‘graft-versus-disease’ effect. Anticoagulant and antithrombotic therapy There are numerous indications for anticoagulant and antithrombotic medications (Box 23.25). The guiding principles are outlined here but management in specific indications is discussed elsewhere in the book. Broadly speaking, antiplatelet medications are of greater efficacy in the prevention of arterial thrombosis and of less value in the prevention of venous thromboembolism (VTE). Thus, antiplatelet agents, such as aspirin, clopidogrel and, increasingly, ticagrelor, are the drugs of choice in acute coronary events (p. 498) and in ischaemic cerebrovascular 23.26 Modes of action of anticoagulant and antithrombotic drugs Mode of action Drug Antiplatelet drugs Cyclo-oxygenase (COX) inhibition Aspirin Adenosine diphosphate (ADP) receptor inhibition Clopidogrel Prasugrel Ticagrelor Glycoprotein IIb/IIIa inhibition Abciximab Tirofiban Eptifibatide Phosphodiesterase inhibition Dipyridamole Oral anticoagulants Vitamin K antagonism Warfarin/coumarins Direct thrombin inhibition Dabigatran Direct Xa inhibition Rivaroxaban Apixaban Edoxaban Injectable anticoagulants Antithrombin-dependent inhibition of thrombin and Xa Heparin LMWH Antithrombin-dependent inhibition of Xa Fondaparinux Danaparoid Direct thrombin inhibition Argatroban

Bivalirudin Heparin/LMWH/Fondaparinux • Prevention and treatment of VTE • Percutaneous coronary intervention • Post-thrombolysis for MI • Unstable angina pectoris • Non-Q wave MI • Acute peripheral arterial occlusion • Cardiopulmonary bypass • Haemodialysis and haemofiltration Coumarins (warfarin etc.) • Prevention and treatment of VTE • Arterial embolism • Atrial fibrillation with specific risk factors for stroke (p. 472) • Mobile mural thrombus post-MI • Extensive anterior MI • Dilated cardiomyopathy • Cardioversion • Ischaemic stroke in antiphospholipid syndrome • Mitral stenosis and mitral regurgitation with atrial fibrillation • Recurrent venous thrombosis while on warfarin • Mechanical prosthetic cardiac valves Rivaroxaban • Prevention and treatment of VTE • Atrial fibrillation with risk factors for stroke Dabigatran etexilate • Prevention of VTE • Atrial fibrillation with risk factors for stroke Apixaban • Prevention of VTE • Atrial fibrillation with risk factors for stroke Edoxaban • Treatment of VTE • Atrial fibrillation with risk factors for stroke Therapeutic INR 2.5 INR 3.5 23.25 Indications for anticoagulation (INR = international normalised ratio; LMWH = low-molecular-weight heparin; MI = myocardial infarction; VTE = venous thromboembolism)

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baseline. The count may still be in the reference range. The patient may be asymptomatic, or develop venous or arterial thrombosis and skin lesions, including overt skin necrosis. Affected patients may complain of pain or itch at injection sites and of systemic symptoms, such as shivering, following heparin injections. Patients who have received heparin in the preceding 100 days and who have preformed antibodies may develop acute systemic symptoms and an abrupt fall in platelet count in the first 24 hours after re-exposure. Investigations The pre-test probability of the diagnosis is assessed using the 4Ts scoring system. This assigns a score based on: • the thrombocytopenia • the timing of the fall in platelet count • the presence of new thrombosis • the likelihood of another cause for the thrombocytopenia. Individuals at low risk need no further test. Those with intermediate and high likelihood scores should have the diagnosis confirmed or refuted using an anti-PF4 enzyme-linked immunosorbent assay (ELISA). Management Heparin should be discontinued as soon as HIT is diagnosed and an alternative anticoagulant that does not cross-react with the antibody should be substituted. Argatroban (a direct thrombin inhibitor) and danaparoid (a heparin analogue) are licensed for use in the UK. In asymptomatic patients with HIT who do not receive an alternative anticoagulant, around 50% will sustain a thrombosis in the subsequent 30 days. Patients with established thrombosis have a poorer prognosis. Coumarins Although several coumarin anticoagulants are used around the world, warfarin is the most common. Coumarins inhibit the vitamin K-dependent post-translational carboxylation of factors II (prothrombin), VII, IX and X in the liver (see Fig. 23.6D). This results in anticoagulation due to an effective deficiency of these factors. This is monitored by the INR, a standardised test based on measurement of the prothrombin time (p. 922). Recommended target INR values for specific indications are given in Box 23.25. Warfarin anticoagulation typically takes more than 3–5 days to become established, even using loading doses. Patients who require rapid initiation of therapy may receive higher initiation doses of warfarin. A typical regime in this situation is to give 10 mg warfarin on the first and second days, with 5 mg on the third day; subsequent doses are titrated against the INR. Patients without an urgent need for anticoagulation (e.g. atrial fibrillation) can have warfarin introduced slowly using lower doses. Low-dose regimens are associated with a lower risk of the patient developing a supratherapeutic INR, and hence a lower bleeding risk. The duration of warfarin therapy depends on the clinical indication, and while treatment of deep vein thrombosis

(DVT) or preparation for cardioversion may require a limited duration, anticoagulation to prevent cardioembolic stroke in atrial fibrillation or from heart valve disease is long-term. The major problems with warfarin are: • a narrow therapeutic window • metabolism that is affected by many factors • numerous drug interactions. weight and with a glomerular filtration rate below 30 mL/min). • LMWHs have a half-life of around 4 hours when given subcutaneously, compared with 1 hour for UFH. This permits once-daily dosing by the subcutaneous route, rather than the therapeutic continuous intravenous infusion or twice-daily subcutaneous administration required for UFH. • While rates of bleeding are similar between products, the risk of osteoporosis and heparin-induced thrombocytopenia is much lower for LMWH. UFH is, however, more completely reversed by protamine sulphate in the event of bleeding and at the end of cardiopulmonary bypass, for which UFH remains the drug of choice (Box 23.27). LMWHs are widely used for the prevention and treatment of VTE, the management of acute coronary syndromes and for most other scenarios listed in Box 23.25. In some situations, UFH is still favoured by some clinicians, though there is little evidence that it is advantageous, except when rapid reversibility is required. UFH is useful in patients with a high risk of bleeding, e.g. those who have peptic ulceration or who may require urgent surgery. It is also favoured in the treatment of life-threatening thromboembolism, e.g. major pulmonary embolism with significant hypoxaemia, hypotension and right-sided heart strain. In this situation, UFH is started with a loading intravenous dose of 80 U/ kg, followed by a continuous infusion of 18 U/kg/hr initially. The level of anticoagulation should be assessed by the APTT after 6 hours and, if satisfactory, twice daily thereafter. It is usual to aim for a patient APTT that is 1.5–2.5 times the control time of the test. Monitoring of UFH treatment by APTT is not without difficulties and other assays, such as the specific anti-Xa assay, may provide more accurate guidance. Heparin-induced thrombocytopenia Heparin-induced thrombocytopenia (HIT) is a rare complication of heparin therapy, caused by induction of anti-heparin/PF4 antibodies that bind to and activate platelets via an Fc receptor. This results in platelet activation and a prothrombotic state, with a paradoxical thrombocytopenia. HIT is more common in surgical than medical patients (especially cardiac and orthopaedic patients), with use of UFH rather than LMWH, and with higher doses of heparin. Clinical features Patients present, typically 5–14 days after starting heparin treatment, with a fall in platelet count of more than 30% from 23.27 Treatments for emergencies in haematological practice • Reversal of life- and limb-threatening haemorrhage in anticoagulated patients: Warfarin: prothrombin complex concentrate and IV vitamin K1 Unfractionated heparin: protamine sulphate Dabigatran: idarucizumab • Recognition of thrombotic thrombocytopenic purpura and treatment with plasma exchange • Recognition of coagulopathy associated with acute promyelocytic leukaemia and treatment with all-trans-retinoic acid and fibrinogen replacement • Recognition of chest syndrome and stroke in patients with sickle-cell anaemia and red cell transfusion or exchange transfusion • Recognition of neutropenic sepsis in patients receiving chemotherapy and early treatment with empirical broad-spectrum antibiotics

940 • HAEMATOLOGY AND TRANSFUSION MEDICINE 2–4 hours after oral intake, have very few drug interactions and are all moderately dependent on renal function for their excretion. An initial perceived drawback was the lack of specific reversal agents for these drugs but idarucizumab is a monoclonal antibody now available for the reversal of dabigatran, and andexanet alfa, a site-inactivated Xa molecule, is close to licensing for the reversal of apixaban and rivaroxaban (see Box 23.27). DOACs are now licensed for the prevention of VTE following high-risk orthopaedic surgery (except edoxaban), the acute management and prevention of recurrence of VTE, and the prevention of stroke and systemic embolism in patients with atrial fibrillation with risk factors. The

general perception at present is that in these indications they are at least as efficacious as dose-adjusted coumarin and probably associated with less clinically significant bleeding. Anaemias Around 30% of the total world population is anaemic and half of these, some 600 million people, have iron deficiency. The classification of anaemia by the size of the red cells (MCV) indicates the likely cause (see Figs 23.10 and 23.11). Red cells in the bone marrow must acquire a minimum level of haemoglobin before being released into the blood stream (Fig. 23.17). While in the marrow compartment, red cell precursors undergo cell division, driven by erythropoietin. If red cells cannot acquire haemoglobin at a normal rate, they will undergo more divisions than normal and will have a low MCV when finally released into the blood. The MCV is low because component parts of the haemoglobin molecule are not fully available: that is, iron in iron deficiency, globin chains in thalassaemia, haem ring in congenital sideroblastic anaemia and, occasionally, poor iron utilisation in the anaemia of chronic disease/anaemia of inflammation. In megaloblastic anaemia, the biochemical consequence of vitamin B12 or folate deficiency is an inability to synthesise new bases to make DNA. A similar defect of cell division is seen in the presence of cytotoxic drugs or haematological disease in the marrow, such as myelodysplasia. In these states, cells haemoglobinise normally but undergo fewer cell divisions, resulting in circulating red cells with a raised MCV. The red cell membrane is composed of a lipid bilayer that will freely exchange with the plasma pool of lipid. Conditions such as liver disease, hypothyroidism, hyperlipidaemia and pregnancy are associated with raised lipids and may also cause a raised MCV. Reticulocytes are larger than mature red cells, so when the reticulocyte count is raised – e.g. in haemolysis – this may also increase the MCV.

Iron deficiency anaemia This occurs when iron losses or physiological requirements exceed absorption. **Blood loss** The most common explanation in men and post-menopausal women is gastrointestinal blood loss (p. 780). This may result from occult gastric or colorectal malignancy, gastritis, peptic ulceration, inflammatory bowel disease, diverticulitis, polyps and angiodysplastic lesions. Worldwide, hookworm and schistosomiasis are the most common causes of gut blood loss (pp. 288 and 294). Gastrointestinal blood loss may be exacerbated

Drug interactions are common through protein binding and metabolism by the cytochrome P450 system. Inter-individual differences in warfarin doses required to achieve a therapeutic INR are mostly accounted for by naturally occurring polymorphisms in the CYP2C9 and the VKORC1 genes (which predict the metabolism and function of warfarin, respectively) and dietary intake of vitamin K. Major bleeding is the most common serious side-effect of warfarin and occurs in 1–2% of patients each year. Fatal haemorrhage, which is most commonly intracranial, occurs in about 0.25% per annum. There are scoring systems that predict the annual bleeding risk and these can be used to help compare the risks and benefits of warfarin for an individual patient (Box 23.28). There are also some specific contraindications to anticoagulation (Box 23.28). Management of warfarin includes strategies for over-anticoagulation and for bleeding:

- If the INR is above the therapeutic level, warfarin should be withheld or the dose reduced. If the patient is not bleeding, it may be appropriate to give a small dose of vitamin K either orally or intravenously (1–2.5 mg), especially if the INR is greater than 8.
- In the event of bleeding, withhold further warfarin. Minor bleeding can be treated with 1–2.5 mg of vitamin K IV. Major haemorrhage should be treated as an emergency with vitamin K 5–10 mg slowly IV, combined with coagulation factor replacement (see Box 23.27). This should optimally be a prothrombin complex concentrate (30–50 U/kg) that contains factors II, VII, IX and X; if that is not available, fresh frozen plasma (15–30 mL/kg) should be given.

Direct oral anticoagulants The direct oral anticoagulants (DOACs) offer an alternative to coumarins in the management of VTE and the prevention of stroke and systemic embolism in patients with atrial fibrillation. The DOACs are direct specific inhibitors of key proteases in the common pathway.

Dabigatran inhibits thrombin while rivaroxaban, apixaban and edoxaban inhibit Xa. The key features of these drugs include the fact that they are efficacious in fixed oral doses, have a short half-life of around 10 hours, achieve peak plasma levels 23.28 How to assess risks of anticoagulation Contraindications • Recent surgery, especially to eye or central nervous system • Pre-existing haemorrhagic state, e.g. advanced liver disease, haemophilia, thrombocytopenia • Pre-existing structural lesions, e.g. peptic ulcer • Recent cerebral or gastrointestinal haemorrhage • Uncontrolled hypertension • Cognitive impairment • Frequent falls Bleeding risk score • Several bleeding risk scores exist for different indications for anticoagulation • The validation of most bleeding risk scores has been poor • Many risk factors for thrombosis are also risk factors for bleeding • Following anticoagulant-related bleeding, reassessment of bleeding and thrombosis risk is indicated • In many cases, patients benefit from recommencing anticoagulants after bleeding

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very specific test; a subnormal level is due to iron deficiency or, very rarely, hypothyroidism or vitamin C deficiency. Ferritin levels can be raised in liver disease and in the acute phase response; in these conditions, a ferritin level of up to 100 µg/L may still be compatible with low bone marrow iron stores. by the chronic use of aspirin or non-steroidal anti-inflammatory drugs (NSAIDs), which cause intestinal erosions and impair platelet function. In women of child-bearing age, menstrual blood loss, pregnancy and breastfeeding contribute to iron deficiency by depleting iron stores; in developed countries, one-third of pre-menopausal women have low iron stores but only 3% display iron-deficient haematopoiesis. Very rarely, chronic haemoptysis or haematuria may cause iron deficiency. Malabsorption A dietary assessment should be made in all patients to ascertain their iron intake (p. 716). Gastric acid is required to release iron from food and helps to keep iron in the soluble ferrous state (Fig. 23.18). Achlorhydria in the elderly or that due to drugs such as proton pump inhibitors may contribute to the lack of iron availability from the diet, as may previous gastric surgery. Iron is absorbed actively in the upper small intestine and hence can be affected by coeliac disease (p. 805). Physiological demands At times of rapid growth, such as infancy and puberty, iron requirements increase and may outstrip absorption. In pregnancy, iron is diverted to the fetus, the placenta and the increased maternal red cell mass, and is lost with bleeding at parturition (Box 23.29). Investigations Confirmation of iron deficiency Serum ferritin is a measure of iron stores in tissues and is the best single test to confirm iron deficiency (Box 23.30). It is a Fig. 23.17 Factors that influence the size of red cells in anaemia. In microcytosis, the MCV is < 76 fL. In macrocytosis, the MCV is > 100 fL. (MCV = mean cell volume; RBC = red blood cell) Normal Defective haemoglobinisation Defective DNA synthesis Normal DNA synthesis e.g. Iron deficiency Thalassaemia Sideroblastic anaemia Reticulocyte e.g. ↓ B12 ↓ Folate Cytotoxic drugs Myelodysplasia Marked reticulocytosis Normal-sized RBC Elevated plasma lipid Liver disease Hypothyroidism Alcohol Hyperlipidaemia Pregnancy Normal haemoglobinisation Macrocytosis (↑ MCV) Microcytosis (↓ MCV) Marrow Blood 23.29 Haematological physiology in pregnancy • Full blood count: increased plasma volume (40%) lowers normal haemoglobin (reference range reduced to > 105 g/L at 28 weeks). The mean cell volume (MCV) may increase by 5 fL. A progressive neutrophilia occurs. Gestational thrombocytopenia (rarely < 60 × 10⁹/L) is a benign phenomenon. • Depletion of iron stores: iron deficiency is a common cause of anaemia in pregnancy and, if present, should be treated with oral iron supplement. • Vitamin B12: serum levels are physiologically low in pregnancy but deficiency is uncommon. • Folate: tissue stores may become depleted, and folate supplementation is recommended in all pregnancies (see Box 19.29, p. 712). •

Coagulation factors: from the second trimester, procoagulant factors increase approximately threefold, particularly fibrinogen, von Willebrand factor and factor VIII. This causes activated protein C resistance and a shortened activated partial thromboplastin time (APTT), and contributes to a prothrombotic state. • Anticoagulants: levels of protein C increase from the second trimester, while levels of free protein S fall as C4b binding protein increases.

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“ 90% Iron available for absorption or Amino acids Vitamin C Phytates Tannins Phosphates Dietary iron 7 mg/1000 kcal < 5% ~30% Iron binds to transferrin for delivery to tissues Maximum iron absorption 3.5 mg/day Tissue iron Enzymes (2%) Myoglobin (4%) Ferritin (29%) Haemoglobin (65%) High hepcidin state Low hepcidin state Ferroportin internalised Ferroportin available Gut lumen Fe Fe Fe Fe Fe Fe Fe Fe Blood Enterocyte Ferroportin Hepcidin Inflammatory cytokines induce hepcidin secretion from liver Anaemia Hypoxia Low iron stores suppress hepcidin secretion from liver 23.30 Investigations to differentiate anaemia of chronic disease from iron deficiency anaemia Ferritin Iron TIBC Transferrin saturation Soluble transferrin receptor Iron deficiency anaemia ↓ ↓ ↑ ↓ ↑ Anaemia of chronic disease ↑/Normal ↓ ↓ ↓ ↓/Normal (TIBC = total iron binding capacity)

Megaloblastic anaemia This results from a deficiency of vitamin B12 or folic acid, or from disturbances in folic acid metabolism. Folate is an important substrate of, and vitamin B12 a co-factor for, the generation of the essential amino acid methionine from homocysteine. This reaction produces tetrahydrofolate, which is converted to thymidine monophosphate for incorporation into DNA. Deficiency of either vitamin B12 or folate will therefore produce high plasma levels of homocysteine and impaired DNA synthesis. The end result is cells with arrested nuclear maturation but normal cytoplasmic development: so-called nucleocytoplasmic asynchrony. All proliferating cells will exhibit megaloblastosis; hence changes are evident in the buccal mucosa, tongue, small intestine, cervix, vagina and uterus. The high proliferation rate of bone marrow results in striking changes in the haematopoietic system in megaloblastic anaemia. Cells become arrested in development and die within the marrow; this ineffective erythropoiesis results in an expanded hypercellular marrow. The megaloblastic changes are most evident in the early nucleated red cell precursors, and haemolysis within the marrow results in a raised bilirubin and lactate dehydrogenase (LDH), but without the reticulocytosis characteristic of other forms of haemolysis (p. 945). Iron stores are usually raised. The mature red cells are large and oval, and sometimes contain nuclear remnants. Nuclear changes are seen in the immature granulocyte precursors and a characteristic appearance is that of 'giant' metamyelocytes with a large 'sausage-shaped' nucleus. The mature neutrophils show hypersegmentation of their nuclei, with cells having six or more nuclear lobes. If severe, a pancytopenia may be present in the peripheral blood. Vitamin B12 deficiency, but not folate deficiency, is associated with neurological disease in up to 40% of cases, although advanced neurological disease due to B12 deficiency is now uncommon in the developed world. The main pathological finding is focal demyelination affecting the spinal cord, peripheral nerves, optic nerves and cerebrum. The most common manifestations are sensory, with peripheral paraesthesiae and ataxia of gait. The clinical and diagnostic features of megaloblastic anaemia are summarised in Boxes 23.31 and 23.32, and the neurological features of B12 deficiency in Box 23.33.

Vitamin B12 absorption The average daily diet contains 5–30 µg of vitamin B12, mainly in meat, fish, eggs and milk – well in excess of the 1 µg daily requirement. Management Unless the patient has angina, heart failure or evidence of cerebral hypoxia, transfusion is not necessary and oral iron replacement is appropriate. Ferrous sulphate 200 mg 3 times daily (195 mg of elemental iron per day) is adequate and should be continued for 3–6 months to replete iron stores. Many patients suffer gastrointestinal side-effects with ferrous sulphate, including dyspepsia and altered bowel habit. When this occurs, reduction in dose to 200 mg twice daily or a switch to ferrous gluconate 300 mg twice daily (70 mg of elemental iron per day) or another alternative oral preparation should be tried. Delayed-release preparations are not useful, since they release iron beyond the upper small intestine, where it cannot be absorbed. The haemoglobin should rise by around 10 g/L every 7–10 days and a reticulocyte response will be evident within a week. A failure to respond adequately may be due to non-adherence, continued blood loss, malabsorption or an incorrect diagnosis. Patients with malabsorption, chronic gut disease or inability to tolerate any oral preparation may need parenteral iron therapy. Previously, iron dextran or iron sucrose was used, but new preparations of iron isomaltose and iron carboxymaltose have fewer allergic effects and are preferred. Doses required can be calculated based on the patient's starting haemoglobin and body weight. Observation for anaphylaxis following an initial test dose is recommended.

Anaemia of chronic disease Anaemia of chronic disease (ACD), also known as anaemia of inflammation (AI), is a common type of anaemia, particularly in hospital populations. It occurs in the setting of chronic infection, chronic inflammation or neoplasia. The anaemia is not related to bleeding, haemolysis or marrow infiltration, is mild, with haemoglobin in the range of 85–115 g/L, and is usually associated

with a normal MCV (normocytic, normochromic), though this may be reduced in long-standing inflammation. The serum iron is low but iron stores are normal or increased, as indicated by the ferritin or stainable marrow iron. Pathogenesis It has recently become clear that the key regulatory protein that accounts for the findings characteristic of ACD/AI is hepcidin, which is produced by the liver (see Fig. 23.18). Hepcidin production is induced by pro-inflammatory cytokines, especially IL-6. Hepcidin binds to ferroportin on the membrane of iron-exporting cells, such as small intestinal enterocytes and macrophages, internalising the ferroportin and thereby inhibiting the export of iron from these cells into the blood. The iron remains trapped inside the cells in the form of ferritin, levels of which are therefore normal or high in the face of significant anaemia. Inhibition or blockade of hepcidin is a potential target for treatment of this form of anaemia. Diagnosis and management It is often difficult to distinguish ACD associated with a low MCV from iron deficiency. Box 23.30 summarises the investigations and results. Examination of the marrow may ultimately be required to assess iron stores directly. A trial of oral iron can be given in difficult situations. A positive response occurs in true iron deficiency but not in ACD. Measures that reduce the severity of the underlying disorder generally help to improve the ACD. Trials of higher-dose intravenous iron are under way to try to bypass the hepcidin-induced blockade.

23.31 Clinical features of megaloblastic anaemia

Symptoms • Malaise (90%) • Breathlessness (50%) • Paraesthesiae (80%) • Sore mouth (20%) • Weight loss • Impotence • Poor memory • Depression • Personality change • Hallucinations • Visual disturbance

Signs • Smooth tongue • Angular cheilosis • Vitiligo • Skin pigmentation • Heart failure • Pyrexia

944 • HAEMATOLOGY AND TRANSFUSION MEDICINE not add much in most clinical situations. Levels of cobalamins fall in normal pregnancy. Reference ranges vary between laboratories but levels below 150 ng/L are common and, in the last trimester, 5–10% of women have levels below 100 ng/L. Spuriously low B12 values occur in women using the oral contraceptive pill and in patients with myeloma, in whom paraproteins can interfere with vitamin B12 assays. Causes of vitamin B12 deficiency

Dietary deficiency This occurs only in strict vegans but the onset of clinical features can occur at any age between 10 and 80 years. Less strict vegetarians often have slightly low vitamin B12 levels but are not tissue vitamin B12-deficient. Gastric pathology Release of vitamin B12 from food requires normal gastric acid and enzyme secretion, and this is impaired by hypochlorhydria in elderly patients or following gastric surgery. Total gastrectomy invariably results in vitamin B12 deficiency within 5 years, often combined with iron deficiency; these patients need life-long 3-monthly vitamin B12 injections. After partial gastrectomy, vitamin B12 deficiency only develops in 10–20% of patients by 5 years; an annual injection of vitamin B12 should prevent deficiency in this group.

Pernicious anaemia This is an organ-specific autoimmune disorder in which the gastric mucosa is atrophic, with loss of parietal cells causing intrinsic factor deficiency. In the absence of intrinsic factor, less than 1% of dietary vitamin B12 is absorbed. Pernicious anaemia has an incidence of 25/100 000 population over the age of 40 years in developed countries, but an average age of onset of 60 years. It is more common in individuals with other autoimmune disease (Hashimoto's thyroiditis, Graves' disease, vitiligo or Addison's disease; Ch. 18) or a family history of these or pernicious anaemia. The finding of anti-intrinsic factor antibodies in the context of B12 deficiency is diagnostic of pernicious anaemia without further investigation. Antiparietal cell antibodies are present in over 90% of cases but are also present in 20% of normal females over the age of 60 years; a negative result makes pernicious anaemia less likely but a positive result is not diagnostic. The Schilling test, involving measurement of absorption of radio-labelled B12 after oral administration before and after replacement of intrinsic factor, has fallen out of favour with the

availability of autoantibody tests, greater caution in the use of radioactive tracers, and limited availability of intrinsic factor. Small bowel pathology One-third of patients with pancreatic exocrine insufficiency fail to transfer dietary vitamin B12 from R protein to intrinsic factor. This usually results in slightly low vitamin B12 values but no tissue evidence of vitamin B12 deficiency. Motility disorders or hypogammaglobulinaemia can result in bacterial overgrowth, and the ensuing competition for free vitamin B12 can lead to deficiency. This is corrected to some extent by appropriate antibiotics. A small number of people heavily infected with the fish tapeworm (p. 297) develop vitamin B12 deficiency. Inflammatory disease of the terminal ileum, such as Crohn's disease, may impair the absorption of vitamin B12-intrinsic factor complex, as may surgery on that part of the bowel. requirement. In the stomach, gastric enzymes release vitamin B12 from food and at gastric pH it binds to a carrier protein termed R protein. The gastric parietal cells produce intrinsic factor, a vitamin B12-binding protein that optimally binds vitamin B12 at pH 8. As gastric emptying occurs, pancreatic secretion raises the pH and vitamin B12 released from the diet switches from the R protein to intrinsic factor. Bile also contains vitamin B12 that is available for reabsorption in the intestine. The vitamin B12-intrinsic factor complex binds to specific receptors in the terminal ileum, and vitamin B12 is actively transported by the enterocytes to plasma, where it binds to transcobalamin II, a transport protein produced by the liver, which carries it to the tissues for utilisation. The liver stores enough vitamin B12 for 3 years and this, together with the enterohepatic circulation, means that vitamin B12 deficiency takes years to become manifest, even if all dietary intake is stopped or severe B12 malabsorption supervenes. Blood levels of vitamin B12 (cobalamin) provide a reasonable indication of tissue stores, are usually diagnostic of deficiency and remain the first-line tests for most laboratories. Additional tests have been evaluated, including measurement of methylmalonic acid, holotranscobalamin and plasma homocysteine levels, but do not.

Neurological findings in B12 deficiency

- Peripheral nerves • Glove and stocking paraesthesiae • Loss of ankle reflexes
- Spinal cord • Subacute combined degeneration of the cord
- Posterior columns – diminished vibration sensation and proprioception
- Corticospinal tracts – upper motor neuron signs
- Cerebrum • Dementia • Optic atrophy
- Autonomic neuropathy

Investigations in megaloblastic anaemia

Investigation	Result
Haemoglobin	Often reduced, may be very low
Mean cell volume	Usually raised, commonly > 120 fL
Erythrocyte count	Low for degree of anaemia
Blood film	Oval macrocytosis, poikilocytosis, red cell fragmentation, neutrophil hypersegmentation
Reticulocyte count	Low for degree of anaemia
Leucocyte count	Low or normal
Platelet count	Low or normal
Bone marrow	Increased cellularity, megaloblastic changes in erythroid series, giant metamyelocytes, dysplastic megakaryocytes, increased iron in stores, pathological non-ring sideroblasts
Serum ferritin	Elevated
Plasma lactate dehydrogenase	Elevated, often markedly

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results are available, that treatment should always include both folic acid and vitamin B12. The use of folic acid alone in the presence of vitamin B12 deficiency may result in worsening of neurological features. Rarely, if severe angina or heart failure is present, transfusion can be used in megaloblastic anaemia. The cardiovascular system is adapted to the chronic anaemia present in megaloblastosis, and the volume load imposed by transfusion may result in decompensation and severe cardiac failure. In such circumstances, exchange transfusion or slow administration of 1 U of red cells with diuretic cover may be given. Vitamin B12 deficiency Vitamin B12 deficiency is treated with hydroxycobalamin. In cases of uncomplicated deficiency, 1000 µg IM for 6 doses 2 or 3 days

apart, followed by maintenance therapy of 1000 µg every 3 months for life, is recommended. In the presence of neurological involvement, a dose of 1000 µg on alternate days until there is no further improvement, followed by maintenance as above, is recommended. The reticulocyte count will peak by the 5th–10th day after starting replacement therapy. The haemoglobin will rise by 10 g/L every week until normalised. The response of the marrow is associated with a fall in plasma potassium levels and rapid depletion of iron stores. If an initial response is not maintained and the blood film is dimorphic (i.e. shows a mixture of microcytic and macrocytic cells), the patient may need additional iron therapy. A sensory neuropathy may take 6–12 months to correct; long-standing neurological damage may not improve. Folate deficiency Oral folic acid (5 mg daily for 3 weeks) will treat acute deficiency and 5 mg once weekly is adequate maintenance therapy. Prophylactic folic acid in pregnancy prevents megaloblastosis in women at risk, and reduces the risk of fetal neural tube defects (p. 712). Prophylactic supplementation is also given in chronic haematological disease associated with reduced red cell lifespan (e.g. haemolytic anaemias). There is some evidence that supraphysiological supplementation (400 µg/day) can reduce the risk of coronary and cerebrovascular disease by lowering plasma homocysteine levels. This has led the US Food and Drug Administration to introduce fortification of bread, flour and rice with folic acid.

Haemolytic anaemia Haemolysis indicates that there is shortening of the normal red cell lifespan of 120 days. There are many causes, as shown in Figure 23.19. To compensate, the bone marrow may increase its output of red cells six- to eightfold by increasing the proportion of red cells produced, expanding the volume of active marrow, and releasing reticulocytes prematurely. Anaemia occurs only if the rate of destruction exceeds this increased production rate. There are some general features of haemolysis and other specific features that help to identify the reason for haemolysis. Results of investigations that establish the presence of haemolysis are shown in Box 23.36. Red cell destruction overloads pathways for haemoglobin breakdown in the liver (p. 850), causing a modest rise in unconjugated bilirubin in the blood and mild jaundice. Increased reabsorption of urobilinogen from the gut results in an increase in urinary urobilinogen (pp. 860 and 915). Red cell destruction releases LDH into the serum. The bone

Folate Folate absorption Foliates are produced by plants and bacteria; hence dietary leafy vegetables (spinach, broccoli, lettuce), fruits (bananas, melons) and animal protein (liver, kidney) are a rich source. An average Western diet contains more than the minimum daily intake of 50 µg but excess cooking destroys folates. Most dietary folate is present as polyglutamates; these are converted to monoglutamate in the upper small bowel and actively transported into plasma. Plasma folate is loosely bound to plasma proteins such as albumin and there is an enterohepatic circulation. Total body stores of folate are small and deficiency can occur in a matter of weeks. Folate deficiency The causes and diagnostic features of folate deficiency are shown in Boxes 23.34 and 23.35. The edentulous elderly or psychiatric patient is particularly susceptible to dietary deficiency and this is exacerbated in the presence of gut disease or malignancy. Pregnancy-induced folate deficiency is the most common cause of megaloblastosis worldwide and is more likely in the context of twin pregnancies, multiparity and hyperemesis gravidarum. Serum folate measurement is very sensitive to dietary intake; a single folate-rich meal can normalise it in a patient with true folate deficiency, whereas anorexia, alcohol and anticonvulsant therapy can reduce it in the absence of megaloblastosis. For this reason, red cell folate levels are a more accurate indicator of folate stores and tissue folate deficiency. Management of megaloblastic anaemia If a patient with a severe megaloblastic anaemia is very ill and treatment must be started before vitamin B12 and red cell folate 23.35

Investigation of folic acid deficiency Diagnostic findings • Serum folate levels may be low but are difficult to interpret • Low red cell folate levels indicate prolonged folate deficiency and are

probably the most relevant measure Corroborative findings • Macrocytic dysplastic blood picture • Megaloblastic marrow *Usually only a problem in patients deficient in folate from another cause.* 23.34 Causes of folate deficiency Diet • Poor intake of vegetables Malabsorption • e.g. Coeliac disease, small bowel surgery Increased demand • Cell proliferation, e.g. haemolysis • Pregnancy Drugs • Certain anticonvulsants (e.g. phenytoin) • Contraceptive pill • Certain cytotoxic drugs (e.g. methotrexate)

946 • HAEMATOLOGY AND TRANSFUSION MEDICINE the red cells may give an indication of the likely cause of the haemolysis: • Spherocytes are small, dark red cells that suggest autoimmune haemolysis or hereditary spherocytosis. • Sickle cells suggest sickle-cell disease. • Red cell fragments indicate microangiopathic haemolysis. • Bite cells (normal-sized red cells that look as if they have been partially eaten) suggest oxidative haemolysis. The compensatory erythroid hyperplasia may give rise to folate deficiency, with megaloblastic blood features. The differential diagnosis of haemolysis is determined by the clinical scenario in combination with the results of blood film examination and Coombs testing for antibodies directed against red cells (see below and Fig. 23.19). Extravascular haemolysis Physiological red cell destruction occurs in the reticulo-endothelial cells in the liver or spleen, so avoiding free haemoglobin in the marrow compensation results in a reticulocytosis, and sometimes nucleated red cell precursors appear in the blood. Increased proliferation of the bone marrow can result in a thrombocytosis, neutrophilia and, if marked, immature granulocytes in the blood, producing a leucoerythroblastic blood film. The appearances of Fig. 23.19 Causes and classification of haemolysis. A Inherited causes. B Acquired causes. (CLL = chronic lymphocytic leukaemia; DIC = disseminated intravascular coagulation; EBV = Epstein-Barr virus; G6PD = glucose-6-phosphate dehydrogenase; HUS = haemolytic uraemic syndrome; PK = pyruvate kinase; RA = rheumatoid arthritis; SLE = systemic lupus erythematosus; TTP = thrombotic thrombocytopenic purpura) A B Primary idiopathic Secondary •Autoimmune, e.g. SLE, RA •Drugs, e.g. L-dopa, methyl dopa, mefenamic acid, penicillin, quinidine, fludarabine •Lymphoid malignancy, e.g. CLL, myeloma, lymphoma •Other malignancy, e.g. lung, colon, kidney, ovary, thymoma •Others, e.g. ulcerative colitis, HIV Primary idiopathic Secondary •Infection, e.g. mycoplasma, EBV, syphilis •Lymphoproliferative disorders, e.g. lymphoma Red cell antigen-induced •Transfusion reaction •Haemolytic disease of the newborn Immune Acquired Inherited Non-immune Autoantibodies Alloantibodies Warm antibodies Cold antibodies Mechanical •Prosthetic valves •Microangiopathic, e.g. DIC, HUS, TTP •March haemoglobinuria Infection •Intracellular organisms, e.g. malaria •Toxins, e.g. C. perfringens Chemical/physical •Oxidative drugs, e.g. dapsone, maloprim •Copper (Wilson's disease) •Burns •Drowning

Acquired abnormal membrane •Paroxysmal nocturnal haemoglobinuria Red cell membrane abnormality •Hereditary spherocytosis •Hereditary elliptocytosis Haemoglobin •Deficiency, e.g. thalassaemias •Abnormality, e.g. sickle-cell disease Red cell enzyme deficiency •Glycolytic pathway, e.g. PK •Hexose monophosphate shunt, e.g. G6PD •Pyrimidine 5' nucleotidase 23.36 Investigation results indicating active haemolysis Hallmarks of haemolysis • ↓ Haemoglobin • ↑ Unconjugated bilirubin • ↑ Lactate dehydrogenase • ↑ Reticulocytes • ↑ Urinary urobilinogen Additional features of intravascular haemolysis • ↓ Haptoglobin • ↑ Methaemalbumin • Positive urinary haemosiderin • Haemoglobinuria

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Hereditary spherocytosis This is usually inherited as an autosomal dominant condition, although 25% of cases have no family history and represent new mutations. The incidence is approximately 1 : 5000 in developed countries but this may be an under-estimate, since the disease may present de novo in patients aged over 65 years and is often discovered as a chance finding on a blood count. The most common abnormalities are deficiencies of beta spectrin or ankyrin (see Fig. 23.4). The severity of spontaneous haemolysis varies. Most cases are associated with an asymptomatic compensated chronic haemolytic state with spherocytes present on the blood film, a reticulocytosis and mild hyperbilirubinaemia. Pigment gallstones are present in up to 50% of patients and may cause symptomatic cholecystitis. Occasional cases are associated with more severe haemolysis; these may be due to coincidental polymorphisms in alpha spectrin or co-inheritance of a second defect involving a different protein. These cases tend to present earlier in life with symptomatic, sometimes transfusion-dependent anaemia. The clinical course may be complicated by crises:

- A haemolytic crisis occurs when the severity of haemolysis increases; this is rare, and usually associated with infection.
- A megaloblastic crisis follows the development of folate deficiency; this may occur as a first presentation of the disease in pregnancy.
- An aplastic crisis occurs in association with parvovirus (erythrovirus) infection (p. 237). Parvovirus causes a common exanthem in children, but if individuals with chronic haemolysis become infected, the virus directly invades red cell precursors and temporarily switches off red cell production. Patients present with severe anaemia and a low reticulocyte count.

Investigations The patient and other family members should be screened for features of compensated haemolysis (see Box 23.36). This may be all that is required to confirm the diagnosis. Haemoglobin levels are variable, depending on the degree of compensation. The blood film will show spherocytes but the direct Coombs test (Fig. 23.20) is negative, excluding immune haemolysis. An osmotic fragility test may show increased sensitivity to lysis in hypotonic saline solutions but is limited by lack of sensitivity and specificity. More specific flow cytometric tests, detecting binding of eosin-5-maleimide to red cells, are recommended in borderline cases.

Management Folic acid prophylaxis, 5 mg daily, should be given for life. In severe cases, consideration may be given to splenectomy, which improves but does not normalise red cell survival. Potential indications for splenectomy include moderate to severe haemolysis with complications (anaemia and gallstones), although splenectomy should be delayed where possible until after 6 years of age in view of the risk of sepsis. Guidelines for the management of patients after splenectomy are presented in Box 23.37. Acute, severe haemolytic crises require transfusion support, but blood must be cross-matched carefully and transfused slowly as haemolytic transfusion reactions may occur (p. 935).

Hereditary elliptocytosis This term refers to a heterogeneous group of disorders that produce an increase in elliptocytic red cells on the blood film and a variable degree of haemolysis. This is due to a functional plasma. In most haemolytic states, haemolysis is predominantly extravascular. To confirm the haemolysis, patients' red cells can be labelled with ⁵¹chromium. When re-injected, they can be used to determine red cell survival; when combined with body surface radioactivity counting, this test may indicate whether the liver or the spleen is the main source of red cell destruction. However, it is seldom performed in clinical practice.

Intravascular haemolysis Less commonly, red cell lysis occurs within the blood stream due to membrane damage by complement (ABO transfusion reactions, paroxysmal nocturnal haemoglobinuria), infections (malaria, *Clostridium perfringens*), mechanical trauma (heart valves, DIC) or oxidative damage (e.g. enzymopathies such as glucose-6-phosphate dehydrogenase deficiency, which may be triggered by drugs such as dapsone and maloprim). When intravascular red cell destruction occurs, free haemoglobin is released into the plasma. Free haemoglobin is toxic to cells and binding proteins have evolved to minimise this risk. Haptoglobin

is an α_2 -globulin produced by the liver, which binds free haemoglobin, resulting in a fall in its levels during active haemolysis. Once haptoglobins are saturated, free haemoglobin is oxidised to form methaemoglobin, which binds to albumin, in turn forming methaemalbumin, which can be detected spectrophotometrically in Schumm's test. Methaemoglobin is degraded and any free haem is bound to a second binding protein called haemopexin. If all the protective mechanisms are saturated, free haemoglobin may appear in the urine (haemoglobinuria). When fulminant, this gives rise to black urine, as in severe falciparum malaria infection (p. 274). In smaller amounts, renal tubular cells absorb the haemoglobin, degrade it and store the iron as haemosiderin. When the tubular cells are subsequently sloughed into the urine, they give rise to haemosiderinuria, which is always indicative of intravascular haemolysis (Box 23.36). Causes of haemolytic anaemia These can be classified as inherited or acquired (Fig. 23.19).

- Inherited red cell abnormalities resulting in chronic haemolytic anaemia may arise from pathologies of the red cell membrane (hereditary spherocytosis or elliptocytosis), haemoglobin (haemoglobinopathies), or protective enzymes that prevent cellular oxidative damage, such as glucose-6-phosphate dehydrogenase (G6PD).
- Acquired causes include auto- and alloantibody-mediated destruction of red blood cells and other mechanical, toxic and infective causes.

Red cell membrane defects The structure of the red cell membrane is shown in Figure 23.4. The basic structure is a cytoskeleton 'stapled' on to the lipid bilayer by special protein complexes. This structure ensures great deformability and elasticity; the red cell diameter is 8 μm but the narrowest capillaries in the circulation are in the spleen, measuring just 2 μm in diameter. When the normal red cell structure is disturbed, usually by a quantitative or functional deficiency of one or more proteins in the cytoskeleton, cells lose their elasticity. Each time such cells pass through the spleen, they lose membrane relative to their cell volume. This results in an increase in mean cell haemoglobin concentration (MCHC), abnormal cell shape (see Box 23.2) and reduced red cell survival due to extravascular haemolysis.

948 • HAEMATOLOGY AND TRANSFUSION MEDICINE A characteristic variant of hereditary elliptocytosis occurs in South-east Asia, particularly Malaysia and Papua New Guinea, with stomatocytes and ovalocytes in the blood. This has a prevalence of up to 30% in some communities because it offers relative protection from malaria and thus has sustained a high gene frequency. The blood film is often very abnormal and immediate differential diagnosis is broad. Red cell enzymopathies The mature red cell must produce energy via ATP to maintain a normal internal environment and cell volume while protecting itself from the oxidative stress presented by oxygen carriage. ATP is generated by glycolysis, while the hexose monophosphate shunt produces nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione to protect against oxidative stress. The impact of functional or quantitative defects in the enzymes in these pathways depends on the importance of the steps affected and the presence of alternative pathways. In general, defects in the hexose monophosphate shunt pathway result in periodic haemolysis precipitated by episodic oxidative stress, while those in the glycolysis pathway result in shortened red cell survival and chronic haemolysis. Glucose-6-phosphate dehydrogenase deficiency The enzyme glucose-6-phosphate dehydrogenase (G6PD) is pivotal in the hexose monophosphate shunt pathway. Deficiencies result in the most common human enzymopathy, affecting 10% of the world's population, with a geographical distribution that parallels the malaria belt because heterozygotes are protected from malarial parasitisation. The enzyme is a heteromeric structure made of catalytic subunits that are encoded by a gene on the X chromosome. The deficiency therefore affects males and rare abnormality of one or more anchor proteins in the red cell membrane, e.g. alpha spectrin or protein 4.1 (see Fig. 23.4). Inheritance may be autosomal dominant or recessive. Hereditary

elliptocytosis is less common than hereditary spherocytosis in Western countries, with an incidence of 1/10 000, but is more common in equatorial Africa and parts of South-east Asia. The clinical course is variable and depends on the degree of membrane dysfunction caused by the inherited molecular defect(s); most cases present as an asymptomatic blood film abnormality but occasional cases result in neonatal haemolysis or a chronic compensated haemolytic state. Management of the latter is the same as for hereditary spherocytosis.

23.37 Management of the splenectomised patient

- Vaccinate with pneumococcal, Haemophilus influenzae type B, meningococcal group C and influenza vaccines at least 2–3 weeks before elective splenectomy. Vaccination should be given after emergency surgery but may be less effective
- Pneumococcal re-immunisation should be given at least 5-yearly and influenza annually. Vaccination status must be documented
- Life-long prophylactic penicillin V (500 mg twice daily) is recommended. In penicillin-allergic patients, consider a macrolide
- Patients should be educated regarding the risks of infection and methods of prophylaxis
- A card or bracelet should be carried to alert health professionals to the risk of overwhelming sepsis
- In sepsis, patients should be resuscitated and given IV antibiotics to cover pneumococcus, Haemophilus and meningococcus, according to local resistance patterns
- The risk of cerebral malaria is increased in the event of infection
- Animal bites should be promptly treated with local disinfection and antibiotics, to prevent serious soft tissue infection and sepsis

Fig. 23.20 Direct and indirect antiglobulin tests. Direct antiglobulin test (DAT) (Coombs test) Detects the presence of antibody bound to the red cell surface, e.g.

1. Autoimmune haemolytic anaemia
2. Haemolytic disease of newborn
3. Transfusion reactions Antibodies to human globulin Red cell agglutination Indirect antiglobulin test (IAT) (indirect Coombs test) Detects antibodies in the plasma, e.g.
4. Antibody screen in pre-transfusion testing
5. Screening in pregnancy for antibodies that may cause

haemolytic disease of newborn Red cells with known antigen expression Red cell agglutination Patient's plasma Stage 1 Red cells with Ag – Ab complex on cell surface Stage 2 Antibodies to human globulin Key Red blood cells Red cell antigen Antibody bound to red cell antigen A B

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Pyrimidine 5' nucleotidase deficiency The pyrimidine 5' nucleotidase enzyme catalyses the dephosphorylation of nucleoside monophosphates and is important during the degradation of RNA in reticulocytes. It is inherited as an autosomal recessive trait and is as common as pyruvate kinase deficiency in Mediterranean, African and Jewish populations. The accumulation of excess ribonucleoprotein results in coarse basophilic stippling (see Box 23.2), associated with a chronic haemolytic state. The enzyme is very sensitive to inhibition by lead and this is the reason why basophilic stippling is a feature of lead poisoning.

Autoimmune haemolytic anaemia This results from increased red cell destruction due to red cell autoantibodies. The antibodies may be IgG or IgM, or more rarely IgE or IgA. If an antibody avidly fixes complement, it will cause intravascular haemolysis, but if complement activation is weak, the haemolysis will be extravascular (in the reticulo-endothelial system). Antibody-coated red cells lose membrane to macrophages in the spleen and hence spherocytes are present in the blood. The optimum temperature at which the antibody is active (thermal specificity) is used to classify immune haemolysis:

- Warm antibodies

bind best at 37°C and account for 80% of cases. The majority are IgG and often react against Rhesus antigens. • Cold antibodies bind best at 4°C but can bind up to 37°C in some cases. They are usually IgM and bind complement. To be clinically relevant, they must act within the range of normal body temperatures. They account for the other 20% of cases.

Warm autoimmune haemolysis The incidence of warm autoimmune haemolysis is approximately 1/100 000 population per annum; it occurs at all ages but is more common in middle age and in females. No underlying cause is identified in up to 50% of cases. The remainder are secondary to a wide variety of other conditions (see Fig. 23.19B). **Investigations** There is evidence of haemolysis, spherocytes and polychromasia on the blood film. The diagnosis is confirmed by the direct Coombs or antiglobulin test (see Fig. 23.20). The patient's red cells are mixed with Coombs reagent, which contains antibodies against human IgG/IgM/complement. If the red cells have been coated by antibody in vivo, the Coombs reagent will induce their agglutination and this can be detected visually. The relevant antibody can be eluted from the red cell surface and tested against a panel of typed red cells to determine against which red cell antigen it is directed. The most common specificity is for Rhesus antigens and most often anti-e; this is helpful when choosing blood to cross-match. The direct Coombs test can be negative in the presence of brisk haemolysis. A positive test requires about 200 antibody molecules to attach to each red cell; with a very avid complement-fixing antibody, haemolysis may occur at lower levels of antibody-binding. The standard Coombs reagent will miss IgA or IgE antibodies. Around 10% of all warm autoimmune haemolytic anaemias are Coombs test-negative.

Management If the haemolysis is secondary to an underlying cause, this must be treated and any implicated drugs stopped.

homozygous females (p. 48), but it is carried by females. Carrier heterozygous females are usually only affected in the neonatal period or in the presence of skewed X-inactivation (p. 49). Over 400 subtypes of G6PD are described. The most common types associated with normal activity are the B+ enzyme present in most Caucasians and 70% of Afro-Caribbeans, and the A+ variant present in 20% of Afro-Caribbeans. The two common variants associated with reduced activity are the A– variety in approximately 10% of Afro-Caribbeans, and the Mediterranean or B– variety in Caucasians. In East and West Africa, up to 20% of males and 4% of females (homozygotes) are affected and have enzyme levels of about 15% of normal. The deficiency in Caucasian and East Asian populations is more severe, with enzyme levels as low as 1%. Clinical features and investigation findings are shown in Box 23.38. **Management** aims to stop the intake of any precipitant drugs or foods and treat any underlying infection. **Favism** due to the consumption of fava beans is the classically described precipitant of haemolysis in patients with G6PD deficiency. Acute transfusion support may be life-saving.

Pyruvate kinase deficiency This is the second most common red cell enzyme defect. It results in deficiency of ATP production and a chronic haemolytic anaemia. It is inherited as an autosomal recessive trait. The extent of anaemia is variable; the blood film shows characteristic 'prickle cells' that resemble holly leaves. Enzyme activity is only 5–20% of normal. Transfusion support may be necessary during periods of haemolysis.

23.38 Glucose-6-phosphate dehydrogenase deficiency **Clinical features** • Acute drug-induced haemolysis to (e.g.): Analgesics: aspirin, phenacetin Antimalarials: primaquine, quinine, chloroquine, pyrimethamine Antibiotics: sulphonamides, nitrofurantoin, ciprofloxacin Miscellaneous: quinidine, probenecid, vitamin K, dapsone • Chronic compensated haemolysis • Infection or acute illness • Neonatal jaundice: may be a feature of the B– enzyme • Favism, i.e. acute haemolysis after ingestion of broad beans (*Vicia fava*) **Laboratory features** Non-spherocytic intravascular haemolysis during an attack The blood film will show: • Bite cells (red cells with a 'bite' of membrane missing) • Blister cells (red cells with surface blistering of the membrane) • Irregularly shaped small cells • Polychromasia reflecting the reticulocytosis • Denatured

haemoglobin visible as Heinz bodies within the red cell cytoplasm with a supravital stain such as methyl violet G6PD level • Can be indirectly assessed by screening methods that usually depend on the decreased ability to reduce dyes • Direct assessment of G6PD is made in those with low screening values • Care must be taken close to an acute haemolytic episode because reticulocytes may have higher enzyme levels and give rise to a false normal result

950 • HAEMATOLOGY AND TRANSFUSION MEDICINE antibody is termed the Donath–Landsteiner antibody and has specificity against the P antigen on the red cells. Alloimmune haemolytic anaemia Alloimmune haemolytic anaemia is caused by antibodies against non-self red cells. It has two main causes, occurring after: • unmatched blood transfusion (p. 935) • maternal sensitisation to paternal antigens on fetal cells (haemolytic disease of the newborn, p. 933). Non-immune haemolytic anaemia Endothelial damage Disruption of red cell membrane may occur in a number of conditions and is characterised by the presence of red cell fragments on the blood film and markers of intravascular haemolysis: • Mechanical heart valves. High flow through incompetent valves or periprosthetic leaks through the suture ring holding a valve in place result in shear stress damage. • March haemoglobinuria. Vigorous exercise, such as prolonged marching or marathon running, can cause red cell damage in the capillaries in the feet. • Thermal injury. Severe burns cause thermal damage to red cells, characterised by fragmentation and the presence of microspherocytes in the blood. • Microangiopathic haemolytic anaemia. Fibrin deposition in capillaries can cause severe red cell disruption. It may occur in a wide variety of conditions: disseminated carcinomatosis, malignant or pregnancy-induced hypertension, haemolytic uraemic syndrome (p. 408), thrombotic thrombocytopenic purpura (p. 979) and disseminated intravascular coagulation (p. 978). Infection *Plasmodium falciparum* malaria (p. 274) may be associated with intravascular haemolysis; when severe, this is termed blackwater fever because of the associated haemoglobinuria. *Clostridium perfringens* sepsis (p. 227), usually in the context of ascending cholangitis or necrotising fasciitis, may cause severe intravascular haemolysis with marked spherocytosis due to bacterial production of a lecithinase that destroys the red cell membrane. Chemicals or drugs Dapsone and sulfasalazine cause haemolysis by oxidative denaturation of haemoglobin. Denatured haemoglobin forms Heinz bodies in the red cells, visible on supravital staining with brilliant cresyl blue. Arsenic gas, copper, chlorates, nitrites and nitrobenzene derivatives may all cause haemolysis. Paroxysmal nocturnal haemoglobinuria Paroxysmal nocturnal haemoglobinuria (PNH) is a rare acquired, non-malignant clonal expansion of haematopoietic stem cells deficient in glycosylphosphatidylinositol (GPI) anchor protein. GPI anchors several key molecules to cells and its absence results in clinical outcomes that reflect this, causing intravascular haemolysis and anaemia because of increased sensitivity of red cells to lysis by complement. This happens because key defence mechanisms that protect cells from complement-mediated lysis (CD55 and CD59) are GPI-anchored to red cells under normal circumstances. Episodes of intravascular haemolysis result in haemoglobinuria, most noticeable in early morning urine, which it is usual to treat patients initially with prednisolone (1 mg/kg orally). A response is seen in 70–80% of cases but may take up to 3 weeks; a rise in haemoglobin will be matched by a fall in bilirubin, LDH and reticulocyte levels. Once the haemoglobin has normalised and the reticulocytosis resolved, the glucocorticoid dose can be reduced slowly over several weeks. Glucocorticoids probably work by decreasing macrophage destruction of antibody-coated red cells and reducing antibody production. Transfusion support may be required for life-threatening problems, such as the development of heart failure or rapid unabated falls in haemoglobin. The least incompatible blood should be used but this may still give rise to transfusion reactions or the

development of alloantibodies. If the haemolysis fails to respond to glucocorticoids or can only be stabilised by large doses, then second-line therapies should be considered. These include immunomodulation/suppression and splenectomy. Currently, there are fewer splenectomies than previously and the second-line drug of choice in current UK guidance is the anti-CD20 monoclonal antibody rituximab. Splenectomy is associated with a good response in 50–60% of cases. The operation can be performed laparoscopically with reduced morbidity. If splenectomy is not appropriate, alternative immunosuppressive therapy with azathioprine, ciclosporin, mycophenolate or cyclophosphamide may be considered. There are concerns about all modes of second-line therapy, as long-term immunosuppression carries a risk of malignancy, while splenectomy is associated with an excess of severe infection due to the capsulate organisms pneumococcus and meningococcus (see Box 23.40).

Cold agglutinin disease This is mediated by antibodies, usually IgM, which bind to the red cells at low temperatures and cause them to agglutinate. It may cause intravascular haemolysis if complement fixation occurs. This can be chronic when the antibody is monoclonal, or acute or transient when the antibody is polyclonal. Chronic cold agglutinin disease This typically affects elderly patients and may be associated with an underlying low-grade B-cell lymphoma. It causes a low-grade intravascular haemolysis with cold, painful and often blue fingers, toes, ears or nose (so-called acrocyanosis). The latter is due to red cell agglutination in the small vessels in these colder, exposed areas. The blood film shows red cell agglutination and the MCV may be spuriously high because the automated analysers detect red cell aggregates as single cells. Monoclonal IgM usually has anti-I or, less often, anti-i specificity. Treatment is primarily by transfusion support but may also be directed at any underlying lymphoma. Patients must keep extremities warm, especially in winter. Some patients respond to glucocorticoid therapy and rituximab. Two considerations for patients requiring blood transfusion is that the cross-match sample must be placed in a transport flask at a temperature of 37°C and blood administered via a blood-warmer. All patients should receive folic acid supplementation. Other causes of cold agglutination Cold agglutination can occur in association with *Mycoplasma pneumoniae* or with infectious mononucleosis. Paroxysmal cold haemoglobinuria is a very rare cause seen in children, in association with viral or bacterial infection. An IgG antibody binds to red cells in the peripheral circulation but lysis occurs in the central circulation when complement fixation takes place. This

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haemoglobin. These substitutions often change the charge of the globin chains, producing different electrophoretic mobility, and this forms the basis for the diagnostic use of haemoglobin electrophoresis to identify haemoglobinopathies. Quantitative abnormalities – thalassaemias In quantitative abnormalities (the thalassaemias), there are mutations causing a reduced rate of production of one or other of the globin chains, altering the ratio of alpha to non-alpha chains. In alpha-thalassaemia excess beta chains are present, while in beta-thalassaemia excess alpha chains are present. The excess chains precipitate, causing red cell membrane damage and reduced red cell survival due to haemolysis. Sick-cell anaemia Sick-cell disease results from a single glutamic acid to valine substitution at position 6 of the beta globin polypeptide chain. It is inherited as an autosomal recessive trait (p. 48). Homozygotes only produce abnormal beta chains that make haemoglobin S (HbS, termed SS), and this results in the clinical syndrome of sickle-cell disease. Heterozygotes produce a mixture of normal and abnormal beta chains that make normal HbA and HbS (termed AS), and this results in sickle-cell trait; although this was previously thought of as asymptomatic, it may be associated with an increased risk of sudden and cardiovascular

death. Epidemiology The heterozygote frequency is over 20% in tropical Africa (see Fig. 23.21). In black American populations, sickle-cell trait has a frequency of 8%. Individuals with sickle-cell trait are relatively resistant to the lethal effects of falciparum malaria in early childhood; the high prevalence in equatorial Africa can be explained by the survival advantage it confers in areas where falciparum malaria is endemic. However, homozygous patients with sickle-cell anaemia do not have correspondingly greater resistance to falciparum malaria. Pathogenesis When haemoglobin S is deoxygenated, the molecules of haemoglobin polymerise to form pseudocrystalline structures known as 'tactoids'. These distort the red cell membrane and has a characteristic red-brown colour. The disease is associated with an increased risk of venous and arterial thrombosis in unusual sites, such as the liver or abdomen. PNH clones are also associated with hypoplastic bone marrow failure, aplastic anaemia and myelodysplastic syndrome (pp. 960 and 969). Management is supportive with transfusion and folate supplements and prophylaxis or treatment of thrombosis. Standard care now includes the anti-complement C5 monoclonal antibody eculizumab. This has been shown to be effective in reducing haemolysis, transfusion requirements and thrombotic risk. Eculizumab carries a risk of infection, particularly for *Neisseria meningitidis*, and all treated patients must be vaccinated against this organism. Haemoglobinopathies These diseases are caused by mutations affecting the genes encoding the globin chains of the haemoglobin molecule. Normal haemoglobin is composed of two alpha and two non-alpha globin chains. Alpha globin chains are produced throughout life, including in the fetus, so severe mutations may cause intrauterine death. Production of non-alpha chains varies with age; fetal haemoglobin (HbF- $\alpha\gamma$) has two gamma chains, while the predominant adult haemoglobin (HbA- $\alpha\beta$) has two beta chains. Thus, disorders affecting the beta chains do not present until after 6 months of age. A constant small amount of haemoglobin A2 (HbA2- $\alpha\delta$, usually less than 2%) is made from birth. The geographical distribution of the common haemoglobinopathies is shown in Figure 23.21. The haemoglobinopathies can be classified into qualitative or quantitative abnormalities. Qualitative abnormalities – abnormal haemoglobins In qualitative abnormalities (called the abnormal haemoglobins), there is a functionally important alteration in the amino acid structure of the polypeptide chains of the globin chains. Several hundred such variants are known; they were originally designated by letters of the alphabet, e.g. S, C, D or E, but the more recently described ones are known by names that usually taken from the town or district in which they were first described. The best-known example is haemoglobin S, found in sickle-cell anaemia. Mutations around the haem-binding pocket cause the haem ring to fall out of the structure and produce an unstable Fig. 23.21 The geographical distribution of the haemoglobinopathies. From Hoffbrand AV, Pettit JE. Essential haematology, 3rd edn. Edinburgh: Blackwell Science; 1992. Thalassaemia Sickle-cell anaemia HbC HbD HbE

952 • HAEMATOLOGY AND TRANSFUSION MEDICINE • Stroke. The single most devastating consequence of sickle-cell disease is stroke. Stroke or silent stroke occurs in 10–15% of children with sickle-cell disease. Children at risk of stroke can be identified by screening with transcranial Doppler ultrasound, with fast flow associated with increased stroke risk. These children may be offered strategies such as transfusion or treatment with hydroxycarbamide to reduce the risk of stroke. • Sickle chest syndrome. This may follow a vaso-occlusive crisis and is the most common cause of death in adult sickle-cell disease. Bone marrow infarction results in fat emboli to the lungs, which cause further sickling and infarction, leading to ventilatory failure if not treated. • Sequestration crisis. Thrombosis of the venous outflow from an organ causes loss of function and acute painful enlargement. In children, the spleen is the most common site. Massive splenic

enlargement may result in severe anaemia, circulatory collapse and death. Recurrent sickling in the spleen in childhood results in infarction and adults may have no functional spleen. In adults, the liver may undergo sequestration with severe pain due to capsular stretching. Priapism is a complication seen in affected men. • Aplastic crisis. Infection of adult sicklers with human parvovirus B19 (erythrovirus) may result in a severe but produce characteristic sickle-shaped cells (Fig. 23.22). The polymerisation is reversible when re-oxygenation occurs. The distortion of the red cell membrane, however, may become permanent and the red cell 'irreversibly sickled'. The greater the concentration of sickle-cell haemoglobin in the individual cell, the more easily tactoids are formed, but this process may be enhanced or retarded by the presence of other haemoglobins. Thus the abnormal haemoglobin C variant participates in polymerisation more readily than haemoglobin A, whereas haemoglobin F strongly inhibits polymerisation. Clinical features Sickling is precipitated by hypoxia, acidosis, dehydration and infection. Irreversibly sickled cells have a shortened survival and plug vessels in the microcirculation. This results in a number of acute syndromes, termed 'crises', and chronic organ damage (Fig. 23.22): • Painful vaso-occlusive crisis. Plugging of small vessels in the bone produces acute severe bone pain. This affects areas of active marrow: the hands and feet in children (so-called dactylitis) or the femora, humeri, ribs, pelvis and vertebrae in adults. Patients usually have a systemic response with tachycardia, sweating and a fever. This is the most common form of crisis. Fig. 23.22 Clinical and laboratory features of sickle-cell disease. CNS Subarachnoid bleed Fits Cardiac Sickle myocardium Cardiomegaly Transfusional iron overload Vertebral collapse Osteoporosis Splenic infarction Avascular necrosis Cerebrovascular event Priapism Leg ulceration Background retinopathy Proliferative retinopathy Vitreous bleeds Ocular Sickle chest syndrome Infection Pulmonary hypertension Pulmonary Osteomyelitis Cholelithiasis Hepatic sequestration Dactylitis Enuresis Haematuria Papillary necrosis Chronic renal failure Renal Arthropathy Blood film Electrophoresis gel Nucleated red cell Sickle cell Normal HbC trait HbS trait HbC HbS HbA HbF Autosomal recessive inheritance

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children or chest syndromes in adults. Exchange transfusion, in which a patient is simultaneously venesected and transfused to replace HbS with HbA, may be used in life-threatening crises or to prepare patients for surgery. A high HbF level inhibits polymerisation of HbS and reduces sickling. Patients with sickle-cell disease and high HbF levels have a mild clinical course with few crises. Some agents are able to increase synthesis of HbF and this has been used to reduce the frequency of severe crises. The oral cytotoxic agent hydroxycarbamide has been shown to have clinical benefit with acceptable side-effects in children and adults who have recurrent severe crises. Relatively few allogeneic stem cell transplants from HLA-matched siblings have been performed but this procedure appears to be potentially curative (p. 937). Prognosis In Africa, few children with sickle-cell anaemia survive to adult life without medical attention. Even with standard medical care, approximately 15% die by the age of 20 years and 50% by the age of 40 years. Other abnormal haemoglobins Another beta-chain haemoglobinopathy, haemoglobin C (HbC) disease, is clinically silent but associated with microcytosis and target cells on the blood film. Compound heterozygotes inheriting one HbS gene and one HbC gene from their parents have haemoglobin SC disease, which behaves like a mild form of sickle-cell disease. SC disease is associated with a reduced frequency of crises but is not uncommonly associated with complications in pregnancy and retinopathy. Thalassaemias Thalassaemia is an inherited impairment of haemoglobin production, in which there is partial or complete failure to synthesise a specific type of globin chain. In alpha-thalassaemia,

disruption of one or both alleles on chromosome 16 may occur, with production of some or no alpha globin chains. In betathalassaemia, defective production usually results from disabling point mutations causing no (β^0) or reduced (β^-) beta chain production. Beta-thalassaemia Failure to synthesise beta chains (beta-thalassaemia) is the most common type of thalassaemia, most prevalent in the Mediterranean area. Heterozygotes have thalassaemia minor, a condition in which there is usually mild microcytic anaemia and little or no clinical disability, which may be detected only when iron therapy for a mild microcytic anaemia fails. Homozygotes (thalassaemia major) either are unable to synthesise haemoglobin A or, at best, produce very little; after the first 4–6 months of life, they develop profound transfusion-dependent hypochromic anaemia. The diagnostic features are summarised in Box 23.40. Intermediate grades of severity occur. Management and prevention See Box 23.41. Cure is now a possibility for selected children, with allogeneic HSCT (p. 937). It is possible to identify a fetus with homozygous betathalassaemia by obtaining chorionic villous material for DNA self-limiting red cell aplasia. This results in profound anaemia, which may cause heart failure. Unlike in all other sickle crises, the reticulocyte count is low.

- **Pregnancy.** Pregnancy in sickle-cell disease requires planning and multidisciplinary management. Women with sickle-cell disease have increased pregnancy-related morbidity, which includes painful crisis, placental failure and thrombosis (Box 23.39). Investigations Patients with sickle-cell disease have a compensated anaemia, usually around 60–80 g/L. The blood film shows sickle cells, target cells and features of hyposplenism from a young age. A reticulocytosis is present. The presence of HbS can be demonstrated by exposing red cells to a reducing agent such as sodium dithionite; HbA gives a clear solution, whereas HbS polymerises to produce a turbid solution. This forms the basis of emergency screening tests before surgery in appropriate ethnic groups but cannot distinguish between sickle-cell trait and disease. The definitive diagnosis requires haemoglobin electrophoresis to demonstrate the absence of HbA, 2–20% HbF and the predominance of HbS. Both parents of the affected individual will have sickle-cell trait. Management All patients with sickle-cell disease should receive prophylaxis with daily folic acid, and appropriate management of the hyposplenic state that is uniformly found in these patients from an early age (see Box 23.37). Seasonal vaccination against influenza is also advised in these patients. Vaso-occlusive crises are managed by aggressive rehydration, oxygen therapy, adequate analgesia (which often requires opiates) and antibiotics. Transfusion should be with fully genotyped blood wherever possible. Simple top-up transfusion may be used in a sequestration or aplastic crisis. A regular transfusion programme to suppress HbS production and maintain the HbS level below 30% may be indicated in patients with recurrent severe complications, such as cerebrovascular accidents in 23.39 Sickle-cell disease in pregnancy
- **Pre-conceptual counselling:** advice on the effect of sickle-cell disease on pregnancy, and vice versa, should be offered.
- **Vaccination status:** should be updated before conception.
- **Testing of partner:** testing for haemoglobinopathy status is advised.
- **Folic acid:** should be taken in high dose (5 mg daily) prior to and throughout pregnancy.
- **Hydroxycarbamide:** should be discontinued 3 months prior to conception.
- **Angiotensin-converting enzyme (ACE) inhibitors:** should be discontinued prior to conception.
- **Pulmonary hypertension:** should be excluded prior to conception.
- **Placental failure:** women with sickle-cell disease have increased rates, resulting in pre-eclampsia and intrauterine growth retardation.
- **Aspirin 75 mg:** should be given throughout pregnancy.
- **Thromboprophylaxis after delivery:** all women with sickle-cell disease should receive thromboprophylaxis with low-molecularweight heparin for at least 10 days post vaginal delivery and for 6 weeks post caesarean section. Antenatal thromboprophylaxis should be considered for women with additional risk factors for venous thromboembolism (see Box 23.65).
- **Transfusion:** extended cross-matched blood for Rhesus and Kell status should be provided. Blood should be

cytomegalovirus- negative.

954 • HAEMATOLOGY AND TRANSFUSION MEDICINE analysis sufficiently early in pregnancy to allow termination. This examination is appropriate only if both parents are known to be carriers (beta-thalassaemia minor) and will accept a termination. Alpha-thalassaemia Reduced or absent alpha-chain synthesis is common in Southeast Asia. There are two alpha gene loci on chromosome 16 and therefore each individual carries four alpha gene alleles. • If one is deleted, there is no clinical effect. • If two are deleted, there may be a mild hypochromic anaemia. • If three are deleted, the patient has haemoglobin H disease. • If all four are deleted, the baby is stillborn (hydrops fetalis). Haemoglobin H is a beta-chain tetramer, formed from the excess of beta chains, which is functionally useless, so that patients rely on their low levels of HbA for oxygen transport. Treatment of haemoglobin H disease is similar to that of beta-thalassaemia of intermediate severity, involving folic acid supplementation, transfusion if required and avoidance of iron therapy. Haematological malignancies Haematological malignancies arise when the processes controlling proliferation or apoptosis are corrupted in blood cells because of acquired mutations in key regulatory genes. If mature differentiated cells are involved, the cells will have a low growth fraction and produce indolent neoplasms, such as the low-grade lymphomas or chronic leukaemias, when patients have an expected survival of many years. In contrast, if more primitive stem or progenitor cells are involved, the cells can have the highest growth fractions of all human neoplasms, producing rapidly progressive, lifethreatening illnesses such as the acute leukaemias or high-grade lymphomas. Involvement of pluripotent stem cells produces the most aggressive acute leukaemias. In general, haematological neoplasms are diseases of elderly patients, the exceptions being acute lymphoblastic leukaemia, which predominantly affects children, and Hodgkin lymphoma, which affects people aged 20–40 years. Management of young patients with haematological malignancy is particularly challenging (Box 23.43). Leukaemias Leukaemias are malignant disorders of the haematopoietic stem cell compartment, characteristically associated with increased numbers of white cells in the bone marrow and/or peripheral blood. The course of leukaemia may vary from a few days or weeks to many years, depending on the type. Epidemiology and aetiology The incidence of leukaemia of all types in the population is approximately 10/100 000 per annum, of which just under half are cases of acute leukaemia. Males are affected more frequently than females, the ratio being about 3 : 2 in acute leukaemia, 2 : 1 in chronic lymphocytic leukaemia and 1.3 : 1 in chronic myeloid leukaemia. Geographical variation in incidence does occur, the most striking being the rarity of chronic lymphocytic leukaemia in Chinese and related races. Acute leukaemia occurs at all ages. Acute lymphoblastic leukaemia shows a peak of incidence in children aged 1–5 years. All forms of acute myeloid leukaemia 23.41 Treatment of beta-thalassaemia major Problem Management Erythropoietic failure Allogeneic HSCT from HLA-compatible sibling Transfusion to maintain Hb

“ 100 g/L Folic acid 5 mg daily Iron overload Iron therapy contraindicated Iron chelation therapy Splenomegaly causing mechanical problems, excessive transfusion needs Splenectomy; see Box 23.37 (Hb = haemoglobin; HLA = human leucocyte antigen; HSCT = haematopoietic stem cell transplantation) 23.40 Diagnostic features of beta-thalassaemia Beta-thalassaemia major (homozygotes) • Profound hypochromic anaemia • Evidence of severe red cell

dysplasia • Erythroblastosis • Absence or gross reduction of the amount of haemoglobin A • Raised levels of haemoglobin F • Evidence that both parents have thalassaemia minor Beta-thalassaemia minor (heterozygotes) • Mild anaemia • Microcytic hypochromic erythrocytes (not iron-deficient) • Some target cells • Punctate basophilia • Raised haemoglobin A2 fraction 23.42

Anaemia in old age • Mean haemoglobin: falls with age in both sexes but remains well within the reference range. When a low haemoglobin does occur, it is generally due to disease. • Anaemia can never be considered 'normal' in old age. • Symptoms: may be subtle and insidious. Cardiovascular features such as dyspnoea and oedema, and cerebral features such as dizziness and apathy, tend to predominate. • Ferritin: if lower than 45 µg/L in older people, is highly predictive of iron deficiency. Conversely, ferritin may be raised by chronic disease and so a normal ferritin does not exclude iron deficiency. • Serum iron and transferrin: fall with age because of the prevalence of other disorders, and are not reliable indicators of deficiency. • Most common cause of iron deficiency: gastrointestinal blood loss. • Most common cause of vitamin B12 deficiency: pernicious anaemia, as the prevalence of chronic atrophic gastritis rises in old age. • Neuropsychiatric symptoms associated with vitamin B12 deficiency: well-established association but a causal relationship has not been clearly shown. Dementia associated with vitamin B12 deficiency in the absence of haematological abnormalities is rare. • Anaemia of chronic disease: frequent in old age because of the rising prevalence of diseases that inhibit iron transport.

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Myeloid refers to the other lineages: that is, precursors of red cells, granulocytes, monocytes and platelets (see Fig. 23.2). The diagnosis of leukaemia is usually suspected from an abnormal blood count, often a raised white count, and is confirmed by examination of the bone marrow. This includes the morphology of the abnormal cells, analysis of cell surface markers (immunophenotyping), clone-specific chromosome abnormalities and molecular changes. These results are incorporated in the World Health Organisation (WHO) classification of tumours of haematopoietic and lymphoid tissues; the subclassification of acute leukaemias is shown in Box 23.45. The features in the bone marrow not only provide an accurate diagnosis but also give valuable prognostic information, increasingly allowing therapy to be tailored to the patient's disease.

Acute leukaemia There is a failure of cell maturation in acute leukaemia. Proliferation of cells that do not mature leads to an accumulation of primitive cells that take up more and more marrow space at the expense of the normal haematopoietic elements. Eventually, this proliferation spills into the blood. Acute myeloid leukaemia (AML) is about four times more common than acute lymphoblastic leukaemia (ALL) in adults. In children, the proportions are reversed, the lymphoblastic variety being more common. The clinical features are usually those of bone marrow failure (anaemia, bleeding or infection; pp. 923, 927 and 930). Investigations

Blood examination usually shows anaemia with a normal or raised MCV. The leucocyte count may vary from as low as $1 \times 10^9/L$ to as high as $500 \times 10^9/L$ or more. In the majority of patients, have their lowest incidence in young adult life and there is a striking rise over the age of 50. Chronic leukaemias

occur mainly in middle and old age. The cause of the leukaemia is unknown in the majority of patients. Several risk factors have been identified (Box 23.44). Terminology and classification

Leukaemias are traditionally classified into four main groups:

- acute lymphoblastic leukaemia (ALL)
- acute myeloid leukaemia (AML)
- chronic lymphocytic leukaemia (CLL)
- chronic myeloid leukaemia (CML).

In acute leukaemia, there is proliferation of primitive stem cells, with limited accompanying differentiation, leading to an accumulation of blasts, predominantly in the bone marrow, which causes bone marrow failure. In chronic leukaemia, the malignant clone is able to differentiate, resulting in an accumulation of more mature cells. Lymphocytic and lymphoblastic cells are those derived from the lymphoid stem cell (B cells and T cells).

23.44 Risk factors for leukaemia

- Ionising radiation
- After atomic bombing of Japanese cities (myeloid leukaemia)
- Radiotherapy
- Diagnostic X-rays of the fetus in pregnancy
- Cytotoxic drugs
- Especially alkylating agents (myeloid leukaemia, usually after a latent period of several years)
- Industrial exposure to benzene
- Retroviruses
- Adult T-cell leukaemia/lymphoma (ATLL) caused by human T-cell lymphotropic virus 1 (HTLV-1), most prevalent in Japan, the Caribbean and some areas of Central and South America and Africa
- Genetic
- Identical twin of patients with leukaemia
- Down's syndrome and certain other genetic disorders
- Immunological
- Immune deficiency states (e.g. hypogammaglobulinaemia)

23.43 Consequences of haematological malignancy in adolescence

- Tailored management protocols: the most effective treatment schedules for leukaemia and lymphoma differ between children and adults. Adolescent patients may be most appropriately managed in specialist centres.
- Psychosocial effects: adolescents undergoing treatment for haematological malignancy may suffer significant consequences for their schooling and social development, and require support from a multidisciplinary team.
- 'Late effects': adolescents who have been treated with chemotherapy and/or radiotherapy in childhood may be at risk of a wide range of complications, depending on the region irradiated, radiation dose and the drugs used. Particularly relevant complications in this age group include short stature, growth hormone deficiency, delayed puberty, and cognitive dysfunction affecting schooling (after cranial irradiation). Life-long follow-up is often undertaken to detect and manage these late effects and to deal with consequences such as infertility and second malignancy.

23.45 WHO classification of acute leukaemia*

- Acute myeloid leukaemia (AML) with recurrent genetic abnormalities
- AML with t(8;21)(q22;q22.1), gene product RUNX1-RUNX1T1
- AML with inv(16)(p13.1;q22), gene product CBFB-MYHL1
- Acute promyelocytic leukaemia t(15;17), gene product PML-RARA
- AML with t(9;11)(p21.3;q23.3), gene product MLLT3-KMT2A
- AML with t(6;9)(p23;q34), gene product DEK-NUP214
- AML with inv(3)(q21.3;q26.2) or t(3;3)(q21.3;q26.2), gene products GATA2, MECOM
- AML (megakaryoblastic) with t(1;22)(p13.3;q13.3), gene product RBM15-MKL1
- AML with mutated NPM1
- AML with biallelic mutations of CEBPA
- Acute myeloid leukaemia with myelodysplasia-related changes
- e.g. Following a myelodysplastic syndrome
- Therapy-related myeloid neoplasms
- e.g. Alkylating agent or topoisomerase II inhibitor
- Myeloid sarcoma
- Myeloid proliferations related to Down's syndrome
- Acute myeloid leukaemia not otherwise specified
- e.g. AML with or without differentiation, acute myelomonocytic leukaemia, erythroleukaemia, megakaryoblastic leukaemia
- Acute lymphoblastic leukaemia (ALL)
- B-lymphoblastic leukaemia/lymphoma
- T-lymphoblastic leukaemia/lymphoma

*Updated 2016; major subtypes.

956 • HAEMATOLOGY AND TRANSFUSION MEDICINE of leukaemia. Classification and prognosis are determined by immunophenotyping and chromosome and molecular analysis, as shown in Figure 23.24. Management The first decision must be whether or not to give specific treatment to attempt to achieve remission. This is generally aggressive, has numerous side-effects, and may not be

appropriate for the very elderly or patients with serious comorbidities (Chs 32 and 33). In these patients, supportive treatment can effect considerable improvement in well-being. Low-intensity chemotherapy, such as low-dose cytosine arabinoside or, recently, azacitidine, is frequently used in elderly and more frail patients but only induces remission in less than 20% of patients. Specific therapy Ideally, whenever possible, patients with acute leukaemia should be treated within a clinical trial. If a decision to embark on specific therapy has been taken, the patient should be prepared as recommended in Box 23.46. It is unwise to attempt aggressive management of acute leukaemia unless adequate services are available for the provision of supportive therapy. The aim of treatment is to destroy the leukaemic clone of cells without destroying the residual normal stem cell compartment from which repopulation of the haematopoietic tissues will occur. There are three phases:

- **Remission induction.** In this phase, a fraction of the tumour is destroyed by combination chemotherapy. The patient goes through a period of severe bone marrow hypoplasia lasting 3–4 weeks and requires intensive support and inpatient care from a specially trained multidisciplinary team. The aim is to achieve remission, a state in which the blood counts return to normal and the marrow blast count is less than 5%. Quality of life is highly dependent on achieving remission.
- **Remission consolidation.** If remission has been achieved, residual disease is attacked by therapy during the consolidation phase. This consists of a number of courses of chemotherapy, again resulting in periods of marrow hypoplasia. In poor-prognosis leukaemia, this may include allogeneic HSCT.
- **Remission maintenance.** If the patient is still in remission after the consolidation phase for ALL, a period of maintenance therapy is given, with the individual as an outpatient and treatment consisting of a repeating cycle of drug administration. This may extend for up to 3 years if relapse does not occur. the count is below $100 \times 10^9/L$. Severe thrombocytopenia is usual but not invariable. Frequently, blast cells are seen in the blood film but sometimes the blast cells may be infrequent or absent. A bone marrow examination will confirm the diagnosis. The bone marrow is usually hypercellular, with replacement of normal elements by leukaemic blast cells in varying degrees (but more than 20% of the cells) (Fig. 23.23). The presence of Auer rods in the cytoplasm of blast cells indicates a myeloblastic type Fig. 23.23 Acute myeloid leukaemia. Bone marrow aspirate showing infiltration with large blast cells, which display nuclear folding and prominent nucleoli. Fig. 23.24 Investigation of acute lymphoblastic leukaemia (ALL). A Flow cytometric analysis of blasts labelled with the fluorescent antibodies anti-CD19 (y axis) and anti-CD10 (x axis). ALL blasts are positive for both CD19 and CD10 (arrow). B Chromosome analysis (karyotype) of blasts showing additional chromosomes X, 4, 6, 7, 14, 18 and 21. A

CD10

CD19

CD19- and CD10positive cells B 23.46 Preparation for specific therapy in acute leukaemia • Existing infections identified and treated (e.g. urinary tract infection, oral candidiasis, dental, gingival and skin infections) • Anaemia corrected by red cell concentrate transfusion • Thrombocytopenic bleeding controlled by platelet transfusions • If possible, central venous catheter (e.g. Hickman line) inserted to facilitate access to the circulation for delivery of chemotherapy, fluids, blood products and other supportive drugs • Tumour lysis risk assessed and prevention started: fluids with allopurinol or rasburicase • Therapeutic regimen carefully explained to the patient and informed consent obtained • Consideration of entry into clinical trial

should be given to maintain the platelet count above $10 \times 10^9/L$. Coagulation abnormalities occur and need accurate diagnosis and treatment (p. 971). Infection Fever ($> 38^\circ C$) lasting over 1 hour in a neutropenic patient indicates possible sepsis (see also p. 218). Parenteral broad-spectrum antibiotic therapy is essential. Empirical therapy is given according to local bacteriological resistance patterns, such as with a combination of an aminoglycoside (e.g. gentamicin) and a broad-spectrum penicillin (e.g. piperacillin/tazobactam) or a single-agent beta-lactam (e.g. meropenem). The organisms most commonly associated with severe neutropenic sepsis are Gram-positive bacteria, such as *Staphylococcus aureus* and *Staphylococcus epidermidis*, which are present on the skin and gain entry via cannulae and central lines. Gram-negative infections often originate from the gastrointestinal tract, which is affected by chemotherapy-induced mucositis; organisms such as *Escherichia coli*, *Pseudomonas* and *Klebsiella* spp. are likely to cause rapid clinical deterioration and must be covered with initially empirical antibiotic therapy. Gram-positive infection may require vancomycin or teicoplanin therapy. If fever has not resolved after 3–5 days and there is evidence on CT scanning or sensitive blood tests for a disseminated fungal infection, empirical antifungal therapy (e.g. a liposomal amphotericin B preparation, voriconazole or caspofungin) is added. Patients with ALL are susceptible to infection with *Pneumocystis jirovecii* (p. 318), which causes a severe pneumonia. Prophylaxis with co-trimoxazole is given during chemotherapy. Diagnosis may require either induced sputum, bronchoalveolar lavage or open lung biopsy. Treatment is with high-dose co-trimoxazole, initially intravenously, changing to oral treatment as soon as possible. Oral and pharyngeal *Candida* infection is common. Fluconazole is effective for the treatment of established local infection and for prophylaxis against systemic candidaemia. Prophylaxis against other systemic fungal infections, including *Aspergillus*, using itraconazole or posaconazole, for example, is usual practice during high-risk intensive chemotherapy. This is often used along with sensitive markers of early fungal infection to guide treatment initiation (a 'pre-emptive approach'). For systemic fungal infection with *Candida* or aspergillosis, intravenous liposomal amphotericin, caspofungin or voriconazole is required for at least 3 weeks. In systemic *Candida* infection intravenous catheters should be removed. Reactivation of herpes simplex infection (p. 247) occurs frequently around the lips and nose during ablative therapy for acute leukaemia, and is treated with aciclovir. This may also be prescribed prophylactically to patients with a history of cold sores or elevated antibody titres to herpes simplex. Herpes zoster manifesting as chickenpox or, after reactivation, as shingles (p. 239) should be treated in the early stage with high-dose aciclovir, as it can be fatal in immunocompromised patients. The value of isolation facilities, such as laminar flow rooms, is debatable but may contribute to staff awareness of careful reverse barrier nursing practice. The isolation can be psychologically stressful for the patient. Metabolic problems Frequent monitoring of fluid balance and renal, hepatic and haemostatic function is necessary. Patients are often severely anorexic and diarrhoea is common as a consequence of the side-effects of therapy; they may find drinking difficult and hence require intravenous fluids and electrolytes. Renal toxicity occurs with some antibiotics (e.g. aminoglycosides) and antifungal agents (amphotericin). Cellular breakdown during induction therapy In patients with ALL, it is necessary to give prophylactic treatment to the central nervous system, as this is a sanctuary site where standard therapy does not penetrate. This usually consists of a combination of cranial irradiation, intrathecal chemotherapy and high-dose methotrexate, which crosses the blood–brain barrier. Thereafter, specific therapy is discontinued and the patient observed. The detail of the schedules for these treatments can be found in

specialist texts. The drugs most commonly employed are listed in Box 23.47. Generally, if a patient fails to go into remission with induction treatment, alternative drug combinations may be tried, but the outlook is poor unless remission can be achieved. Disease that relapses during treatment or soon after the end of treatment carries a poor prognosis and is difficult to treat. The longer after the end of treatment that relapse occurs, the more likely it is that further treatment will be effective. In some patients, alternative palliative chemotherapy, not designed to achieve remission, may be used to curb excessive leucocyte proliferation. Drugs used for this purpose include hydroxycarbamide and mercaptopurine. The aim is to reduce the blast count without inducing bone marrow failure. Supportive therapy Aggressive and potentially curative therapy, which involves periods of severe bone marrow failure, would not be possible without appropriate supportive care. The following problems commonly arise. Anaemia Anaemia is treated with red cell concentrate transfusions. Bleeding Thrombocytopenic bleeding requires platelet transfusions, unless the bleeding is trivial. Recent trials have confirmed that in acute leukaemia prophylactic platelet transfusion 23.47 Drugs commonly used in the treatment of acute leukaemia Phase Acute lymphoblastic leukaemia Acute myeloid leukaemia Induction Vincristine (IV) Prednisolone (oral) L-Asparaginase (IM) Daunorubicin (IV) Methotrexate (intrathecal) Imatinib (oral)* Daunorubicin (IV) Cytarabine (IV) Etoposide (IV and oral) Gentuzumab ozogamicin (IV) All-trans retinoic acid (ATRA) (oral) Arsenic trioxide (ATO) Consolidation Daunorubicin (IV) Cytarabine (IV) Etoposide (IV) Methotrexate (IV) Imatinib (oral)* Cytarabine (IV) Amsacrine (IV) Mitoxantrone (IV) Maintenance Prednisolone (oral) Vincristine (IV) Mercaptopurine (oral) Methotrexate (oral) Imatinib (oral)* Relapse Fludarabine Cytarabine Idarubicin Fludarabine Cytarabine Arsenic trioxide (ATO) Idarubicin *If Philadelphia chromosome-positive.

958 • HAEMATOLOGY AND TRANSFUSION MEDICINE in acute promyelocytic leukaemia, which has greatly reduced induction deaths from bleeding in this good-risk leukaemia. A chemotherapy-free schedule of ATRA and ATO has recently produced cure rates of 90% in patients with low-risk acute promyelocytic leukaemia. Current trials aim to improve survival, especially in standard and poor-risk disease, with strategies that include better use of allogeneic HSCT and targeted therapies such as anti-CD33 monoclonal antibodies (Mylotarg) and FLT3 inhibitors. FLT3 is a cytokine receptor often expressed on AML blast cells and whose expression is associated with a poorer prognosis. Chronic myeloid leukaemia Chronic myeloid leukaemia (CML) is a myeloproliferative stem cell disorder resulting in proliferation of all haematopoietic lineages but manifesting predominantly in the granulocytic series. Maturation of cells proceeds fairly normally. The disease occurs chiefly between the ages of 30 and 80 years, with a peak incidence at 55 years. It is rare, with an annual incidence in the UK of 1.8/100 000, and accounts for 20% of all leukaemias. It is found in all races. The defining characteristic of CML is the chromosome abnormality known as the Philadelphia (Ph) chromosome. This is a shortened chromosome 22 resulting from a reciprocal translocation of material with chromosome 9. The break on chromosome 22 occurs in the breakpoint cluster region (BCR). The fragment from chromosome 9 that joins the BCR carries the *abl* oncogene, which forms a fusion gene with the remains of the BCR. This BCR ABL fusion gene codes for a 210 kDa protein with tyrosine kinase activity, which plays a causative role in the disease as an oncogene (p. 1318), influencing cellular proliferation, differentiation and survival. In some patients in whom conventional chromosomal analysis does not detect a Ph chromosome, the BCR ABL gene product is detectable by molecular techniques. Natural history The disease has three phases: • A chronic phase, in which the disease is responsive to treatment and is easily controlled, which used to last 3–5 years. With the introduction of imatinib therapy, this phase has been prolonged to encompass

a normal life expectancy in many patients. • An accelerated phase (not always seen), in which disease control becomes more difficult. • Blast crisis, in which the disease transforms into an acute leukaemia, either myeloblastic (70%) or lymphoblastic (30%), which is relatively refractory to treatment. This is the cause of death in the majority of patients; survival is therefore dictated by the timing of blast crisis, which cannot be predicted. Prior to imatinib therapy (see below), approximately 10% of patients per year would transform. In those treated with imatinib for up to 10 years, only between 0.5 and 2.5% have transformed each year. Clinical features Symptoms at presentation may include lethargy, weight loss, abdominal discomfort, gout and sweating, but about 25% of patients are asymptomatic at diagnosis. Splenomegaly is present in 90%; in about 10%, the enlargement is massive, extending to over 15 cm below the costal margin. A friction rub may be heard in cases of splenic infarction. Hepatomegaly occurs in about 50%. Lymphadenopathy is unusual. (tumour lysis syndrome; p. 1328) releases intracellular ions and nucleic acid breakdown products, causing hyperkalaemia, hyperuricaemia, hyperphosphataemia and hypocalcaemia. This may lead to renal failure. Allopurinol and intravenous hydration are given to try to prevent this. In patients at high risk of tumour lysis syndrome, prophylactic rasburicase (a recombinant urate oxidase enzyme) is used. Occasionally, dialysis may be required. Psychological problems Psychological support is a key aspect of care. Patients should be kept informed, and their questions answered and fears allayed as far as possible. A multidisciplinary approach to patient care involves input from many services, including psychology. Key members of the team include haematology specialist nurses, who are often the central point of contact for patients and families throughout the illness. Haematopoietic stem cell transplantation This is described on page 936. In patients with high-risk acute leukaemia, allogeneic HSCT can improve 5-year survival from 20% to around 50%. Reduced-intensity conditioning has allowed HSCT to be delivered to a higher proportion of patients with acute leukaemias, up to the age of about 65 years. Prognosis Without treatment, the median survival of patients with acute leukaemia is about 5 weeks. This may be extended to a number of months with supportive treatment. Patients who achieve remission with specific therapy have a better outlook. Around 80% of adult patients under 60 years of age with ALL or AML achieve remission, although remission rates are lower for older patients. However, the relapse rate continues to be high. Box 23.48 shows the survival in ALL and AML and the influence of prognostic features. The level of detectable leukaemia cells, called minimal residual disease (MRD), measured after induction therapy in ALL by sensitive laboratory techniques, has been shown to be a powerful prognostic tool that is now used routinely to direct subsequent consolidation therapy. Advances in treatment have led to steady improvement in survival from leukaemia. They include the introduction of drugs such as all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) 23.48 Outcome in adult acute leukaemia Disease/risk Risk factors 5-year overall survival Acute myeloid leukaemia (AML) Good risk Promyelocytic leukaemia t(15;17) 90% t(8;21) 65% inv 16 or t(16;16) 70% Poor risk Cytogenetic abnormalities 21% -5, -7, del 5q, abn(3q), complex (> 5) Intermediate risk AML with none of the above 48% Acute lymphoblastic leukaemia (ALL) Poor risk Philadelphia chromosome 20% High white count > 100 × 10⁹/L Abnormal short arm of chromosome 11 t(1;19) Standard ALL with none of the above 37%

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to stop TKI therapy and this is being investigated in clinical trials. For those failing to respond or who lose their response and progress on first-line therapy, options include switching to a different TKI (Box 23.49). Some patients develop detectable mutations in the BCR ABL gene, which renders

them resistant to one or more of the TKIs. The T315I mutation has been particularly problematic, as this provides wide-ranging resistance. The third-generation TKI ponatinib is effective, however. Allogeneic HSCT (p. 937) is now reserved for patients who fail TKI therapy. Hydroxycarbamide and interferon were previously used for control of disease. Hydroxycarbamide is still useful in palliative situations and interferon is used in women planning pregnancy. Accelerated phase and blast crisis Management is more difficult. For patients in accelerated phase, TKI therapy is indicated, most commonly with nilotinib or dasatinib. When blast transformation occurs, the type of blast cell should be determined. Response to appropriate acute leukaemia treatment (see Box 23.49) is better if disease is lymphoblastic than if myeloblastic. Second- or third-generation TKIs such as dasatinib are used in combination with chemotherapy to try and achieve remission. In younger and fitter patients an allogeneic HSCT is appropriate therapy if a return to chronic phase is achieved. Hydroxycarbamide can be an effective single agent and low-dose cytarabine can also be used palliatively in older patients.

Chronic lymphocytic leukaemia Chronic lymphocytic leukaemia (CLL) is the most common variety of leukaemia, accounting for 30% of cases. The male-to-female ratio is 2 : 1 and the median age at presentation is 65–70 years. In this disease, B lymphocytes, which would normally respond to antigens by transformation and antibody formation, fail to do so. An ever-increasing mass of immuno-incompetent cells accumulates, to the detriment of immune function and normal bone marrow haematopoiesis.

Clinical features The onset is usually insidious. Indeed, in around 70% of patients, the diagnosis is made incidentally on a routine FBC. Presenting problems may be anaemia, infections, painless lymphadenopathy, and systemic symptoms such as night sweats or weight loss; these more often occur later in the course of the disease.

Investigations The diagnosis is based on the peripheral blood findings of a mature lymphocytosis ($> 5 \times 10^9/L$) with characteristic morphology and cell surface markers. Immunophenotyping reveals the lymphocytes to be monoclonal B cells expressing the B-cell antigens CD19 and CD23, with either kappa or lambda immunoglobulin light chains and, characteristically, an aberrant T-cell antigen CD5. On flow cytometry, some people are shown to have circulating CLL cells at a level less than $5 \times 10^9/L$. This is known as monoclonal B lymphocytosis of uncertain significance. Other useful investigations in CLL include a reticulocyte count and a direct Coombs test, as autoimmune haemolytic anaemia may occur (p. 949). Serum immunoglobulin levels should be estimated to establish the degree of hypogammaglobulinaemia, which is common and progressive. Bone marrow examination by aspirate and trephine is not essential for the diagnosis of CLL, but may be helpful in difficult cases, for prognosis (patients with diffuse marrow involvement have a poorer prognosis) and to monitor response to therapy. The main prognostic factor is

Investigations FBC results are variable between patients. There is usually a normocytic, normochromic anaemia. The leucocyte count can vary from 10 to $600 \times 10^9/L$. In about one-third of patients, there is a very high platelet count, sometimes as high as $2000 \times 10^9/L$. In the blood film, the full range of granulocyte precursors, from myeloblasts to mature neutrophils, is seen but the predominant cells are neutrophils and myelocytes (see Fig. 23.3). Myeloblasts usually constitute less than 10% of all white cells. There is often an absolute increase in eosinophils and basophils, and nucleated red cells are common. If the disease progresses through an accelerated phase, the percentage of more primitive cells increases. Blast transformation is characterised by a dramatic increase in the number of circulating blasts. In patients with thrombocytosis, very high platelet counts may persist during treatment, in both chronic and accelerated phases, but usually drop dramatically at blast transformation. Basophilia tends to increase as the disease progresses. Bone marrow should be obtained to confirm the diagnosis and phase of disease by morphology, chromosome analysis to demonstrate the presence of the Ph chromosome, and RNA analysis to demonstrate the presence

of the BCR ABL gene product. Blood LDH levels are elevated and the uric acid level may be high due to increased cell breakdown. Management Chronic phase There are now five available tyrosine kinase inhibitors (TKIs) for the treatment of CML (Box 23.49). These specifically inhibit BCR ABL tyrosine kinase activity. Imatinib, nilotinib and dasatinib are recommended as first-line therapy in chronic phase CML; they usually normalise the blood count within a month and within 3–6 months produce complete cytogenetic response (disappearance of the Ph chromosome) in some 90% of patients. A sample of bone marrow is taken at 6 months to confirm complete cytogenetic response, and patients are subsequently monitored by 3-monthly real-time quantitative polymerase chain reaction (PCR) for BCR ABL mRNA transcripts in blood. The aim is to reduce the BCR ABL transcript levels by 3–5 logs from baseline and this is called major molecular response (MR3–MR5). A proportion of patients achieve a complete molecular response where the transcripts are not detectable by PCR. It may be possible for patients with a complete or major molecular response

23.49 Tyrosine kinase inhibition in chronic myeloid leukaemia Agents First-line • Imatinib • Nilotinib • Dasatinib Second-line • Imatinib • Nilotinib • Dasatinib • Bosutinib • Ponatinib* Outcomes • 90% achieve complete cytogenetic response • Responses faster with nilotinib and dasatinib • Median survival comparable to normal population *For patients with T315I kinase domain mutations use ponatinib.

960 • HAEMATOLOGY AND TRANSFUSION MEDICINE Prognosis The majority of clinical stage A patients have a normal life expectancy but patients with advanced CLL are more likely to die from their disease or infectious complications. Survival is influenced by prognostic features of the leukaemia, particularly TP53 mutation status, and whether patients can tolerate and respond to fludarabine-based treatment. In those able to be treated with chemotherapy and rituximab, 90% are alive 4 years later. Rarely, CLL transforms to an aggressive high-grade lymphoma, called Richter's transformation. Prolymphocytic leukaemia Prolymphocytic leukaemia (PLL) is a variant of chronic lymphocytic leukaemia found mainly in males over the age of 60 years; 25% of cases are of the T-cell variety. There is typically massive splenomegaly with little lymphadenopathy and a very high leucocyte count, often in excess of $400 \times 10^9/L$. The characteristic cell is a large lymphocyte with a prominent nucleolus. Treatment is generally unsuccessful and the prognosis very poor. Leukapheresis, splenectomy and chemotherapy may be tried. The anti-CD52 antibody alemtuzumab, when given intravenously, has produced responses in some 90% of patients with T-PLL. Hairy cell leukaemia This is a rare chronic B-cell lymphoproliferative disorder. The male-to-female ratio is 6 : 1 and the median age at diagnosis is 50 years. Presenting symptoms are general ill health and recurrent infections. Splenomegaly occurs in 90% but lymph node enlargement is unusual. Severe neutropenia, monocytopenia and the characteristic hairy cells in the blood and bone marrow are typical. These cells usually have a B-lymphocyte immunotype but they also characteristically express CD25 and CD103. Recently, all patients with hairy cell leukaemia have been found to have a mutation in the BRAF gene. Over recent years, a number of treatments, including cladribine and deoxycoformycin, have been shown to produce long-lasting remissions. Myelodysplastic syndromes Myelodysplastic syndromes (MDSs) constitute a group of clonal haematopoietic disorders with the common features of ineffective blood cell production and a tendency to progress to AML. As such, they are pre-leukaemic and represent genetic steps in the development of leukaemia. These genetic abnormalities have been identified and are present as a manifestation of clonal haematopoiesis in about 3% of patients over the age of 80, at a time when their blood counts are normal (clonal haematopoiesis of indeterminate potential, CHIP). MDS presents with consequences of bone marrow failure (anaemia, recurrent infections or bleeding),

usually in older people (median age at diagnosis is 73 years). The overall incidence is 4/100 000 in the population, rising to more than 30/100 000 in the over-seventies. The blood film is characterised by cytopenias and abnormal-looking (dysplastic) blood cells, including macrocytic red cells and hypogranular neutrophils with nuclear hyper- or hyposegmentation. The bone marrow is hypercellular, with dysplastic changes in at least 10% of cells of one or more cell lines. Blast cells may be increased but do not reach the 20% level that indicates acute leukaemia. Chromosome analysis frequently reveals abnormalities, particularly stage of disease (Box 23.50); however, loss of chromosome 17p or mutation in the TP53 gene, which resides at this genetic locus, is a powerful prognostic marker and predictor of response to therapy. A mutation in TP53 is present in < 10% of patients at presentation but rises to 30% of cases at relapse. This test should be performed in all patients prior to the initiation of therapy. Management No specific treatment is required for most clinical stage A patients, unless progression occurs. Life expectancy is usually normal in older patients. The patient should be offered clear information about CLL and be reassured about the indolent nature of the disease, as the diagnosis of leukaemia inevitably causes anxiety. Treatment is required only if there is evidence of bone marrow failure, massive or progressive lymphadenopathy or splenomegaly, systemic symptoms such as weight loss or night sweats, a rapidly increasing lymphocyte count, autoimmune haemolytic anaemia or thrombocytopenia. Initial therapy for those requiring treatment (progressive stage A and stages B and C) is based on the age and fitness of the patient and the TP53 mutation status. For patients who are under 70 years, fit and TP53 mutation-negative, fludarabine in combination with the alkylating agent cyclophosphamide and the anti-CD20 monoclonal antibody rituximab (FCR) is standard care. For older, less fit patients, rituximab is combined with gentler chemotherapy: bendamustine or oral chlorambucil. Recently, a more potent type 2 anti-CD20 antibody, obinutuzumab, has become available and produces better responses in combination with chlorambucil than rituximab. CLL cells are dependent on abnormal and persistent signalling through the B-cell receptor (BCR) pathway. Drugs that can inhibit this pathway are now available and show great promise. Ibrutinib inhibits Bruton's tyrosine kinase and idelalisib inhibits PI3 kinase, both components of the BCR pathway. Ibrutinib and idelalisib are licensed for relapsed CLL but crucially are licensed and effective in TP53-mutated disease at all stages and are quickly becoming standard care in TP53-mutated CLL. Bone marrow failure or autoimmune cytopenias may respond to glucocorticoid treatment. Supportive care is increasingly required in progressive disease, such as transfusions for symptomatic anaemia or thrombocytopenia, prompt treatment of infections and, for some patients with hypogammaglobulinaemia, immunoglobulin replacement. Radiotherapy may be used for lymphadenopathy that is causing discomfort or local obstruction, and for symptomatic splenomegaly. Splenectomy may be required to improve low blood counts due to autoimmune destruction or to hypersplenism, and can relieve massive splenomegaly.

23.50 Staging of chronic lymphocytic leukaemia

Clinical stage A (60% patients) • No anaemia or thrombocytopenia and fewer than three areas of lymphoid enlargement

Clinical stage B (30% patients) • No anaemia or thrombocytopenia, with three or more involved areas of lymphoid enlargement

Clinical stage C (10% patients) • Anaemia and/or thrombocytopenia, regardless of the number of areas of lymphoid enlargement

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with isolated del(5q) responds well to the immunomodulatory drug lenalidomide, with two-thirds of anaemic patients becoming transfusion-independent for up to 2 years. Allogeneic stem cell

transplantation may afford a cure in patients with a good performance status and is considered in high-risk patients (IPSS-R high and very high) and some low-risk patients. More recently, the hypomethylating agent azacytidine has improved survival by a median of 9 months for high-risk patients, and in the UK is a recommended standard of care for those not eligible for transplantation.

Lymphomas These neoplasms arise from lymphoid tissues, and are diagnosed from the pathological findings on biopsy as Hodgkin or nonHodgkin lymphoma. The majority are of B-cell origin. Non-Hodgkin lymphomas are classified as low- or high-grade tumours on the basis of their proliferation rate. The normal architecture of the lymph node is outlined in Figure 23.25.

- High-grade tumours divide rapidly, are typically present for a matter of weeks before diagnosis, and may be lifethreatening with frequent risk of extranodal involvement.
- Low-grade tumours divide slowly, may be present for many months before diagnosis, and typically behave in an indolent fashion.

Hodgkin lymphoma The histological hallmark of Hodgkin lymphoma (HL) is the presence of Reed–Sternberg cells: large, malignant lymphoid cells of B-cell origin (Fig. 23.26). They are often present only in small numbers but are surrounded by large numbers of reactive non-malignant T cells, plasma cells and eosinophils. The epidemiology of HL is shown in Box 23.53 and its histological WHO classification in Box 23.54. Nodular lymphocyte-predominant HL is slow-growing, localised and rarely fatal. It has biological features, such as CD20-positive Hodgkin cells, and clinical features that make it more akin to a low-grade B-cell non-Hodgkin lymphoma. Classical HL is divided into four histological subtypes from the appearance of the of chromosome 5 or 7. The WHO classification of MDS is shown in Box 23.51.

Prognosis The natural history of MDS is progressive worsening of dysplasia leading to fatal bone marrow failure or progression to AML in 30% of cases. The time to progression varies (from months to years) with the subtype of MDS, being slowest in MDS with ring sideroblasts and single-lineage dysplasia and most rapid in MDS with excess blasts. The revised International Prognostic Scoring System (IPSS-R) predicts clinical outcome based on karyotype and cytopenias in blood, as well as percentage of bone marrow blasts (Box 23.52). There are five prognostic groups. The median survival for low-risk patients (IPSS-R very low and low) is 5–9 years, that for the intermediate group is 3 years and that for high-risk patients (IPSS-R high and very high) is 1–1.5 years.

Management For the vast majority of patients who are elderly, the disease is incurable, and supportive care with red cell and platelet transfusions is the mainstay of treatment. A trial of erythropoiesis stimulating agents (ESA) and granulocyte-colony-stimulating factor (G-CSF) is recommended in some patients with lowrisk MDS (IPSS-R very low, low and intermediate) to improve haemoglobin or neutrophil counts. A rare subtype called MDS 23.51

WHO classification of myelodysplastic syndromes (MDSs)

Disease

Bone marrow findings

MDS with single-lineage dysplasia < 5% blasts and single-lineage dysplasia only

MDS with ring sideroblasts (MDS-RS)

“ 15% ring sideroblasts, or 6–14% and presence of SF3B1 gene mutation MDS with multilineage dysplasia < 5% blasts and dysplasia in 2 or more lineages MDS with excess blasts 5–19% blasts MDS with isolated del(5q) Myelodysplastic syndrome associated with a del(5q) cytogenetic abnormality < 5% blasts Often normal or increased blood platelet count MDS, unclassifiable None of the above or inadequate material *The IPSS-R is based on three prognostic factors: the blast percentage in bone marrow; karyotype; and number and degree of blood cytopenias. A score is derived from which patients can be stratified into five risk*

categories for survival and leukaemic transformation. 23.52 Revised International Prognostic Scoring System and outcomes in myelodysplasia

Risk category	Overall score	Median survival (years)	25% progression to acute myeloid leukaemia (years)
Very low	≤ 1.5	8.8	Not reached
Low	1.5–3	5.3	10.8
Intermediate	3–4.5	3.0	3.2
High	4.5–6	1.6	1.4
Very high	6	0.8	0.73

Fig. 23.25 Schema of lymph node architecture. Different lymphocyte populations reside in different areas of the node: B cells in the follicles, T cells in the paracortex and plasma cells in the medulla. B cells are selected for antigen in the follicle centre. Errors during this process result in B-cell lymphomas, which are by far the most common type. Germinal centre B-cell follicle Mantle zone Marginal zone Afferent lymph Paracortex Cortex Efferent lymph Medulla Blood vessels Capsule

962 • HAEMATOLOGY AND TRANSFUSION MEDICINE but may cause dry cough and some breathlessness. Isolated subdiaphragmatic nodes occur in fewer than 10% at diagnosis. Hepatosplenomegaly may be present but does not always indicate disease in those organs. Spread is contiguous from one node to the next, and extranodal disease, such as bone, brain or skin involvement, is rare. Investigations Treatment of HL depends on the stage at presentation; investigations therefore aim not only to diagnose lymphoma but also to determine the extent of disease (Box 23.55). • FBC may be normal. If a normochromic, normocytic anaemia or lymphopenia is present, this is a poor prognostic factor. An eosinophilia or a neutrophilia may be present. • ESR may be raised. • Renal function tests are required to ensure function is normal prior to treatment. • Liver function may be abnormal in the absence of disease or may reflect hepatic infiltration. An obstructive pattern may be caused by nodes at the porta hepatis. • LDH measurements showing raised levels are an adverse prognostic factor. • Chest X-ray may show a mediastinal mass. • CT scan of chest, abdomen and pelvis permits staging. Bulky disease (> 10 cm in a single node mass) is an adverse prognostic feature. • Positron emission tomography (PET) scanning identifies nodes involved with HL, which are 18fluorodeoxyglucose (FDG)-avid, and this allows more accurate staging and monitoring of response (Fig. 23.27). • Lymph node biopsy may be undertaken surgically or by percutaneous needle biopsy under radiological guidance (Fig. 23.28). Management Clinical trials have shown that patients with early-stage disease (stages IA and IIA) have better outcomes if limited cycles of chemotherapy are combined with radiotherapy, rather than using radiotherapy alone. 23.55 Clinical stages of Hodgkin lymphoma (Ann Arbor classification) Stage Definition I Involvement of a single lymph node region (I) or extralymphatic* site (IE) II Involvement of two or more lymph node regions (II) or an extralymphatic site and lymph node regions on the same side of (above or below) the diaphragm (IIE) III Involvement of lymph node regions on both sides of the diaphragm with (IIIE) or without (III) localised extralymphatic involvement or involvement of the spleen (IIIs), or both (IIISE) IV Diffuse involvement of one or more extralymphatic tissues, e.g. liver or bone marrow Each stage is subclassified: A No systemic symptoms B Weight loss > 10%, drenching sweats, fever *The lymphatic structures are defined as the lymph nodes, spleen, thymus, Waldeyer's ring, appendix and Peyer's patches. 23.54 WHO pathological classification of Hodgkin lymphoma (HL) Type Histology classification Proportion of HL Nodular lymphocytetpredominant HL 5% Classical HL Nodular sclerosing 70% Mixed cellularity 20% Lymphocyte-rich 5% Lymphocyte-depleted Rare 23.53 Epidemiology and aetiology of Hodgkin lymphoma Incidence • Approximately 4 new cases/100 000 population/year Sex ratio • Slight male

excess (1.5 : 1) Age • Median age 31 years; first peak at 20–35 years and second at 50–70 years
 Aetiology • Unknown • More common in patients from well-educated backgrounds and small families • Three times more likely with a past history of infectious mononucleosis but no definitive causal link to Epstein–Barr virus infection proven Fig. 23.26 Hodgkin lymphoma. In the centre of this lymph node biopsy is a large typical Reed–Sternberg cell with two nuclei containing a prominent eosinophilic nucleolus. Reed–Sternberg cells and surrounding reactive cells. The nodular sclerosing type is more common in young patients and in women. Mixed cellularity is more common in the elderly. Lymphocyte-rich HL usually presents in men. Lymphocyte-depleted HL is rare and probably represents large-cell or anaplastic non-Hodgkin lymphoma. Clinical features There is painless, rubbery lymphadenopathy, usually in the neck or supraclavicular fossae; the lymph nodes may fluctuate in size. Young patients with nodular sclerosing disease may have large mediastinal masses that are surprisingly asymptomatic

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rate and decades of life ahead of them. Recent randomised trial data from the UK RAPID study have suggested that early-stage patients without bulk disease who have a negative PET scan after three cycles of ABVD can safely omit radiotherapy. Young women receiving breast irradiation during the treatment of chest disease have an increased risk of breast cancer and should participate in a screening programme. Patients continuing to smoke after lung irradiation are at particular risk of lung cancer. ABVD chemotherapy can cause cardiac and pulmonary toxicity, due to doxorubicin and bleomycin, respectively. The incidence of infertility and secondary myelodysplasia/AML is low with this regimen. Patients with advanced-stage disease are most commonly managed with chemotherapy alone. Standard treatment in the UK is 6–8 cycles of ABVD, followed by an assessment of response. The recent UK RATHL trial has confirmed previous data showing that achieving a PET-negative response after two cycles of ABVD (interim PET-2 response) predicts a very good outcome from continuing with up to six cycles of ABVD. Indeed, the same outcome can be achieved by omitting the bleomycin from the last four cycles and using just AVD, thus reducing the risk of lung toxicity. Patients who are PET-positive after two cycles, however, have a very high relapse risk if they continue with ABVD, only 13% being relapse-free at 2 years. The RATHL and other studies have demonstrated that changing to a more intensive regimen, BEACOPP (bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine (oncovin), procarbazine, prednisolone), in these patients improves the relapse-free survival to approximately 65%. Patients with relapsed disease that responds to salvage chemotherapy and ideally becomes PET-negative should be considered for autologous stem cell transplantation (p. 937). Those with resistant disease might benefit from an allogeneic A Fig. 23.27 Positron emission tomography (PET) scans in Hodgkin lymphoma, demonstrating response to treatment. A Chest X-ray from a young man with Hodgkin lymphoma at presentation, showing a left-sided anterior–superior mediastinal mass with tracheal deviation to the right. B Fused PET-CT image showing intense fluorodeoxyglucose (FDG) uptake (avidity) in the mass at presentation. C Fused PET-CT image showing no FDG uptake (PET negativity), representing complete response at the end of treatment. B C Fig. 23.28 CT-guided percutaneous needle biopsy of retroperitoneal nodes involved by lymphoma. Biopsy needle Enlarged lymph nodes The ABVD regimen (doxorubicin, bleomycin, vinblastine and dacarbazine) is widely used in the UK. Standard therapy for early-stage patients without additional risk factors, such as bulk disease or high ESR, is two cycles of ABVD combined with 20 Gy radiotherapy to the involved sites of disease. Standard therapy for early-stage patients

with additional risk factors is four cycles of ABVD combined with 30 Gy radiotherapy. Careful planning of radiotherapy is required to limit the doses delivered to normal tissues and new planning techniques continue to improve targeting of radiotherapy. Nevertheless, the long-term risks of second cancers and heart and lung disease within the radiation fields remain a concern, especially for young people with a high cure

964 • HAEMATOLOGY AND TRANSFUSION MEDICINE even years before presentation, runs an indolent course, but is not curable by conventional therapy. Of all cases of NHL in the developed world, over two-thirds are either diffuse large B-cell NHL (high-grade) or follicular NHL (low-grade) (Fig. 23.29). Other forms of NHL, including Burkitt lymphoma, mantle cell lymphoma, mucosa-associated lymphoid tissue (MALT) lymphomas and T-cell lymphomas, are less common. Clinical features Unlike Hodgkin lymphoma, NHL is often widely disseminated at presentation, including in extranodal sites. Patients present with lymph node enlargement (Fig. 23.30), which may be associated with systemic upset: weight loss, sweats, fever and itching. Hepatosplenomegaly may be present. Sites of extranodal involvement include the bone marrow, gut, thyroid, lung, skin, testis, brain and, more rarely, bone. Bone marrow involvement is more common in low-grade (50–60%) than high-grade (10%) disease. Compression syndromes may occur, including gut obstruction, ascites, superior vena cava obstruction and spinal cord compression. The same staging system (see Box 23.55) is used for both HL and NHL, but NHL is more likely to be stage III or IV at presentation. Investigations These are as for HL, but in addition the following should be performed: • Bone marrow aspiration and trephine to identify bone marrow involvement. • Immunophenotyping of surface antigens to distinguish T-cell from B-cell tumours. This may be done on blood, marrow or nodal material. stem cell transplant. Brentuximab vedotin is an antibody-drug conjugate directed against CD30 on the Reed–Sternberg cell surface. This antibody delivers the antimetabolic toxin monomethyl auristatin E to the Hodgkin cells and, as a single agent, can produce good responses in patients who have failed, or are not suitable for, an autologous transplant and can be a ‘bridge’ to an allogeneic transplant. Prognosis Over 90% of patients with early-stage HL achieve complete remission when treated with chemotherapy followed by involved field radiotherapy, and the great majority are cured. The major challenge is how to reduce treatment intensity, and hence long-term toxicity, without reducing the excellent cure rates in this group. Omitting radiotherapy in the majority of PET-negative patients is one major step forward in this regard. Historically, between 50 and 70% of those with advanced-stage HL were cured. The Hasenclever index (Box 23.56) can be helpful in assigning approximate chances of cure when discussing treatment plans with patients. More recent data using the PET scanner to direct therapy suggests that long-term survival is improving to beyond 80%. Patients who fail to respond to initial chemotherapy or relapse within a year of initial therapy have a poor prognosis but some may achieve long-term survival after autologous HSCT. Patients relapsing after 1 year may obtain long-term survival with further chemotherapy alone, but fit patients frequently proceed to autologous HSCT. Non-Hodgkin lymphoma Non-Hodgkin lymphoma (NHL) represents a monoclonal proliferation of lymphoid cells of B-cell (90%) or T-cell (10%) origin. The incidence of these tumours increases with age, to 62.8/million population per annum at age 75 years, and the overall rate is increasing at about 3% per year. The epidemiology of NHL is shown in Box 23.57. Previous classifications were based principally on histological appearances. The current WHO classification stratifies according to cell lineage (T or B cells) and incorporates clinical features, histology, chromosomal abnormalities and concepts related to the biology of the lymphoma. Clinically, the most important factor is grade, which is a reflection of proliferation rate. High-grade NHL has high proliferation rates, rapidly

produces symptoms, is fatal if untreated, but is potentially curable. Low-grade NHL has low proliferation rates, may be asymptomatic for many months or 23.57 Epidemiology and aetiology of non-Hodgkin lymphoma Incidence • 12 new cases/100 000 people/year Sex ratio • Slight male excess Age • Median age 65–70 years Aetiology • No single causative abnormality described • Lymphoma is a late manifestation of HIV infection (p. 322) • Specific lymphoma types are associated with viruses: e.g. Epstein-Barr virus (EBV) with post-transplant NHL, human herpesvirus 8 (HHV8) with a primary effusion lymphoma, and human T-cell lymphotropic virus (HTLV-1) with adult T-cell leukaemia lymphoma • Gastric lymphoma can be associated with *Helicobacter pylori* infection • Some lymphomas are associated with specific chromosomal translocations: The t(14;18) in follicular lymphoma results in the dysregulated expression of the BCL-2 gene product, which inhibits apoptotic cell death The t(8;14) found in Burkitt lymphoma and the t(11;14) in mantle cell lymphoma alter function of c-myc and cyclin D1, respectively, resulting in malignant proliferation • Lymphoma occurs in congenital immunodeficiency states and in immunosuppressed patients after organ transplantation 23.56 The Hasenclever prognostic index for advanced Hodgkin lymphoma Score 1 for each of the following risk factors present at diagnosis: • Age > 45 years • Male gender • Serum albumin < 40 g/L • Hb < 105 g/L • Stage IV disease • White blood cell count > 15 × 10⁹/L • Lymphopenia < 0.6 × 10⁹/L Score 5-year rate of freedom from progression (%) 5-year rate of overall survival (%) 0–1

“ 2

“ 3

“ 4

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Fig. 23.29 Histology of non-Hodgkin lymphoma. A (Low-grade) follicular or nodular pattern. B (High-grade) diffuse pattern. A B Fig. 23.30 Bulky axillary lymphadenopathy with distended superficial veins in a patient presenting with high-grade lymphoma. From Howard MR, Hamilton PJ. Haematology: An illustrated colour text, 4th edn. Edinburgh: Elsevier Ltd; 2013. • Cytogenetic analysis to detect chromosomal translocations and molecular testing for T-cell receptor or immunoglobulin gene rearrangements. • Immunoglobulin determination. Some lymphomas are associated with IgG or IgM paraproteins, which serve as markers for treatment response. • Measurement of uric acid levels. Some very aggressive high-grade NHLs are associated with very high urate levels, which can precipitate renal failure when treatment is started. • HIV testing. HIV is a risk factor for some lymphomas and affects treatment decisions. • Hepatitis B and C testing. This should be done prior to therapy with rituximab. Management Low-grade NHL The majority of patients (80%) present with advanced stage disease and will run a relapsing and remitting course over several years. Asymptomatic patients may not require therapy and are managed by ‘watching

and waiting'. Indications for treatment include marked systemic symptoms, lymphadenopathy causing discomfort or disfigurement, bone marrow failure or compression syndromes. In follicular lymphoma, the options are:

- Radiotherapy. This can be used for localised stage I disease, which is rare.
- Chemotherapy. Most patients will respond to oral therapy with chlorambucil, which is well tolerated but not curative. More intensive intravenous chemotherapy in younger patients produces better quality of life but no survival benefit.
- Monoclonal antibody therapy. Humanised monoclonal antibodies ('biological therapy'; p. 960) can be used to target surface antigens on tumour cells and to induce tumour cell apoptosis directly. The anti-CD20 antibody rituximab has been shown to induce durable clinical responses in up to 60% of patients when given alone, and acts synergistically when given with chemotherapy. Rituximab (R) in combination with cyclophosphamide, vincristine and prednisolone (R-CVP), cyclophosphamide, doxorubicin, vincristine, prednisolone (R-CHOP) or bendamustine (R-bendamustine) is commonly used as first-line therapy. Randomised trials have also confirmed that 2 years of maintenance therapy with single-agent rituximab, following achievement of first or second response, delays relapse and the time to next treatment. As yet, however, rituximab maintenance has not shown a survival benefit. New and more potent monoclonal antibodies are also in development and trials of obinutuzumab (p. 960) have been completed.
- Kinase inhibitors. Idelalisib is approved for relapsed follicular lymphoma and ibrutinib (p. 960) is approved for relapsed mantle cell lymphoma, a poor-prognosis lymphoma with low-grade histology but aggressive clinical behaviour. These targeted therapies are likely to become more widely used in low-grade lymphomas in the near future.
- Transplantation. High-dose chemotherapy and autologous HSCT can produce long remissions in patients with relapsed disease. Decisions on the timing of such treatment are complex in the context of rituximab maintenance and newer targeted therapies. However, younger patients with short first or second remissions or who relapse during rituximab maintenance should be considered.

966 • HAEMATOLOGY AND TRANSFUSION MEDICINE present in the blood but there are no other features of myeloma, Waldenström macroglobulinaemia (see below), lymphoma or related disease. It is a common condition associated with increasing age; a paraprotein can be found in 1% of the population aged over 50 years, increasing to 5% over 80 years. Clinical features and investigations Patients are usually asymptomatic, and the paraprotein is found on blood testing for other reasons. The routine blood count and biochemistry are normal, the paraprotein is usually present in small amounts with no associated immune paresis, and there are no lytic bone lesions. The bone marrow may have increased plasma cells but these usually constitute less than 10% of nucleated cells. Prognosis After follow-up of 20 years, only one-quarter of cases will progress to myeloma or a related disorder (i.e. around 1% per annum). There is no certain way of predicting progression in an individual patient. However, an abnormal ratio of kappa to lambda light chains (serum free light chain ratio, SFLR) increases the risk of progression. Patients with an abnormal ratio should be monitored for progression on an annual basis. Waldenström macroglobulinaemia This is a low-grade lymphoplasmacytic lymphoma associated with an IgM paraprotein, causing clinical features of hyperviscosity syndrome. It is a rare tumour occurring in the elderly and more commonly affects males. Patients classically present with features of hyperviscosity, such as nosebleeds, bruising, delirium and visual disturbance. However, presentation may be with anaemia, systemic symptoms, splenomegaly or lymphadenopathy, or may be asymptomatic, with an IgM paraprotein detected on routine screening. Patients are found on investigation to have an IgM paraprotein associated with a raised plasma viscosity. The bone marrow has a characteristic appearance, with infiltration of lymphoid cells, plasma cells and sometimes prominent mast cells. A high proportion of patients

have a mutation in the MYD88 gene. Management If patients show symptoms of hyperviscosity and anaemia, plasmapheresis is required to remove IgM and make blood transfusion possible. Chemotherapy with alkylating agents, such as chlorambucil, has been the mainstay of treatment, controlling disease in over 50%. Fludarabine may be more effective in this disease but has more side-effects. Rituximab in combination with chemotherapy is most commonly used; ibrutinib is very effective and has recently been licensed for use. Rituximab alone can cause a rapid release of IgM and increase in viscosity. The median survival is 5 years.

Multiple myeloma

This is a malignant proliferation of plasma cells. Normal plasma cells are derived from B cells and produce immunoglobulins that contain heavy and light chains. Normal immunoglobulins are polyclonal, which means that a variety of heavy chains are produced and each may be of kappa or lambda light chain type (p. 68). In myeloma, plasma cells produce immunoglobulin of a single heavy and light chain, a monoclonal protein commonly referred to as a paraprotein. In most cases an excess of light chain is produced, and in some cases only light chain is produced;

High-grade NHL

Patients with diffuse large B-cell NHL need treatment at initial presentation:

- Chemotherapy. The majority (> 90%) are treated with intravenous combination chemotherapy, typically with the CHOP regimen (cyclophosphamide, doxorubicin, vincristine and prednisolone).
- Monoclonal antibody therapy. When combined with CHOP chemotherapy, rituximab (R) increases the complete response rates and improves overall survival. R-CHOP is currently recommended as first-line therapy for those with stage II or higher diffuse large B-cell lymphoma.
- Radiotherapy. Stage I patients without bulky disease are treated with four cycles of CHOP or R-CHOP, followed by involved site radiotherapy. Radiotherapy is also indicated for a residual localised site of bulk disease after chemotherapy, and for spinal cord and other compression syndromes.
- HSCT. Autologous HSCT (p. 937) benefits patients with relapsed disease that is sensitive to salvage immunochemotherapy. As with HL, achieving PET negativity prior to autologous transplantation is desirable.

Prognosis Low-grade NHL

runs an indolent remitting and relapsing course, with an overall median survival of 12 years. Transformation to a high-grade NHL occurs in 3% per annum and is associated with poor survival. In diffuse large B-cell NHL treated with R-CHOP, some 75% of patients overall respond initially to therapy and 50% will have disease-free survival at 5 years. The prognosis for patients with NHL is further refined according to the international prognostic index (IPI). For high-grade NHL, 5-year survival ranges from over 75% in those with low-risk scores (age < 60 years, stage I or II, one or fewer extranodal sites, normal LDH and good performance status) to 25% in those with high-risk scores (increasing age, advanced stage, concomitant disease and a raised LDH). Relapse is associated with a poor response to further chemotherapy (< 10% 5-year survival), but in patients under 65 years HSCT improves survival.

Paraproteinaemias

A gammopathy refers to over-production of one or more classes of immunoglobulin. It may be polyclonal in association with acute or chronic inflammation, such as infection, sarcoidosis, autoimmune disorders or some malignancies. Alternatively, a monoclonal increase in a single immunoglobulin class may occur in association with normal or reduced levels of the other immunoglobulins. Such monoclonal proteins (also called M-proteins, paraproteins or monoclonal gammopathies) occur as a feature of myeloma, lymphoma and amyloidosis, in connective tissue disease such as rheumatoid arthritis or polymyalgia rheumatica, in infection such as HIV, and in solid tumours. In addition, they may be present with no underlying disease. Gammopathies are detected by plasma immunoelectrophoresis.

Monoclonal gammopathy of uncertain significance

In monoclonal gammopathy of uncertain significance (MGUS, also known as benign monoclonal gammopathy), a paraprotein is

60–70 years and the disease is more common in Afro-Caribbeans. The clinical features are demonstrated in Figure 23.31. Diagnosis of myeloma requires two of the following criteria to be fulfilled: • increased malignant plasma cells in the bone marrow • serum and/or urinary M-protein • skeletal lytic lesions. Bone marrow aspiration, plasma and urine electrophoresis, and a skeletal survey are thus required. Normal immunoglobulin levels, i.e. the absence of immunoparesis, should cast doubt on the diagnosis. Paraproteinaemia can cause an elevated ESR but this is a non-specific test; only approximately 5% of patients with a persistently elevated ESR above 100 mm/hr have underlying myeloma. Management If patients are asymptomatic with no evidence of end-organ damage (e.g. to kidneys, bone marrow or bone), treatment may not be required. So-called asymptomatic myeloma should be monitored closely for the development of end-organ damage. Immediate support • High fluid intake to treat renal impairment and hypercalcaemia (p. 661). • Analgesia for bone pain. this appears in the urine as Bence Jones proteinuria and can be measured in the urine or serum as free light chain. The frequency of different isotypes of monoclonal protein in myeloma is shown in Box 23.58. Although a small number of malignant plasma cells are present in the circulation, the majority are present in the bone marrow. The malignant plasma cells produce cytokines, which stimulate osteoclasts and result in net bone reabsorption. The resulting lytic lesions cause bone pain, fractures and hypercalcaemia. Marrow involvement can result in anaemia or pancytopenia. Clinical features and investigations The incidence of myeloma is 4/100 000 new cases per annum, with a male-to-female ratio of 2 : 1. The median age at diagnosis is Fig. 23.31 Clinical and laboratory features of multiple myeloma. (ESR = erythrocyte sedimentation rate; NSAIDs = non-steroidal anti-inflammatory drugs) Spinal cord compression Bony collapse Extradural mass Amyloid 'Panda' eyes Nephrotic syndrome Carpal tunnel syndrome Abnormal blood tests Bone pain/fracture Retinal bleeds Bruising Heart failure Cerebral ischaemia Engorged retinal veins in hyperviscosity Hyperviscosity Renal failure due to: Paraprotein deposition Hypercalcaemia Infection NSAIDs Amyloid Lytic lesions Lytic lesions in skull Anaemia Normo- or macrocytic Pancytopenia Raised ESR Bone marrow Plasmacytosis > 10% Bence Jones proteinuria Serum free light chains Paraproteinaemia Immune paresis Plasma cells in bone marrow Lytic lesion eroding right superior pubic ramus and acetabulum Hypercalcaemia Renal impairment 23.58 Classification of multiple myeloma Type of monoclonal (M)-protein Relative frequency (%) IgG

IgA

Light chain only

Others (D, E, non-secretory)

968 • HAEMATOLOGY AND TRANSFUSION MEDICINE emergency treatment of spinal cord compression complicating extradural plasmacytomas. Bisphosphonates Long-term bisphosphonate therapy reduces bone pain and skeletal events. These drugs protect bone (p. 1047) and may cause apoptosis of malignant plasma cells. There is evidence that intravenous zoledronate in combination with anti-myeloma therapy confers a survival advantage over oral bisphosphonates. Osteonecrosis of the jaw may be associated with long-term use or poor oral hygiene and gum sepsis; regular dental review, including a check before starting therapy, is therefore important. Prognosis The international staging system (ISS) identifies poor prognostic features, including a high β 2-

microglobulin and low albumin at diagnosis (ISS stage 3, median survival 29 months). Those with a normal albumin and a low β 2-microglobulin (ISS stage 1) have a median survival of 62 months. Increasingly, cytogenetic analysis is used to identify poor-risk patients, e.g. t(4;14), del(17/17p), t(14;16), t(14;20), non-hyperdiploidy and gain(1q). Use of autologous HSCT and advances in drug therapy with the newer agents have increased survival. Over one-third of patients are now surviving for 5 years, compared with only one-quarter 10 years ago. The outlook may improve further with new drugs and combinations of treatments.

Aplastic anaemias

Primary idiopathic acquired aplastic anaemia This is a rare disorder in Europe and North America, with 2–4 new cases per million population per annum. The disease is much more common in certain other parts of the world, e.g. east Asia. The basic problem is failure of the pluripotent stem cells because of an autoimmune attack, producing hypoplasia of the bone marrow with a pancytopenia in the blood. The diagnosis rests on exclusion of other causes of secondary aplastic anaemia (see below) and rare congenital causes, such as Fanconi's anaemia.

Clinical features and investigations Patients present with symptoms of bone marrow failure, usually anaemia or bleeding, and less commonly, infections. An FBC demonstrates pancytopenia, low reticulocytes and often macrocytosis. Bone marrow aspiration and trephine reveal hypocellularity. The severity of aplastic anaemia is graded according to the Camitta criteria (Box 23.60).

- Bisphosphonates for hypercalcaemia and to delay other skeletal related events (p. 1047).
- Allopurinol to prevent urate nephropathy.
- Plasmapheresis, if necessary, for hyperviscosity.

Chemotherapy with or without HSCT Myeloma therapy has improved with the addition of novel agents, initially thalidomide and more recently the proteasome inhibitor bortezomib and the second-generation immunomodulatory drug lenalidomide. For first-line therapy in older patients, thalidomide combined with the alkylating agent melphalan and prednisolone (MPT) has increased the median overall survival to more than 4 years. Lenalidomide is approved first-line treatment for patients not eligible for transplantation and who are intolerant of, or unsuitable for, thalidomide. Thalidomide and lenalidomide both have anti-angiogenic effects against tumour blood vessels and immunomodulatory effects. Both can cause somnolence, constipation, peripheral neuropathy and thrombosis, though lenalidomide has a better side-effect profile. It is vital that females of child-bearing age use adequate contraception, as thalidomide and lenalidomide are teratogenic. Treatment is administered until paraprotein levels have stopped falling. This is termed 'plateau phase' and can last for weeks or years. In younger, fitter patients, standard treatment includes firstline therapies, such as cyclophosphamide, thalidomide and dexamethasone (CTD) or bortezomib (Velcade), thalidomide and dexamethasone (VTD) to maximum response, and then autologous HSCT, which improves quality of life and prolongs survival but does not cure myeloma. In all patients who have achieved maximal response, lenalidomide maintenance has been shown to prolong the response. When myeloma progresses, treatment is given to induce a further plateau phase. In the UK, the proteasome inhibitor bortezomib and lenalidomide have been used as second- and third-line therapy, as appropriate. As they have been used more frequently in the first or second line with prognostic benefit, however, subsequent relapses are more difficult to treat. A second-generation proteasome inhibitor, carfilzomib, and the anti-CD38 antibody daratumumab show promise in relapsed/ refractory disease. Responding patients may benefit from a second autologous HSCT.

Radiotherapy This is effective for localised bone pain not responding to simple analgesia and for pathological fractures. It is also useful for the 23.59

Haematological malignancy in old age

- Median age: approximately 70 years for most haematological malignancies.
- Poor-risk biological features: adverse cytogenetics or the presence of a multidrug resistance phenotype are more frequent.
- Prognosis: increasing age is an independent adverse variable in acute leukaemia and aggressive lymphoma.
-

Chemotherapy: may be less well tolerated. Older people are more likely to have antecedent cardiac, pulmonary or metabolic problems, tolerate systemic infection less well and metabolise cytotoxic drugs differently. • Cure rates: similar to those in younger patients, in those who do tolerate treatment. • Decision to treat: should be based on the individual's biological status, the level of social support available, and the patient's wishes and those of the immediate family, but not on chronological age alone. 23.60 Camitta criteria Severe AA (SAA) • Marrow cellularity < 25% (or 25–50% with < 30% residual haematopoietic cells), plus at least two of: Neutrophils < $0.5 \times 10^9/L$ Platelets < $20 \times 10^9/L$ Reticulocyte count < $20 \times 10^9/L$ Very severe AA (VSAA) • As for SAA but neutrophils < $0.2 \times 10^9/L$ Non-severe AA (NSAA) • AA not fulfilling the criteria for SAA or VSAA

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rubra vera (PRV), essential thrombocythaemia and myelofibrosis are the non-leukaemic myeloproliferative neoplasms. Although the majority of patients are classifiable as having one of these disorders, some have overlapping features and there is often progression from one to another, e.g. PRV to myelofibrosis. The recent discovery of the molecular basis of these disorders will lead to changes in classification and treatment; a mutation in the gene on chromosome 9 encoding the signal transduction molecule JAK-2 has been found in more than 90% of PRV cases and 50% of those with essential thrombocythaemia and myelofibrosis. Mutations in the calreticulin gene (CALR), which produces a chaperone protein that protects proteins moving from the endoplasmic reticulum to the cytoplasm, have been found in a further 25% of patients with essential thrombocythaemia. Less commonly, mutations can be detected in the thrombopoietin receptor gene MPL. Myelofibrosis In myelofibrosis, the marrow is initially hypercellular, with an excess of abnormal megakaryocytes that release growth factors, such as platelet-derived growth factor, to the marrow microenvironment, resulting in a reactive proliferation of fibroblasts. As the disease progresses, the marrow becomes fibrosed. Most patients present over the age of 50 years, with lassitude, weight loss and night sweats. The spleen can be massively enlarged due to extramedullary haematopoiesis (blood cell formation outside the bone marrow), and painful splenic infarcts may occur. The characteristic blood picture is leucoerythroblastic anaemia, with circulating immature red blood cells (increased reticulocytes and nucleated red blood cells) and granulocyte precursors (myelocytes). The red cells are shaped like teardrops (teardrop poikilocytes), and giant platelets may be seen in the blood. The white count varies from low to moderately high and the platelet count may be high, normal or low. Urate levels may be high due to increased cell breakdown, and folate deficiency is common. The marrow is often difficult to aspirate and a trephine biopsy shows an excess of megakaryocytes, increased reticulin and fibrous tissue replacement. The presence of a JAK-2 mutation supports the diagnosis. Management and prognosis Median survival is 4 years from diagnosis, but ranges from 1 year to over 20 years. Treatment is directed at control of symptoms, e.g. red cell transfusions for anaemia. Folic acid should be given to prevent deficiency. Cytotoxic therapy with hydroxycarbamide may help control spleen size, the white cell count or systemic symptoms. Splenectomy may be required for a grossly enlarged spleen or symptomatic pancytopenia secondary to splenic pooling of cells and hypersplenism. HSCT may be considered for younger patients. Ruxolitinib, an inhibitor of JAK-2, is now licensed in myelofibrosis and is effective at reducing systemic symptoms and splenomegaly. Essential thrombocythaemia Uncontrolled proliferation of megakaryocytes results in a raised level of circulating platelets that are often dysfunctional. Prior to a diagnosis of essential thrombocythaemia being made, reactive causes of thrombocytosis must be excluded (see Box

23.15). The presence of a JAK-2, CALR or, rarely, MPL mutation supports the diagnosis but is not universal. Patients present at a median age of 60 years with vascular occlusion or bleeding, or with an asymptomatic isolated raised platelet count. A small Management All patients will require blood product support and aggressive management of infection. The prognosis of severe aplastic anaemia managed with supportive therapy only is poor and more than 50% of patients die, usually in the first year. The curative treatment for patients under 35 years of age with severe idiopathic aplastic anaemia is allogeneic HSCT if there is an available sibling donor (p. 937). Older patients (35–50) may be candidates if they have no comorbidities (p. 937). Those with a compatible sibling donor should proceed to transplantation as soon as possible; they have a 75–90% chance of long-term cure. In older patients and those without a suitable donor, immunosuppressive therapy (IST) with anti-thymocyte globulin (ATG) and ciclosporin is the treatment of choice and gives 5-year survival rates of 75%. Unrelated donor allografts are considered for suitable patients who fail IST. The thrombopoietin receptor agonist eltrombopag (p. 971) has produced trilineage responses in patients who fail IST and is licensed for this indication. Non-transplanted patients may relapse or other clonal disorders of haematopoiesis may evolve, such as paroxysmal nocturnal haemoglobinuria (p. 950), myelodysplastic syndrome (p. 960) and AML (p. 955). Patients with aplastic anaemia must be followed up long-term. Secondary aplastic anaemia Causes of this condition are listed in Box 23.61. It is not practical to list all the drugs that have been suspected of causing aplasia. It is important to check the reported side-effects of all drugs taken over the preceding months. In some instances, the cytopenia is more selective and affects only one cell line, most often the neutrophils. Frequently, this is an incidental finding, with no ill health. It probably has an immune basis but this is difficult to prove. 23.61 Causes of secondary aplastic anaemia • Drugs: Cytotoxic drugs Antibiotics – chloramphenicol, sulphonamides Antirheumatic agents – penicillamine, gold, phenylbutazone, indometacin Antithyroid drugs – carbimazole, propylthiouracil Anticonvulsants Immunosuppressants – azathioprine • Chemicals: Benzene, toluene solvent misuse – glue-sniffing Insecticides – chlorinated hydrocarbons (DDT), organophosphates and carbamates (pp. 145 and 146) • Radiation • Viral hepatitis • Pregnancy • Paroxysmal nocturnal haemoglobinuria The clinical features and methods of diagnosis are the same as for primary idiopathic aplastic anaemia. An underlying cause should be treated or removed, but otherwise management is as for the idiopathic form. Myeloproliferative neoplasms These make up a group of chronic conditions characterised by clonal proliferation of marrow precursor cells. Polycythaemia

970 • HAEMATOLOGY AND TRANSFUSION MEDICINE (p. 929), von Willebrand disease (p. 974), and also in platelet function disorders and diseases affecting the vessel wall. Vessel wall abnormalities Vessel wall abnormalities may be: • congenital, such as hereditary haemorrhagic telangiectasia • acquired, as in a vasculitis (p. 1040) or scurvy. Hereditary haemorrhagic telangiectasia Hereditary haemorrhagic telangiectasia (HHT) is a dominantly inherited condition caused by mutations in the genes encoding endoglin and activin receptor-like kinase, which are endothelial cell receptors for transforming growth factor-beta (TGF- β), a potent angiogenic cytokine. Telangiectasia and small aneurysms are found on the fingertips, face and tongue, and in the nasal passages, lung and gastrointestinal tract. A significant proportion of these patients develop larger pulmonary arteriovenous malformations (PAVMs) that cause arterial hypoxaemia due to a right-to-left shunt. These predispose to paradoxical embolism, resulting in stroke or cerebral abscess. All patients with HHT should be screened for PAVMs; if these are found, ablation by percutaneous embolisation should be considered. Patients present either with recurrent bleeds, particularly epistaxis, or with iron deficiency due to occult gastrointestinal bleeding. Treatment can be difficult because of the

multiple bleeding points but regular iron therapy often allows the marrow to compensate for blood loss. Local cautery or laser therapy may prevent single lesions from bleeding. A variety of medical therapies have been tried but none has been found to be universally effective. Ehlers–Danlos disease Vascular Ehlers–Danlos syndrome (type 4) is a rare autosomal dominant disorder (1/100 000) caused by a defect in type 3 collagen that results in fragile blood vessels and organ membranes, leading to bleeding and organ rupture. Classical joint hypermobility (p. 1059) is often limited in this form of the disease but skin changes and facial appearance are typical. The diagnosis should be considered when there is a history of bleeding with normal laboratory tests. Scurvy Vitamin C deficiency affects the normal synthesis of collagen and results in a bleeding disorder characterised by perifollicular and petechial haemorrhage, bruising and subperiosteal bleeding. The key to diagnosis is the dietary history (p. 715). Platelet function disorders Bleeding may result from thrombocytopenia (see Box 23.14, p. 929) or from congenital or acquired abnormalities of platelet function. The most common acquired disorders are iatrogenic, resulting from the use of aspirin, clopidogrel, ticagrelor, dipyridamole and the glycoprotein IIb/IIIa inhibitors to prevent arterial thrombosis (see Box 23.26, p. 938). Inherited platelet function abnormalities are relatively rare. Congenital abnormalities may be due to deficiency of the membrane glycoproteins, e.g. Glanzmann's thrombasthenia (IIb/IIIa) or Bernard-Soulier syndrome (Ib), or due to the presence of defective platelet granules, e.g. a deficiency of dense (delta) granules (see Fig. 23.7, p. 920) giving rise to storage pool disorders. The congenital macrothrombocytopathies that are due to mutations in the myosin heavy chain gene MYH-9 are characterised by large platelets, percentage (around 5%) will transform to acute leukaemia and others to myelofibrosis. It is likely that most patients with essential thrombocythaemia benefit from low-dose aspirin to reduce the risk of occlusive vascular events. Low-risk patients (age < 40 years, platelet count < $1500 \times 10^9/L$ and no bleeding or thrombosis) may not require treatment to reduce the platelet count. For those with a platelet count above $1500 \times 10^9/L$, with symptoms, or with other risk factors for thrombosis such as diabetes or hypertension, treatment to control platelet counts should be given. Agents include oral hydroxycarbamide or anagrelide, an inhibitor of megakaryocyte maturation. Intravenous radioactive phosphorus (^{32}P) may be useful in old age and interferon- α has a role in younger patients. Polycythaemia rubra vera PRV occurs mainly in patients over the age of 40 years and presents either as an incidental finding of a high haemoglobin, or with symptoms of hyperviscosity, such as lassitude, loss of concentration, headaches, dizziness, blackouts, pruritus and epistaxis. Some patients present with manifestations of peripheral arterial or cerebrovascular disease. Venous thromboembolism may also occur. Peptic ulceration is common, sometimes complicated by bleeding. Patients are often plethoric and many have a palpable spleen at diagnosis. Investigation of polycythaemia is discussed on page 925. The diagnosis of PRV now rests on the demonstration of a high haematocrit and the presence of the JAK-2 V617F mutation (positive in 95% of cases). In the occasional JAK-2-negative cases, a raised red cell mass and absence of causes of a secondary erythrocytosis must be established. The spleen may be enlarged and neutrophil and platelet counts are frequently raised, an abnormal karyotype may be found in the marrow, and in vitro culture of the marrow can be used to demonstrate autonomous growth in the absence of added growth factors. Management and prognosis Aspirin reduces the risk of thrombosis. Venesection gives prompt relief of hyperviscosity symptoms. Between 400 and 500 mL of blood (less if the patient is elderly) are removed and the venesection is repeated every 5–7 days until the haematocrit is reduced to below 45%. Less frequent but regular venesection will maintain this level until the haemoglobin remains reduced because of iron deficiency. Suppression of marrow proliferation with hydroxycarbamide or interferon- α may reduce the risk of vascular occlusion, control spleen size

and reduce transformation to myelofibrosis. Intravenous 32P, which is reserved for older patients as it increases the risk of transformation to acute leukaemia by 6–10-fold, is rarely used now in Europe and North America. Median survival after diagnosis in treated patients exceeds 10 years. Some patients survive more than 20 years; however, cerebrovascular or coronary events occur in up to 60% of patients. The disease may convert to another myeloproliferative disorder, with about 15% developing acute leukaemia or myelofibrosis.

Bleeding disorders

Disorders of primary haemostasis

The initial formation of the platelet plug (see Fig. 23.6A, p. 918; also known as 'primary haemostasis') may fail in thrombocytopenia

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phagocytosis of sensitised platelets by reticulo-endothelial cells. Administration of intravenous immunoglobulin can raise the platelet count by blocking antibody receptors on reticulo-endothelial cells, and is combined with glucocorticoid therapy if there is severe haemostatic failure, especially with evidence of significant mucosal bleeding or a slow response to glucocorticoids alone. Persistent or potentially life-threatening bleeding should be treated with platelet transfusion in addition to the other therapies. The condition may become chronic, with remissions and relapses. Relapses should be treated by re-introducing glucocorticoids. If a patient has two relapses or primary refractory disease, second-line therapies are considered. The options for second-line therapy include the thrombopoietin receptor agonists (TPO-RA) eltrombopag and romiplostim, splenectomy and immunosuppression. Where splenectomy is considered, the precautions shown in Box 23.40 need to be in place. Splenectomy produces complete remission in about 70% of patients and improvement in a further 20–25% in favourable cases. The TPO-RAs induce response in around 75% of cases, usually within 10–14 days. Low-dose glucocorticoid therapy and immunosuppressants such as rituximab, ciclosporin, mycophenolate and tacrolimus may also produce remissions. The order in which therapies should be used is not entirely clear, although the TPO-RAs are licensed for this indication while the immunosuppressive agents are not.

Coagulation disorders

Normal coagulation is explained in Figure 23.6 (p. 918). Coagulation factor deficiency may be congenital or acquired, and may affect one or several of the coagulation factors (Box 23.62). Inherited disorders are almost uniformly related to decreased synthesis, as a result of mutation in the gene encoding a key protein in coagulation. Von Willebrand disease is the most common inherited bleeding disorder. Haemophilia A and B are the most common single coagulation factor deficiencies but inherited deficiencies of all the other coagulation factors are seen. Acquired disorders may be due to under-production (e.g. in liver failure), increased consumption (e.g. in DIC) or inhibition of function of coagulation factors (such as heparin therapy or immune inhibitors of coagulation, e.g. acquired haemophilia A).

Haemophilia A

Factor VIII deficiency resulting in haemophilia A affects 1/10 000 individuals. It is the most common congenital coagulation factor deficiency. Factor VIII is primarily synthesised by the liver and endothelial cells and has a half-life of about 12 hours. It is protected from proteolysis in the circulation by binding to von Willebrand factor (vWF).

Genetics

The factor VIII gene is located on the X chromosome. Haemophilia is associated with a range of mutations in the factor VIII gene; these include major inversions, large deletions and missense, nonsense and splice site abnormalities. As the factor VIII gene is on the X chromosome, haemophilia A is a sex-linked disorder (p. 48). Thus all daughters of a patient with haemophilia are obligate carriers and they, in turn, have a 1 in 4 chance of each pregnancy resulting in the birth of an affected male baby, a normal male baby, a carrier female or a normal female. Antenatal diagnosis by chorionic villous sampling is possible in families with a

known mutation. inclusion bodies in the neutrophils (Döhle bodies) and a variety of other features, including sensorineural deafness and renal abnormalities. Other familial thrombocytopathies are important, as they can be associated with somatic features, and some are associated with a propensity for development of bone marrow failure or dysplasia (e.g. RUNX-1-associated thrombocytopenia). Apart from Glanzmann's thrombasthenia, these conditions are mild disorders, with bleeding typically occurring after trauma or surgery, but rarely spontaneous. Glanzmann's thrombasthenia is an autosomal recessive condition associated with a variable but often severe bleeding disorder. These conditions are usually managed by local mechanical measures, but antifibrinolytics, such as tranexamic acid, may be useful and, in severe bleeding, platelet transfusion may be required. Recombinant VIIa is licensed for the treatment of resistant bleeding in Glanzmann's thrombasthenia.

Thrombocytopenia Thrombocytopenia occurs in many disease processes, as listed in Box 23.14 (p. 929), many of which are discussed elsewhere in this chapter.

Idiopathic thrombocytopenic purpura Idiopathic thrombocytopenic purpura (ITP) is immune-mediated with involvement of autoantibodies, most often directed against the platelet membrane glycoprotein IIb/IIIa, which sensitise the platelet, resulting in premature removal from the circulation by cells of the reticulo-endothelial system. It is not a single disorder; some cases occur in isolation while others are associated with underlying immune dysregulation in conditions such as connective tissue diseases, HIV infection, B-cell malignancies, pregnancy and certain drug therapies. The clinical presentation and pathogenesis are similar, however, whatever the cause of ITP.

Clinical features and investigations The presentation depends on the degree of thrombocytopenia. Spontaneous bleeding typically occurs only when the platelet count is below $20 \times 10^9/L$. At higher counts, the patient may complain of easy bruising or sometimes epistaxis or menorrhagia. Many cases with counts of more than $50 \times 10^9/L$ are discovered by chance. In adults, ITP more commonly affects females and may have an insidious onset. Unlike ITP in children, it is unusual for there to be a history of a preceding viral infection. Symptoms or signs of a connective tissue disease may be apparent at presentation or emerge several years later. Patients aged over 65 years should be considered for a bone marrow examination to look for an accompanying B-cell malignancy, and appropriate autoantibody testing performed if a diagnosis of connective tissue disease is likely. HIV testing should be considered because a positive result will have major implications for appropriate therapy. The peripheral blood film is normal, apart from a greatly reduced platelet number, while the bone marrow reveals an obvious increase in megakaryocytes.

Management Many patients with stable compensated ITP and a platelet count of more than $30 \times 10^9/L$ do not require treatment to raise the platelet count, except at times of increased bleeding risk, such as surgery and biopsy. First-line therapy for patients with spontaneous bleeding is with high doses of glucocorticoids, either prednisolone (1 mg/kg daily) or dexamethasone (40 mg daily for 4 days), to suppress antibody production and inhibit

972 • HAEMATOLOGY AND TRANSFUSION MEDICINE (factor VIII levels < 0.01 U/mL) present with spontaneous bleeding into skin, muscle and joints. Retroperitoneal and intracranial bleeding is also a feature. Babies with severe haemophilia have an increased risk of intracranial haemorrhage and, although there is insufficient evidence to recommend routine caesarean section for these births, it is appropriate to avoid head trauma and to perform imaging of the newborn within the first 24 hours of life. Individuals with moderate and mild haemophilia (factor VIII levels 0.01–0.4 U/mL) present with the same pattern of bleeding but usually after trauma or surgery, when bleeding is disproportionate to the severity of the insult. The major morbidity of recurrent bleeding in severe haemophilia is musculoskeletal. Bleeding is typically into large joints, especially knees, elbows,

ankles and hips. Muscle haematomas are also characteristic, most commonly in the calf and psoas muscles. If early treatment is not given to arrest bleeding, a hot, swollen and very painful joint or muscle haematoma develops. Recurrent bleeding into joints leads to synovial hypertrophy, destruction of the cartilage and chronic haemophilic arthropathy (Fig. 23.32). Complications of muscle haematomas depend on their location. A large psoas bleed may extend to compress the femoral nerve; calf haematomas may increase pressure within the inflexible fascial sheath, causing a compartment syndrome with ischaemia, necrosis, fibrosis, and subsequent contraction and shortening of the Achilles tendon.

Management The key to the management of severe haemophilia A (and B; p. 974) in more affluent countries is prophylactic coagulation factor replacement. The aim of this treatment is to maintain trough levels of factor VIII (or IX in the case of haemophilia B) above 0.02 U/mL. Doing this substantially reduces the number of bleeding episodes for men with severe haemophilia and so reduces the rate of deterioration of joints, which is the major long-term morbidity. Prophylaxis can be provided in many different ways: daily, on alternate days, or on information from pharmacokinetic studies that inform on the best way of scheduling prophylaxis. Practice in haemophilia A and B is in the process of changing somewhat due to the introduction of a variety of recombinant factor concentrates that have been manipulated to alter their half-life. In addition to standard half-life recombinant factor VIII, there are new products produced by Fc fusion and pegylation/ glycopegylation that extend the half-life of factor VIII to the degree that it can be used to alter dosing schedules for prophylaxis. The alternative approach, which still needs to be used in less affluent countries, is to treat on demand. In severe haemophilia A, bleeding episodes should be treated by raising the factor VIII level, usually by intravenous infusion of factor VIII concentrate. Factor VIII concentrates are freeze-dried and stable at 4°C and can therefore be stored in domestic refrigerators, allowing patients to treat themselves at home at the earliest indication of bleeding. Factor VIII concentrate prepared from blood donor plasma is now screened for HBV, HCV and HIV, and undergoes two separate virus inactivation processes during manufacture; these preparations have a good safety record. However, factor VIII concentrates prepared by recombinant technology are now widely available and, although more expensive, are perceived as being safer than those derived from human plasma in relation to infection risk. In addition to raising factor VIII concentrations, resting of the bleeding site with either bed rest or a splint reduces continuing haemorrhage. Once bleeding has settled, the patient should be mobilised and physiotherapy used to restore strength to the surrounding muscles. All non-immune potential recipients

Haemophilia ‘breeds true’ within a family; all members have the same factor VIII gene mutation and a similarly severe or mild phenotype. Female carriers of haemophilia may have reduced factor VIII levels because of random inactivation of their normal X chromosome in the developing fetus (p. 49). This can result in a mild bleeding disorder; thus all known or suspected carriers of haemophilia A should have their factor VIII level measured.

Clinical features The extent and patterns of bleeding are closely related to residual factor VIII levels (Box 23.63). Patients with severe haemophilia

Severity	Factor VIII or IX level	Clinical presentation
Severe	< 0.01 U/mL	Spontaneous haemarthroses and muscle haematomas
Moderate	0.01–0.05 U/mL	Mild trauma or surgery causes bleeding
Mild	0.05–0.4 U/mL	Major injury or surgery results in excess bleeding

“ 0.05–0.4 U/mL Major injury or surgery results in excess bleeding (ISTH = International Society on Thrombosis and Haemostasis) 23.62 Causes of coagulopathy Congenital X-linked • Haemophilia A and B Autosomal • Von

Willebrand disease • Factor II, V, VII, X, XI and XIII deficiencies • Combined II, VII, IX and X deficiency • Combined V and VIII deficiency • Hypofibrinogenaemia • Dysfibrinogenaemia Acquired Under-production • Liver failure • Vitamin K deficiency Increased consumption • Coagulation activation: Disseminated intravascular coagulation (DIC) • Immune-mediated: Acquired haemophilia and von Willebrand disease • Others: Acquired factor X deficiency (in amyloid) Acquired von Willebrand disease in Wilms' tumour Acquired factor VII deficiency in sepsis Drug-induced • Inhibition of function: Heparins Argatroban Bivalirudin Fondaparinux Rivaroxaban Apixaban Dabigatran Edoxaban • Inhibition of post-translational modification: Warfarin

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have evidence of HBV exposure, and 60% became HIV-positive. Management is described in Chapters 22 and 12. Concern that the infectious agent that causes vCJD (p. 1127) might be transmissible by blood and blood products has been confirmed in recipients of red cell transfusion (p. 931), and in one recipient of factor VIII. Pooled plasma products, including factor VIII concentrate, are now manufactured from plasma collected in countries with a low incidence of bovine spongiform encephalopathy. Another serious complication of factor VIII infusion is the development of anti-factor VIII antibodies, which arise in about 20% of those with severe haemophilia. Such antibodies rapidly neutralise therapeutic infusions, making treatment relatively ineffective. Infusions of activated clotting factors, e.g. VIIa or factor VIII inhibitor bypass activity (FEIBA), may stop bleeding. Haemophilia B (Christmas disease) Aberrations of the factor IX gene, which is also present on the X chromosome, result in a reduction of the plasma factor IX level, giving rise to haemophilia B. This disorder is clinically indistinguishable from haemophilia A but is less common. The of pooled blood products should be offered hepatitis A and B immunisation. The vasopressin receptor agonist desmopressin (p. 688) raises the vWF and factor VIII levels 3–4-fold, which is useful in arresting bleeding in patients with mild or moderate haemophilia A. The dose required for this purpose is higher than that used in diabetes insipidus, usually 0.3 µg/kg, and is given intravenously or subcutaneously. Alternatively, the same effect can be achieved by intranasal administration of 300 µg. Following repeated administration of desmopressin, patients need to be monitored for evidence of water retention, which can result in significant hyponatraemia. Desmopressin is contraindicated in patients with a history of severe arterial disease because of a propensity to provoke a thrombotic event, and in young children where hyponatraemia can result in fits. Complications of coagulation factor therapy Before 1986, coagulation factor concentrates from human plasma were not virally inactivated and many patients became infected with HIV and HBV/HCV. In patients with haemophilia treated with pooled concentrates that were not virally inactivated before 1988, infection with HCV is almost universal, 80–90% Fig. 23.32 Clinical manifestations of haemophilia. On the knee X-ray, repeated bleeds have led to broadening of the femoral epicondyles, and there is no cartilage present, as evidenced by the close proximity of the femur and tibia (A); sclerosis (B), osteophyte (C) and bony cysts (D) are present. (HCV = hepatitis C virus) Inset (Massive bruising) From Hoffbrand VA. Color atlas of clinical hematology, 3rd edn. Philadelphia: Mosby, Elsevier Inc.; 2000. D B C A Haemophilia B in the descendants of Queen Victoria Albert Victoria

Numerical value = age at death Affected with haemophilia (male) Carrier for haemophilia (female)
 Chronic haemophilic arthropathy with joint swelling and muscle wasting on left Left thigh muscle
 haematoma in severe haemophilia Massive bruising X-ray of advanced haemophilic arthropathy
 Massive retroperitoneal haemorrhage Hepatoma in cirrhotic liver secondary to HCV infection
 contracted from coagulation factor concentrate X-linked inheritance of haemophilia B

974 • HAEMATOLOGY AND TRANSFUSION MEDICINE assays that include functional and antigenic measures of vWF, multimeric analysis of the protein, and specific tests of function to determine binding to platelet glycoprotein Ib (RIPA) and factor VIII (Box 23.64). In addition, analysis for mutations in the vWF gene is informative in most cases. Management Many episodes of mild haemorrhage can be successfully treated by local means or with desmopressin, which raises the vWF level, resulting in a secondary increase in factor VIII. Tranexamic acid may be useful in mucosal bleeding. For more serious or persistent bleeds, haemostasis can be achieved with selected factor VIII concentrates, which contain considerable quantities of vWF in addition to factor VIII. Young children and patients with severe arterial disease should not receive desmopressin, and patients with type 2B disease develop thrombocytopenia that may be troublesome following desmopressin. Bleeding in type 3 patients responds only to factor VIII/vWF concentrate. Rare inherited bleeding disorders Severe deficiencies of factor VII, X and XIII occur as autosomal recessive disorders. They are rare but are associated with severe bleeding. Typical features include haemorrhage from the umbilical stump and intracranial haemorrhage. Factor XIII deficiency in women is typically associated with recurrent fetal loss. Factor XI deficiency may occur in heterozygous or homozygous individuals. Bleeding is very variable and is not accurately predicted by coagulation factor levels. In general, severe bleeding is confined to patients with levels below 15% of normal. Acquired bleeding disorders DIC is an important cause of bleeding that begins with exaggerated and inappropriate intravascular coagulation. It is discussed under thrombotic disease on page 978. frequency of bleeding episodes is related to the severity of the deficiency of the plasma factor IX level. Treatment is with a factor IX concentrate, used in much the same way as factor VIII for haemophilia A. The new extended half-life recombinant factor IX products made by Fc fusion, albumin fusion and pegylation offer the possibility of prophylaxis on a once-weekly or even two-weekly schedule. Although factor IX concentrates shared the problems of virus transmission seen with factor VIII, they do not commonly induce inhibitor antibodies (< 1% patients); when this does occur, however, it may be heralded by the development of a severe allergic-type reaction. Von Willebrand disease Von Willebrand disease is a common but usually mild bleeding disorder caused by a quantitative (types 1 and 3) or qualitative (type 2) deficiency of von Willebrand factor (vWF). This protein is synthesised by endothelial cells and megakaryocytes, and is involved in both platelet function and coagulation. It normally forms a multimeric structure that is essential for its interaction with subendothelial collagen and platelets (see Fig. 23.7, p. 920). vWF acts as a carrier protein for factor VIII, to which it is non-covalently bound; deficiency of vWF lowers the plasma factor VIII level. vWF also forms bridges between platelets and subendothelial components (e.g. collagen; see Fig. 23.6B, p. 918), allowing platelets to adhere to damaged vessel walls; deficiency of vWF therefore leads to impaired platelet plug formation. Blood group antigens (A and B) are expressed on vWF, reducing its susceptibility to proteolysis; as a result, people with blood group O have lower circulating vWF levels than individuals with non-O groups. This needs to be borne in mind when making a diagnosis of von Willebrand disease. Most patients with von Willebrand

disease have a type 1 disorder, characterised by a quantitative decrease in a normal functional protein. Patients with type 2 disorders inherit vWF molecules that are functionally abnormal. The type of abnormality depends on the site of the mutation in the vWD gene and how it affects binding to platelets, collagen and factor VIII. Patients with type 2A disease have abnormalities in vWF-dependent platelet adhesion; those with mutations in the platelet glycoprotein 1b binding site, resulting in increased affinity for glycoprotein 1b, have type 2B disease; those with mutations in the factor VIII binding site have type 2N disease; and those with other abnormalities in platelet binding but with normal vWF multimeric structure have type 2M disease. The patterns of laboratory abnormality accompanying these types are described in Box 23.64. The gene for vWF is located on chromosome 12 and the disease is usually autosomal dominantly inherited, except in type 2N and type 3, where inheritance is autosomal recessive. Clinical features Patients present with haemorrhagic manifestations similar to those in individuals with reduced platelet function. Superficial bruising, epistaxis, menorrhagia and gastrointestinal haemorrhage are common. Bleeding episodes are usually much less frequent than in severe haemophilia, and excessive haemorrhage may be observed only after trauma or surgery. Within a single family, the disease has variable penetrance, so that some members may have quite severe and frequent bleeds, whereas others are relatively asymptomatic. Investigations The disorder is characterised by reduced activity of vWF and factor VIII. The disease can be classified using a combination of 23.64

Classification of von Willebrand disease	Type	Defect	Inheritance	Investigations/patterns
Partial quantitative AD	Parallel decrease in vWF:Ag, RiCoF and VIII:c	2A	Qualitative AD	Absent HWM of vWF
Ratio of vWF activity to antigen < 0.7	2B	Qualitative AD	Reduced HWM of vWF	Enhanced platelet agglutination (RIPA)
2M	Qualitative AD	Ratio of vWF activity to antigen $\propto$ 0.7	Normal multimers of vWF	Abnormal vWF/platelet interactions
2N	Qualitative AR	Defective binding of vWF to VIII	Low VIII	

Severe quantitative AR or CH Very low vWF and VIII:c activity Absent multimers (AD = autosomal dominant; AR = autosomal recessive; CH = compound heterozygote; HWM = high-weight multimers of vWF; RiCoF = ristocetin co-factor; RIPA = ristocetin-induced platelet agglutination; VIII:c = coagulation factor VIII activity in functional assay; vWF = von Willebrand factor; vWF:Ag = vWF antigen measured by ELISA)

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Management of VTE The mainstay of treatment for all forms of VTE is anticoagulation. This can be achieved in several ways. One option is to use LMWH followed by a coumarin anticoagulant, such as warfarin. Treatment of acute VTE with LMWH should continue for a minimum of 5 days. Patients treated with warfarin should achieve a target INR of 2.5 (range 2–3; pp. 922 and 938) with LMWH continuing until the INR is above 2. Alternatively, patients may be treated with a DOAC. Rivaroxaban and apixaban may be used immediately from diagnosis without the need for LMWH, while the licences for dabigatran and edoxaban include initial treatment with LMWH for a minimum of 5 days before commencing the DOAC. In patients with active cancer and VTE, there is evidence that maintenance anticoagulation with LMWH is associated with a lower recurrence rate than warfarin. Patients who have had VTE and have a strong contraindication to anticoagulation and those who continue to have new pulmonary emboli despite therapeutic anticoagulation should have an inferior vena cava (IVC) filter inserted to prevent life-threatening PE (p. 619). The optimal

initial period of anticoagulation is between 6 weeks and 6 months. Patients with a provoked VTE in the Liver disease Although, traditionally, severe parenchymal liver disease (Ch. 22) has been described as a state associated with an excess of bleeding, it is now clear that these patients also have an increased risk of venous thrombosis. Although there is reduced hepatic synthesis of procoagulant factors, this is balanced to a degree by the reduced production of natural anticoagulant proteins and reduced fibrinolytic activity in patients with advanced liver disease. In severe parenchymal liver disease, bleeding may arise from many different causes. Pathological sources of potential major bleeding, such as oesophageal varices or peptic ulcer, are common. There is reduced hepatic synthesis, for example, of factors V, VII, VIII, IX, X, XI, prothrombin and fibrinogen. Clearance of plasminogen activator is reduced. Thrombocytopenia may occur secondary to hypersplenism in portal hypertension. In cholestatic jaundice, there is reduced vitamin K absorption, leading to deficiency of factors II, VII, IX and X, but also of proteins C and S. Treatment with plasma products or platelet transfusion should be reserved for acute bleeds or to cover interventional procedures such as liver biopsy. Vitamin K deficiency can be readily corrected with parenteral administration of vitamin K. Renal failure The severity of the haemorrhagic state in renal failure is proportional to the plasma urea concentration. Bleeding manifestations are those of platelet dysfunction, with gastrointestinal haemorrhage being particularly common. The causes are multifactorial and include anaemia, mild thrombocytopenia and the accumulation of low-molecular-weight waste products, normally excreted by the kidney, that inhibit platelet function. Treatment is by dialysis to reduce the urea concentration. Rarely, in severe or persistent bleeding, platelet concentrate infusions and red cell transfusions are indicated. Increasing the concentration of vWF, either by cryoprecipitate or by desmopressin, may promote haemostasis. Thrombotic disorders Venous thromboembolic disease (venous thromboembolism) While the most common presentations of venous thromboembolism (VTE) are deep vein thrombosis (DVT) of the leg (p. 186) and/or pulmonary embolism (PE; see also p. 619), similar management principles apply to rarer manifestations such as jugular vein thrombosis, upper limb DVT, cerebral sinus thrombosis (p. 1128) and intra-abdominal venous thrombosis (e.g. Budd–Chiari syndrome; p. 898). VTE has an annual incidence of approximately 1 : 1000 in Western populations. The relative incidence of DVT:PE is approximately 2 : 1. Mortality 30 days after DVT is approximately 10%, compared to 15% for PE. All forms of VTE are increasingly common with age and many of the deaths are related to coexisting medical conditions, such as active cancer or inflammatory disease, which predispose the patient to thrombosis in the first place. Risk factors for VTE are often present (Box 23.65) and it is appropriate to seek evidence of these risk factors in determining the long-term management strategy. Figure 23.33 illustrates some of the causes and consequences of VTE. The diagnosis of DVT and PE are discussed on pages 187 and 619, respectively.

23.65 Factors predisposing to venous thrombosis

Patient factors

- Increasing age
- Obesity
- Varicose veins
- Previous deep vein thrombosis
- Family history, especially of unprovoked venous thromboembolism when young

Transient additional risk factors:

- Pregnancy/puerperium
- Oestrogen-containing oral contraceptives and hormone replacement therapy
- Immobility, e.g. long-distance travel (> 4 hrs)
- Intravenous drug use involving the femoral vein
- Surgery (see below)
- Medical illnesses (see below)

Surgical conditions

- Major surgery, especially if > 30 mins' duration
- Abdominal or pelvic surgery, especially for cancer
- Major lower limb orthopaedic surgery, e.g. joint replacement and hip fracture surgery

Medical conditions

- Myocardial infarction/heart failure
- Inflammatory bowel disease
- Malignancy (anti-cancer chemotherapy increases the risk of venous thromboembolism compared with cancer alone)
- Nephrotic syndrome
- Chronic obstructive pulmonary disease
- Pneumonia
- Neurological conditions associated with immobility, e.g. stroke, paraplegia,

Guillain-Barré syndrome • Any high-dependency admission Haematological disorders • Polycythaemia rubra vera • Essential thrombocythaemia • Deficiency of natural anticoagulants: antithrombin, protein C, protein S • Paroxysmal nocturnal haemoglobinuria • Gain-of-function prothrombotic mutations: factor V Leiden, prothrombin gene G20210A • Myelofibrosis Antiphospholipid syndrome

976 • HAEMATOLOGY AND TRANSFUSION MEDICINE The management of DVT of the leg should also include elevation and analgesia; in limb-threatening DVT, thrombolysis may also be considered. Thrombolysis for PE is discussed on page 621. Post-thrombotic syndrome is due to damage of venous valves by the thrombus. It occurs in around 30% of patients who sustain a proximal lower limb DVT and results in persistent leg swelling, heaviness and discoloration. The most severe complication of this syndrome is ulceration around the medial malleolus (Fig. 23.33). Recent trial evidence suggests that use of elastic compression stockings following a DVT does not reduce the incidence of post-thrombotic syndrome. Prophylaxis of VTE All patients admitted to hospital should be assessed for their risk of developing VTE and appropriate prophylactic measures should be put in place. Both medical and surgical patients are at increased risk. A summary of the risk categories is given in Box 23.66. Early mobilisation of patients is important to prevent DVT, and those at medium or high risk require additional antithrombotic measures; these may be pharmacological or mechanical. There presence of a temporary risk factor, which is then removed, can usually be treated for short periods (e.g. 3 months), and indeed anticoagulation for more than 6 months does not alter the rate of recurrence following discontinuation of therapy. If there are ongoing risk factors that cannot be alleviated, such as active cancer, long-term anticoagulation is usually recommended, provided that the risk of bleeding is not deemed excessive. For patients with unprovoked VTE, the optimum duration of anticoagulation can be difficult to establish. Recurrence of VTE is about 2–3% per annum in patients who have a temporary medical risk factor at presentation and about 7–10% per annum in those with apparently unprovoked VTE. This plateaus at around 30–40% recurrence at 5 years. As such, many patients who have had unprovoked episodes of VTE will benefit from long-term anticoagulation. Several factors predict risk of recurrence following an episode of unprovoked VTE. The strongest predictors of recurrence are male sex and a positive D-dimer assay measured 1 month after stopping anticoagulant therapy. These factors are incorporated into scoring systems to predict recurrence such as the DASH score and the Vienna prediction model. Fig. 23.33 Causes and consequences of venous thromboembolic disease and its treatment. (DVT = deep vein thrombosis; IVC = inferior vena cava) Lateral sinus thrombosis is an uncommon form of venous thrombosis at an unusual site c i n e g o r t a l l a c i g o l o h t a P Fatal intracerebral haemorrhage is the most common cause of haemorrhagic death in patients on warfarin Postmortem fatal massive pulmonary embolism Absent IVC predisposes to lower limb DVT Inferior vena cava Common iliac vein Common femoral vein Superficial femoral vein Popliteal vein External and internal iliac veins Profunda femoris vein Gastrocnemius vein Anterior tibial vein Soleus muscle sinus Massive haemorrhage may complicate heparin therapy. This is particularly problematic in patients with renal failure on haemodialysis Iliac vein thrombosis IVC filter Post-thrombotic syndrome complicates 30% of cases of lower limb DVT. Severe cases are complicated by ulceration

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anticoagulation, as discussed on page 975. Patients who are deemed to be at high risk of thrombosis, e.g. those with antithrombin deficiency in pregnancy, should receive treatment or prophylactic doses of heparin to cover the period of risk only.

Antithrombin deficiency Antithrombin (AT) is a serine protease inhibitor (SERPIN) that inactivates the activated coagulation factors IIa, IXa, Xa and XIa. Heparins and fondaparinux achieve their therapeutic effect by potentiating the activity of AT. Familial deficiency of AT is inherited in an autosomal dominant manner; homozygosity for mutant alleles is not compatible with life. Around 70% of affected individuals will have an episode of VTE before the age of 60 years and the relative risk for thrombosis compared with the background population is 10–20. Pregnancy is a high-risk period for VTE and this requires fairly aggressive management with doses of LMWH that are greater than the usual prophylactic doses (≥ 100 U/kg/day). AT concentrate (either plasma-derived or recombinant) is available; this is required for cardiopulmonary bypass and may be used as an adjunct to heparin in surgical prophylaxis and in the peripartum period.

Protein C and S deficiencies Protein C and its co-factor protein S are vitamin K-dependent natural anticoagulants involved in switching off coagulation factor activation (factors Va and VIIIa) and thrombin generation (see Fig. 23.6F, p. 919). Inherited deficiency of either protein C or S results in a prothrombotic state with a fivefold relative risk of VTE compared with the background population.

Factor V Leiden Factor V Leiden results from a gain-of-function, single-base-pair mutation which prevents the cleavage and hence inactivation of activated factor V. This results in a relative risk of venous thrombosis of 5 in heterozygotes and 50 or more in rare homozygotes. The mutation is found in about 5% of Northern Europeans, 2% of Hispanics, 1.2% of African-Americans, 0.5% of Asian-Americans and 1.25% of Native Americans, and is rare in Chinese and Malay people.

Prothrombin G20210A This gain-of-function mutation in the non-coding 3' end of the prothrombin gene is associated with an increased plasma level of prothrombin. It is present in about 2% of Northern Europeans but is rare in native populations of Korea, China, India and Africa. In the heterozygous state, it is associated with a 2–3-fold increase in risk of VTE compared with the background population.

Antiphospholipid syndrome Antiphospholipid syndrome (APS) is a clinicopathological entity in which a constellation of clinical conditions, alone or in combination, is found in association with a persistently positive test for an antiphospholipid antibody. The antiphospholipid antibodies are heterogeneous and typically are directed against proteins that bind to phospholipids (Box 23.67). Although causal roles for these antibodies have been proposed, the mechanisms underlying the clinical features of APS are not clear. In clinical practice, two types of test are used, which detect:

- antibodies that bind to negatively charged phospholipid on an ELISA plate (called an anticardiolipin antibody test). These assays usually contain β 2-glycoprotein 1 (β 2-GP1) is increasing evidence in high-risk groups, such as patients who have had major lower limb orthopaedic surgery and abdominal or pelvic cancer surgery, for protracted thromboprophylaxis for as long as 30 days or so after the procedure. Particular care should be taken with the use of pharmacological prophylaxis in patients with a high risk of bleeding or with specific risks of haemorrhage related to the site of surgery or the use of spinal or epidural anaesthesia.

Inherited and acquired thrombophilia and prothrombotic states Several inherited conditions predispose to VTE (see Box 23.65), and have several points in common that are worth noting:

- None of them is strongly associated with arterial thrombosis.
- All are associated with a slightly increased incidence of adverse outcome of pregnancy, including recurrent early fetal loss, but there are no data to indicate that any specific intervention changes that outcome.
- Apart from in antithrombin deficiency and homozygous factor V Leiden, most carriers of these genes will never have an episode of VTE; if they do, it will be associated with the presence of an additional temporary risk factor.
- There is little evidence that detection of these abnormalities predicts

recurrence of VTE. • None of these conditions per se requires treatment with anticoagulants. Patients with thrombosis should receive 23.66 Antithrombotic prophylaxis Indications Patients in the following categories should be considered for specific antithrombotic prophylaxis: Moderate risk of DVT • Major surgery: In patients > 40 years or with other risk factor for VTE • Major medical illness, e.g.: Heart failure Myocardial infarction with complications Sepsis Inflammatory conditions, including inflammatory bowel disease Active malignancy Nephrotic syndrome Stroke and other conditions leading to lower limb paralysis High risk of DVT • Major abdominal or pelvic surgery for malignancy or with history of DVT or known thrombophilia (see Box 23.4, p. 923) • Major hip or knee surgery • Neurosurgery Methods of VTE prophylaxis Mechanical • Intermittent pneumatic compression • Mechanical foot pumps • Graduated compression stockings Pharmacological • LMWHs • Unfractionated heparin • Fondaparinux • Dabigatran • Rivaroxaban • Apixaban • Warfarin (DVT = deep vein thrombosis; VTE = venous thromboembolism)

978 • HAEMATOLOGY AND TRANSFUSION MEDICINE • those that interfere with phospholipid-dependent coagulation tests like the APTT or the dilute Russell viper venom time (DRVVT; called a lupus anticoagulant test). The term antiphospholipid antibody encompasses both a lupus anticoagulant and an anticardiolipin antibody/ anti- β 2-GP1; individuals may be positive for one, two or all three of these activities. It has been shown that patients who are 'triple-positive' have an increased likelihood of thrombotic events. Clinical features and management APS may present in isolation (primary APS) or in association with one of the conditions shown in Box 23.67, most typically systemic lupus erythematosus (secondary APS). Most patients present with a single manifestation and APS is now most frequently diagnosed in women with adverse outcomes of pregnancy. It is extremely important to make the diagnosis in patients with APS, whatever the manifestation, because it affects the prognosis and management of arterial thrombosis, VTE and pregnancy. Arterial thrombosis, typically stroke, associated with APS should probably be treated with warfarin, as opposed to aspirin. APS-associated VTE is one of the situations in which the predicted recurrence rate is high enough to indicate long-term anticoagulation after a first event. In women with obstetric presentations of APS, intervention with heparin and aspirin is almost routinely prescribed, although there is little evidence from clinical trials that it is an effective therapy in increasing the chance of a successful pregnancy outcome. Disseminated intravascular coagulation Disseminated intravascular coagulation (DIC) may complicate a range of illnesses (Box 23.68). It is characterised by systemic activation of the pathways involved in coagulation and its regulation. This may result in the generation of intravascular fibrin clots causing multi-organ failure, with simultaneous coagulation factor and platelet consumption, causing bleeding. The systemic coagulation activation is induced either through cytokine pathways, which are activated as part of a systemic inflammatory 23.67 Antiphospholipid syndrome (APS) Clinical manifestations • Adverse pregnancy outcome Recurrent first trimester abortion (≥ 3) Unexplained death of morphologically normal fetus after 10 weeks' gestation Severe early pre-eclampsia • Venous thromboembolism • Arterial thromboembolism • Livedo reticularis, catastrophic APS, transverse myelitis, skin necrosis, chorea Conditions associated with secondary APS • Systemic lupus erythematosus • Rheumatoid arthritis • Systemic sclerosis • Behçet's disease • Temporal arteritis • Sjögren's syndrome Targets for antiphospholipid antibodies • β 2-glycoprotein 1 • Protein C • Annexin V • Prothrombin (may result in haemorrhagic presentation) 23.68 Disseminated intravascular coagulation (DIC) Underlying conditions • Infection/sepsis • Trauma • Obstetric, e.g. amniotic fluid embolism, placental abruption, pre-eclampsia • Severe liver failure • Malignancy, e.g. solid tumours and leukaemias • Tissue destruction, e.g. pancreatitis, burns • Vascular

abnormalities, e.g. vascular aneurysms, liver haemangiomas • Toxic/immunological, e.g. ABO incompatibility, snake bites, recreational drugs ISTH scoring system for diagnosis of DIC Presence of an associated disorder Essential Platelets ($\times 10^9/L$)

“ 100 = 0 < 100 = 1 < 50 = 2 Elevated fibrin degradation products No increase = 0 Moderate = 2 Strong = 3 Prolonged prothrombin time < 3 secs = 0 3 secs but < 6 secs = 1 6 secs = 2 Fibrinogen 1 g/L = 0 < 1 g/L = 1 Total score ≥ 5 = Compatible with overt DIC < 5 = Repeat monitoring over 1-2 days (ISTH = International Society for Thrombosis and Haemostasis) 23.69 Haemostasis and thrombosis in old age • Thrombocytopenia: not uncommon because of the rising prevalence of disorders in which it may be a secondary feature, and also because of the greater use of drugs that can cause it. • ‘Senile’ purpura: presumed to be due to an age-associated loss of subcutaneous fat and the collagenous support of small blood vessels, making them more prone to damage from minor trauma. • Thrombosis: incidence of thromboembolic disease rises with increasing age. This may be due to stasis and concurrent illness, to which older people are prone; some studies show increased platelet aggregation with age, and others age-associated hyperactivity of the haemostatic system, which could contribute to a prothrombotic state. • Thromboprophylaxis: should be considered in all older patients who are immobile as a result of acute illness. Prophylaxis is not required in chronic immobility without a medical cause, as there is no associated increase in thromboembolism. • Anticoagulation: older patients are more sensitive to the anticoagulant effects of warfarin, partly due to the concurrent use of other drugs and the presence of other pathology. Life-threatening or fatal bleeds on warfarin are significantly more common in those over 80 years. response, or by the release of procoagulant substances such as tissue factor. In addition, suboptimal function of the natural anticoagulant pathways and dysregulated fibrinolysis contribute to DIC. There is consumption of platelets, coagulation factors (notably factors V and VIII) and fibrinogen. The lysis of fibrin

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• thrombocytopenia • microangiopathic haemolytic anaemia • neurological sequelae • fever • renal impairment. It is an acute autoimmune disorder mediated by antibodies against ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif). This enzyme normally cleaves vWF multimers to produce normal functional units, and its deficiency results in large vWF multimers that cross-link platelets. The features are of microvascular occlusion by platelet thrombi affecting key organs, principally brain and kidneys. It is a rare disorder (1 in 750 000 per annum), which may occur alone or in association with drugs (ticlopidine, ciclosporin), HIV, shiga toxins (p. 263) and malignancy. It should be treated by emergency plasma exchange. Glucocorticoids, aspirin and rituximab also have a role in management. Untreated mortality rates are 90% in the first 10 days, and even with appropriate therapy, the mortality rate is 20–30% at 6 months. Further information Websites bcshguidelines.com British Committee for Standards in Haematology

guidelines. cibmtr.org International Bone Marrow Transplant Registry. transfusionguidelines.org.uk Contains the UK Transfusion Services' Handbook of Transfusion Medicine and links to other relevant sites. ukhcdo.org UK Haemophilia Centre Doctors' Organisation. clot results in production of fibrin degradation products (FDPs), including D-dimers. Investigations DIC should be suspected when any of the conditions listed in Box 23.68 are met. Measurement of coagulation times (APTT and PT; p. 920), along with fibrinogen, platelet count and FDPs, helps in the assessment of prognosis and aids clinical decision-making with regard to both bleeding and thrombotic complications. Management Therapy is primarily aimed at the underlying cause. These patients will often require intensive care to deal with concomitant issues, such as acidosis, dehydration, renal failure and hypoxia. Blood component therapy, such as fresh frozen plasma, cryoprecipitate and platelets, should be given if the patient is bleeding or to cover interventions with a high bleeding risk, but should not be prescribed routinely based on coagulation tests and platelet counts alone. Prophylactic doses of heparin should be given, unless there is a clear contraindication. Established thrombosis should be treated cautiously with therapeutic doses of unfractionated heparin, unless clearly contraindicated. Patients with DIC should not, in general, be treated with antifibrinolytic therapy, e.g. tranexamic acid. Thrombotic thrombocytopenic purpura Like DIC and also heparin-induced thrombocytopenia (p. 938), thrombotic thrombocytopenic purpura (TTP) is a disorder in which thrombosis is accompanied by paradoxical thrombocytopenia. TTP is characterised by a pentad of findings, although few patients have all five components:

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