Haematology at a Glance

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Contents

Preface to the second edition

We were pleased that the first edition of *Haematology at a Glance* has been widely read both in its English version and in the French translation. Informal feedback from medical students, nurses and medical technologists has all been favourable.

The new second edition has additional chapters on Haematological Effects of Drugs, Haematology of Pregnancy and Infancy, Red Cell Disorders and Practical Procedures. The style of the book has also been altered to bring it more in line with the 'At a Glance' format. Some figures have been redrawn to add clarity and a number of new figures have been added. New diagnostic techniques e.g. DNA micro-array have also been included. In order to avoid expansion of the book, we have removed all non-essential material and in places simplified the text. We are grateful to our Publishers, and particularly Geraldine Jeffers, for their support and expertise in the preparation of this second edition and to Ms June Elliott and Miss Avani Mehta for their expert secretarial help.

Atul Mehta
Victor Hoffbrand
2005

Preface to the first edition

With the ever-increasing complexity of the medical undergraduate curriculum, we feel that there is a need for a concise introduction to clinical and laboratory haematology for medical students. The 'At a Glance' format has allowed us to divide the subject into easily digestible slices or bytes of information.

We have tried to emphasize the importance of basic scientific and clinical mechanisms, and common diseases as opposed to rare syndromes. The clinical features and laboratory findings are summarized and illustrated; treatment is briefly outlined.

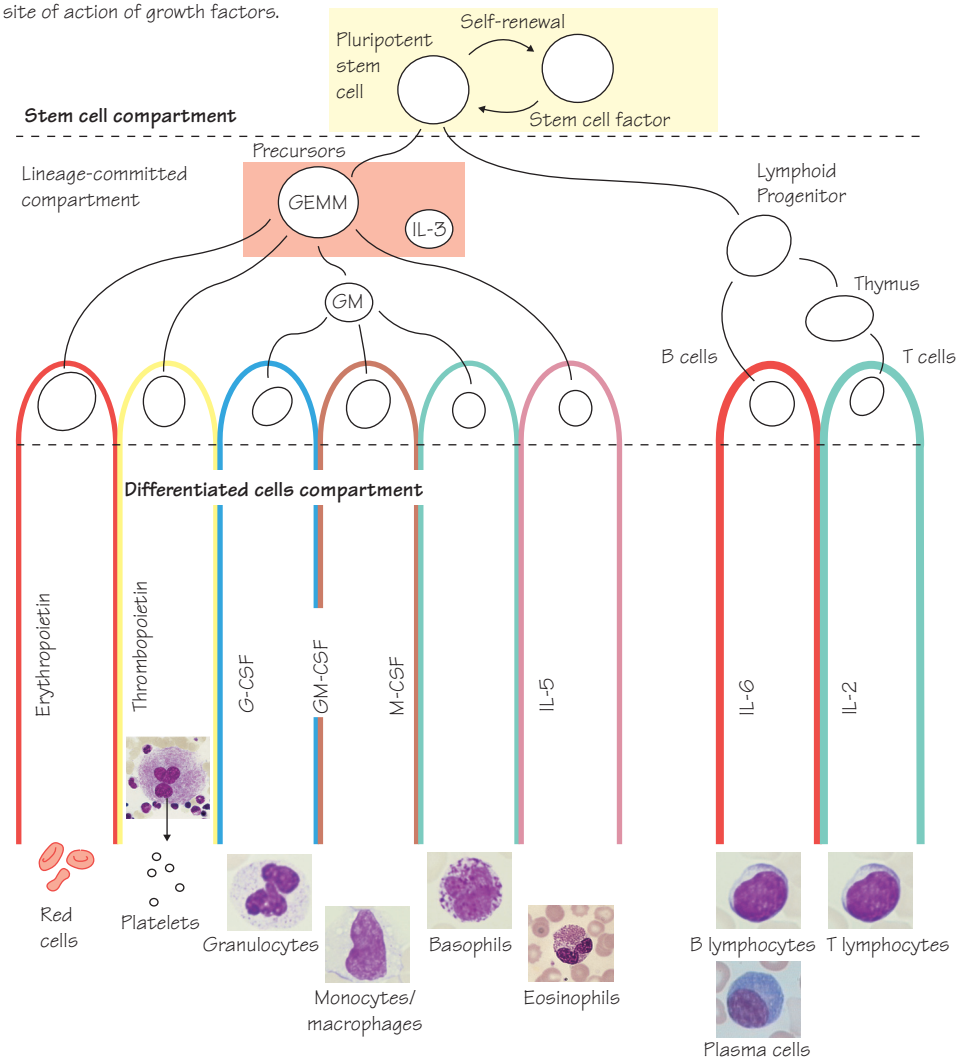
This book is intended for medical students, but will be useful to anyone who needs a concise and up-to-date introduction to haematology, for example nurses, medical laboratory scientists and those in professions supplementary to medicine.

We particularly wish to thank June Elliott who has patiently word-processed the manuscript through many revisions and Jonathan Rowley and his colleagues at Blackwell Science.

Atul Mehta
Victor Hoffbrand
January 2000

1 Haemopoiesis: physiology and pathology

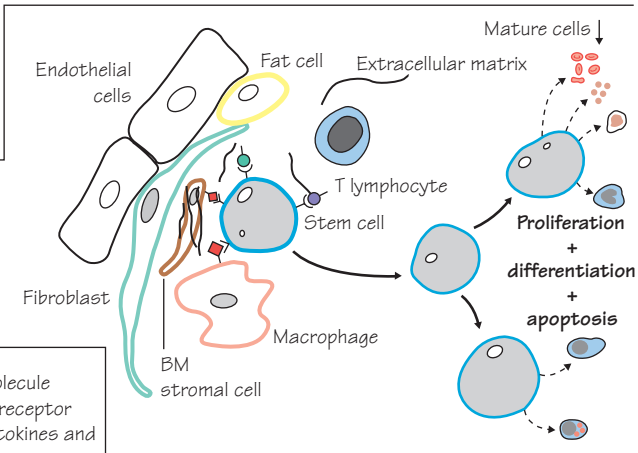
(a) Haemopoiesis. Showing site of action of growth factors.



Key

GEMM = Granulocyte/erythroid/monocyte/megakaryocyte precursor
GM = Granulocyte/monocyte precursor
GM-CSF = Granulocyte - macrophage colony - stimulating factor
G - CSF = Granulocyte colony - stimulating factor
IL-2 = Interleukin 2
IL-3 = Interleukin 3
IL-5 = Interleukin 5
IL-6 = Interleukin 6

(b) Haemopoiesis occurs within bone marrow microenvironment where haemopoietic stem cells are brought into contact with a range of other cell types. Cell–cell communication is binding, via cell surface receptors, to adhesion molecules and to fixed or secreted cytokines and growth factors. This binding triggers signal transduction which regulates gene transcription leading to proliferation, differentiation and apoptosis.



Key

Adhesion molecule
Cell surface receptor
Secreted cytokines and growth factors

Definition and sites

Haemopoiesis is the process whereby blood cells are made (Fig. 1a). The yolk sac, and later the liver and spleen, are important in fetal life but after birth normal haemopoiesis is restricted to the bone marrow. Infants have haemopoietic marrow in all bones but in adults it is in the central skeleton and proximal ends of long bones (normal fat to haemopoietic tissue ratio of about 50 : 50; Fig. 44d). Expansion of haemopoiesis down the long bones may occur, e.g. in leukaemias and chronic haemolytic anaemias. The liver and spleen can resume extramedullary haemopoiesis when there is marrow replacement, e.g. in myelofibrosis, or excessive demand, e.g. in severe haemolytic anaemias.

Stem cell and progenitor cells

A common primitive stem cell in the marrow has the capacity to self replicate, proliferate and differentiate to increasingly specialized progenitor cells which, after many cell divisions within the marrow, form mature cells (red cells, granulocytes, monocytes, platelets and lymphocytes) of the peripheral blood (Fig. 1a). The earliest recognizable red cell precursor is a pronormoblast and the granulocyte or monocyte precursor a myeloblast. An early lineage division is between lymphoid and myeloid cells. Stem and progenitor cells cannot be recognized morphologically; they resemble lymphocytes. Progenitor cells can be detected by *in vitro* assays in which they form colonies. Stem and progenitor cells also circulate in the peripheral blood. The stromal cells of the marrow (fibroblasts, endothelial cells, macrophages, fat cells) have adhesion molecules which react with corresponding ligands on the stem cells and maintain their viability. The haemopoietic stem cells may be 'plastic' i.e. capable of forming cells of other tissues e.g. liver, heart, nervous system but this is controversial. The marrow also contains mesenchymal stem cells that can form cartilage, fibrous tissue, bone and endothelial cells.

Growth factors

Haemopoiesis is regulated by growth factors (GFs) (Table 1.1) which usually act in synergy. These are glycoproteins produced by stromal cells, T lymphocytes, the liver and, for erythropoietin, the kidney. While some GFs act mainly on receptors on primitive cells, others act on later cells already committed to a particular lineage. GFs also affect the function of mature cells. GFs inhibit apoptosis (programmed cell death) of their target cells. Growth factors in clinical use include erythropoietin (EPO) and granulocyte colony-stimulating factor (G-CSF).

Table 1.1 Haemopoietic growth factors.

Act on stromal cells
IL-1 {Stimulate production of other growth factors}
TNF
Act on pluripotential cells
Stem cell factor
Act on early multipotential cells
IL-3
IL-4
IL-6
GM-CSF
Act on committed progenitor cells*
G-CSF
M-CSF
IL-5 (eosinophil CSF)
Erythropoietin
Thrombopoietin

*These growth factors (especially G-CSF and thrombopoietin) also act on earlier cells.
G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; M-CSF, monocyte colony-stimulating factor; TNF, tumour necrosis factor.

Signal transduction

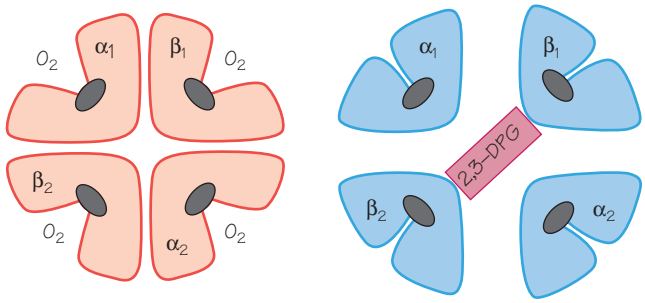
The binding of a GF with its surface receptor on the haemopoietic cell activates by phosphorylation a complex series of biochemical reactions by which a message is transmitted to the nucleus (Fig. 1b). The signal activates transcription factors which in turn activate or inhibit gene transcription. The signal may activate pathways which cause the cell to enter cell cycle (replicate), differentiate, maintain viability (inhibition of apoptosis) or increase functional activity (e.g. enhancement of cell killing by neutrophils).

Assessment of haemopoiesis

Haemopoiesis can be assessed clinically by performing a full blood count on peripheral blood (see Appendix 1). Bone marrow aspiration also allows assessment of the later stages of maturation of haemopoietic cells (Fig. 44c). Trephine biopsy provides a core of bone and bone marrow to show architecture (Fig 44b).

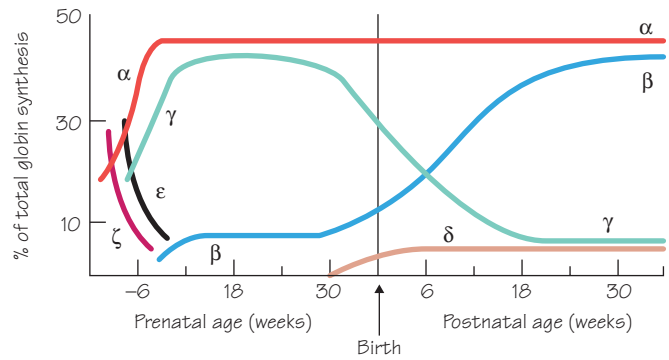
2 Normal blood cells I: red cells

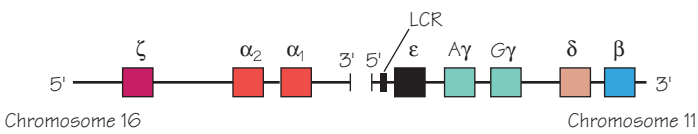
(a) Normal adult haemoglobin contains four globin (polypeptide) chains (α_1 α_2, β_1, β_2), each with its own haem molecule. These chains undergo conformational change and move with respect to each other when binding O_2 and CO_2. 2,3 diphosphoglycerate (2,3-DPG) binds between the β chains to reduce affinity for O_2 and allow O_2 release to the tissues.



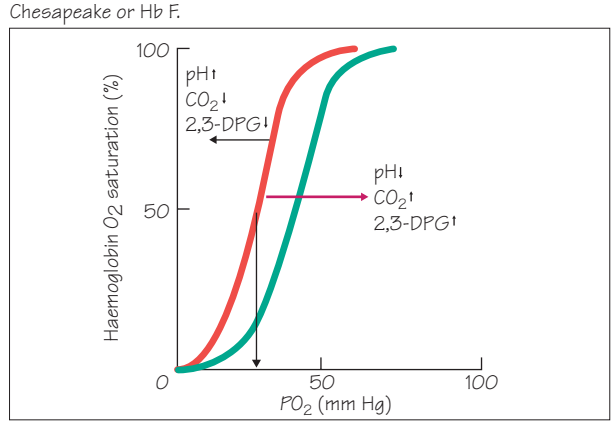
Oxyhaemoglobin Deoxyhaemoglobin

(b) The globin genes are located on chromosomes 16 (ζ, α) and 11 (ε, γ, δ, β). A 5' locus control region (LCR) is important in regulating γ and β globin gene expression. Different genes are transcribed during pre- and postnatal life, and the chains are synthesized independently and then combine to produce the different haemoglobins. The γ genes differ to produce either a glutamic acid (Gγ) or alanine (Aγ) residue at position 136. Whereas haemopoiesis occurs in yolk sac, liver and spleen prenatally, it is confined to marrow postnatally.





(c) The p50 is the partial pressure of oxygen at which haemoglobin is 50% saturated (red curve, normally 27mmHg). Decreased oxygen affinity, with increasing p50 (green curve) occurs as carbon dioxide concentration increases or pH decreases (Bohr effect) or 2,3-DPG levels rise. Increased oxygen affinity occurs during the opposite circumstances or may be a characteristic of a variant haemoglobin, which may lead to polycythaemia (see Chapter 30), e.g. Hb Chesapeake or Hb F.



(d) Red cell metabolism.

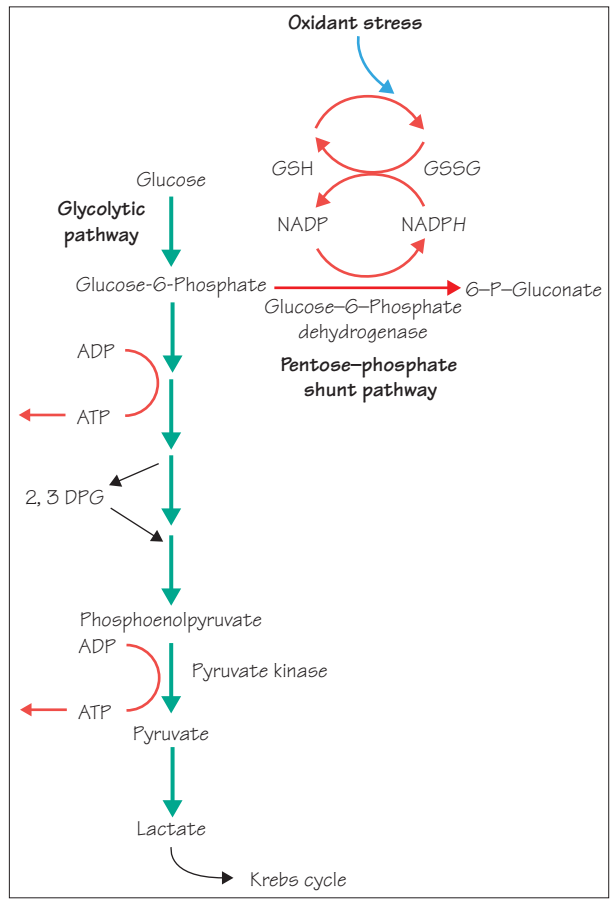


Table 2.1 Normal haemoglobins.

	Hb A	Hb A2	Hb F
Structure	$\alpha_2\beta_2$	$\alpha_2\delta_2$	$\alpha_2\gamma_2$
Normal adult (%)	96–98	1.5–3.5	0.5–0.8

Peripheral blood cells

Normal peripheral blood contains mainly mature cells which do not undergo further division.

Red cells (erythrocytes)

Red cells contain haemoglobin (Hb) which allows them to carry oxygen (O_2) and carbon dioxide (CO_2). Haemoglobin is composed of four polypeptide globin chains each with an iron-containing haem molecule (Fig. 2a). Embryonic haemoglobins (Portland, Gower I and II) are present in early fetal life, fetal haemoglobin (Hb F) dominates by late fetal life. A switch occurs at 3–6 months in the neonatal period to normal adult haemoglobin (Hb A) (Fig. 2b). Low levels of Hb F ($\alpha_2\gamma_2$) and the minor adult haemoglobin Hb A_2 ($\alpha_2\delta_2$) are present in normal adults (Table 2.1). The ability of haemoglobin to bind O_2 is measured as the haemoglobin–O_2 dissociation curve. Raised concentrations of 2,3-DPG, H^+ ions or CO_2 decrease O_2 affinity, allowing more O_2 delivery to tissues (Fig. 2c). Hb F has a higher, and sickle Hb (Hb S) a lower, O_2 affinity than Hb A. **Erythropoietin** controls the production of red cells. It is produced in the peritubular complex of the kidney (90%), liver and other organs. Erythropoietin stimulates mixed lineage and red cell progenitors as well as pronormoblasts and early erythroblasts to proliferate, differentiate and produce haemoglobin (Table 2.1). Erythropoietin secretion is stimulated by reduced O_2 supply to the kidney receptor (see Table 30.1).

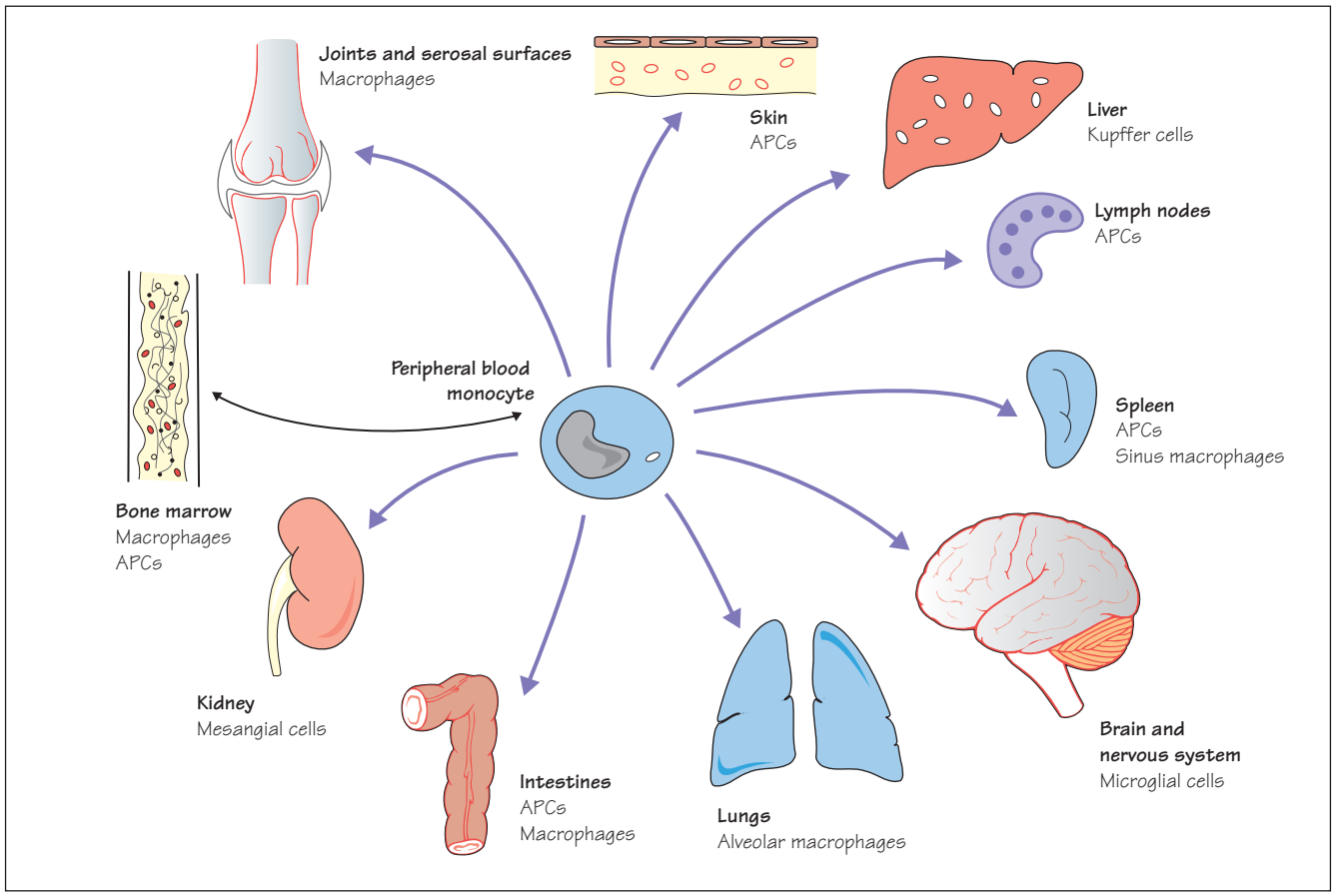
Developing red cells in the marrow (erythroblasts) are nucleated; the nucleus condenses with maturation, to be extruded prior to red cell release into the circulation. **Reticulocytes** are young non-nucleated red cells which retain RNA (stainable by supravital stains) (Fig. 9b). They are a measure of red cell production. They increase in number following acute haemorrhage, treatment of haematinic deficiency and in haemolytic anaemias. Between 10 and 15% of developing erythroblasts die within the marrow without producing mature red cells. This 'ineffective erythropoiesis' is increased in, for example, thalassaemia major, myelofibrosis and megaloblastic anaemia.

The glycolytic pathway (Fig. 2d) is the main source of energy (ATP) required to maintain red cell shape and deformability. The **hexose monophosphate** 'shunt' pathway provides the main source of reduced nicotinamide adenine dinucleotide phosphate (NADPH), which maintains reduced glutathione (GSH) and protects haemoglobin and the membrane proteins against oxidant damage. Mature red cells have no nucleus, ribosomes or mitochondria. They survive for about 120 days and are removed by macrophages of the reticuloendothelial system (see Chapter 4).

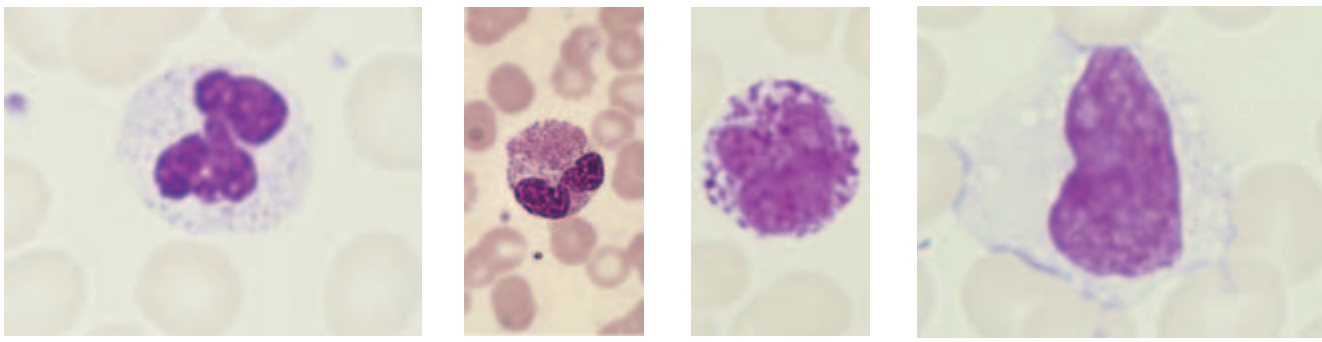
The red cell membrane is a bipolar lipid layer which anchors surface antigens. It has a protein skeleton (spectrin, actin, protein 4.1 and ankyrin) which maintains the red cell's biconcave shape and deformability.

3 Normal blood cells II: granulocytes, monocytes and the reticuloendothelial system

(a) The reticuloendothelial system. APC, antigen presenting cell.



(b) Normal peripheral blood cells (May Grunewald Giemsa stain): (i) neutrophil; (ii) eosinophil; (iii) basophil; (iv) monocyte.



(i) (ii) (iii) (iv)

Granulocyte and monocyte production occurs in the bone marrow and is controlled by growth factors (see Table 1.1). External stimuli (e.g. infection, fever, inflammation, allergy, trauma) act on cytokine networks to increase production of these growth factors e.g. IL-1 and TNF. The earliest recognizable granulocyte precursors are promyelocytes. These undergo further division and maturation into myelocytes, metamyelocytes and, finally, granulocytes (neutrophils, eosinophils and basophils). Primary granules, present in promyelocytes, contain lysosomal enzymes. Secondary granules containing other enzymes (peroxidase, lysosyme, alkaline phosphatase and lactoferrin) appear later. Basophil granules contain histamine and heparin.

Function of white cells

The primary function of white cells is to protect the body against infection. They work closely with proteins of the immune response, immunoglobulins and complement. Neutrophils, eosinophils, basophils and monocytes are all phagocytes; they ingest and destroy pathogens and cell debris. Phagocytes are attracted to bacteria at the site of inflammation by chemotactic substances released from damaged tissues or by complement components. Opsonization is the coating of cells or foreign particles by immunoglobulin or complement; this aids phagocytosis (engulfment) because phagocytes have immunoglobulin Fc and complement C3b (see below) receptors. Killing involves reduction of pH within the phagocytic vacuole, the release of granule contents and the production of antimicrobial oxidants and superoxides (the 'respiratory burst').

Neutrophils

Neutrophils (polymorphs) (Fig. 3b(i)) are the most numerous peripheral blood leucocyte. They have a short lifespan of around 10 hours in the circulation. About 50% of neutrophils in peripheral blood are attached to the walls of blood vessels (marginating pool). Neutrophils enter tissues by migrating in response to chemotactic factors. Migration, phagocytosis and killing are energy-dependent functions. The concentration of neutrophils in the blood may be lower in certain racial populations, e.g. Negro, Middle Eastern.

Eosinophils

Eosinophils have similar kinetics of production, differentiation and circulation to neutrophils; the growth factor IL-5 is important in regulating their production. They have a bilobed nucleus (Fig. 3b(ii)) and red-orange staining granules (containing histamine). They are particularly important in the response to parasitic and allergic diseases. Release of their granule contents onto larger pathogens (e.g. helminths) aids their destruction and subsequent phagocytosis.

Basophils

Basophils are closely related to mast cells (small darkly staining cells in the bone marrow and tissues). Both are derived from granulocyte precursors in the bone marrow. They are the least numerous peripheral blood leucocytes and have large dark purple granules which may obscure the nucleus (Fig. 3b(iii)). The granule contents include histamine and heparin and are released following binding of IgE to surface receptors. They play an important part in immediate hypersensitivity reactions. Mast cells also have an important role in defence against allergens and parasitic pathogens.

Monocytes

Monocytes (Fig. 3b(iv)) circulate for 20–40 hours and then enter tissues as macrophages where they mature and carry out their principal functions of phagocytosis and killing. Within tissues they survive for many days, possibly months. They have variable morphology in peripheral blood, but are mononuclear, have greyish cytoplasm with vacuoles and small granules. Within tissues, they often have long cytoplasmic projections allowing them to communicate widely with other cells.

Reticuloendothelial system (RES)

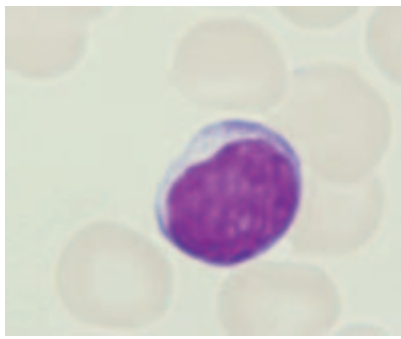
This is used to describe monocyte-derived cells (Fig. 3a) which are distributed throughout the body in multiple organs and tissues. The system includes Kupffer's cells in the liver, alveolar macrophages in the lung, mesangial cells in the kidney, microglial cells in the brain and macrophages within the bone marrow, spleen, lymph nodes, skin and serosal surfaces. The principal functions of the RES are to:

- phagocytose and destroy pathogens and cellular debris;
- process and present antigens to lymphoid cells (the antigen presenting cells react principally with T cells with whom they 'interdigitate' in lymph nodes, spleen, thymus, bone marrow and tissues);
- produce cytokines (e.g. IL-1) which regulate and participate within cytokine and growth factor networks governing haemopoiesis, inflammation and cellular responses.

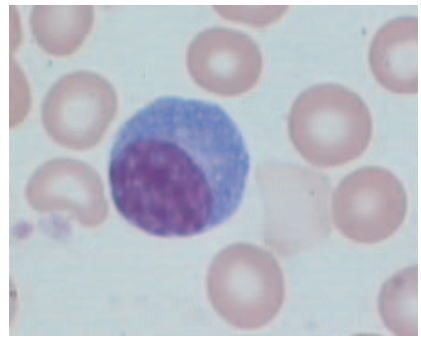
The cells of the RES are particularly localized in tissues which may come into contact with external allergens or pathogens. The main organs of the RES allow its cells to communicate with lymphoid cells, and include the liver, spleen, lymph nodes, bone marrow, thymus and intestinal tract associated lymphoid tissue.

4 Normal blood cells III: lymphocytes

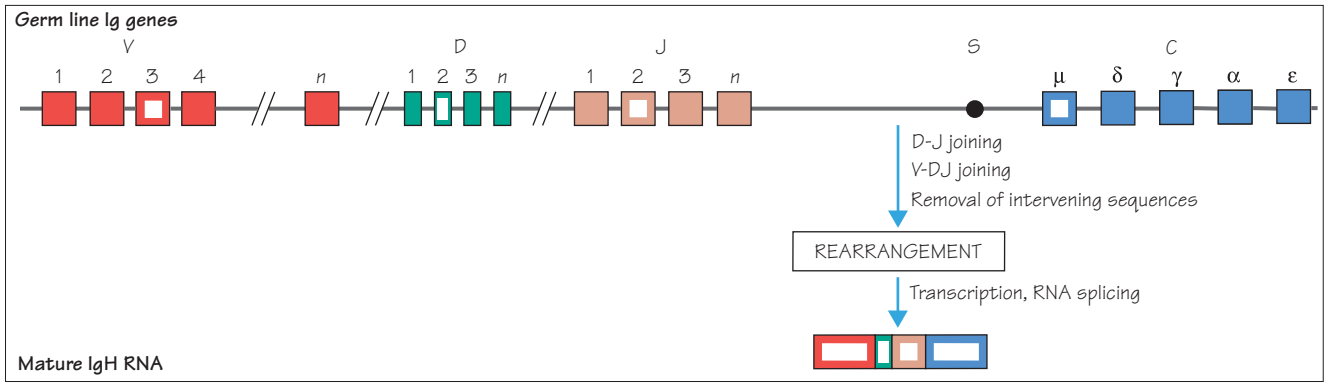
(a) Lymphocyte.



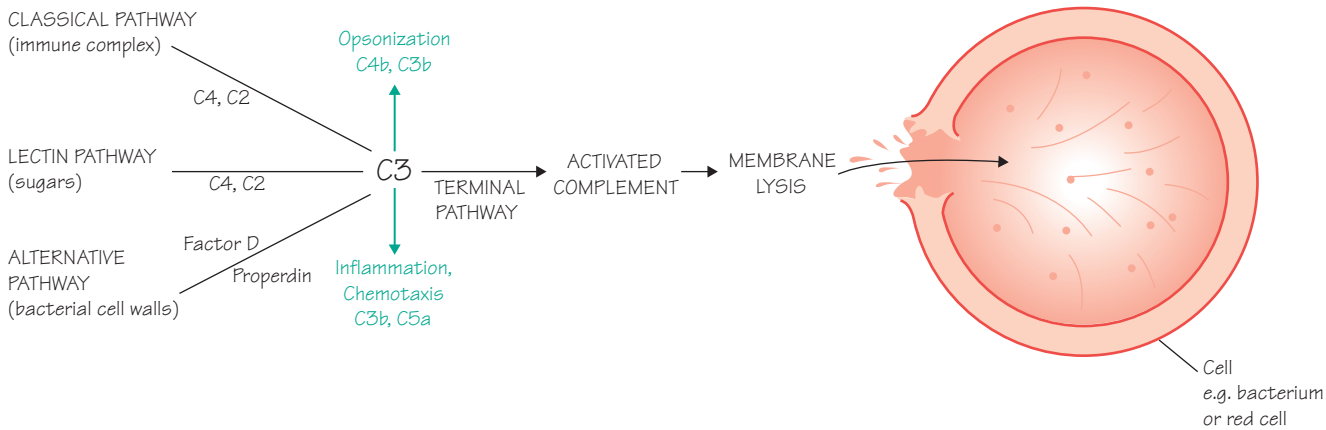
(b) Plasma cell.



(c) Immunoglobulin gene rearrangement and transcription. Immunoglobulin heavy chain genes occur as segments for variable (V), diversity (D), joining (J) and constant (C) regions. The class of the immunoglobulin depends on which of the constant region genes (μ, δ, γ, α, ε) is transcribed. The switch region (S) allows switching between classes. Diversity occurs through variability of which V segment joins which D and which J segment; the enzyme terminal deoxynucleotidyl transferase (TdT) randomly inserts new bases into D region DNA to generate additional diversity. Recombinase enzymes join up rearranged segments of DNA and intervening sequences are deleted. Similar rearrangements occur at the immunoglobulin light chain (κ and λ) and T cell receptor (α/β, γ/δ) loci. In the example shown, V_3 joins D_2, J_2 and μ.



(d) The complement cascade may be activated by antibody or immune complexes (classical pathway) sugars (lectin pathway) or bacterial cell walls (alternative pathway). Activation leads to generation of mediators of inflammation, phagocytosis and membrane damage.



Lymphocytes (Fig. 4a) are an essential component of the immune response and are derived from haemopoietic stem cells. A common lymphoid stem cell undergoes differentiation and proliferation to give rise to B cells, which mediate humoral or antibody-mediated immunity, and T cells (processed in the thymus), mediating cell-mediated immunity. Mature lymphocytes are small mononuclear cells with scanty blue cytoplasm (Fig. 4a). The majority of peripheral blood lymphocytes (70%) are T cells, which may have more cytoplasm than B cells and may contain granules.

Lymphocyte maturation occurs principally in bone marrow for B cells and in the thymus for T cells but also involves the lymph nodes, liver, spleen and other parts of the RES. The antigens expressed on the surface of a cell react with monoclonal antibody reagents. The cluster of differentiation (CD) nomenclature system has evolved as a means of classifying these antigens (see Appendix II). Lymphocytes have the longest lifespan of any leucocyte, and some (e.g. 'memory' B cells) may live for many years.

Immune response

Specificity of the immune response derives from amplification of antigen-selected T and B cells. The T cell receptor (TCR) on T cells and surface membrane immunoglobulin (sIg) on B cells are receptor molecules which have a variable and a constant portion. The variability ensures that a specific antigen is recognized by a lymphocyte with a matching variable receptor region. The genetic mechanisms required to generate the required diversity are common to T and B cells (Fig. 4c). They involve rearrangement of variable, joining, diversity and constant region genes to generate genes coding for surface receptors (Ig or TCR) capable of reacting specifically with one of an enormous array of antigens.

The immune response involves interaction between antigens and T cells, B cells and antigen presenting cells (APCs). Mature T cells are of three main types: helper cells expressing the CD4 antigen; suppressor cells expressing CD8; and cytotoxic cells also expressing CD8. Developing T cells are 'educated' in the thymus only to react to foreign antigens, and to develop tolerance to self human leucocyte antigens (HLA). B cells can also interact directly with antigen. Adhesion molecules mediate these cellular interactions. Reaction between antigen and appropriate receptor (sIg or TCR) leads to cellular proliferation and differentiation.

Natural killer (NK) cells

NK cells are neither T nor B cells, though are often $CD8^+$. They characteristically have prominent granules and are often large granular lymphocytes. These cells are not governed by MHC restriction and can kill target cells by direct adhesion.

Immunoglobulins

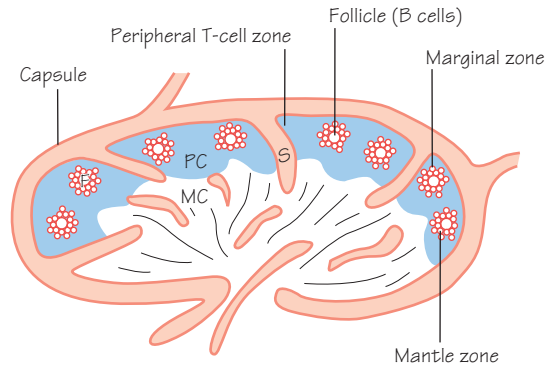
These are gammaglobulins produced by plasma cells (Fig. 4b). There are five main groups: IgG, IgM, IgA, IgD and IgE. Each is composed of light and heavy chains, and each chain is made up of variable, joining and constant regions.

Complement

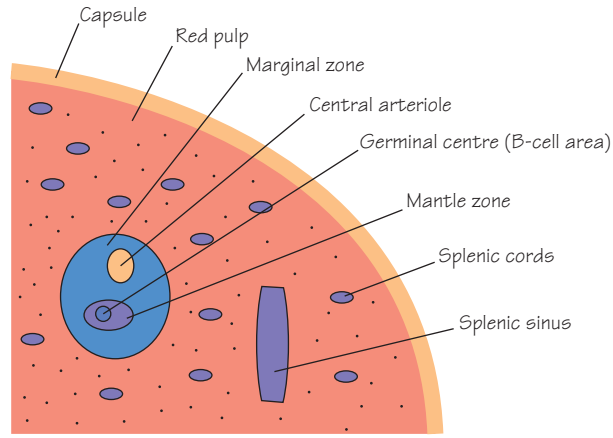
This is a group of plasma proteins and cell surface receptors which, if activated, interact with cellular and humoral elements in the inflammatory response (Fig. 4d). The complete molecule is capable of direct lysis of cell membranes and of pathogens sensitized by antibody. The C3b component coats cells making them sensitive to phagocytosis by macrophages. C3a and C5a may also activate chemotaxis by phagocytes and activate mast cells and basophils to release mediators of inflammation.

5 Lymph nodes, the lymphatic system and the spleen

(a) Diagramatic section through a lymph node. The marginal zone is a thin rim around the mantle. F, follicle and germinal centre; MC, medullary cords; PC, paracortex (interfollicular area); S, sinus.



(b) The splenic arterioles are surrounded by a periarteriolar sheath—the 'white pulp'. This is composed of T cells, antigen presenting interdigitating cells. Blood flows through the meshwork of the red pulp to find its way into sinuses which are the venous outflow. Part of the splenic blood flow bypasses this filtration process to pass directly through to the veins.



The lymph nodes and spleen are important organs of the body's immune system and reticuloendothelial system (RES). They are key areas where antigen (processed by the cells of the RES) can be presented in close proximity to the cells of the immune system (B cells and T cells). The anatomy of lymph nodes and spleen are illustrated in Figs 5a and 5b. In the spleen blood is filtered from the arteriolar circulation to the densely cellular areas (the white pulp) and then through the endothelial meshwork of the red pulp to the sinuses of the venous circulation. This filtration process allows removal of unwanted particulate matter (e.g. opsonized bacteria), effete cells or unwanted material from within deformable red cells (e.g. nuclear remnants, iron granules).

Lymph and the lymphatic system

Lymph is derived from blood as a filtrate and circulates around the body (including lymph nodes, liver, spleen and serosal surfaces) in lymph vessels (the lymphatic system). Lymph is rich in lymphocytes, which are returned to the blood circulation via the azygous vein and thoracic duct which returns lymph to the right atrium. Obstruction of the lymph vessels (e.g. by external compression or as a result of pathology within lymph nodes) leads to swelling (oedema or lymphoedema).

Causes of lymph node enlargement are listed in Table 5.1.

Table 5.1 Causes of lymphadenopathy.

Local
Localized bacterial/viral infection
Skin condition—e.g. trauma, eczema
Malignant—secondary carcinoma, lymphoma
General
Infection
bacterial—e.g. endocarditis, tuberculosis
viral—e.g. HIV, infectious mononucleosis, cytomegalovirus
other—e.g. toxoplasmosis, malaria
Malignancy
e.g. lymphoma, lymphoid leukaemias
Inflammatory disorders
e.g. sarcoidosis, connective tissue diseases
Generalized allergic conditions
e.g. widespread eczema

Functions of the spleen

The spleen is a specialized organ with an anatomical structure designed to allow close proximity between the portal circulation, systemic circulation and cells of the reticuloendothelial and haemopoietic systems (Fig. 5b). The functions of the spleen are:

- to allow antigens to be processed and presented to lymphoid cells;
- to manufacture antibody;
- to allow antibody-coated cells to be phagocytosed by the interaction with macrophages via their surface Fe receptors;
- to temporarily sequester red cells (especially reticulocytes) and allow removal of nuclear remnants, siderotic granules and other inclusions;
- haemopoiesis in early fetal life; and (rarely) in some pathological states, e.g. myelofibrosis.

Impaired splenic function or splenectomy reduce ability to make antibody, particularly to capsulated organisms, reduces clearance of

Table 5.2 Causes of splenomegaly.

Haemolytic anaemia
Hereditary spherocytosis, autoimmune haemolytic anaemia, thalassaemia major or intermedia, sickle cell anaemia (before infarction occurs)
Haematological malignancies
Lymphoma, CLL, ALL, AML, CML*
Polycythaemia vera, myelofibrosis*
Myelodysplasia
Storage diseases
Gaucher's*
Amyloid
Liver disease and portal hypertension
Congestive cardiac failure
Infection
Malaria*
Leishmaniasis*
Bacterial endocarditis
Viral infections, e.g. infectious mononucleosis

*Causes of massive splenomegaly.
ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia.

intracellular organisms (e.g. parasitized red cells) and impairs defence against organisms and toxins in the portal circulation.

Splenomegaly

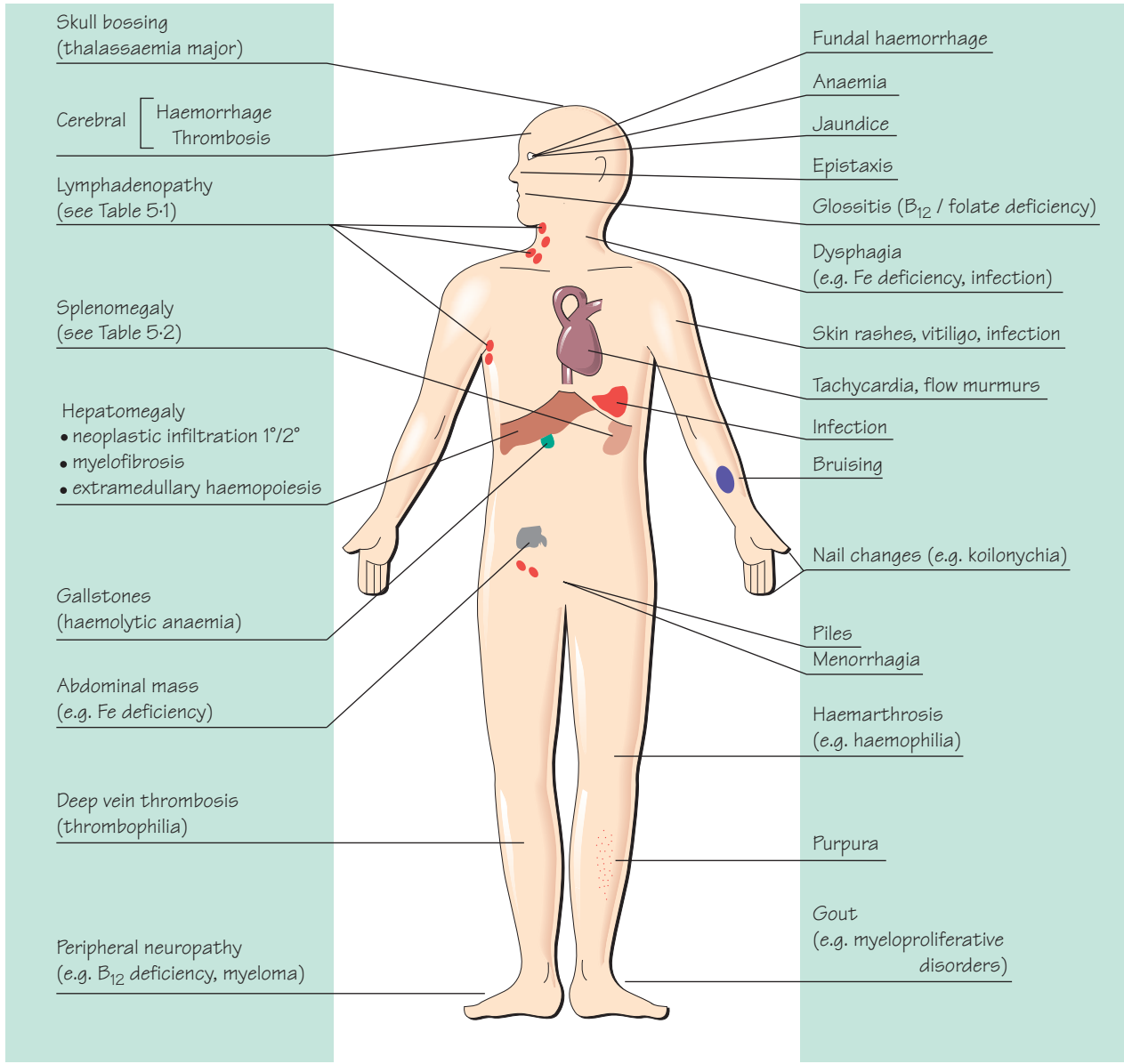
Causes of an enlarged spleen are listed in Table 5.2. Inflammation and infection typically cause an increase in the white cell areas (white pulp) while congestion causes an increase in the red cell areas (red pulp). Proliferation of malignant cells, storage disease and extramedullary haemopoiesis are other causes of enlargement.

Splenectomy

Splenectomy is beneficial in a number of haematological conditions, particularly when the spleen is the site of excessive destruction of peripheral blood cells (e.g. selected patients with haemolytic anaemia and thrombocytopenia (particularly autoimmune), myelofibrosis). The spleen has an important role in removing opsonized bacteria (see Chapter 3) and splenectomy (or hyposplenism due to disease, e.g. sickle cell disease) can lead to an increased susceptibility to infection. The operation should be avoided in children under 5 years and should be preceded by vaccination against pneumococcus, *Haemophilus influenzae* type B and meningococcus. Patients who are hyposplenic or post-splenectomy should take prophylactic antibiotics indefinitely (e.g. oral penicillin V 250 mg or erythromycin at low dose) and should at all times carry a card informing them and their carers of their condition. Malaria is likely to be more severe.

6 Clinical assessment

(a) Physical signs in haematological disease.



History

Anaemia

- Symptoms: shortness of breath on exertion, tiredness, headache or angina, more marked if anaemia is severe, of rapid onset and in older subjects.
- Causes: e.g. bleeding, dietary deficiency, malabsorption, systemic illness, haemolysis, bone marrow failure, inherited abnormalities of red cells.

Leucopenia

- Neutropenia, particularly if neutrophils are $<0.5 \times 10^9$/L, frequently leads to bacterial or fungal infection in skin, mouth, throat and chest.
- Pus is lacking.
- Infection is often atypical, caused by organisms non-pathogenic for normal individuals, rapidly progressive and difficult to treat.

• Lymphopenia predisposes to viral infection (e.g. herpes zoster), tuberculosis.
• Functional defects of neutrophils and lymphocytes also predispose to infection.

Thrombocytopenia
• Spontaneous bruising (ecchymoses) or petechiae, mucosal bleeding, e.g. epistaxis, menorrhagia. Bleeding following trauma is increased with platelets $<50 \times 10^9$/L. Spontaneous bleeding occurs when platelets $<10 \times 10^9$/L.
• Functional platelet defects also predispose to bleeding.

Coagulation factor defects
• Easy bleeding after trauma (e.g. circumcision, dental treatment); spontaneous haemorrhage in deep tissues (e.g. muscles, joints); family history.
• Acquired coagulation defects often accompanied by thrombocytopenia; spontaneous skin bleeding and excessive bleeding in response to trauma.
NB: Combination of anaemia, excessive bleeding and/or infection suggest pancytopenia caused by bone marrow failure (see Chapter 19).

Other symptoms (Fig. 6a)
• Weight loss, fever, pruritus and skin rash—lymphoma or myeloproliferative disorder.
• Bone pain, symptoms of hypercalcaemia (thirst, polyuria, constipation)—myeloma.
• Left hypochondrial pain—splenomegaly.
• Painless lymphadenopathy.
• Joint pains—gout caused by hyperuricaemia.

Family history
Inherited anaemia (e.g. genetic disorders of haemoglobin), coagulation disorders (e.g. haemophilia) and certain leucocyte disorders.

Drug history
Haemolytic anaemia in G6PD deficiency; disordered platelet function caused by aspirin; drug-induced agranulocytosis; macrocytosis of red cells caused by alcohol.

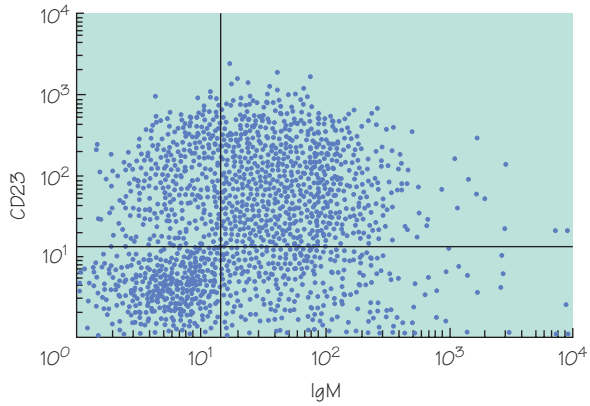
Operations
Gastrectomy, intestinal resection may lead to iron or vitamin B_{12} deficiency.

Examination
• Pallor of mucous membranes, if Hb <9 g/dL.
• Tachycardia, systolic murmur.
• Jaundice (haemolytic or megaloblastic anaemia); pigment (gallstones).
• Lymphadenopathy (generalized or localized) (Table 5.1).
• Skin changes, e.g. purpura caused by thrombocytopenia, vitiligo associated with pernicious anaemia, melanin pigmentation in iron overload, ankle ulcers in haemolytic anaemia, rashes caused by tumour infiltration.
• Nail changes (e.g. koilonychia in iron deficiency).
• Signs of infection (mouth, throat, skin, perineum, chest) associated with neutropenia.
• Mouth, e.g. angular cheilosis in iron deficiency, glossitis in B_{12} or folate deficiency.
• Hepatomegaly or splenomegaly (Table 5.2).
• Nervous system examination, e.g. B_{12} neuropathy, peripheral neuropathy in myeloma, amyloidosis, malignant infiltration in central nervous system leukaemia.
• Optic fundi, e.g. haemorrhage in severe anaemia, hyperviscosity in polycythaemia.

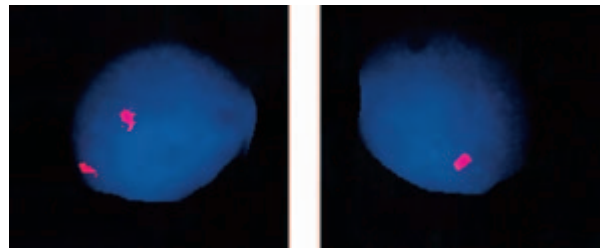
Special investigations
Haematological diseases are often multisystem disorders and a range of special investigations (X-ray, ultrasound, CT/MRI imaging, endoscopy, etc.) are frequently required to define the extent and stage of the disease. Nuclear medicine studies allow study of organ function, and tests useful to haematologists include the following:
• Isotope labelling of cells followed by scanning, e.g. autologous red cells can be labelled with radioactive chronium/technetium, reinjected, the red cell mass determined and their lifespan measured, loss detected in stools and destruction in liver/spleen detected by surface counting. Labelled white cell (gallium) scans can detect occult infection or lymphoma.
• Positron emission tomography (PET) measures metabolic activity of tissue and is able to distinguish active tumour, e.g. lymphoma (positive), from inactive scar tissue (negative).
• Multiple gated acquisition (MUGA) scanning to assess ventricular function.

7 Laboratory assessment

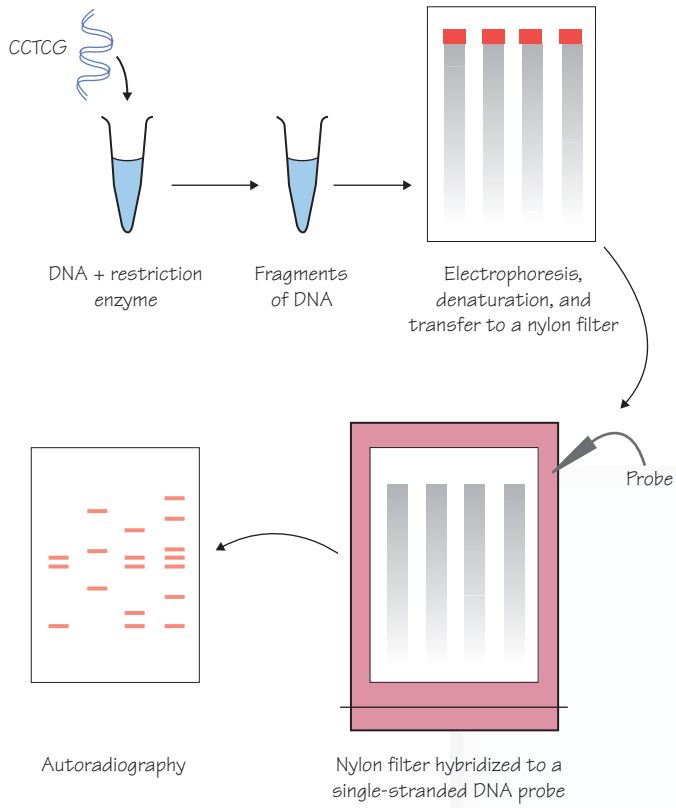
(a) Flow cytometry. In this example, cells are simultaneously tested for expression of CD23 and IgM. The x and y scales indicate the number of cells detected expressing that antigen.



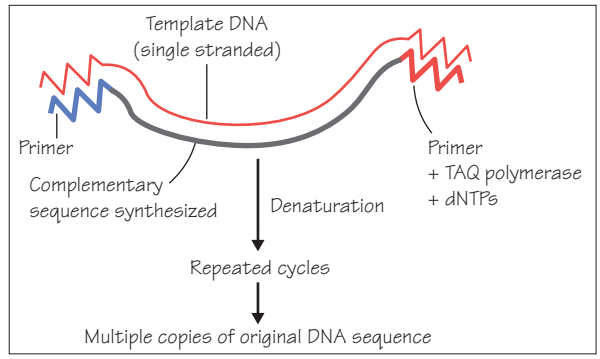
(b) Fluorescent *in situ* hybridization (FISH). Slides have been prepared from a cytogenetic preparation and hybridized with a fluorescent-labelled probe specific for chromosome 7. (a) Normal control showing two signals; (b) a patient with myelodysplasia who has monosomy 7



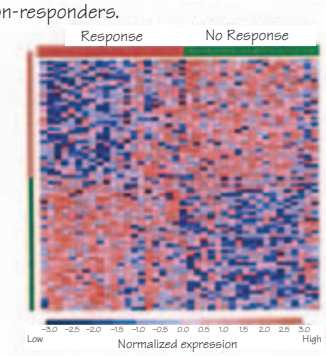
(c) Southern blot technique. Restriction enzymes are bacterial enzymes which recognize specific 3–6 nucleotide sequences (e.g. CCTCG) and cleave DNA whenever that sequence occurs. The fragments of DNA are separated by gel electrophoresis. DNA is then denatured to make it single-stranded and transferred by capillary action to a nylon filter. The filter is then incubated with a single stranded probe, which will hybridize to those DNA fragments with which there is base-pair homology. The Northern blot technique is a way of analysing RNA species by probe hybridization.



(d) The polymerase chain reaction (PCR) can be used to amplify a DNA segment (typically 0.1–2 kb). The template DNA is denatured and incubated with oligonucleotides (15–20 bp which hybridize specifically to sequences in template DNA and not anywhere else in the genome). The enzyme TAQ polymerase, in the presence of deoxynucleotides (dNTP), allows formation of a complete DNA chain complementary to the template sequence. This process is repeated 40 or so times. Modifications include amplifying a mRNA sequence into DNA and using nucleotides which only bind to a mutant and not a wild-type DNA sequence so that only mutant sequences are amplified.



(e) DNA microarray. DNA probes corresponding to different genes are immobilized in horizontal lanes. Fluorescent-labelled patient RNA or cDNA is added in vertical lanes. In this example, patients who subsequently responded to a new chemotherapy approach have a different pattern of gene expression to patients subsequently found to be non-responders.



Routine tests

Full blood count (FBC) (see Appendix I)

Blood sample in sequestrene (ethylenediaminetetra-acetate, EDTA) anticoagulant tested by an automated analyser (see Chapter 9). Analysers provide the following:

- haemoglobin concentration, haematocrit, red cell count, red cell indices (see Chapter 9);
- white cell count and differential (three-part: neutrophils, lymphocytes, monocytes; or five-part to include eosinophils and basophils);
- platelet count and size;
- analysers increasingly provide automated reticulocyte counts and enumerate immature platelets ('platelet reticulocytes').

Blood film

Blood film is used to assess red cell size/shape; white cell appearance and differential; abnormal cells; platelet size and morphology; detection of parasites, e.g. malaria.

Special laboratory tests

Investigations of haemolytic anaemia, haemoglobin disorders, haematinic deficiency, malignant diseases and coagulation disorders are discussed in the relevant chapters.

Erythrocyte sedimentation rate (ESR), plasma/whole blood viscosity and C-reactive protein

The ESR measures the rate of fall of a column of red cells in plasma in 1 hour. It is largely determined by plasma concentrations of proteins, especially fibrinogen and globulins. It is raised in anaemia. The normal range rises with age. A raised ESR is a non-specific indicator of an acute phase response and is of value in monitoring disease activity (e.g. rheumatoid arthritis). A raised ESR occurs in inflammatory disorders, infections, malignancy, myeloma, anaemia and pregnancy. The *plasma viscosity* gives comparable information and is increasingly favoured as it can be easily automated. *Whole blood viscosity* is also influenced by the cell counts, and is therefore raised when the red cell count (erythrocrit), white cells count (leucocrit) or platelet count is grossly raised. *C-reactive protein* (CRP) is raised in an acute phase response and is valuable in monitoring this.

Bone marrow aspiration and trephine biopsy

See Chapter 44.

Specialized tests

Flow cytometry

Flow cytometry (Fig. 7a) is an automated technique whereby a population of cells is incubated with specific monoclonal antibodies which are conjugated to a fluorochrome. The labelled cells are then passed in a fluid stream across a laser light source which allows quantitative analysis of antigen expression on the cell population. The technique is important in leukaemia diagnosis and assessment of residual malignant disease.

Chromosomal analysis

Normal individuals have 46 chromosomes: 44 autosomes (22 from each parent) and two sex chromosomes (46 XY = male, 46 XX = female). Chromosomal analysis is made initially by special stains of cells in division. Loss or gain of whole chromosomes, chromosome breaks, and loss, inversion or translocation of a part of a chromosome can be detected. *Fluorescent* in situ *hybridization* (FISH) is a sensitive technique for detecting chromosome abnormalities (Fig. 7b) which involves the use of a fluorescent DNA probe which hybridizes selectively to a particular chromosome segment, allowing sensitive detection of deletion, translocation and duplication of that segment, or fusion with another chromosome.

DNA abnormalities

DNA abnormality as a cause of haematological disease may be inherited or acquired. *Inherited* haematological diseases are most commonly autosomal recessive, requiring an individual to inherit two mutant copies (alleles) of a gene (one from each parent) for expression of the disease (homozygotes). Carriers (heterozygotes) have one normal and one mutant allele and may express minor abnormalities. Autosomal dominant diseases are rarer, and require only one mutant allele for full expression of the disease. Sex-linked diseases arise if the mutant gene is on the X chromosome; males, having only one X chromosome, are affected whereas females are usually carriers. *Acquired* DNA abnormalities are frequently present in clones of malignant cell populations and serve as disease markers and clues to pathogenesis (see Chapter 20).

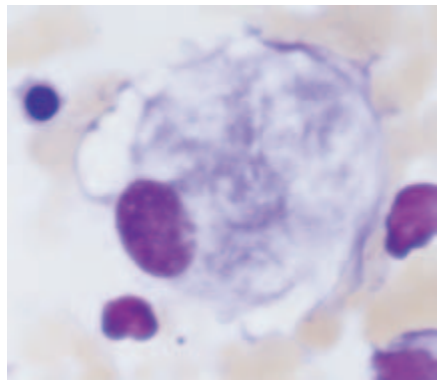
Molecular techniques

These include:

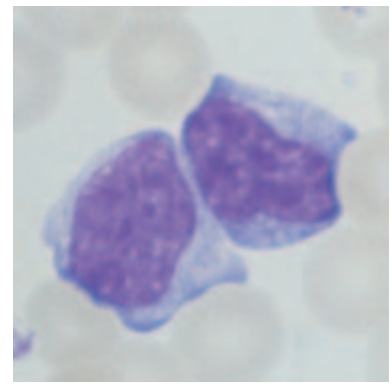
- Southern blotting (Fig. 7c), which allows assessment of deletion, rearrangement, inversion or duplication of DNA segments. Single base mutations will only be detected, however, if they alter the recognition sequence of a restriction enzyme.
- Polymerase chain reaction (PCR) (Fig. 7d) can be used to amplify a DNA segment which can then be sequenced or fractionated by size using gel electrophoresis. PCR can also be used to selectively amplify a particular sequence which may, for example, characterize a clone of malignant cells (minimal residual disease, see Chapter 20).
- Gene expression is studied by analysing RNA extracted from fresh cells, e.g. by gel electrophoresis (Northern blot). It can be semi-quantitated by using the enzyme reverse transcriptase to generate a DNA copy and then applying a modified PCR technique.
- DNA microarray analyses expression of multiple cellular genes (Fig. 7e). Fluorescent labelled cell RNA or cDNA is hybridized to DNA probes immobilized on a solid support. The pattern of mRNA expression is obtained and this is characteristic of the different leukaemia or lymphoma subtype and often gives important prognostic information.

8 Benign disorders of white cells: granulocytes, monocytes, macrophages and lymphocytes

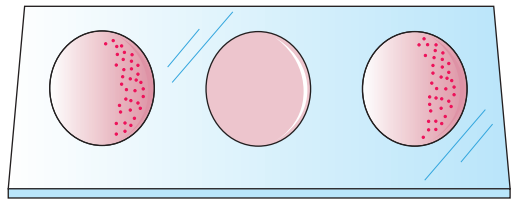
(a) Histiocyte laden with glucocerebroside to give a fibrillar cytoplasmic pattern (Gaucher's cell).



(b) Peripheral blood lymphocytes (activated T cells) in infectious mononucleosis.



(c) Screening test for infectious mononucleosis (IM). In the monospot slide test a drop of patient serum is added to red cell particles sensitized with a bovine mononucleosis antigen.



Granulocytes and monocytes

Inflammation commonly causes a neutrophil leucocytosis (neutrophilia) (Table 8.1). In addition, neutrophil granules may stain intensely (toxic granulation) and Doehle bodies (cytoplasmic RNA) may be present. A leukaemoid reaction is a profound reactive neutrophilia in which granulocyte precursors (e.g. myelocytes) appear in the blood. Neutropenia (reduced number of circulating neutrophils; Table 8.2) increases susceptibility to infection, particularly bacterial. The normal neutrophil count is lower in black and Middle Eastern subjects than in white people; this has no clinical consequences. Causes of **eosinophilia** are listed in Table 8.3. **Basophilia** (increase in blood basophils to $>0.1 \times 10^9$/L) is uncommon but occurs in myeloproliferative disorders. **Monocytosis** (increase in circulating monocytes to $>1.0 \times 10^9$/L) may occur in chronic infections (bacterial and protozoal, particularly in patients who cannot mount a neutrophil response), in malignancy and in myelodysplasia (see Chapter 22). **Disorders of neutrophil function** may be congenital or acquired and affect neutrophil interaction with immunoglobulin/complement, migration, phagocytosis and microbicidal activity. *Chronic granulomatous disease* is a rare inherited (X-linked) condition in which the cells are able to phagocytose but not kill. Acquired defects occur more commonly, e.g. in diabetes, myelodysplasia and corticosteroid therapy.

Table 8.1 Causes of neutrophilia (neutrophils $>7.5 \times 10^9$/L).

Bacterial infections
Inflammation, e.g. collagen diseases, Crohn's disease
Trauma/surgery
Tissue necrosis/infarction
Neoplasia
Haemorrhage and haemolysis
Metabolic, e.g. diabetic ketoacidosis
Myeloproliferative disorders
Pregnancy
Drugs, e.g. steriods, G-CSF, corticosteroids

G-CSF, granulocyte colony-stimulating factor.

Lysosomal storage disease

Hereditary deficiencies of enzymes required for glycolipid metabolism lead to the accumulation of ceramide components in various cells and tissues. *Gaucher's disease* is the most common (autosomal

Table 8.2 Causes of neutropenia (neutrophils $<1.5 \times 10^9$/L). Normal black and Middle Eastern subjects have lower counts.

1 Decreased production
(a) General bone marrow failure, e.g. aplastic anaemia, megaloblastic anaemia, myelodysplasia, acute leukaemia, chemotherapy, replacement by tumour (see Chapter 19)
(b) Specific failure of neutrophil production
Congenital, e.g. Kostman's syndrome
Cyclical
Drug-induced, e.g. sulfonamides, chlorpromazine, clozaril, diuretics, neomercazole, gold
2 Increased destruction
(a) General, e.g. hypersplenism
(b) Specific, e.g. autoimmune—alone or in association with connective tissue disorder, rheumatoid arthritis (Felty's syndrome)

Table 8.3 Causes of eosinophilia (eosinophils $>0.4 \times 10^9$/L).

Allergic diseases, e.g. asthma, hay fever, eczema, pulmonary hypersensitivity reaction (e.g. Loeffler's syndrome)
Parasitic disease
Skin diseases, e.g. psoriasis, drug rash
Drug sensitivity
Connective tissue disease
Haematological malignancy (e.g. Hodgkin lymphoma)
Idiopathic hypereosinophilia
Eosinophilic leukaemia (rare)

recessive) and is caused by mutations in the gene encoding glucocerebrosidase. Type I (most common) occurs especially among Ashkenazic Jews (age of presentation from infancy to middle age) and does not involve the central nervous system (CNS); types II and III are rarer and do involve the CNS. Clinical features (enlarged liver and spleen, bone defects) and haematological features (anaemia, thrombocytopenia) result from accumulation of Gaucher's cells (Fig. 8a) in the spleen, liver, skeleton, marrow and in types II and III in the CNS. Treatment is principally by enzyme replacement therapy.

Histiocyte disorders

Histiocytes are the terminally differentiated cells of the monocyte macrophage system and are widely distributed throughout all tissues. Langerhans' cells are macrophages present in epidermis, spleen, thymus, bone, lymph nodes and mucosal surfaces. *Langerhans' cell histiocytosis* (LCH) is a rare single organ or system or multisystem disease occurring principally in childhood (<10 years). Clinical features include skin rash, bone pain/swelling, lymphadenopathy, hepatosplenomegaly, endocrine changes (e.g. diabetes insipidus as a result of posterior pituitary involvement). Malignant histiocyte disorders include monocytic variants of acute leukaemia (see Chapter 23) and some types of non-Hodgkin lymphoma (see Chapter 28).

Haemophagocytic syndromes

In these syndromes the bone marrow shows increased numbers of histiocytes which contain ingested blood cells, leading to pancytopenia. The mechanism is poorly understood and prognosis is usually poor. Causes include infection (viral, bacterial, tuberculosis), especially in an immunosuppressed host, tumours (e.g. lymphoma) (see Fig. 40a) or a rare familial type.

Lymphocyte disorders

Lymphocytosis occurs in viral infections, some bacterial infections (e.g. pertussis) and in lymphoid neoplasia.

Lymphopenia (reduction in circulating lymphocytes to $<1.5 \times 10^9$/L) occurs in viral infection (e.g. HIV), lymphoma, connective tissue disease, severe bone marrow failure and with immunosuppressive drug therapy.

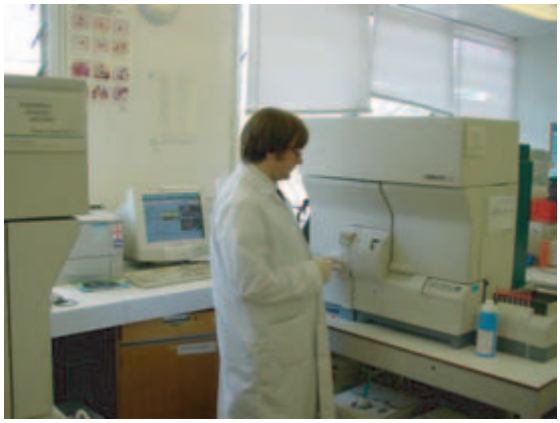
Infectious mononucleosis (*glandular fever*) is caused by Epstein–Barr virus (EBV) infection of B lymphocytes. Atypical circulating lymphocytes are reactive T cells. Cytomegalovirus, other viruses and toxoplasma infections cause a similar blood picture (Fig. 8b). Clinical features include onset usually in young adults (age 15–40 years), sore throat, lymphadenopathy, fever, morbilliform rash—particularly following treatment with amoxicillin—and jaundice, hepatomegaly and tender splenomegaly in a minority. Complications include autoimmune thrombocytopenia and/or haemolytic anaemia, myocarditis, encephalitis, hepatitis and postviral fatigue syndrome. The Paul–Bunnell test, modified as the monospot slide test (Fig. 8c), detects heterophile antibodies (antibodies against cells of a different species). The test is positive from 1 week after infection and persists for up to 2 months. Viral culture from sputum/saliva and specific IgM and IgG antibody tests against EBV nuclear and capsular antigens are sometimes useful in diagnosis.

Immunodeficiency

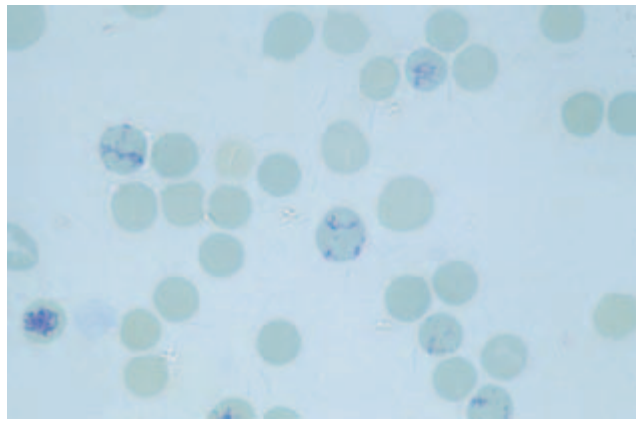
Depressed humoral immunity may be congenital (e.g. X-linked agammaglobulinaemia) or acquired (e.g. myeloma and chronic lymphocytic leukaemia (CLL)) and characteristically leads to recurrent pyogenic bacterial infections. Depressed cell-mediated immunity may be congenital (e.g. Di George's syndrome) or acquired (e.g. HIV infection, lymphoma, CLL) and causes susceptibility to viral, protozoal and fungal infections, anergy and a secondary defect in humoral immunity. Mixed B and T cell deficiency is common.

9 Red cell disorders

(a) Automatic cell counter.



(b) Reticulocyte (brilliant cresyl blue stain) with blue strands of RNA.



(c) Red cell morphology.

Red cell abnormalities	Causes	Red cell abnormalities	Causes
Normal		Spherocyte	Hereditary spherocytosis autoimmune haemolytic anaemia, septicaemia
Macrocyte	Liver disease, alcoholism. Oval in megaloblastic anaemia	Fragments	DIC, microangiopathy, HUS, TTP, burns, cardiac valves
Target cell	Iron deficiency, liver disease, haemoglobinopathies, post-splenectomy	Elliptocyte	Hereditary elliptocytosis
Stomatocyte	Liver disease, alcoholism	Tear drop poikilocyte	Myelofibrosis, extramedullary haemopoiesis
Pencil cell	Iron deficiency	Basket cell	Oxidant damage–e.g. G6PD deficiency, unstable haemoglobin
Ecchinocyte	Liver disease, post-splenectomy	Howell–Jolly body	Hyposplenism, post-splenectomy
Acanthocyte	Liver disease, abetalipoprotein-aemia, renal failure	Basophilic stippling	Haemoglobinopathy, lead poisoning, myelodysplasia, haemolytic anaemia
Sickle cell	Sickle cell anaemia	Malarial parasite	Malaria. Other intra-erythrocytic parasites include *Bartonella bacilliformis*, babesiosis
Microcyte	Iron deficiency, haemoglobinopathy	Siderotic granules (Pappenheimer bodies)	Disordered iron metabolism e.g. sideroblastic anaemia, post-splenectomy

(d) High performance liquid chromatography. (i) HPLC printout showing a patient with β-thalassaemia trait — major band of HbA with increased concentrations of HbA2 and HbF. (ii) A patient with sickle cell trait and two bands corresponding to HbA and HbF.

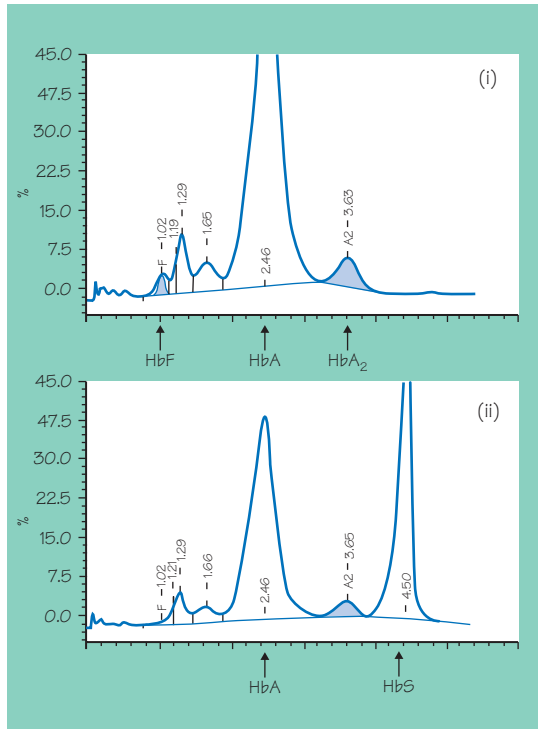


Table 9.1 Classification of anaemia.

Macrocytic (MCV > 98 fL)
Megaloblastic
Vitamin B_{12} or folate deficiency
Other
See Table 13.1
Normocytic (MCV 78–98 fL)
Most haemolytic anaemias
Secondary anaemias
Mixed cases
Microcytic (MCV <78 fL; MCH usually also <27 pg/L)
Iron deficiency
Thalassaemia (α or β)
Other haemoglobin defects
Anaemia of chronic disorders (some cases)
Congenital sideroblastic anaemia (some cases)

Assessment of anaemia

Anaemia is defined as a reduction in haemoglobin concentration. The normal range (Appendix I) differs according to age and sex. The cause of anaemia must be established before treatment is commenced. This is done by clinical assessment (history, physical examination) and appropriate use of special investigations. This chapter gives an overview of the laboratory tests which are useful in determining the cause of anaemia. A classification of anaemia is given in Table 9.1 and further details on the specific types of anaemia are given in the appropriate chapters.

The full blood count (FBC) must be performed using an automatic cell counter (Fig. 9a) as an initial investigation. The red cell indices (mean corpuscular volume = MCV; mean corpuscular haemoglobin = MCH; mean corpuscular haemoglobin concentration = MCHC; red cell distribution width = RDW) and red cell count ($RBC \times 10^{12}/L^{-1}$) will give indicators of the type of anaemia (e.g. iron deficiency or macrocytic).

Reticulocytes (Fig. 9b) are immature, non-nucleated red cells which retain RNA. They may be quantified by a manual differential count of a specially stained slide and expressed as a percentage of red cells (normal = 1–3%); or counted automatically by the cell counter and expressed as an absolute number (normal range = $50–150 \times 10^9$/L). They increase in number, providing marrow function is intact, following increased red cell loss (e.g. haemorrhage) or destruction (e.g. haemolysis) or following treatment of haematinic deficiency.

A **blood film** is made by spreading a drop of blood on a glass slide, staining with a Romanowsky stain, and examining the film microscopically initially at low power (×10) and then at higher power (×40).

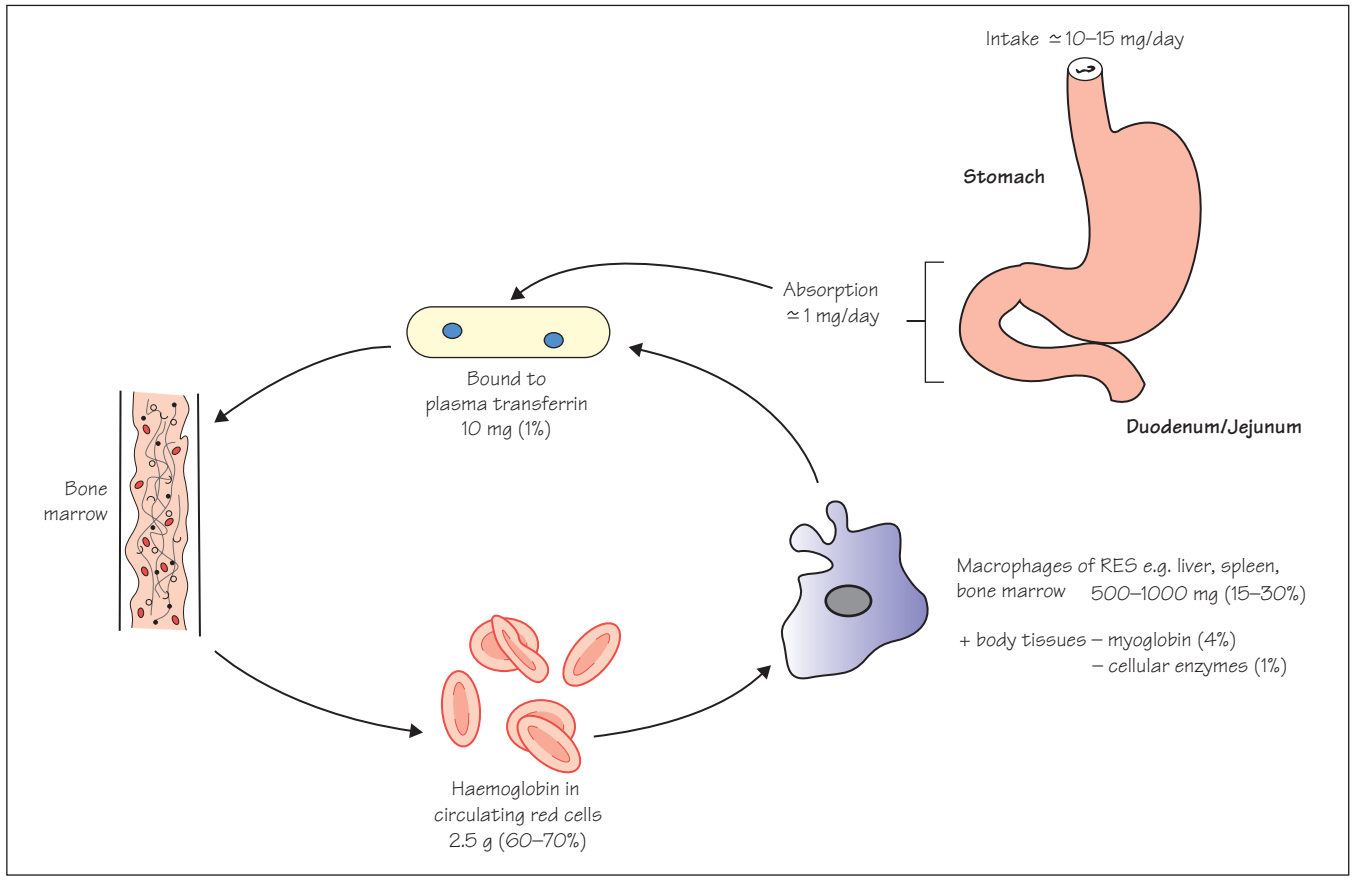
A stained blood film is an excellent way of examining red cell morphology as a clue to underlying pathology (Fig. 9c). The blood film also allows estimation of the white cell differential count, though this is now usually performed automatically by the cell counter. The blood film also allows examination of morphology of white cells, platelets and any circulating non-haemopoietic cells (e.g. parasites).

Haemoglobin disorders (see Chapters 17–18) are among the most commonly inherited conditions in mankind. Haemoglobin electrophoresis is a simple technique whereby red cells are lysed to release haemoglobin, and the lysate is applied to a gel across which an electric current is applied (see Fig. 18d). High performance liquid chromatography (HPLC) is an automated technique that is increasingly used in place of haemoglobin electrophoresis (Fig. 9d). These techniques allow detection of an abnormal haemoglobin; and the relative proportions of the different normal haemoglobins (Hb A, A_2, F)

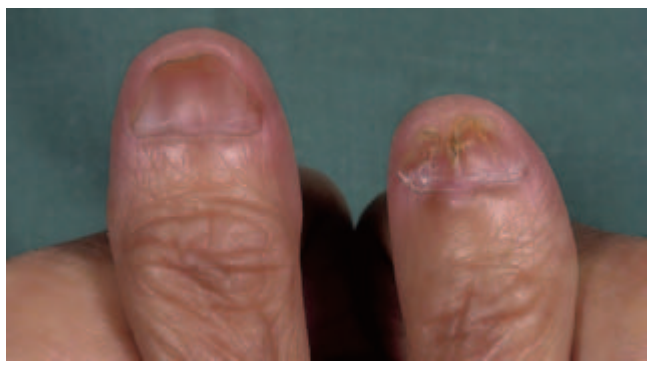
Haematinic levels (i.e. serum vitamin B_{12}, folate, ferritin, iron and iron binding capacity) are performed by analysers using immunoassay. The results may indicate the underlying cause of anaemia. Normal ranges are given in Appendix I.

10 Iron I: physiology and deficiency

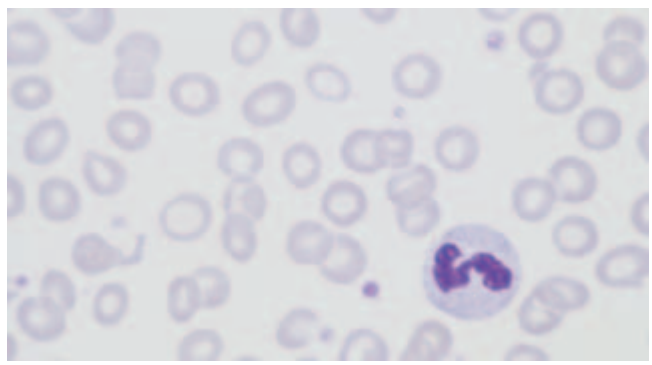
(a) Iron metabolism.



(b) Nail changes in chronic iron deficiency include brittle nails, ridged nails and spoon-shaped nails (koilonychia).



(c) Iron deficiency. Peripheral blood film showing hypochromic microcytic cells, with variation in cell size (anisocytosis) and abnormally shaped cells (poikilocytosis).



Distribution of body iron

Iron is contained in haemoglobin, the reticuloendothelial system (RES) (as ferritin and haemosiderin), muscle (myoglobin), plasma (bound to transferrin) and cellular enzymes (e.g. cytochromes, catalase) (Fig. 10a). RE cells (macrophages) gain iron from the haemoglobin of effete red cells and release it to plasma transferrin which transports iron to bone marrow and other tissues with transferrin receptors. Transferrin is capable of binding two iron atoms per mol-

ecule and is reutilized after giving up iron to cells. The iron-responsive-element-binding protein (IRE-BP) is an RNA-binding protein which binds to specific messenger RNA sequences and is a mechanism whereby the body's iron content regulates uptake and storage of iron by cells of the RES. When iron is in excess, transferrin receptor synthesis, and therefore iron uptake, is reduced and ferritin synthesis is increased. Iron deficiency has the opposite effects.

Iron intake, absorption and loss

The average Western diet contains 10–15 mg of iron daily, of which 5–10% (about 1 mg) is normally absorbed through the upper small intestine. Absorption is adjusted to body needs (increased in iron deficiency and pregnancy, reduced in iron overload). Absorption is regulated by DMT-1 at the villous tip, and HFE and ferroportin 1 at the basolateral surfaces of the enterocyte. Hepcidin also has an important regulatory role. Hepcidin is a large polypeptide released by the liver in response to iron content or inflammation. It reduces iron absorption and iron release from macrophages to plasma.

Iron in animal products is more easily absorbed than vegetable iron; inorganic iron in ferrous form is absorbed more than ferric form. Vitamin C enhances absorption; phytates inhibit it. Dietary intake makes up for daily loss (about 1 mg) in hair, skin, urine, faeces and menstrual blood loss in women. Infants, children and pregnant women need extra iron to expand their red cell mass and, in pregnancy, for transfer to the fetus.

Iron deficiency

Causes (Table 10.1)

- Blood loss (500 mL of normal blood contains 200–250 mg iron)—dominant cause in Western countries.
- Malabsorption—rarely a main cause.
- Poor dietary intake—a contributory cause, especially in children, menstruating females or pregnancy, especially in developing countries.

Clinical features

- General features of anaemia (see Chapter 6).
- Special features (minority of patients): koilonychia (Fig. 10b) or ridged brittle nails, glossitis, angular cheilosis (sore corners of mouth), pica (abnormal appetite), hair thinning and pharyngeal web formation (Paterson–Kelly syndrome).
- Features resulting from an underlying cause.

Laboratory findings

- Hypochromic microcytic anaemia.
- Raised platelet count.
- Blood film appearances (Fig. 10c) include hypochromic/microcytic cells, aniso/poikilocytosis, target cells and 'pencil' cells.
- Bone marrow—not needed for diagnosis: erythroblasts show ragged irregular cytoplasm; absence of iron from stores and erythroblasts.

Table 10.1 Causes of iron deficiency.

Chronic blood loss	
Uterine, e.g. menorrhagia or post-menopausal bleeding	
Gastrointestinal, e.g. oesophageal varices, hiatus hernia, atrophic gastritis, peptic ulcer, ingestion of aspirin (or other non-steroidal anti-inflammatory drugs), gastrectomy, carcinoma (stomach, caecum, colon or rectum), hookworm, angiodysplasia, colitis, diverticulosis, piles	
Rarely, haematuria, haemoglobinuria, pulmonary haemosiderosis, self-inflicted blood loss	
Increased demands	
Prematurity Growth Pregnancy	Deficiency occurs if these are associated with poor diet
Malabsorption	
Postgastrectomy, gluten-induced enteropathy	
Poor diet	
Rarely the sole cause in developed countries	

- Serum ferritin reduced, serum iron low with raised transferrin and unsaturated iron binding capacity.
- Serum soluble transferrin receptors increased.

Other investigations

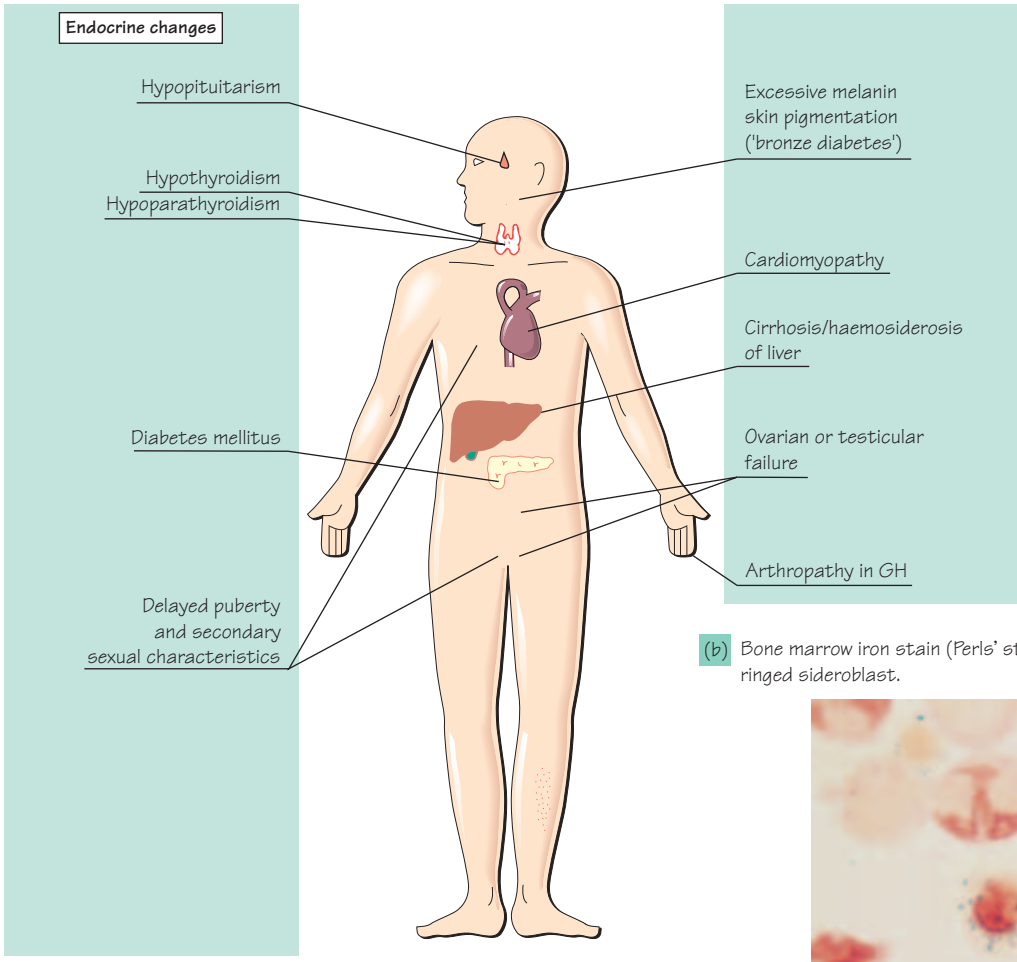
- History (especially for blood loss, diet, malabsorption). Tests for cause (especially in males and postmenopausal females) include occult blood tests, upper and lower gastrointestinal endoscopy or radiology, tests for hookworm, malabsorption and urine haemosiderin.
- Haemoglobin electrophoresis and/or globin gene DNA analysis to exclude thalassaemia trait or other haemoglobin defects.

Treatment

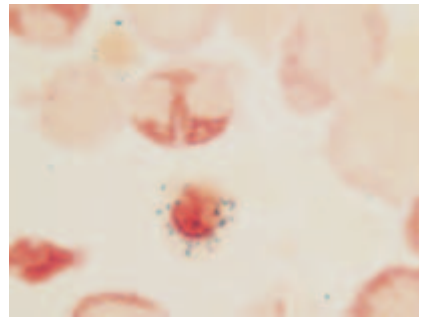
- Oral iron—ferrous sulphate is best (200 mg, 67 mg iron per tablet) before meals three times daily.
- A reticulocyte response begins in 7 days but treatment should be continued for 4–6 months to replenish stores.
- Side effects (e.g. abdominal pain, diarrhoea or constipation) require a lower dose, taking iron with food, or a different preparation (e.g. ferrous gluconate 300 mg, 37 mg iron per tablet).
- Poor response may be due to continued bleeding, incorrect diagnosis, malabsorption or poor compliance.
- Prophylactic oral iron, often combined with folic acid, is given in pregnancy.
- Intramuscular or intravenous iron is used in patients with malabsorption or who are unable to take oral iron. Intravenous iron, e.g. iron sucrose (Venofer) or iron-dextran (Cosmofer), is useful to replenish iron stores and in renal dialysis patients receiving erythropoietin therapy.

11 Iron II: overload and sideroblastic anaemia

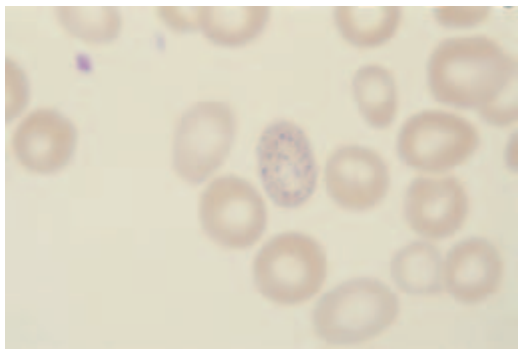
(a) Iron overload: clinical features.



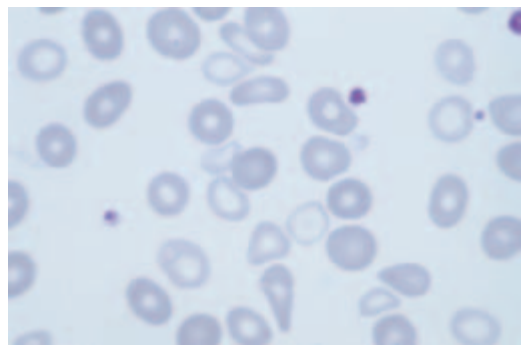
(b) Bone marrow iron stain (Perls' stain, Prussian blue) showing a ringed sideroblast.



(c) Basophilic stippling in red cells of a patient with lead poisoning.



(d) Sideroblastic anaemia: dimorphic blood film. Dual population of well-haemoglobinized cells and hypochromic cells.



Iron overload

Iron overload is the pathological state in which total body stores of iron are increased, often with organ dysfunction as a result of iron deposition.

Causes

- Primary (genetic) haemochromatosis (GH) is an autosomal recessive condition associated with excessive iron absorption. Ninety per cent of cases are homozygous for a mutation in the HFE gene situated close to the HLA complex on chromosome 6. Rarer causes include mutations in genes for hepcidin, hemojuvelin and transferrin receptor 2. It is thought that all these mutations lead to reduced hepcidin levels and therefore increased iron absorption.
- African iron overload; dietary and genetic components.
- Excess dietary iron.
- Ineffective erythropoiesis with increased iron absorption (e.g. thalassaemia intermedia).
- Repeated blood transfusions in patients with severe refractory anaemia, e.g. thalassaemia major, myelodysplasia. Each unit of blood contains 250 mg iron.

Clinical features

- These are mainly caused by organ dysfunction as a result of iron deposition (Fig. 11a).
- Cardiomyopathy gives rise to dysrhythmias and congestive heart failure: major cause of death.
- Growth/sexual development are reduced in children: delayed puberty, diabetes mellitus, hypothyroidism and hypoparathyroidism are frequent.
- The liver may show haemosiderosis and cirrhosis. The liver abnormality in transfusional iron overload is, however, often a result of hepatitis B or C infection.
- Excessive melanin skin pigmentation.
- Excessive infections.
- Arthropathy in GH caused by pyrophosphate deposition.

Laboratory features

- Raised serum iron and transferrin saturation.
- Raised serum ferritin.
- Increased iron in liver (histology or chemical estimation).
- Abnormal liver function tests.
- Low serum hepcidin.
- Increased urinary iron excretion in response to iron chelator therapy.
- Cardiomyopathy causes abnormal echocardiographic findings.
- Endocrine abnormalities, e.g. raised blood glucose.
- MRI can detect increased iron in heart, liver.

Treatment

- Genetic haemochromatosis: regular venesections to reduce iron level to normal, assessed by serum ferritin, serum iron and total iron binding capacity and by liver biopsy or MRI.
- Transfusional iron overload: iron chelation using subcutaneous desferrioxamine (DFX) over 8–12 hours on 5–7 nights each week. Vitamin C enhances iron excretion. An orally active iron chelator (deferiprone) is available for those unable to take DFX effectively. It is superior to DFX at removing cardiac iron. It rarely causes agranulocytosis. The drugs may be combined.

Sideroblastic anaemia

Definition

Sideroblastic anaemia is a refractory anaemia in which the marrow shows increased iron present as granules arranged in a ring around the nucleus in developing erythroblasts ('ringed sideroblasts', Fig. 11b). At least 15% of erythroblasts show this in the primary forms. A defect of haem synthesis is present.

Classification

- The most common form is primary acquired type (a type of myelodysplasia; see Chapter 22).
- An X-linked genetic defect in haem synthesis (usually due to mutation of δ-amino laevulinic acid synthase, ALA-S), a key enzyme in haem synthesis, underlies some congenital forms, usually in males.
- Ringed sideroblasts may also occur with other haematological disorders and with alcohol, isoniazid therapy and lead poisoning.

Clinical and laboratory features

The congenital anaemia is sometimes mild (haemoglobin 8–10 g/dL) but may become more severe with age. Leucopenia and thrombocytopenia may occur in patients with myelodysplasia. The blood film may be dimorphic (Fig. 11d). The MCV is usually raised in acquired and low in the inherited variety.

Treatment

Usually symptomatic. Regular blood transfusion and iron chelation is often required. Patients with inherited forms may respond to pyridoxine (vitamin B_6), a co-factor for ALA-S.

Lead poisoning

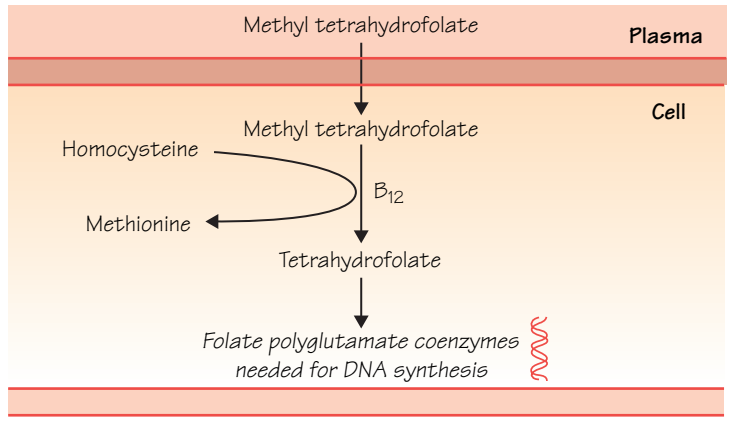
Clinically this presents with abdominal pain, constipation, anaemia, peripheral neuropathy and a blue (lead) line of the gums. The blood film shows punctate basophilia (blue staining dots as a result of undegraded RNA) (Fig. 11c) and features of haemolysis. The marrow may show ringed sideroblasts.

Acute iron poisoning

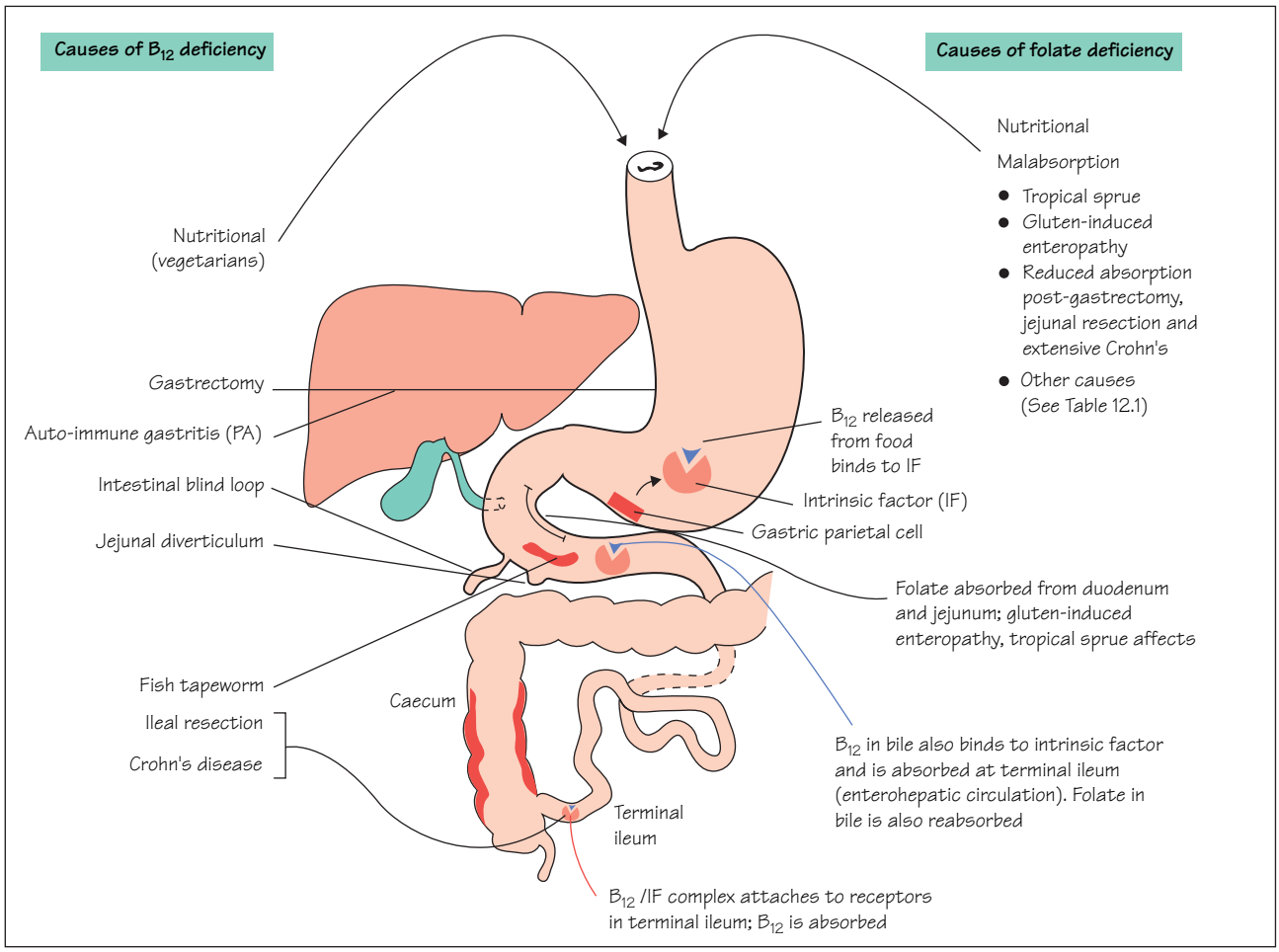
This is commonest in childhood and is usually accidental. Clinical features include nausea, abdominal pain, diarrhoea, gastrointestinal bleeding and abnormalities of liver function. Immediate treatment is with gastric lavage (within 1–2 hours); whole bowel irrigation may be indicated. Intravenous desferrioxamine is valuable in chelating iron and reducing the risk of liver damage.

12 Megaloblastic anaemia I: vitamin B_{12} and folate deficiency—biochemical basis, causes

(a) The role of vitamin B_{12} in conversion of 5-methyltetrahydrofolate to tetrahydrofolate required as substrate for folate polyglutamate synthesis. The reaction methionine synthase involves conversion of homocysteine to methionine which is converted to S-adenosylmethionine involved in numerous methylation reactions. 5,10-Methylene tetrahydrofolate, a folate polyglutamate coenzyme, plays a key part in DNA synthesis by acting as coenzyme for synthesis of thymidine monophosphate from deoxyuridine monophosphate.



(b) The gastrointestinal tract in B_{12} or folate deficiency.



Megaloblastic anaemia (MA) is associated with an abnormal appearance of the bone marrow erythroblasts in which nuclear development is delayed and nuclear chromatin has a lacy open appearance. There is a defect in DNA synthesis usually caused by deficiency of vitamin B_{12} (B_{12}, cobalamin) or folate.

Biochemical basis

Folate is an essential coenzyme for the synthesis of thymidine monosphosphate (TMP) and therefore of DNA. B_{12} is a coenzyme for methionine synthase, a reaction needed in the demethylation of the form of folate, 5-methyltetrahydrofolate (methyl THF), which enters the cells from plasma. The demethylation provides THF which acts as substrate for synthesis of intracellular folate polyglutamates, the coenzyme forms of folate needed in cells for DNA synthesis.

B_{12} physiology (Fig. 12a)

- The adult daily requirement for B_{12} is 1 μg (normal mixed diet contains 10–15 μg). B_{12} is present only in foods of animal origin: meat, fish, eggs, milk and butter; it is absent from vegetables, cereals and fruit, unless these are contaminated by microorganisms. Normal body stores of B_{12}, largely in the liver with an enterohepatic circulation, are sufficient to last for 2–4 years.
- Dietary B_{12} after release from food and gastric 'R' binder (see below) combines with intrinsic factor (IF) secreted by gastric parietal cells (GPC). The IF–B_{12} complex attaches to ileal receptors and B_{12} is absorbed.
- Passive absorption (about 0.1% of oral B_{12}) occurs through buccal, gastric and duodenal mucosae.
- Absorbed B_{12} attaches to transcobalamin (TC) II which carries B_{12} in plasma to the liver, bone marrow, brain and other tissues. Most B_{12} in plasma is attached to a second B_{12} binding protein, TC I (haptocorrin), and is functionally inactive. TC I is synthesized by granulocytes and their precursors. Similar glycoproteins ('R' proteins) occur in saliva, gastric juice and milk.

Causes of B_{12} deficiency (Fig. 12b)

Inadequate diet

Vegans may develop B_{12} deficiency, although the intact enterohepatic circulation of a few micrograms of B_{12} daily delays its onset. Infants born to B_{12}-deficient mothers and breastfed by them may present with failure to thrive and MA resulting from B_{12} deficiency.

Malabsorption

Gastric causes

- Pernicious anaemia (PA) is characterized by an autoimmune gastritis, and reduced gastric secretion of IF and acid. It is often associated with other organ-specific autoimmune diseases (e.g. myxoedema, thyrotoxicosis, vitiligo, Addison's disease and hypoparathyroidism). Antibodies to IF and gastric parietal cells occur in serum (50 and 90%, respectively) of patients. Pernicious anaemia is also associated with early greying of hair, blue eyes, blood group A, a family history of PA or related autoimmune disease and a two- to three-fold increased incidence of carcinoma of the stomach. It occurs in all races and has a female/male incidence of 1.6 : 1. The peak age of incidence is 60 years.
- Gastrectomy (total or subtotal) leads to B_{12} deficiency.
- Congenital IF deficiency or abnormality is rare.

Table 12.1 Causes of folate deficiency.

Nutritional
Especially old age, institutions, poverty, famine
Malabsorption
Gluten-induced enteropathy, dermatitis herpetiformis, tropical sprue
Excess utilization
Physiological
Pregnancy and lactation, prematurity
Pathological
Haematological diseases: haemolytic anaemias, myelofibrosis
Malignant diseases: carcinoma, lymphoma, myeloma
Inflammatory diseases: Crohn's disease, rheumatoid arthritis, extensive psoriasis, exfoliative dermatitis, malaria
Excess urinary folate loss
Congestive heart failure, chronic dialysis
Drugs
Anticonvulsants, sulfasalazine
Mixed
Liver disease, alcoholism (spirit drinkers)

Intestinal causes

These include bacterial (rarely fish tapeworm) colonization of small intestine, stagnant loop syndromes, congenital and acquired defects of the ileum (e.g. ileal resection, Crohn's disease). Congenital B_{12} malabsorption with proteinuria is rare. Less severe malabsorption of food B_{12} may occur with (simple) atrophic gastritis, *Helicobacter* infection, gluten-induced enteropathy, with drugs, e.g. metformin.

Folate physiology

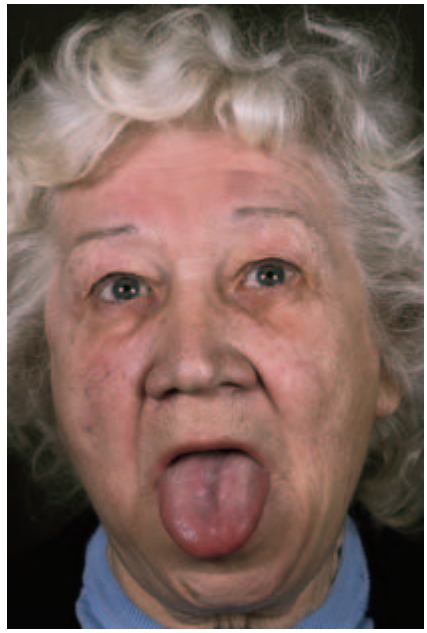
- Folates consist of a large number of compounds derived from the parent compound pteroylglutamic (folic) acid by reduction, addition of single carbon groups, e.g. methyl or formyl, and, in cells, addition of extra glutamate moieties usually four, five or six.
- Folates occur in most foods, especially liver and green vegetables. Normal daily diet contains 200–250 μg, of which about 50% is absorbed.
- Daily adult requirements are about 100 μg; body stores are sufficient for 4 months.
- Absorbed through the upper small intestine with conversion of all natural forms to 5-methyl tetrahydrofolate (methyl THF).

Causes of folate deficiency (Table 12.1, Fig. 12b)

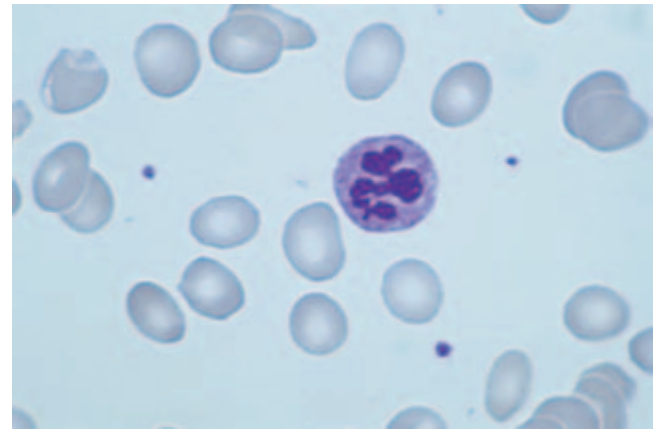
- The most common cause is poor dietary intake, either alone or in association with increased folate utilization (e.g. pregnancy or haemolytic anaemia).
- Malabsorption occurs in gluten-induced enteropathy or tropical sprue.
- Increased utilization: increased cell turnover and DNA synthesis causes breakdown of folates; the most common causes include pregnancy, haemolytic anaemia, severe chronic inflammatory and malignant diseases, and anticonvulsant drugs.
- Folate is loosely bound to protein in plasma and is easily removed by dialysis.

13 Megaloblastic anaemia II: clinical features, treatment and other macrocytic anaemias

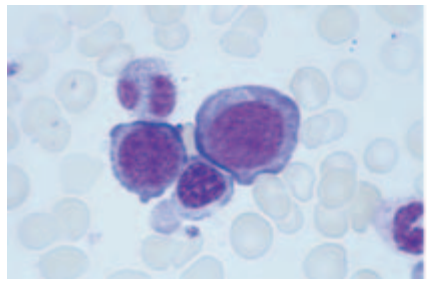
(a) Pernicious anaemia in a female aged 65 years. She is wearing a wig to disguise premature greying of the hair. There is a tinge of jaundice in the conjunctivae and skin, blue eyes and an enlarged fleshy sore tongue.



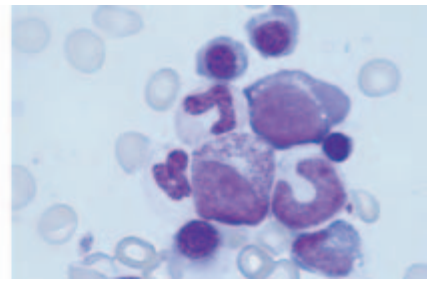
(b) Peripheral blood in megaloblastic anaemia, showing a hypersegmented neutrophil, oval macrocytes and poikilocytosis.



(c) (i) Bone marrow in megaloblastic anaemia showing megaloblasts. These are nucleated erythroid cells with open lacy chromatin and delayed nuclear maturation. (ii) Megaloblasts with developing myeloid cells; the cell with a C-shaped nucleus is a giant metamyelocyte.



(i)



(ii)

B_{12} deficiency

Clinical features

- Gradual onset of features of anaemia.
- Mild jaundice, caused by ineffective erythropoiesis.
- Glossitis (Fig. 13a) and angular cheilosis and, if severe, sterility (either sex) and reversible melanin skin pigmentation.
- B_{12} deficiency causes a symmetrical neuropathy affecting the pyramidal tracts and posterior columns of the spinal cord (subacute combined degeneration of the cord) and the peripheral nerves. Patients present with tingling in the feet (more than the hands), difficulty in gait, visual or psychiatric disorders.
- B_{12} or folate deficiency are associated with increased plasma homocysteine and, in pregnancy, with an increased incidence of fetal neural tube defects.
- Patients may be asymptomatic and detected by a routine blood test.

Laboratory findings

- Macrocytic anaemia with oval macrocytes and hypersegmented neutrophils (>5 nuclear lobes) (Fig. 13b).
- Moderate reduction in leucocyte and platelet counts (severe cases).
- Biochemical tests show raised serum bilirubin (indirect), lactate dehydrogenase.

• In B_{12} deficiency, the serum B_{12} is low, serum folate is normal or raised and red cell folate is normal or low.
• Bone marrow is hypercellular, increased proportion of early cells, megaloblastic erythropoiesis and giant metamyelocytes (Fig. 13c).
• Raised serum methylmalonic acid (B_{12} deficiency), raised serum homocysteine (either B_{12} or folate deficiency).

Tests for causes of B_{12} deficiency

These include history (diet, previous surgery), tests for intrinsic factor (IF) and parietal cell antibodies, upper gastrointestinal endoscopy and radioactive B_{12} absorption studies.

Radioactive B_{12} absorption studies distinguish gastric from intestinal causes of B_{12} malabsorption. The amount of radioactive B_{12} absorbed is measured in a 24-hour urine sample after an oral radioactive dose given with a 'flushing' dose of 1 mg unlabelled B_{12}. The test can be repeated (Part II) with the labelled B_{12} given with IF. A combined urinary excretion test using two isotopic forms of B_{12}, one bound to IF, can be used (Dicopac test). B_{12} absorption is low and corrected by IF in PA but is low and not corrected by IF in intestinal diseases.

Treatment

Treatment for B_{12} deficiency is 1 mg hydroxocobalamin intramuscularly, repeated every 2–3 days until six injections have been given; then one injection every 3 months for life unless the cause of deficiency has been corrected.

Folate deficiency

The clinical features of folate deficiency are the same as B_{12} deficiency, but folate deficiency does not cause a similar neuropathy. Folic acid therapy in early pregnancy reduces the incidence of neural tube defects (NTD) (anencephaly, spina bifida, encephalocoele) in the fetus.

Tests for causes of folate deficiency

These include history (diet, previous surgery, drug therapy, alcohol, other associated diseases), anti-endomysial and anti-transglutaminase antibodies, and tests for malabsorption (e.g. duodenal biopsy). The serum folate is low, red cell folate low and serum B_{12} is normal or slightly reduced.

Treatment

Treatment is 5 mg folic acid daily for 4 months, then decide whether to continue folic acid, e.g. 5 mg folic acid once weekly or 400 µg daily indefinitely. Folate therapy corrects the anaemia but not the neuropathy of B_{12} deficiency. Indeed, administration of large (>1 mg) doses of folic acid to a severely B_{12}-deficient individual may precipitate or worsen B_{12} neuropathy. Pregnant women are given about 400 µg daily to reduce the incidence of megaloblastic anaemia and of NTDs in the fetus. Dietary fortification with folate is used in many countries (not UK) to reduce the incidence of NTDs.

Table 13.1 Causes of raised MCV other than megaloblastic anaemia.

Alcohol
Liver disease
Myxoedema
Reticulocytosis
Cytoxic drugs
Aplastic anaemia
Pregnancy
Myelodysplastic syndromes
Myeloma
Neonatal

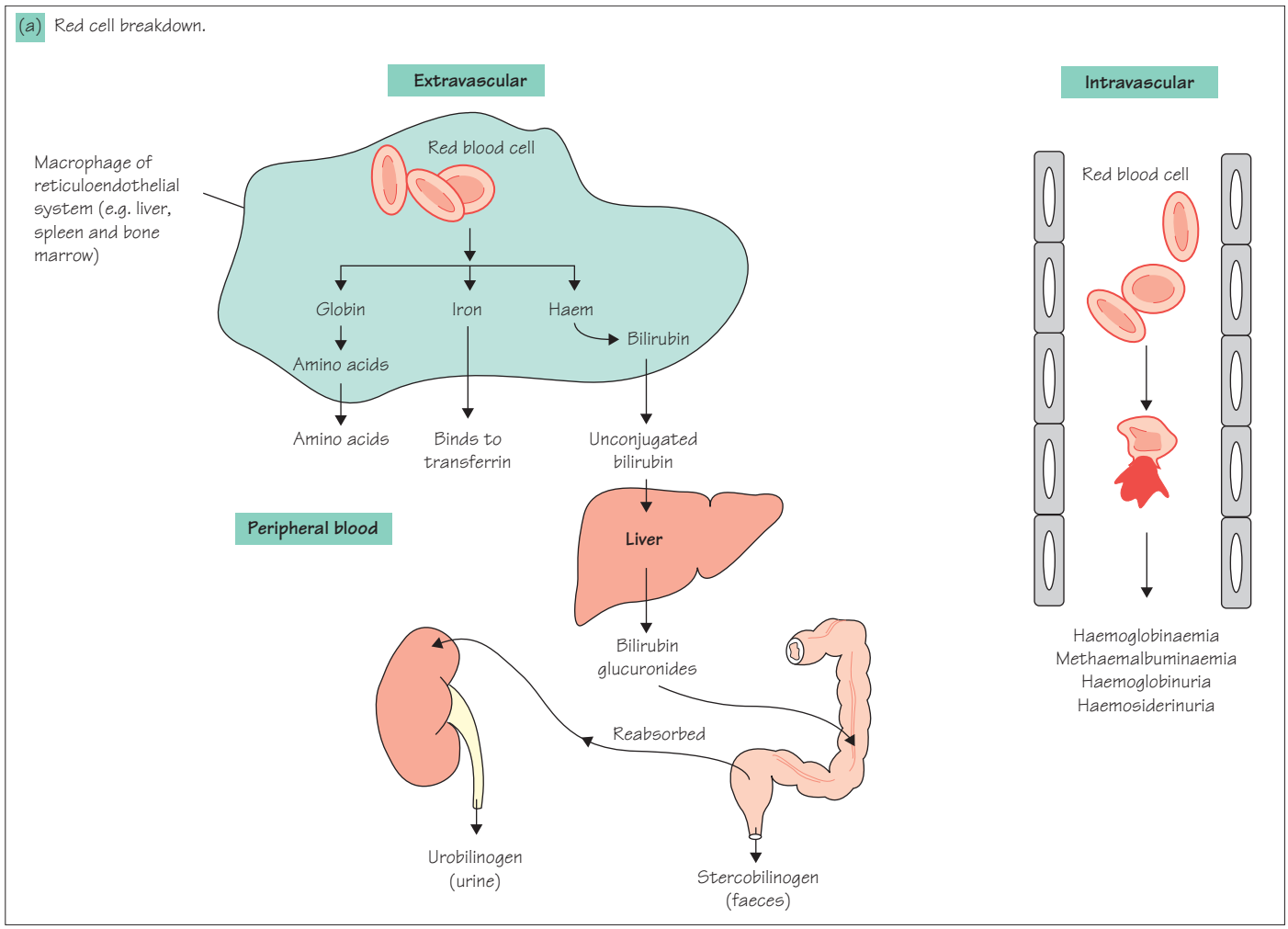
Other causes of megaloblastic anaemia

Defects of B_{12} or folate metabolism include congenital transcobalamin (TC) II deficiency which leads to B_{12} malabsorption and to failure of B_{12} to enter cells resulting in megaloblastic anaemia (MA) in early infancy. N_2O anaesthesia reversibly inactivates body B_{12} and prolonged or repeated exposure may cause megaloblastic anaemia or B_{12} neuropathy. Antifolate drugs include the inhibitors of dihydrofolate reductase (methotrexate, pyrimethamine and trimethoprim) which have progressively less activity against the human compared to the bacterial enzyme. Folinic acid (5-formyl-THF) is used to overcome methotrexate or co-trimoxazole toxicity. Megaloblastic anaemia also occurs with cytotoxic drug therapy (e.g. 6-mercaptopurine, cytosine arabinoside or hydroxyurea (hydroxycarbamide)) or, rarely, inborn errors, e.g. orotic aciduria.

Causes of macrocytosis

Alcohol is the most frequent cause (Table 13.1). MCV is not usually as high as in severe MA in these conditions. White cell and platelet counts are normal unless the underlying marrow disease affects these, the red cells are circular rather than oval, hypersegmented neutrophils are absent and the marrow is normoblastic.

14 Haemolytic anaemias I: general



Haemolytic anaemias are caused by a shortened red cell lifespan; the normal mean red cell life (MRCL) is 120 days. Red cell production can be increased six to eight times by normal bone marrow and haemolytic anaemia (HA) occurs if MRCL falls to 15 days or less, particularly in the presence of ineffective erythropoiesis, haematinic deficiency or marrow disease. Haemolysis may be caused by a fault in the red cell, usually inherited, or an abnormality in its environment, usually acquired (Table 14.1).

Physiology of red cell destruction

(Fig. 14a)

Red cell destruction is normally extravascular in the macrophages of the reticuloendothelial system (RES), in bone marrow, liver and spleen. Globin is degraded to amino acids, haem to protoporphyrin, carbon monoxide and iron. Protoporphyrin is metabolized to bilirubin, conjugated to a glucuronide in the liver, excreted in faeces (as stercobilinogen) and, after reabsorption, in urine as urobilinogen. Iron is recycled to plasma and combined to transferrin. Some iron remains in the macrophages as ferritin and haemosiderin. Haptoglobins are plasma proteins which bind haemoglobin to form a complex which is removed by the liver; their level is reduced in haemolysis as well as in liver disease. Pathological red cell destruction may occur intravascularly (Table 14.2). Some haemoglobin is then excreted in the urine unchanged; it is also partly reabsorbed by the renal tubules and broken down to haemosiderin.

Clinical features

- Anaemia (unless fully compensated haemolysis).
- Jaundice (usually mild) caused by unconjugated bilirubin in plasma; bilirubin is absent from the urine.
- An increased incidence of pigment gallstones.
- Splenomegaly—in many types.
- Ankle ulcers, especially sickle cell anaemia, thalassaemia intermedia and hereditary spherocytosis.
- Expansion of marrow with, in children, bone expansion, e.g. frontal bossing in β-thalassaemia major.
- Aplastic crises caused by parvovirus infection.
- Megaloblastic anaemia caused by folate deficiency.

Table 14.1 Classification of haemolytic anaemia.

Hereditary	Acquired
Membrane	**Immune**
	Autoimmune
Hereditary spherocytosis, hereditary elliptocytosis	Warm antibody type
	Idiopathic or secondary to SLE, CLL, drugs, e.g. methyldopa, fludarabine
South-East Asian ovalocytosis	Cold antibody type
	Idiopathic or secondary to infections (e.g. mycoplasma, infectious mononucleosis), lymphoma, paroxysmal cold haemoglobinuria
Metabolism	
G6PD deficiency	*Alloimmune*
Pyruvate kinase deficiency	Haemolytic transfusion reactions
Other rare enzyme deficiencies e.g. triose phosphate isomerase deficiency	Haemolytic disease of newborn
Haemoglobin	**Red cell fragmentation syndromes**
Haemoglobin defect (HbS, HbC, unstable) see Chapter 18	Cardiac valve, 'March' haemoglobinuria
	Thrombotic thrombocytopenia purpura
	Haemolytic uraemic syndrome
	Disseminated intravascular coagulation
	Infections
	e.g. malaria, clostridia
	Chemical and physical agents
	e.g. drugs, industrial/domestic substances, burns
	Secondary
	e.g. liver and renal disease
	Paroxysmal nocturnal haemoglobinuria (PNH)

CLL, chronic lymphocytic leukaemia; SLE, systemic lupus erythematosus.

Table 14.2 Causes of intravascular haemolysis.

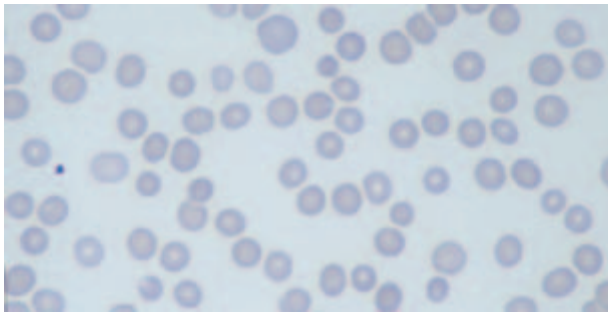
Mismatched blood transfusion (usually ABO)
G6PD deficiency with oxidant stress
Red cell fragmentation syndromes
Some autoimmune haemolytic anaemias
Some drug- and infection-induced haemolytic anaemias
Paroxysmal nocturnal haemoglobinuria
March haemoglobinuria
Unstable haemoglobin

Laboratory features

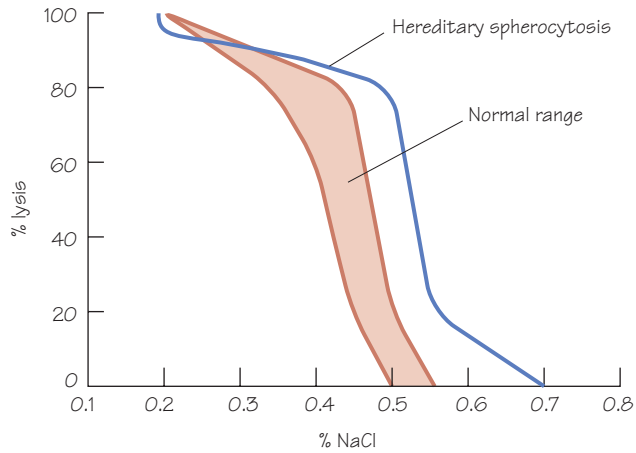
- Haemoglobin level may be normal or reduced.
- Raised reticulocyte count.
- Blood film may show polychromasia (blue staining in young red cells), altered red cell shape, e.g. spherocytes, elliptocytes, sickle cells or fragmented cells.
- Bone marrow shows increased erythropoiesis.
- Serum indirect (unconjugated) bilirubin is raised.
- Serum haptoglobins absent.
- Radioactive chromium (^{51}Cr) labelling of red cells measures lifespan and assesses the sites of red cell destruction by surface counting. Useful in predicting value of splenectomy.
- Intravascular haemolysis leads to raised plasma and urine haemoglobin, positive serum (Schumm's) test for methaemalbumin, urine haemosiderin.

15 Haemolytic anaemias II: inherited membrane and enzyme defects

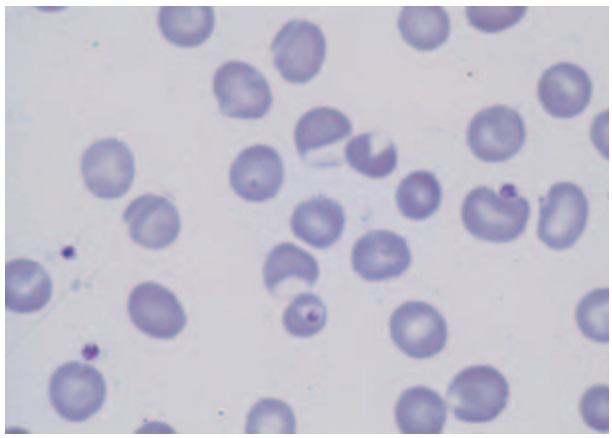
(a) Hereditary spherocytosis: peripheral blood film.



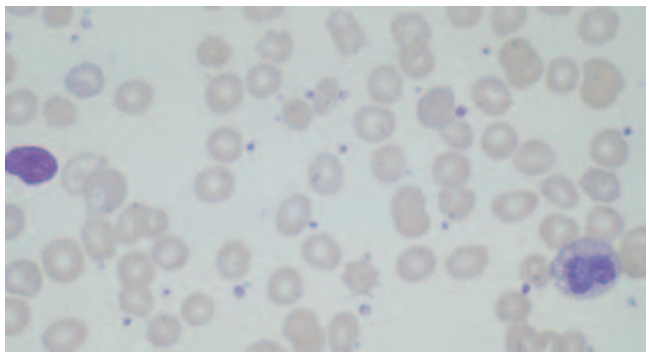
(b) Osmotic fragility test. Red cells are suspended in saline of increasing concentration and the degree of haemolysis is assessed by spectrophotometry. The red cells of patients with hereditary spherocytosis have an increased volume/surface area ratio and are more susceptible to lysis than normal red blood cells.



(c) Glucose-6-phosphate dehydrogenase deficiency: peripheral blood film showing red cells which have 'blistered' cytoplasm, and where haemoglobin in cytoplasm is contracted and pulled away from the membrane to give a 'basket' cell.



(d) Pyruvate kinase deficiency: peripheral blood film, postsplenectomy, showing irregularly contracted and crenated 'spicule' cells or 'prickle' cells—an extreme type of ecchinocyte.



Membrane abnormalities

Hereditary spherocytosis (HS)

This is the most common inherited haemolytic anaemia (HA) in white people. It is autosomal dominant with variable severity and may present as severe neonatal HA, as symptomatic HA later in life or it may be an incidental finding. The defect is in a red cell membrane protein, e.g. ankyrin; 25% of cases are new mutations. Affected red cells lose membrane during passage through the RES, especially the spleen. The cells become progressively more spherical (decreased surface area/volume ratio) and microcytic. They are destroyed prematurely, mainly in the spleen. Hereditary elliptocytosis (HE) is a similar, usually milder, abnormality.

Clinical features

Clinical features are those generally associated with HA. The spleen is usually enlarged.

Laboratory features

- Blood film: microspherocytes and polychromasia (Fig. 15a).
- Haemoglobin level variable.
- Tests for HA are positive (see Chapter 14).
- Special tests: osmotic fragility increased (Fig. 15b), autohaemolysis increased and corrected by addition of glucose.
- Direct antiglobulin test is negative (excluding warm autoimmune HA which can cause a similar blood picture).

Treatment

- Splenectomy corrects the decrease in lifespan although spherocytosis persists; may not be needed in mild cases; defer if possible in children until over the age of 6 years.
- Give folic acid prophylactically for severe cases.
- Pigment gallstones may cause cholecystitis.

Table 15.1 Agents which may cause haemolytic anaemia in G6PD deficiency.

Infections and other acute illnesses, e.g. diabetic ketoacidosis
Drugs
Antimalarials, e.g. primaquine
Sulfonamides and sulfones, e.g. co-trimoxazole, sulfanilamide, dapsone, salazopyrin
Other antibacterial agents, e.g. nitrofurans, chloramphenicol
Analgesics, e.g. aspirin (moderate doses are safe)
Antihelminths, e.g. β-naphthol, stibophen, niridazole
Miscellaneous, e.g. vitamin K analogues, naphthalene (mothballs), probenecid
Fava beans (possibly other vegetables)

• If cholecystectomy required, perform splenectomy also to reduce risk of recurrent gallstones.

South-East Asian ovalocytosis

This is an inherited red cell membrane protein defect (band 3), in which carriers have a degree of protection against malaria.

Enzyme abnormalities

Glucose-6-phosphate dehydrogenase (G6PD) deficiency

G6PD is the first enzyme in the hexose monophosphate pathway (see Fig. 2d) which generates reducing power as reduced nicotinamide adenine dinucleotide phosphate (NADPH). Deficiency results in red cells being susceptible to oxidant stress. The gene is on the X chromosome so inheritance is sex-linked. Many mutant enzymes occur. Affected males develop HA when the red cells are exposed to oxidant stress, especially by drugs, infections, ingestion of fava beans and during the neonatal period (Table 15.1). Deficiency is common in black, mediterranean, Middle Eastern and oriental populations. Individuals with G6PD deficiency have a degree of protection against malaria.

Clinical and laboratory features

- Blood count and film normal between crises.
- During crises features of acute intravascular haemolysis.
- Blood film in a crisis (Fig. 15c) shows red cells with absent haemoglobin ('bite' and 'blister' cells) and polychromasia. Heinz bodies (denatured haemoglobin) may be seen in a reticulocyte preparation with supravital staining.
- Haemolysis is usually self-limited because of the increased G6PD activity in reticulocytes.
- Chronic non-spherocytic HA (CNSHA) occurs rarely with certain mutant enzymes.
- Neonatal jaundice is frequent.
- Screening tests for red cell G6PD deficiency measure the generation of NADPH. The enzyme may also be characterized by electrophoresis, assay of activity and DNA analysis. Diagnosis should, when possible, be undertaken in the steady state as reticulocytes generally have higher enzyme activity and the raised reticulocyte count following haemolysis may lead to a false normal result.

Management

- Stop offending drugs or fava bean ingestion.
- Treat infection if present.
- Transfuse packed red cells if necessary.
- Splenectomy may ameliorate HA in rare CNSHA.

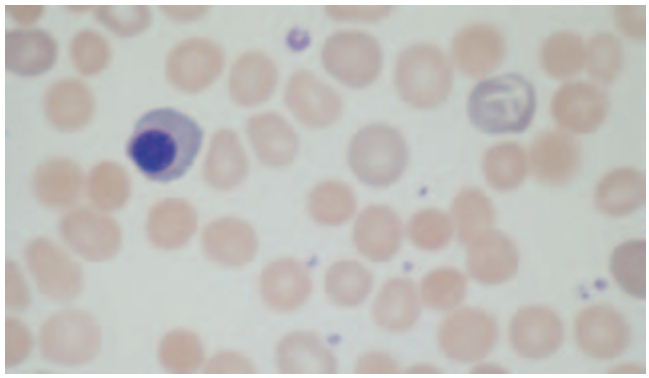
Pyruvate kinase deficiency

Pyruvate kinase (PK) deficiency is the most frequent enzyme deficiency in the Embden–Meyerhof (glycolytic) pathway to cause CNSHA (Fig. 15d). Inheritance is autosomal recessive. The O_2-dissociation curve is shifted to the right so symptoms are mild in comparison to the degree of anaemia. Splenectomy partly improves the anaemia.

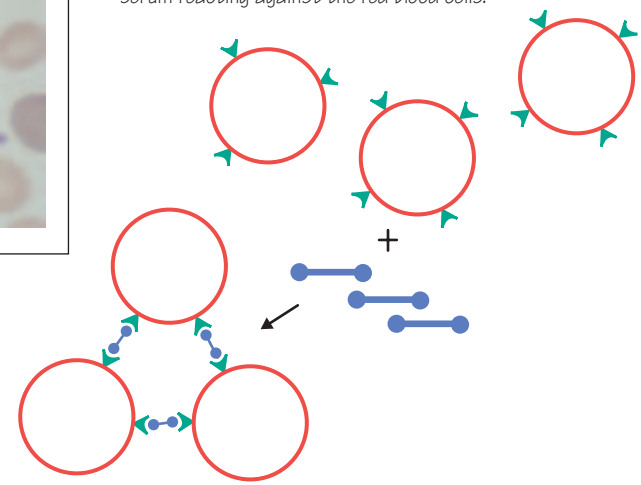
Other enzyme deficiencies are rare causes of CNSHA and are frequently associated with musculoskeletal disease.

16 Haemolytic anaemias III: acquired

(a) Warm autoimmune haemolytic anaemia: peripheral blood film. There is a circulating nucleated red blood cell (NRBC), polychromasia and microspherocytes.



(b) Direct antiglobulin (Coombs') test (DAT) is a means of detecting immunoglobulin and/or complement coating the red blood cells. Red blood cells are washed and anti-human globulin is added. This may be of broad specificity or specific, e.g. for IgG, IgA, IgM or complement. If agglutination occurs, then the red blood cells must have been coated; if no agglutination occurs, the red blood cells were not coated. In the indirect antiglobulin test, red cells are first incubated with serum at 37°C for 30min; a DAT is then performed, which will be positive if there are antibodies in the serum reacting against the red blood cells.



Autoimmune haemolytic anaemia

This is caused by autoantibodies against the red cell membrane. It is divided into warm and cold antibody types and each may be idiopathic or secondary to other diseases (see Table 15.1 and p. 103).

Warm autoimmune haemolytic anaemia

Antibody, typically IgG, has maximum activity at 37°C.

Clinical and laboratory features

- Presents at any age, in either sex, with features of extravascular haemolytic anaemia of varying severity.
- The spleen is often enlarged.
- Blood film shows microspherocytes (Fig. 16a), polychromasia, anisocytosis, ±circulating nucleated red blood cells.
- Direct antiglobulin test (DAT) is positive (Fig. 16b).
- Antibody may be non-specific or directed against antigens in the Rh system.
- IgG or IgG + complement (C3d) is detected on the red cell.
- Free antibody may be present in the serum.
- May be associated with immune thrombocytopenia (Evans' syndrome).
- Antibody-coated red cells are destroyed in the RES especially the spleen.

Treatment

- Corticosteroids, e.g. prednisolone 1 mg/kg orally with subsequent gradual reduction.
- Blood transfusion if necessary.
- Consider splenectomy if steroid therapy fails.
- Other immunosuppressive drugs, e.g. azathioprine, ciclosporin, cyclophosphamide, mycophenolate, rituximab (anti CD20).
- Remove cause, e.g. drug.
- Treat underlying disease, e.g. chronic lymphocytic leukaemia, systemic lupus erythematosus.

Cold autoimmune haemolytic anaemia

Antibody, typically IgM, has maximum activity at 4°C.

Clinical and laboratory features

- Raynaud's phenomenon affecting the fingers, toes, nose and ears.
- Positive DAT with C3d on red cells.
- Cold agglutinins, usually IgM and directed against I or i antigen (especially in infectious mononucleosis) on red cells are present in serum, often to titres of 1:4000 or more. In primary form (cold haemagglutinin disease) the antibody is monoclonal and the patient may ultimately develop non-Hodgkin lymphoma.
- Paroxysmal cold haemoglobinuria is a rare syndrome, precipitated by infections. Intravascular haemolysis is caused by the Donath–Landsteiner antibody which binds red cells in the cold but causes lysis at 37°C.

Treatment

- Keep the patient warm.

• Consider immunosuppression with chlorambucil or cyclophosphamide.
• Consider plasma exchange to lower antibody titre.

Alloimmune haemolytic anaemia

This is caused when antibody produced by one individual reacts against red cells of another. The three important situations are:

1 mismatched blood transfusions (see Chapter 41);
2 haemolytic disease of the newborn (see Chapter 45);
3 following marrow or solid organ transplantation.

Drug-induced immune haemolytic anaemia

See Chapter 46.

Red cell fragmentation syndromes

These occur when red cells are exposed to an abnormal surface (e.g. non-endothelialized artificial heart valve or arterial graft), or flow through small vessels containing fibrin strands (e.g. in disseminated intravascular coagulation) or damaged small vessels. This is termed microangiopathic haemolytic anaemia (MAHA) and occurs in thrombotic thrombocytopenic purpura, haemolytic uraemic syndrome, widespread adenocarcinoma, malignant hypertension, pre-eclampsia and meningococcal septicaemia. Haemolysis is both extra- and intravascular; the blood film shows deeply staining fragmented red cells (see Fig. 33e).

Infections

These may cause haemolysis by:

• direct damage to red cells (e.g. malaria);
• toxin production (e.g. *Clostridium perfringens*);
• oxidant stress in G6PD-deficient individuals;
• MAHA (e.g. meningococcal septicaemia);
• autoantibody formation (e.g. infectious mononucleosis);
• extravascular destruction (e.g. malaria).

Chemical and physical agents

Some drugs, e.g. dapsone, or chemicals, e.g. chlorate, cause haemolysis by oxidation even with normal G6PD levels. Severe burns and snake bites may also cause haemolysis.

Paroxysmal nocturnal haemoglobinuria

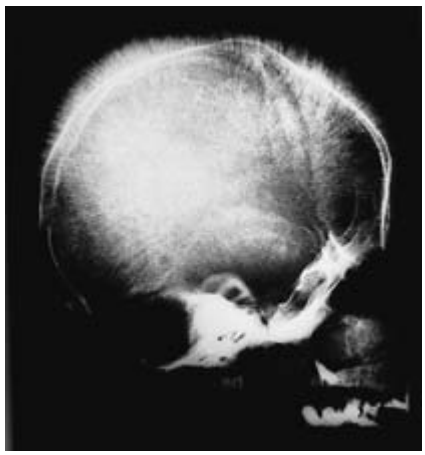
This is a clonal disorder in which haemolysis is caused by a rare acquired mutation of the PIG-A gene in haemopoietic stem cells. This results in a defect of the phosphatidyl inositol anchor which tethers a large number of proteins to the cell membrane. The cells become abnormally sensitive to complement-mediated haemolysis (lack of proteins that protect against complement, e.g. CD55 (DAF), CD59 (MIRL)). It is often associated with a hypoplastic marrow with neutropenia and thrombocytopenia. The clinical course is frequently complicated by recurrent venous thromboses, especially of large veins, e.g. hepatic or portal, also by iron deficiency and infections. Diagnosis is made by a positive acid lysis (Ham's) test and the presence of red cells lacking the CD55 or CD59 antigens.

Treatment

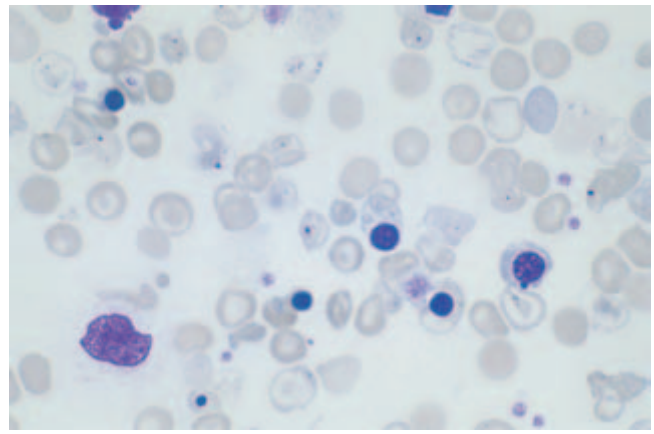
• Iron is given for iron deficiency resulting from chronic intravascular haemolysis.
• Transfusion of leucodepleted red cells may be necessary.
• Warfarin may be needed for the patient's life to prevent thrombosis.
• A monoclonal antibody, Eculizumab, that inhibits the activation of terminal complement components by binding to C5 has been used to reduce haemolysis.
• Allogeneic stem cell transplant for serious cases in young adults.

17 Haemolytic anaemias IV: genetic defects of haemoglobin

(a) β-thalassaemia major: skull X-ray showing expansion of the medullary cavity giving rise to a 'hair on end' appearance.



(b) β-thalassaemia major: peripheral blood film showing hypochromic microcytic red cells, target cells, poikilocytes and nucleated red blood cells. The few well-haemoglobinized cells are transfused red cells.



Genetic disorders of haemoglobin comprise:
1 disorders of globin chain synthesis (the thalassaemias);
2 structural defects of haemoglobin which give rise to haemolysis (e.g. sickle cell anaemia, haemoglobin C);
3 unstable haemoglobins (rare);
4 structural disorders giving rise to polycythaemia or methaemoglobinaemia (rare).
The first and second have a wide global prevalence, particularly where malaria is, or was, common, as the carrier states give some protection against falciparum malaria. Frequent compound heterozygotes of a thalassaemic allele and a haemoglobin structural variant allele include sickle/β-thalassaemia and Hb E/β-thalassaemia.

Thalassaemia

These autosomal recessive syndromes divide into α- and β-thalassaemia depending on whether there is reduced synthesis of α- or β-globin (Table 17.1).

Table 17.1 Classification of thalassaemia.

Clinical phenotype	Thalassaemia (thal) syndrome
Hydrops fetalis	Homozygous α-globin gene deletions→ complete lack of α globin
Thalassaemia major	Homozygous β or doubly heterozygous thal major → complete or almost complete lack of β globin
Thalassaemia intermedia	See p. 41
Thalassaemia trait	Heterozygous β-thalassaemia (β-thal minor, lack of one functional β-globin gene*) Heterozygous α-thalassaemia (α-thal minor, lack of one or two α-globin genes†)

* Normal individual has two (one from each parent/on each allele).
† Normal individual has four (two from each parent on each allele).

α-thalassaemia

Normally there are four α-globin genes, two on each chromosome 16 (see Fig. 2b). Severity of α-thalassaemia depends on the number of α-genes deleted or, less frequently, dysfunctional.

Hydrops fetalis

In hydrops fetalis all four α genes are inactive. The fetus is unable to make either fetal ($\alpha_2\gamma_2$) or adult Hb A ($\alpha_2\beta_2$) haemoglobin. Death occurs *in utero* or neonatally.

Haemoglobin H disease

This is caused by deletion or functional inactivity of three of the four α-genes. There is markedly microcytic hypochromic anaemia (Hb 6–11.0 g/dL); splenomegaly is usual. Bone deformities and features of iron overload do not occur. Haemoglobin electrophoresis shows 4–10% haemoglobin H (β_4) and supravital staining shows 'golf ball' cells.

α-thalassaemia trait

This is a one or two α-gene deletion or dysfunction with microcytic, hypochromic red cells with raised red cell count (>5.5 × 10^{12}/L). Mild anaemia occurs in some cases with two α-genes deleted.

β-thalassaemia

Thalassaemia major

This is complete (β^0) or almost complete (β^+) failure of β-globin chain synthesis resulting from one of nearly 200 different point mutations or deletions in the β-globin gene or its controlling

sequences on chromosome 11. There is severe imbalance of α:β chains with deposition of α chains in erythroblasts, ineffective erythropoiesis, severe anaemia and extramedullary haemopoiesis.

Clinical features

- Anaemia presents at the age of 3–6 months when the switch from γ- to β-chain synthesis normally occurs. Milder cases present later (up to age 4 years).
- Failure to thrive, intercurrent infection, pallor, mild jaundice.
- Enlargement of the liver and spleen, expansion of the bones—especially of the skull—with bossing and a 'hair on end' appearance on X-ray (Fig. 17a); thalassaemic facies, caused by expansion of skull and facial bones.
- Features of iron overload as a result of blood transfusions include melanin pigmentation, growth/endocrine defects, e.g. diabetes mellitus, hypothyroidism, hypoparathyroidism, failure of sexual development, cardiac failure or arrhythmia, liver abnormality (see Chapter 11).

Laboratory findings

- Severe anaemia (Hb 2–6 g/dL) with reduced MCV and MCH.
- Blood film (Fig. 17b) shows hypochromic microcytic cells, target cells, erythroblasts and, often, myelocytes.
- Bone marrow is hypercellular with erythroid hyperplasia.
- Globin chain synthesis studies show absent, or severely deficient, β-chain synthesis. Fetal haemoglobin variably increased.
- DNA analysis reveals the specific mutations or deletions.

Management

- Regular transfusions of packed red cells to maintain haemoglobin above 9–10 g/dL, leucodepleted to reduce risk of HLA sensitization and of transmission of disease, e.g. cytomegalovirus.
- Iron chelation therapy with subcutaneous desferrioxamine (DFX) over 8–12 hours on 5–7 nights weekly. Additional DFX may be given intravenously at the time of blood transfusion via a separate bag. Oral vitamin C increases iron excretion with DFX. An orally active chelator, deferiprone, is also available for those inadequately chelated by DFX (see also Chapter 11). It is more effective at removing cardiac iron. Side-effects include agranulocytosis and arthralgia. A second oral iron chelator KL 670 is in trial.
- Hepatitis B is prevented by early immunization. Patients who already have chronic active hepatitis caused by hepatitis C may need α-interferon + ribavirin therapy.
- Splenectomy is necessary if blood requirements are excessively high. Defer if possible until the age of 5 years, precede by immunization (see Chapter 5) and follow by oral penicillin therapy for life. If the platelet count remains raised, low-dose aspirin reduces the risk of thromboembolism.
- Bone marrow transplantation from an HLA-matching sibling may give long-term disease-free survival of up to 90% in good-risk patients, but nearer 50% in poor risk (previously poorly chelated with iron overload and liver fibrosis).
- Treat complications of iron overload: heart, endocrine organs, liver damage.
- Osteoporosis may occur as a result of marrow expansion, endocrine deficiencies.

Thalassaemia intermedia

A variable syndrome milder than thalassaemia major, with later onset, and characterized by moderately severe (Hb 6–10 g/dL), hypochromic microcytic anaemia requiring either few or no transfusions. There is milder imbalance of α:β+γ globin chain synthesis than in thalassaemia major, due to milder β-chain defects, increased γ-chain or reduced α-chain synthesis. Hepatosplenomegaly, extramedullary haemopoiesis, anaemia and bone deformities may occur. Iron overload occurs as a result of irregular blood transfusions and increased gastrointestinal iron absorption.

β-thalassaemia trait

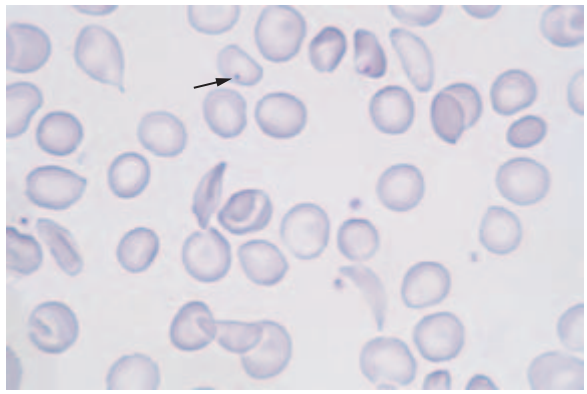
A mild hypochromic microcytic anaemia with a raised red cell count (>5.5 × 10^{12}/L) and raised haemoglobin A_2 ($\alpha_2\,\delta_2$) level (>3.5%). Iron stores are normal. Accurate diagnosis allows genetic counselling and avoidance of inappropriate iron therapy.

Prenatal diagnosis of haemoglobin defects

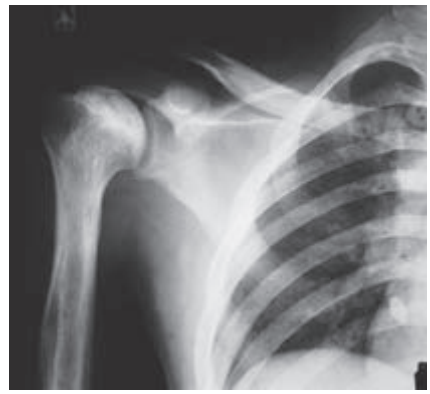
Prenatal diagnosis is available using either DNA (chorionic villous or amniotic fluid) or fetal blood. Carriers must first be identified (screening by blood count in ethnic minority groups, at preconception counselling or in the antenatal clinic). If a mother is a carrier, her partner must be tested. If both are carriers, there is a one in four chance that the fetus is homozygous, or doubly heterozygous, and a one in two chance that the fetus is a carrier. Fetal DNA is then usually amplified by use of the polymerase chain reaction and the DNA mutations are detected. If the fetus is severely affected the couple should be counselled and termination of pregnancy, if appropriate, offered.

18 Haemolytic anaemias V: inherited defects of haemoglobin—sickle cell disease

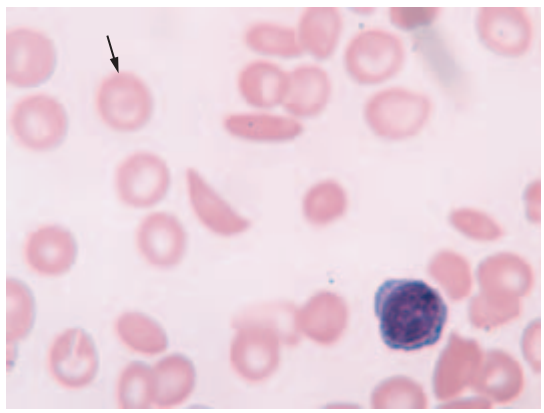
(a) Sickle cell anaemia (Hb SS): peripheral blood film showing sickle cells, anisocytois and changes of hyposplenism (target cells, Howell–Jolly bodies, arrow).



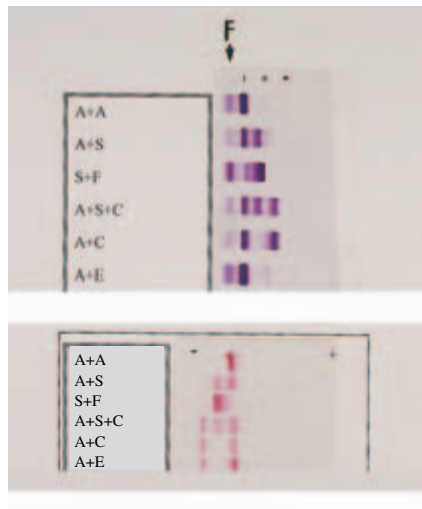
(b) Sickle cell anaemia: bony changes. X-ray showing avascular necrosis of the head of the humerus.



(c) Sickle cell/β-thalassaemia (Hb S/β-thal): peripheral blood film. Sickle cells, target cells (arrow), hypochromasia and a low mean corpuscular volume (MCV) are characteristic.



(d) Haemoglobin electrophoresis (Hb Ep). A lysate of red cells is applied to a gel and an electric current is applied. The upper panel shows migration of different haemoglobins in acid agar gel (pH 6.0) and the lower panel shows migration in cellulose acetate at alkaline pH. Haemoglobins S and F and haemoglobins A2, C and E run together on cellulose acetate and must be distinguished by acid agar Hb Ep.



Sickle cell disease

Sickle cell disease (homozygous sickle cell anaemia) is a chronic haemolytic anaemia caused by a point mutation in the β-globin gene causing substitution of valine for glutamic acid in the sixth position of the β-globin chain. This causes insolubility of Hb S in its deoxygenated state. The insoluble chains crystallize in the red cells causing sickling (Fig. 18a) and vascular occlusion. The disease is most common in Africans (1 in 5 West Africans are carriers—they have some protection against falciparum malaria). The mutant gene also occurs in other parts of the world where malaria is or was prevalent, e.g. the Middle East, Far East and the Indian subcontinent.

Clinical features

These resemble those of other chronic haemolytic anaemias, punctuated with different types of crisis.

- **Vaso-occlusive crisis with blockage of small vessels** is caused by increased sickling; common precipitants are infection, dehydration, acidosis and deoxygenation. Abdominal pain is caused by infarction affecting abdominal organs; bone pain may occur in the back, pelvis, ribs and long bones. Infarction may affect the central nervous system—causing a stroke or fits—lungs, spleen or kidneys. In children the 'hand–foot syndrome' is caused by infarction of the metaphyses of the small bones.
- **Visceral sequestration crisis** is caused by sickling with pooling

of red cells in the liver, spleen or lungs. Sequestration in the lungs is partly responsible for the acute chest syndrome, though infarction and infection contribute.

• **Aplastic crisis** occurs following infection by B19 parvovirus. This causes temporary arrest of erythropoiesis which in healthy individuals is of no consequence but in patients with reduced red cell survival, such as Hb SS, can rapidly cause severe anaemia requiring blood transfusion.

• Increased susceptibility to infection. Splenic function is reduced because infarction leads to autosplenectomy. Pneumococcal infections may lead to pneumonia and meningitis. Infarction of intestinal mucosa predisposes to *Salmonella* infection and osteomyelitis may result.

• Other clinical features include pigment gallstones with cholecystitis, chronic leg ulcers, avascular necrosis of the femoral and humeral heads (Fig. 18b) or other bones, cardiomyopathy, proliferative retinopathy and renal papillary necrosis (leading to polyuria, failure to concentrate urine and tendency to dehydration).

Laboratory features

• Haemoglobin level is 7–9 g/dL but symptoms of anaemia are usually mild (the O_2 dissociation curve of Hb S is shifted to the right).

• Blood film shows sickle cells, target cells and often features of splenic atrophy (Fig. 18a).

• Screening tests for sickling demonstrate increased turbidity of the blood after deoxygenation (e.g. with dithionate or Na_2HPO_4). Haemoglobin electrophoresis (Fig. 18d) shows haemoglobin with an abnormal migration. In Hb SS there is absence of Hb A. The Hb F level is usually mildly raised (5–10%).

Treatment

• General—avoid known precipitants of sickle cell crisis, especially dehydration and infections. Give folic acid, pneumococcal, HIB and meningococcal vaccination and oral penicillin indefinitely to compensate for splenic atrophy.

• Vaso-occlusive crisis is treated with hydration, usually intravenous normal saline, analgesia (e.g. diamorphine subcutaneous infusion); O_2 if there is hypoxia; antibiotics if there is infection.

• Red cell transfusion for severe anaemia (sequestration or aplastic crisis) or as a 3–12-month programme of therapy for patients with frequently recurring crises or for 2–3 years following central nervous system crisis.

• Severe sickling or sequestration crisis (e.g. 'chest syndrome' and stroke) is treated acutely with exchange transfusion to reduce Hb S levels to <30%. Pregnant patients and those undergoing general anaesthesia may need transfusion to reduce Hb S levels to <30%.

• Oral hydroxyurea (20–40 mg/kg/day) reduces both the frequency and duration of sickle cell crises. Although its precise mode of action is not known, it increases Hb F production, decreases intracellular Hb S concentration by increasing MCV, lowers the neutrophil count and inhibits prothrombotic interactions between sickle cells and the endothelium.

• Bone marrow transplantation in selected cases.

• Joint replacement surgery may be required for avascular necrosis (hips and shoulder).

• Iron chelation therapy for patients with iron overload caused by multiple transfusions.

Sickle cell trait

Sickle cell trait is a benign condition without anaemia and is usually asymptomatic. Occasionally haematuria or overt crisis occurs. Genetic counselling should be offered to carriers.

Other sickling disorders

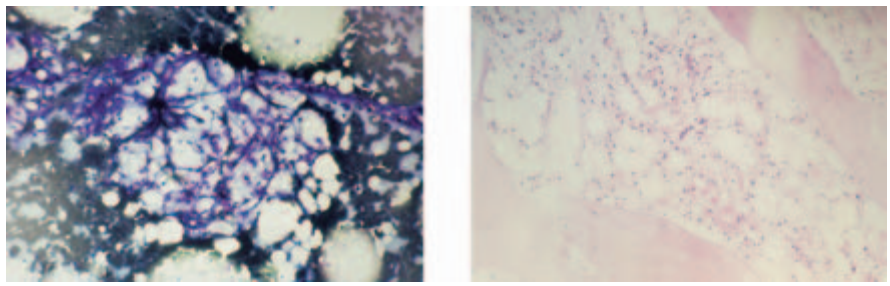
Haemoglobin S may occur in combination with other genetic defects of haemoglobin (compound heterozygotes). Hb S/β-thalassaemia (Fig. 18c) clinically resembles Hb SS but the spleen usually remains enlarged and MCV is reduced. Hb SC disease varies in severity from mild to indistinguishable from sickle cell anaemia; thrombotic complications are particularly common.

Other structural haemoglobin abnormalities

Many other mutations of the α- or β-chain genes have been identified. Most that have a significant population frequency are not associated with clinical symptoms. Haemoglobin E gives a mild thalassaemia-like blood picture.

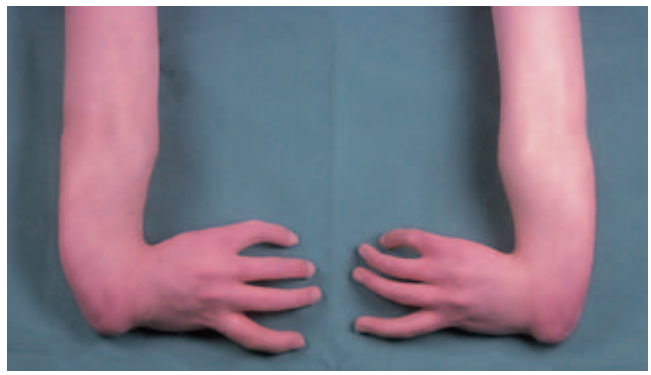
19 Bone marrow failure

(a) Aplastic anaemia: (i) bone marrow aspirate; and (ii) trephine biopsy showing reduced cellularity with increased fat spaces.



(i) (ii)

(b) Aplastic anaemia: Fanconi's anaemia, with multiple skeletal deformities of upper limbs.



Bone marrow failure is the failure of the bone marrow to produce sufficient red cells, white cells and platelets. Causes are listed in Table 19.1. The bone marrow may be hypoplastic or aplastic, with a reduction in haemopoietic cells and an increase in fat spaces. Alternatively, the haemopoietic cells may be replaced by abnormal cells or malignant cells either arising in the marrow (primary) or infiltrating it (secondary), or marrow production may be ineffective.

Clinical features

- Symptoms and signs of anaemia, infections and easy bruising or bleeding.
- Symptoms and signs as a result of the underlying cause, e.g. side effects of chemotherapy.

Laboratory findings

- Anaemia, leucopenia and thrombocytopenia (pancytopenia) of varying severity.
- Blood film typically shows no abnormal cells. It may show circulating red cell and white precursors (leucoerythroblastic) caused by bone marrow infiltration (see Chapter 38) or may show evidence of primary haematological malignancy, e.g. circulating leukaemic blast cells.
- Bone marrow aspirate and trephine biopsy are required to define cause (Fig. 19a).

Table 19.1 Bone marrow failure.

Primary reduction in haemopoietic cells
Aplastic anaemia
Replacement of marrow by malignant cells
Primary —leukaemia, myeloma, lymphoma
Secondary —e.g. carcinoma
Infiltration by abnormal tissue
Myelofibrosis
Rarely, Gaucher's disease, amyloidosis, osteopetrosis
Ineffective haemopoiesis
Myelodysplasia, megaloblastic anaemia

Differential diagnosis

- Pancytopenia (reduction in all three haemopoietic cell lines) can also result from accelerated destruction of cells (e.g. as a result of splenomegaly or autoimmune destruction) or pooling of cells (e.g. within an enlarged spleen).

Treatment

- Remove any known cause, e.g. drugs.
- Support care with appropriate blood components (see Chapter 41) and antimicrobials (see Chapter 43).

Table 19.2 Causes of aplastic anaemia.

Congenital
Fanconi
Other, e.g. dyskeratosis congenita
Acquired
Idiopathic
Secondary
Inevitable (cytotoxic drugs, radiation)
Idiosyncratic
Drugs, e.g. chlorampenicol, sulfonamides, gold, chlorpromazine, carbimazole
Chemical agents/toxins, e.g. benzene
Infection, e.g. viral hepatitis (non-A, non-B, non-C)
Associated with haematological malignancy, e.g. acute lymphoblastic leukaemia
Other, e.g. in association with paroxysmal nocturnal haemoglobinuria

• Specific therapy is considered separately with the specific diseases.

Aplastic anaemia

This is a chronic pancytopenia associated with a hypoplastic bone marrow. There are reduced marrow stem cells, increased fat spaces (fat/haemopoiesis ratio >75 : 25%) and no evidence of malignancy. The marrow microenvironment is intact.

Aetiology and pathogenesis

• The disease may be congenital or acquired (Table 19.2).
• Congenital aplastic anaemia may be inherited as an autosomal recessive (Fanconi type); rarely associated with dyskeratosis congenita.
• Acquired aplastic anaemia has an identifiable cause (viral infection, radiation or drug exposure) in about 50% of cases. In the remainder the cause is unknown, but may involve an immune reaction against marrow stem cells.

Clinical features

• May occur at any age, in either sex, incidence of 2–5 cases/million population.
• Onset rapid (over a few days) or slow (over weeks or months).
• Symptoms and signs are caused by bone marrow failure (see above).
• Liver, spleen and lymph nodes are not enlarged.
• Fanconi's anaemia (Fig. 19b) usually presents in childhood. Associated findings may include skeletal and renal tract defects, microcephaly and altered skin pigmentation. In dyskeratosis congenita there are skin, hair and nail changes.

Laboratory findings

• Anaemia is normocytic or mildly macrocytic with a low reticulocyte count.
• Leucopenia is usual with neutrophils below 1.5×10^9/L ($<0.2 \times 10^9$/L in severe cases).
• Thrombocytopenia ($<10 \times 10^9$/L in severe cases).
• Bone marrow is hypoplastic with >75% fat spaces. Remaining haemopoietic cells are of normal appearance.
• Megakaryocytes are particularly reduced.
• In Fanconi's anaemia lymphocyte chromosomes show random breaks. Acute leukaemia may occur.

Specific therapy

• Immunosuppression, e.g. antilymphocyte globulin (ALG), horse or rabbit, given intravenously over several days, and ciclosporin (alone or with ALG) improve marrow function in 50–70% of severe cases. Corticosteroids are given with ALG to prevent serum sickness.
• Androgens (e.g. oxymetholone) may benefit Fanconi's anaemia and acquired aplastic anaemia.
• Bone marrow transplantation offers a cure in severe cases, providing there is an HLA matching sibling to act as donor. Results are best (60–70% cure) in younger patients (<20 years).
• Haemopoietic growth factors, G-CSF or GM-CSF may raise the neutrophil count temporarily but have no long-term benefit on the underlying bone marrow defect.
• Blood product support (see Chapter 41).

Red cell aplasia

Red cell aplasia is anaemia caused by selective reduction of red cell production by the bone marrow. There is absence or severe reduction of developing erythroblasts in the marrow and of reticulocytes in the peripheral blood, with no abnormality in other cell lines.

Clinical and laboratory features

A rare congenital form (Diamond–Blackfan anaemia) is frequently associated with other somatic malformations. Acquired red cell aplasia may occur as a result of drugs (e.g. azathioprine, isoniazid), in association with autoimmune diseases (e.g. systemic lupus erythematosus), haematological malignancy (e.g. chronic lymphocytic leukaemia) or with a thymoma (see Chapter 38).

Transient red cell aplasia occurs following infection with B19 parvovirus and can lead to a profound but temporary reduction of red cell production with severe anaemia in patients with a haemolytic disorder (e.g. 'aplastic crisis' in hereditary spherocytosis or sickle cell anaemia).

Treatment

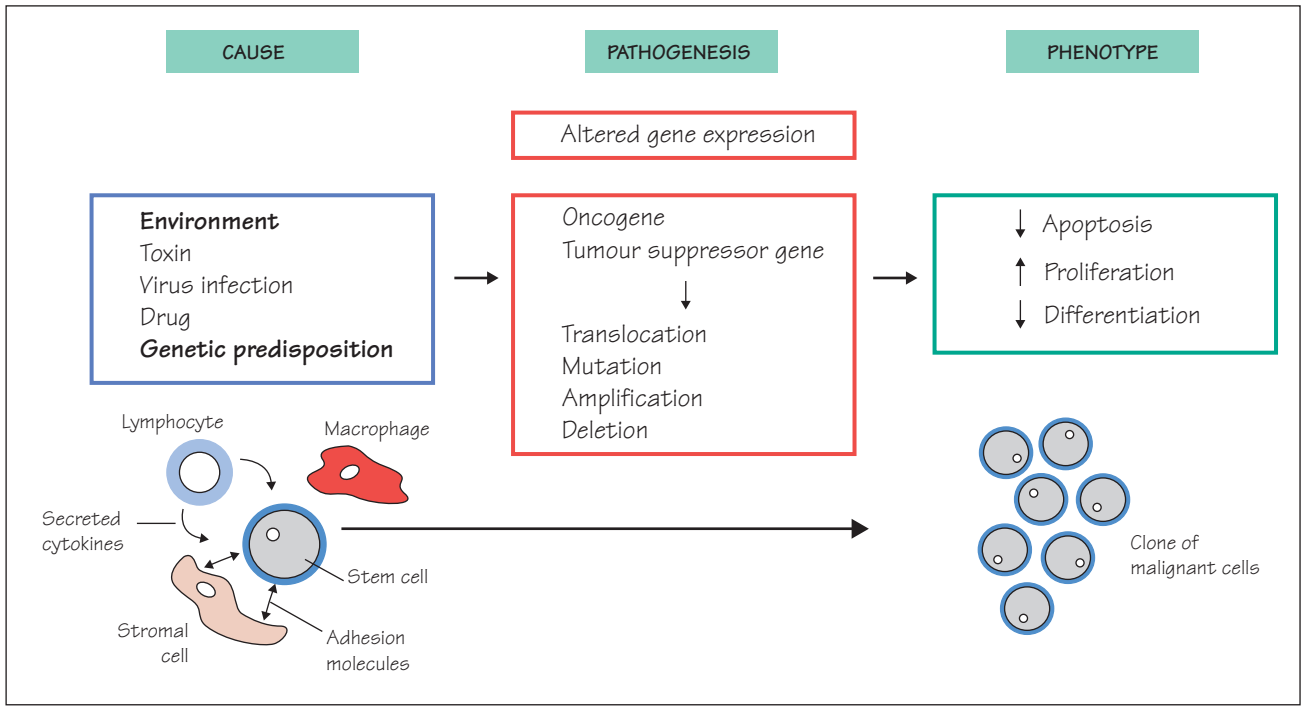
Treatment of the underlying disorder (e.g. removal of a thymoma) is required. Red cell transfusion and iron chelation therapy may be required. Immunosuppressive therapy (e.g. prednisolone, ciclosporin, ALG) is useful in selected patients with either congenital or acquired red cell aplasia.

Congenital dyserythopoietic anaemias

Congenital dyserythropoietic anaemias are a rare group of recessively inherited conditions in which chronic anaemia results from abnormal maturation of erythroid cells in the marrow. Red cell precursors usually show marked morphological abnormalities, e.g. bi- and trinucleated normoblasts.

20 Haematological malignancy: basic mechanisms

(a) Pathogenesis of haematological malignancy.



(b) Minimal residual disease. Morphologically obvious disease (e.g. in AML) is present at presentation (A). At remission (B), when there is no morphological evidence of disease, there are still substantial numbers of malignant cells and disease is detectable by immunological and molecular techniques. At (C) there is no disease detectable, but there may well be minimal residual disease. At (D) disease is once more detectable and, in this example, precedes overt relapse (E).

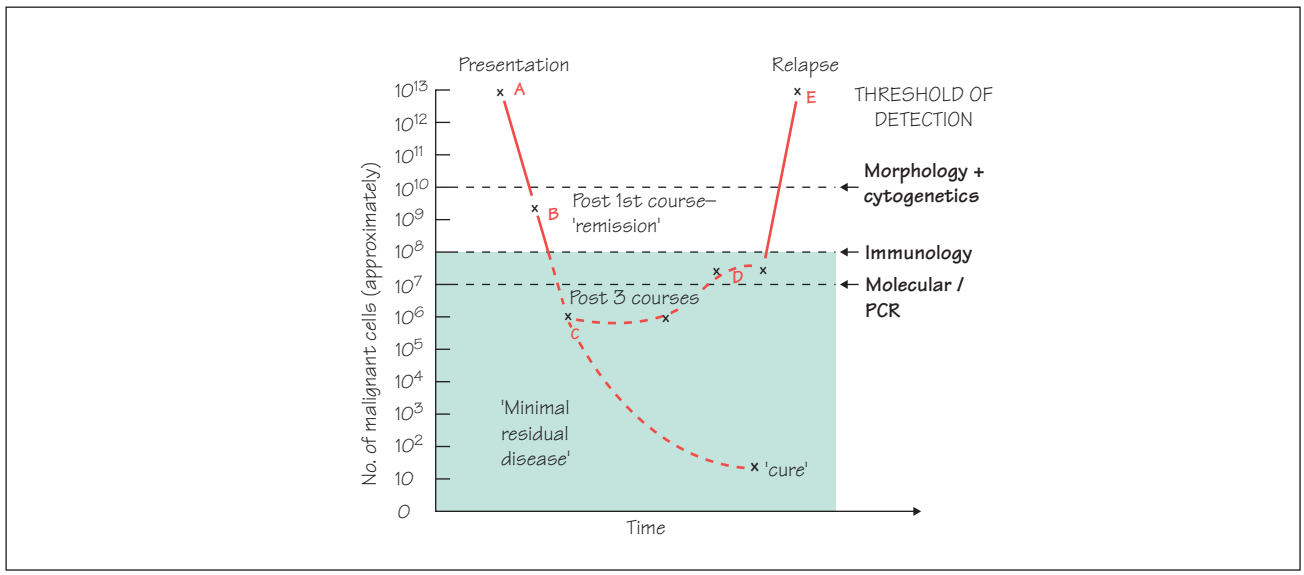


Table 20.1 Classification of haematological malignancies.

	Acute	Chronic
Lymphoid	Acute lymphoblastic leukaemia (ALL) and subtypes	Chronic lymphocytic leukaemia Non-Hodgkin lymphoma (NHL) Hodgkin lymphoma (HL) Multiple myeloma and variants
Myeloid	Acute myeloid leukaemia (AML) and subtypes	Chronic myeloid leukaemia (CML) Myelodysplasia (MDS) Myeloproliferative disorders

Neoplasia

Haematological malignancies (Table 20.1) are thought to arise from a single cell in the bone marrow, thymus or peripheral lymphoid system. This cell has undergone genetic change (somatic mutation) leading to malignant *transformation*. Successive mitotic divisions give rise to a clone of cells derived from the parent cell. Further mutations may give rise to subclones (clonal evolution). Transformed cells proliferate excessively and/or are resistant to apoptosis. They are often 'frozen' at a particular stage of differentiation.

Causes of neoplasia

Neoplasia is caused by a complex interaction between genetic and environmental mechanisms (Fig. 20a).

1 Genetic predisposition. Certain inherited conditions (e.g. Down's syndrome, trisomy 21) and conditions associated with defective DNA repair (e.g. Fanconi's anaemia) or immune suppression (e.g. ataxia telangiectasia).

2 Viral infection. Human T-cell leukaemia virus (HTLV-1) incorporates into T-lymphoid-cell genome and underlies adult T-cell leukaemia lymphoma (see Chapter 28). Other viruses predispose to malignancy by immune suppression (e.g. HIV). Epidemiological evidence implicates Epstein–Barr virus in Burkitt's lymphoma and less strongly with Hodgkin's disease. *Helicobacter pylori* infection of the stomach predisposes to gastric lymphoma.

3 Ionizing radiation causes DNA mutation and increases the risk of haematological neoplasia.

4 Toxins/chemicals, e.g. benzene and organochemicals, may predispose to leukaemia and myelodysplasia (MDS).

5 Drugs. Alkylating agents (e.g. melphalan, mustine) and other forms of chemotherapy predispose to MDS or acute myeloid leukaemia.

Mechanism of malignant transformation

Altered expression of three types of gene underlies multistep pathogenesis of haematological malignancy.

Oncogenes

Oncogenes, whose protein products cause malignant transformation, are derived from normal cellular genes (proto-oncogenes). These code for proteins usually involved in one or other stage in cell signal transduction, gene transcription, cell cycle, cell survival/apoptosis or differentiation. Activation of proto-oncogenes to become oncogenes may occur by **amplification**, **point mutation** or **translocation** (most frequent in haematological malignancies) from one chromosomal location to another. Translocation may lead to a quantitative change in expression (e.g. MYC translocation to the immunoglobulin heavy chain locus in lymphoid neoplasia, t(8;14)) or qualitative change by joining all or part of the oncogene to another gene to form a fusion gene (e.g. ABL translocation from chromosome 9 to the breakpoint cluster region (BCR) on chromosome 22 to form BCR-ABL in CML, t(9;22)) (see Fig. 21a).

Anti-oncogenes

Anti-oncogenes, or tumour suppressor genes, are genes encoding proteins which have a critical role in suppressing cell growth. Chromosome deletion may obliterate tumour suppressor genes on one allele; deletion or mutation of the remaining allele may allow uncontrolled cell growth.

Inhibition of apoptosis

Malignant cells often show resistance to apoptosis. The BCL-2 gene product inhibits apoptosis and its expression is increased in some chromosome translocations (e.g. in follicular lymphoma).

Evidence of clonality

A population of cells is considered clonal (derived from a single cell by mitotic division) if they have some or all the following:

- The same acquired chromosome abnormality, e.g. Ph chromosome (see Fig. 21a) or point mutation within an individual gene.
- Clonal rearrangement of an immunoglobulin or T cell receptor gene in lymphoid neoplasia.
- Restriction in a B-lymphoid neoplasm to expression of only λ or only κ light chains, but not both as in polyclonal B cells.
- Restriction fragment length polymorphism in which the size of a restriction fragment of DNA on the X chromosome is analysed. In females, two fragments derived from the two X chromosomes will be found in polyclonal populations, both fragments being hypomethylated and transcriptionally active. In tumours only one size of fragment is hypomethylated as only one X chromosome is active.

Minimal residual disease (Fig. 20b)

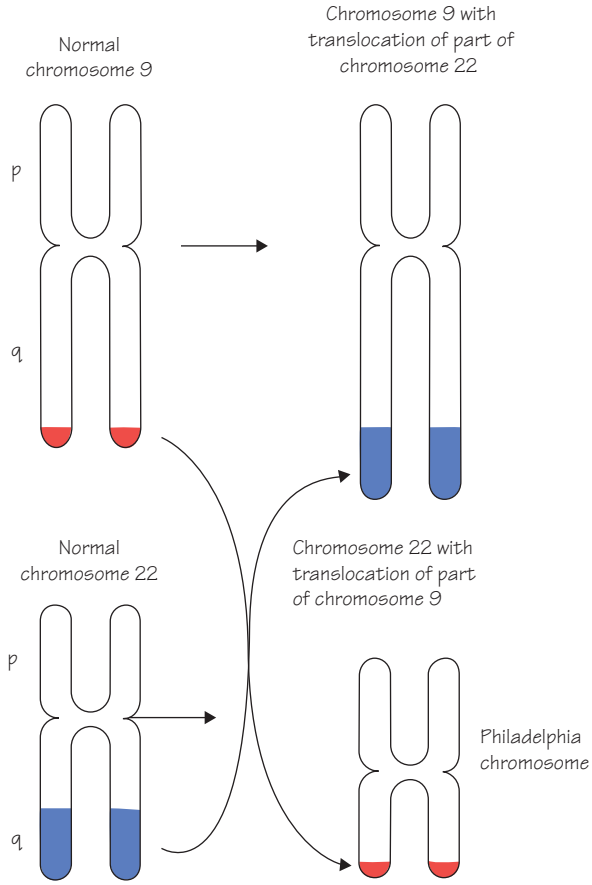
At the time of diagnosis of a haematological neoplasm, the patient will have approximately 10^{13}–10^{14} malignant cells. Even if treatment results in 1000-fold reduction of tumour cells, there remain 10^{10} cells, which may be below the microscopic level of detection. Using immunological or molecular techniques, residual malignant cells may be detected in blood or marrow of patients who clinically and by conventional light microscopy are in complete remission.

The techniques to detect minimal residual disease include:

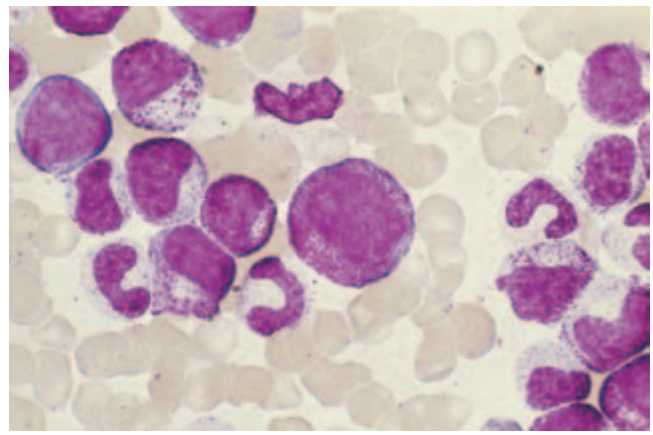
- immunological, particularly if residual malignant cells carry a distinctively abnormal phenotype (pattern of antigen expression);
- chromosomal analysis and FISH (see p. 20);
- molecular using the polymerase chain reaction, which are the most sensitive (will detect one malignant cell in up to 10^6 normal cells).

21 Chronic myeloid leukaemia

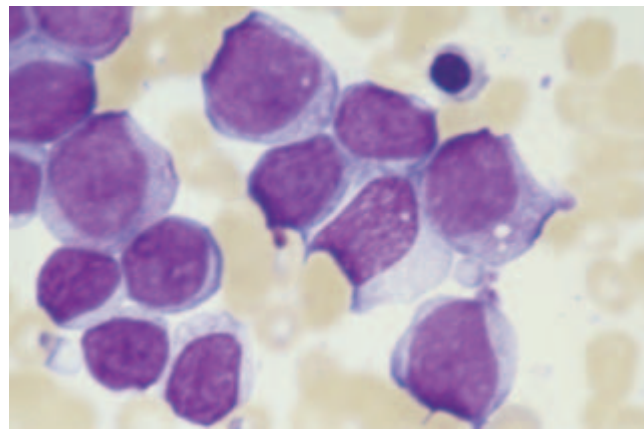
(a) The Philadelphia chromosome is an abnormal chromosome 22 caused by translocation of part of long arm (q) of chromosome 22 to chromosome 9, and reciprocal translocation of part of chromosome 9, including the ABL oncogene, to a specific breakpoint cluster region (BCR) of chromosome 22. A fusion gene results on the derived chromosome 22 which leads to the synthesis of an abnormal protein with tyrosine protein kinase activity that is much greater than that of the normal ABL protein.



(b) Chronic myeloid leukaemia (chronic phase): peripheral blood film, showing immature granulocytes (myelocytes, metamyelocytes) in the peripheral blood.



(c) Chronic myeloid leukaemia: blast transformation. There is replacement by homogenous blast cells.



This is a clonal myeloproliferative disorder characterized by an increase in neutrophils and their precursors in the peripheral blood with increased cellularity of the marrow as a result of an excess of granulocyte precursors. The leukaemic cells of >95% of patients have a reciprocal translocation between the long arms of chromosomes 9 and 22,t(9;22). The derived chromosome 22 is termed the Philadelphia (Ph) chromosome (Fig. 21a). The disease usually transforms from a relatively stable chronic phase to an acute leukaemia phase (blast transformation).

Aetiology and pathophysiology

Aetiology is unknown. Exposure to ionizing radiation is a risk factor. The ABL oncogene is translocated from chromosome 9 into the breakpoint cluster region (BCR) on chromosome 22 to form the BCR-ABL fusion gene. This fusion gene encodes a 210-kDa protein with greatly increased tyrosine kinase activity compared to the normal ABL product. The disease is of stem cell origin as the Ph chromosome is present in erythroid, granulocytic, megakaryocytic and T-lymphoid precursors. Rare cases show variant translocations, or are Ph-negative but show the BCR-ABL fusion gene. The Ph chromosome abnormality may also occur in acute lymphoblastic leukaemia (ALL; see Chapter 23).

Clinical features

- Occurs at all ages (peak age 25–45 years, male/female ratio equal, incidence of 5–10 cases/million population).

• Patients usually present in the chronic phase.
• Presenting symptoms include weight loss, night sweats, itching, left hypochondrial pain, gout.
• Priapism, visual disturbance and headaches caused by hyperviscosity (WBC >250 × 10^9/L) are less frequent.
• Splenomegaly, often massive, occurs in over 90% of cases.
• Some cases are discovered on routine blood test.

Laboratory findings

• Raised white cell count (often 50 × 10^9/L or more), mainly neutrophils and myelocytes (Fig. 21b).
• Basophils may be prominent.
• Platelet count may be raised, normal or low and anaemia may be present.
• Low leucocyte alkaline phosphatase score.
• Raised serum uric acid.
• Bone marrow is hypercellular with a raised myeloid/erythroid ratio.
• Cytogenetic analysis of bone marrow cells shows the Philadelphia chromosome in >95% of metaphases. The BCR-ABL fusion gene is detectable by FISH (see p. 20) and its RNA product by PCR (see p. 20).

Course and progress

Patients are typically well during the 'chronic phase'. The main cause of death is transformation into acute leukaemia (Fig. 21c) (80% AML, 20% ALL, with a proportion showing a mixed blast cell population), which may occur at any stage, even at presentation. Median survival is currently about 4 years. Staging to predict prognosis uses age, spleen size, blood blast cell and platelet counts. There may be an accelerated phase of variable duration in which anaemia, thrombocytopenia, splenic enlargement and marrow fibrosis occur. Transformation is usually accompanied by additional morphological and chromosome abnormalities.

Treatment

Chronic phase

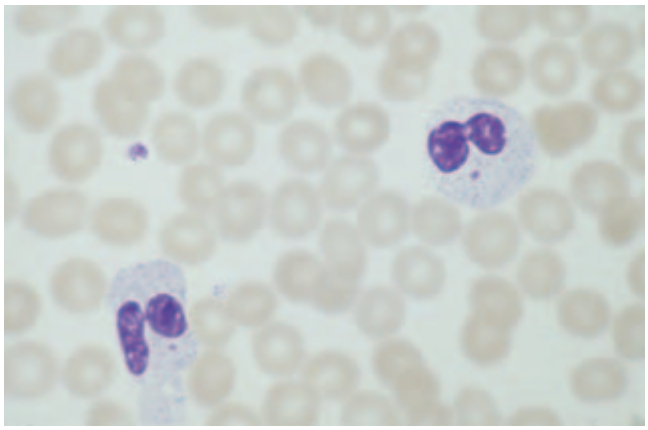
• Imatinib (Glivec). This is a specific inhibitor of the tyrosine kinase encoded by BCR-ABL. It controls the blood count and causes the marrow to become Ph negative in a high proportion of cases, though nearly all remain positive for the BCR-ABL fusion messenger RNA when tested by PCR. The chronic phase is prolonged and the rate of acute transformation is reduced. Side effects include nausea, skin rashes and muscle pains. Imatinib in combination with other drugs is also valuable in the therapy of Ph+ALL and blast transformation of CML.
• Hydroxyurea will control the raised white cell count.
• α-interferon (IFN) may also control the white cell count and may delay the onset of acute transformation, prolonging overall survival by 1–2 years. The best responders to IFN become Ph-negative, but usually remain BCR-ABL-positive, and have the best prognosis.
• Allopurinol to prevent hyperuricaemia.
• Allogeneic stem cell transplantation (SCT) before the age of 50 from an HLA matching sibling offers a 70% chance of cure in the chronic phase but 30% or less once acceleration has occurred. HLA-matched unrelated donor (MUD) SCT is less successful in curing the disease because of higher morbidity and mortality. Transfusion of donor lymphocytes may be valuable in eliminating BCR-ABL-positive cells in cases of relapse post-SCT.

Acute phase

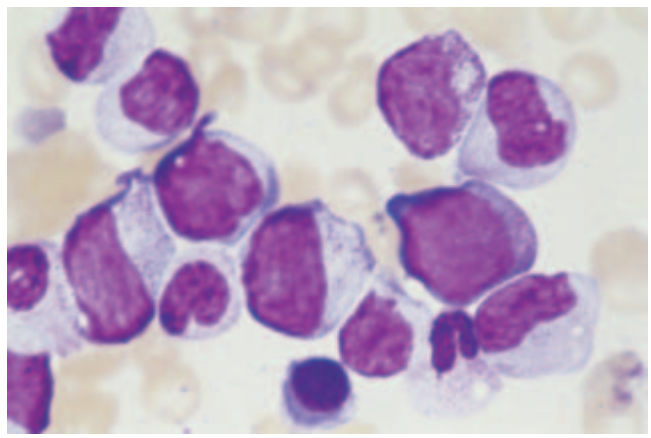
Therapy as for acute leukaemia, AML or ALL with the addition of imatinib may be given, but the prognosis is poor.

22 Myelodysplasia

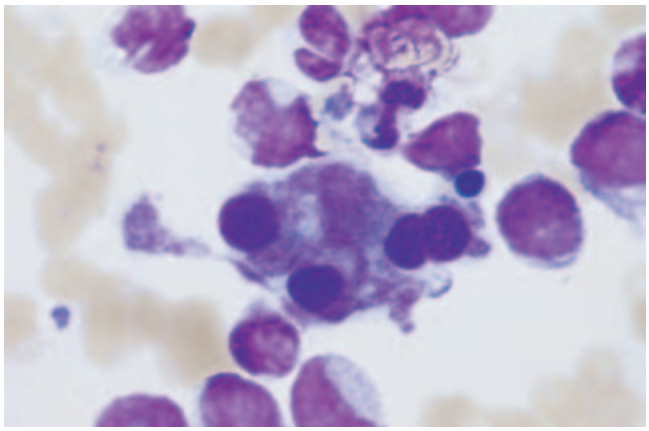
(a) Myelodysplasia: peripheral blood film showing hypogranular neutrophils with bilobed nuclei (pseudo-Pelger cells).



(b) Myelodysplasia: bone marrow aspirate showing a granular blast with blue cytoplasm and hypogranular maturing myeloid cells.



(c) Myelodysplasia: bone marrow aspirate showing mononuclear and binuclear micromegakaryocytes.



This is a clonal haemopoietic stem cell disorder characterized by peripheral blood cytopenias affecting more than one lineage usually in association with a cellular marrow, indicating ineffective haemopoiesis.

Aetiology and pathogenesis

Myelodysplasia (MDS) may be primary (*de novo*) or a consequence of previous chemotherapy/radiotherapy (secondary). Various chromosome and oncogene abnormalities occur, e.g. complete or partial deletions of chromosomes 5 or 7, point mutations in RAS oncogenes. The disease is divided into five subgroups (Table 22.1). It may transform to acute myeloid leukaemia (AML) (>20% blasts in the marrow).

Clinical features

- Most frequent in the elderly but young adults or even children may be affected.

Table 22.1 Classification of the myelodysplastic syndromes.

Disease	Bone marrow	Approximate survival (months)
1 RA*	Blasts <5%	50
2 RA with ring sideroblasts Ring sideroblasts >15% of total erythroblasts	Blasts <5%	50
3 RAEB	Blasts 5–20%	11
4 CMML	As any of the above with promonocytes	11
5 5q-isolated syndrome	Normal or megakarocytes increased <5% blasts	>60

* In some cases neutropenia or thrombocytopenia is present without anaemia. These cases are termed refractory cytopenia.
CMML, chronic myelomonocytic leukaemia; RA, refractory anaemia; RAEB, RA with excess blasts.

• Bone marrow failure (see Chapter 19) with anaemia and/or leucopenia and/or thrombocytopenia.
• In chronic myelomonocytic leukaemia (CMML), the spleen may be enlarged.
• The 5q-syndrome is a subgroup, occurring particularly in elderly females with a high platelet count, macrocytosis and good prognosis.

Laboratory findings

• Anaemia is usually macrocytic.
• Neutropenia is frequent and neutrophils may be hypogranular with pseudo-Pelger forms (Fig. 22a). Monocytes are increased in CMML to $>1.0 \times 10^9$/L.
• Bone marrow is usually hypercellular but may be hypocellular and/or fibrotic.
• Characteristic morphological changes are seen in all three lineages (Figs 22a–22c).

Differential diagnosis

This is very broad, particularly when only one lineage is involved in an elderly person. Thus, other causes of anaemia must be excluded. Thrombocytopenia or leucopenia may be caused by drugs, immune destruction or hypersplenism. The hallmark of MDS is involvement of more than one—typically all three—lineage(s). Nevertheless, distinction between MDS, myelofibrosis and aplasia may be difficult in patients with pancytopenia. The finding of a cytogenetic abnormality greatly strengthens what may otherwise be a difficult morphological diagnosis.

Course and prognosis

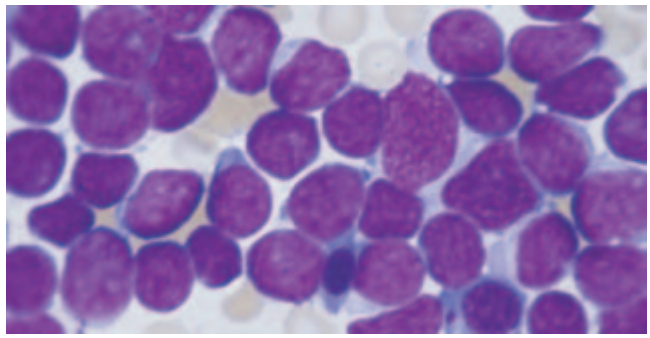
This depends on the type of MDS (Table 22.1). The degree of cytopenia influences the incidence of complications and treatment, while the percentage of blast cells is predictive of the risk of developing acute leukaemia. The presence of complex cytogenetic changes is also associated with a poor prognosis. Scoring systems have been devised whereby the degree of cytopenia, proportion of blasts and nature of cytogenetic changes are used to estimate prognosis. Death may be caused by infection, haemorrhage, iron overload from multiple transfusions or from transformation into AML.

Treatment

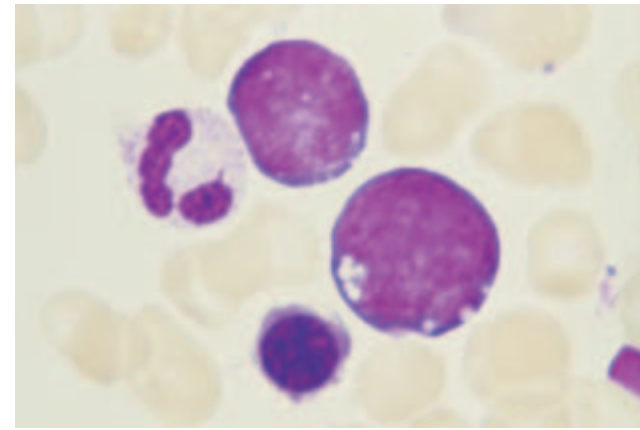
• Support care with red cell or platelet transfusions and antimicrobials may be required.
• Iron chelation therapy may be needed for multiply transfused iron-loaded patients with an otherwise good prognosis.
• G-CSF may be used temporarily to increase neutrophil production; erythropoietin produces a rise in haemoglobin in about 5–15% of patients with refractory anaemia (RA).
• Chemotherapy with low-dose ara-C, etoposide, thioguanine or 6-mercaptopurine is used to control excess blast proliferation in patients unsuitable for high-dose chemotherapy. Newer agents include azacytidine and thalidomide analogues.
• Younger patients with refractory anaemia with excess blasts (RAEB) may be treated as AML. Fludarabine, Ara-C, G-CSF ± idarubicin (FLAG ± Ida) is a useful form of combination chemotherapy. Complete remissions are less frequent than in *de novo* AML. Allogeneic SCT (sibling or MUD) may cure younger patients.

23 Acute leukaemia I: classification and diagnosis

(a) T-cell acute lymphoblastic leukaemia (T-ALL): bone marrow showing large numbers of lymphoblasts with a high nuclear/cytoplasmic ratio.



(b) B-cell ALL: bone marrow showing large blasts with characteristic vacuoles and blue cytoplasm.



(c) Common ALL: cerebrospinal fluid cytocentrifuge specimen showing lymphoblasts.

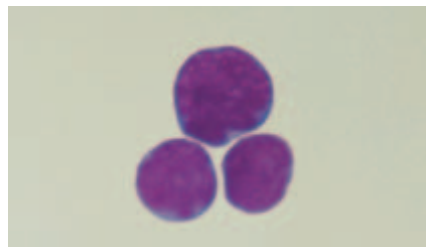


Table 23.1 French–American–British (FAB) morphological classification of acute leukaemia.

Myeloid		Lymphoid
M0	Undifferentiated by morphology + cytochemistry, myeloid immunophenotype	L1 Small cells, high nuclear/cytoplasmic ratio
M1	Little differentiation, >90% blasts	L2 Larger cells, lower nuclear/cytoplasmic ratio
M2	Differentiated, 20–90% blasts	L3 Vacuolated, basophilic blast cells
M3	Promyelocytic: intensely granular, variant form is microgranular	
M4	Myelomonocytic	
M5a	Monocytic without differentiation	
M5b	Monocytic with differentiation	
M6	Erythroid differentiation, >50% of mononuclear cells are erythroid	
M7	Megakaryoblastic	

* All subtypes have >20% blast cells in the bone marrow.

Acute leukaemia is a malignant disorder in which haemopoietic blast cells constitute >20% of bone marrow cells. The primitive cells usually also accumulate in the blood, infiltrate other tissues and cause bone marrow failure.

Classification

There are two main groups: acute lymphoblastic (ALL) and acute myeloid (myeloblastic) leukaemia (AML). Rare cases are undifferentiated or mixed. Subclassification of ALL or AML depends on morphological, immunological, cytochemical and cytogenetic criteria (Tables 23.1–23.4).

Aetiology and pathogenesis

The malignant cells typically show a chromosome translocation or other DNA mutation affecting oncogenes (see Chapter 19). AML may follow previous myeloproliferative or myelodysplastic diseases. In some cases of childhood B lineage (common) ALL there is evidence from identical twin studies that the first event, a chromosomal translocation, may occur *in utero* and subsequent events (? infection) precipitate the onset of ALL.

Incidence

Approximately 1000 new cases (20–25/million population) each of AML and ALL per year in the UK. ALL is the most common malignancy in childhood (peak age 4 years) but also occurs in adults.

Table 23.2 WHO Classification for acute myeloid leukaemia (AML)

AML with recurrent cytogenetic translocations
AML with t(8, 21), (q22, q22)
AML with t(15, 17), (q22, q11–12) + variants = APL
AML with abnormal bone marrow eosinophils inv (16)(p13, q22) or t(16, 16)
AML with 11q23 (mixed lineage leukaemia) abnormalities
AML with multilineage dysplasia
With prior myelodysplastic syndrome (MDS)
Without prior MDS
AML and MDS, therapy-related
Alkylating agent-related
Epipodophyllotoxin-related (some may be lymphoid)
Other
Acute biphenotypic leukaemias

Table 23.3 Immunophenotypes of acute leukaemia.

Disease	Immunophenotype
AML	CD33, CD13, CD117 Monocytic cells: CD14, CD61 Megakaryoblasts: CD41, CD61 Erythroid: glycophorin, transferrin receptor (CD71)
ALL	
Early B-precursors (Pro-B)	CD19, TdT
Common ALL	CD10, CD19, cyt CD22, TdT
Pre B-ALL	cyIg, CD19, cyt CD22, TdT
B-ALL	sMIg, CD19, CD20
T-ALL	CD7, cytCD3, TdT

CD34 is a marker of haemopoietic stem cells and may be positive in both AML and ALL.
ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; B-ALL, B cell ALL; CyIg, cytoplasmic immunoglobin; sMIg, surface membrane immunoglobuli; T-ALL, T cell ALL; TdT, terminal deoxynucleotidyl transferase.

Table 23.4 Cytogenetic changes in acute myeloid leukaemia.

Good risk
M2: t(8,21)
M3: t(15,17)
M4 eosinophilia: inversion 16
Poor risk
Monosomy 5, monosomy 7
Complex karyotypes
11q 23 abnormalities
Mutation of FLt-3
Standard risk
All other cases. These cases may be further subdivided into prognostic groups using DNA microarray techniques

Table 23.5 Prognostic factors in acute lymphoblastic leukaemia.

	Good	Bad
Sex	Female	Male
Age	2–9 years	Adult
White cell count	Low ($<10 \times 10^9$/L)	High ($>50 \times 10^9$/L)
Chromosomes	Hyperdiploid	t(9;22), t(4;11)
Extramedullary disease	Absent	Present
Speed of remission	4 weeks	>4 weeks
Clearance of peripheral blood blasts	1 week	>1 week
Loss of minimal residual disease in bone marrow	1–3 months	> 3 months

AML occurs at all ages but is rarer than ALL in childhood, being most common in the elderly.

Clinical features

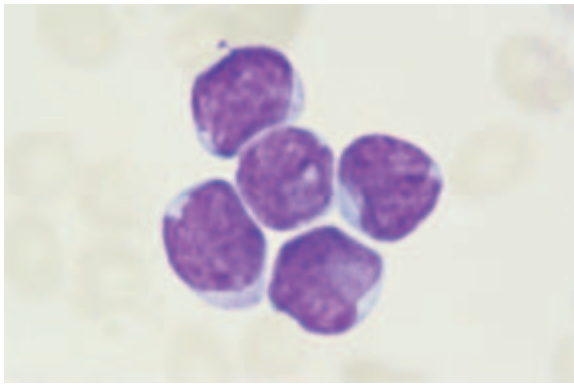
- Short (<3-month) history of symptoms due to bone marrow failure (e.g. of anaemia, abnormal bruising/bleeding or infection). Disseminated intravascular coagulation (DIC) with bleeding is particularly common in acute promyelocytic leukaemia (AML M3).
- Increased cellular catabolism may cause sweating, fever and general malaise.
- Lymphadenopathy and hepatosplenomegaly are frequent, especially in ALL.
- Tissue infiltration, e.g. of meninges, testes (more common in ALL), skin, bones, gums with hypertrophy (AML M5 or M4).

Laboratory features

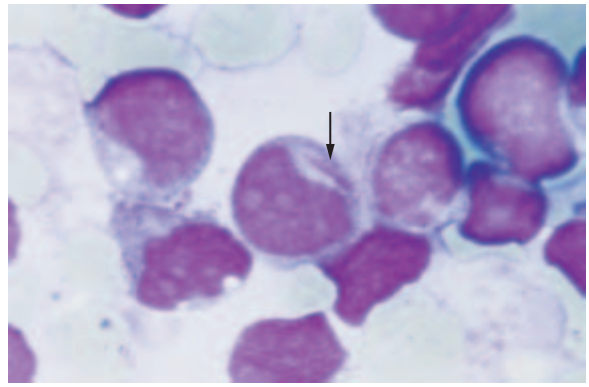
- Anaemia, thrombocytopenia and often neutropenia.
- Leucocytosis caused by blast cells in the blood usually occurs. Leucopenia is less frequent.
- The bone marrow shows infiltration by blast cells (>20% and often 80–90% of marrow cells).
- Coagulation may be abnormal and DIC can occur, especially with AML M3.
- Serum uric acid, lactate dehydrogenase (LDH) may be raised.
- Morphological analysis (Table 23.1; ALL, Figs 23a–c; AML, Figs 24a–f) usually reveals cytoplasmic granules or Auer rods (condensations of granules) in AML. Cytochemical stains are helpful—AML blasts have granules positive by Sudan black, myeloperoxidase and chloroacetate esterase, while monoblasts are positive for non-specific and butyrate esterase. B-lineage lymphoblasts show blocks of positive material with periodic acid–Schiff (PAS) stain, and in T-lineage ALL with acid phosphatase.
- Immunophenotype analysis involves the use of antibodies to identify cell antigens (many termed clusters of differentiation or CD, see Appendix II) which correlate with lineage and maturity (Table 23.3). Other antigens, e.g. TdT, and cytoplasmic immunoglobulin may be also be detected.
- Cytogenetic and DNA microarray analysis gives diagnostic and prognostic information (Tables 23.4, 23.5).

24 Acute leukaemia II: treatment and prognosis

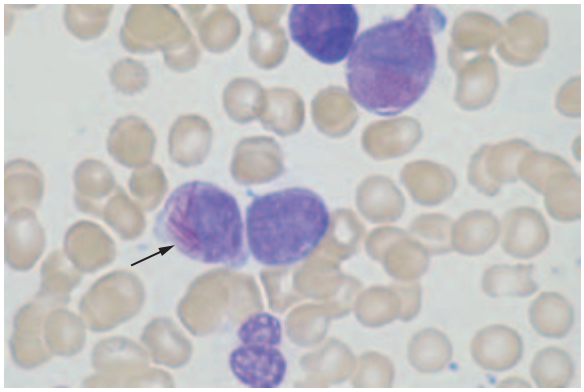
(a) Acute myeloid leukaemia: bone marrow—M0.



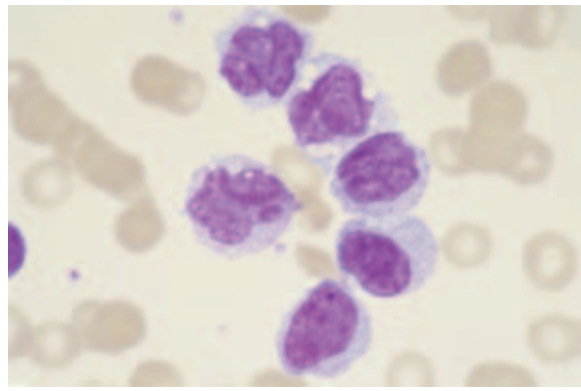
(b) Acute myeloid leukaemia: bone marrow—M2. Note Auer rod (arrow) in myeloblast.



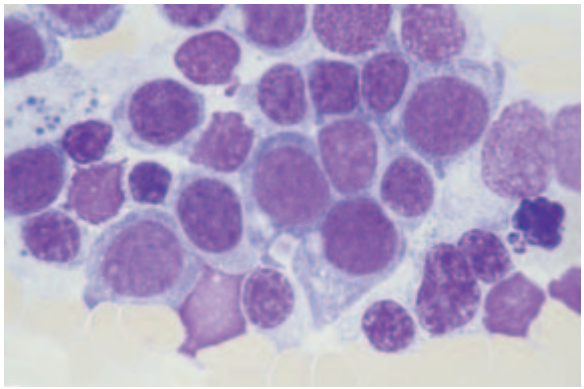
(c) Acute myeloid leukaemia: bone marrow—M3. Note cell with multiple Auer rods (arrow).



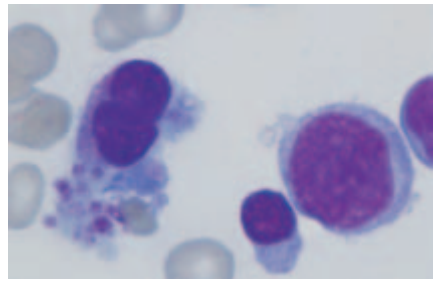
(d) Acute myeloid leukaemia: bone marrow—M5. Showing vacuolated monoblasts.



(e) Acute myeloid leukaemia: bone marrow—M6. Showing abnormal erythroid cells, erythroblasts and myeloblasts.



(f) Acute myeloid leukaemia: bone marrow—M7. Showing myeloblasts and megakaryoblasts, with platelets budding from the cytoplasm.



Treatment

The first phase of therapy (remission-induction) is with high-dose intensive combination chemotherapy to reduce or eradicate leukaemic cells from the bone marrow and re-establish normal haemopoiesis. Further therapy is postinduction chemotherapy: this may be intensive ('intensification' or 'consolidation' chemotherapy) or less intensive (maintenance chemotherapy). Each course of intensive treatment typically requires 4–6 weeks in hospital. Complications of chemotherapy and supportive care are considered in Chapter 43; blood component therapy is considered in Chapter 41.

Acute myeloid leukaemia

Remission induction regimes usually comprise an anthracycline (e.g. daunorubicin), cytosine arabinoside and in some protocols etoposide. All trans retinoic acid (ATRA) is given concurrently in acute promyelocytic leukaemia (AML M3) to induce differentiation. More than 80% of patients under the age of 60 years achieve remission, defined as a normal full blood count and <5% blasts in bone marrow, with one course, and >85% patients with two courses. Older patients and those with preceding MDS or AML secondary to another disease (e.g. myeloproliferative disorder, MPD) have lower remission rates. Three further courses are given as post-induction therapy, and other agents used include mitoxantrone, M-AMSA, idarubicin and high dose ara-c. Tumour lysis syndrome may occur (see p. 95). Anti-CD33 combined with a toxin is undergoing trials. Fludarabine combined with high dose of Ara-C and G-CSF (FLAG) may be used for induction especially in older patients. Some older patients may be considered medically unfit for intensive chemotherapy and only given support care with or without single agent chemotherapy.

Acute lymphoblastic leukaemia

Remission induction regimes comprise vincristine, prednisolone and L-asparaginase often with daunorubicin, cyclophosphamide. Post-remission therapy is with two or three 'intensification' blocks with additional drugs. Patients then receive maintenance chemotherapy for a further 2–3 years with daily mercaptopurine, weekly methotrexate and monthly vincristine and dexamethasone. Treatment protocols may be modified according to whether minimal residual disease can be detected at various time points in therapy.

Central nervous system involvement is common in ALL in children and adults, and normal practice is to give multiple intrathecal injections and courses of high-dose systemic chemotherapy with methotrexate or ara-C, or cranial radiotherapy to prevent or treat this complication.

Stem cell transplantation (see Chapter 42)

Allogeneic stem cell transplantation (SCT) is usually recommended for adult patients (<60 years) in first remission of AML and for adults with ALL (>20 years, <60 years) who have a histocompatible sibling. Matched unrelated transplants are becoming safer and more used. However, good prognosis AML (see Table 23.3) and ALL (see Table 23.4) cases are not given SCT in first remission.

Prognosis

Childhood acute lymphoblastic leukaemia

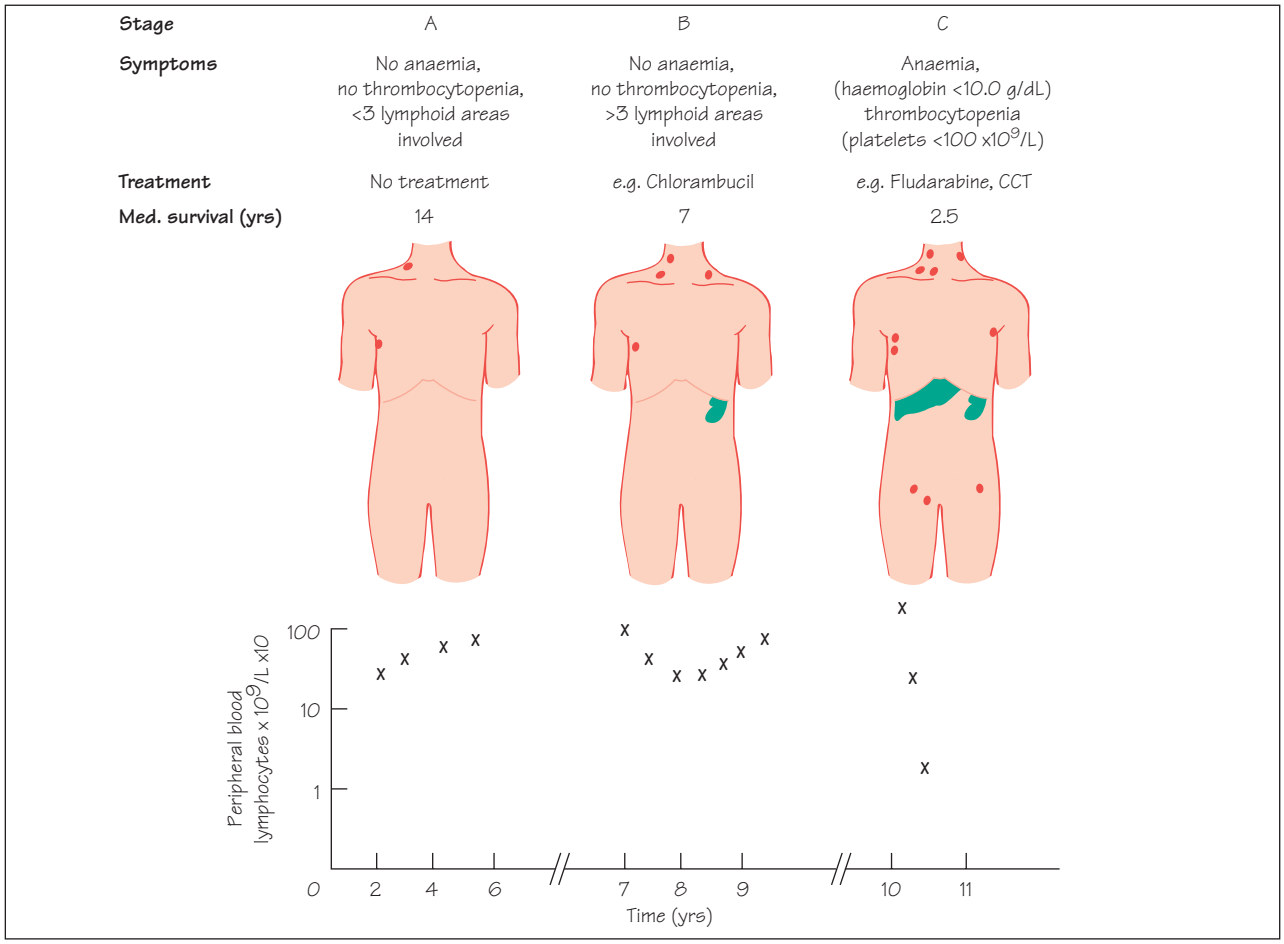
Overall 70% of children with ALL are cured, the best responses being in girls, aged 2–12 years with low presenting white cell count ($<10 \times 10^9$/L) and favourable cytogenetics (see Table 23.4).

Acute myeloid leukaemia and adult acute lymphoblastic leukaemia

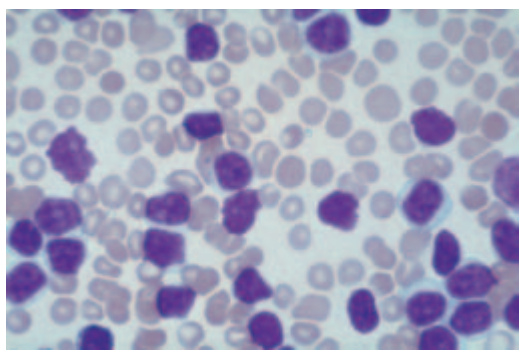
Approximately 30–40% of patients less than 60 years old are cured. This varies widely according to age and prognostic features.

25 Chronic lymphocytic leukaemia

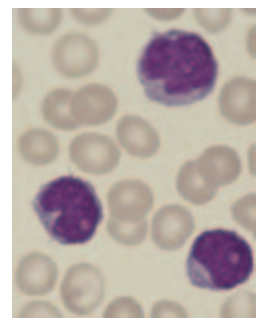
(a) The clinical course of chronic lymphocytic leukaemia (CLL). The Binet staging system evaluates enlargement of the following: lymph nodes, whether unilateral or bilateral, in the head and neck, axillae and inguinal regions; spleen and liver. Stage A patients are usually asymptomatic and do not require treatment. The peripheral blood lymphocyte count may rise progressively. Stage B patients often require treatment (e.g. with oral chlorambucil). Stage C patients will often respond better to more intensive therapy (fludarabine, combination chemotherapy (CCT)).



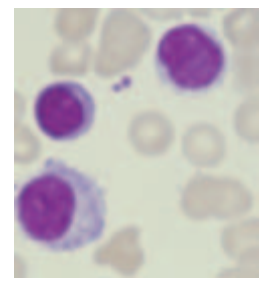
(b) Chronic lymphocytic leukaemia: peripheral blood film showing large numbers of mature lymphoid cells and some 'smear' cells.



(c) (i) Prolymphocytic leukaemia: blood film. (ii) Hairy cell leukaemia: blood film.



(i)



(ii)

Chronic lymphocytic leukaemia (CLL) is a B-cell clonal lymphoproliferative disease in which lymphocytes accumulate in the blood, bone marrow and often in the lymph nodes and spleen (absolute lymphocyte count >5.0 × 10^9/L). A disease of older patients (peak age 72), it is the commonest leukaemia in Western countries (over 70 new cases/million population/year in the UK, male/female ratio 2 : 1) but is rare in Asia.

Aetiology and pathophysiology

The cause is unknown. Commonest chromosome changes are trisomy 12, a 13q deletion and deletions of 11q including the ataxia telangiectasia gene. Oncogene mutations or deletions occur which may prevent cells from undergoing apoptosis.

Clinical features

- Stage depends on clinical and laboratory findings (Fig. 25a).
- Many cases (Stage A) are symptomless and diagnosed on routine blood test.
- Presenting features include lymphadenopathy (typically symmetrical, painless and discrete), night sweats, loss of weight, symptoms of bone marrow failure.
- Spleen is often moderately enlarged.
- Hypogammaglobulinaemia and reduced cell-mediated immunity predispose to bacterial and viral infection.
- Autoimmune haemolytic anaemia in 15–25% of cases.

Laboratory findings

- Increased peripheral blood lymphocytes (Fig. 25b usually 10–30 × 10^9/L at presentation) which are B cells (CD19, CD22 and also CD5 positive).
- They have weak expression of surface IgM which is monoclonal (expressing only κ or only λ light chains).
- Serum immunoglobulins are depressed.
- Anaemia and thrombocytopenia may occur due to marrow infiltration or as a result of auto-antibodies.
- Expression of a protein kinase ZAP-70 is increased in some (poorer prognostic) cases.
- Degree of somatic mutation in IgH immunoglobulin gene relates to prognosis (Table 25.1).

Course and prognosis

Many patients present at an early stage, and subsequently remain stationary or progress. Others present with late-stage disease. Some patients need no treatment for 10 years or more whilst in others the disease follows an aggressive course. Local immunoblastic transformation (Richter's syndrome) may be a terminal event. The natural history correlates with the maturity of the cell of origin, post-germinal centre (good) or pre-germinal centre (bad).

Table 25.1 Prognostic features of CLL.

	Favourable	Unfavourable
Sex	Female	Male
Stage	A	B,C
Lymphocyte doubling time	>1 year	<6 months
ZAP-70	Negative	Positive
Somatic mutation	Mutated	Germline
Cytogenetics	13q deletions	Trisomy 12, p53 deletions

Treatment

- Observation only for asymptomatic Stage A patients.
- Oral chlorambucil to lower the lymphocyte count and reduce lymph node and spleen size.
- Corticosteroids for bone marrow failure due to infiltration and for autoimmune haemolytic anaemia or thrombocytopenia.
- The purine analogue fludarabine is valuable, either alone or in combination with cyclophosphamide as initial or subsequent therapy.
- Combination chemotherapy e.g. CHOP (see Chapter 29).
- Monoclonal antibodies, rituximab (anti-CD20) or Alemtuzimab (Campath10, antiCD52) may be used in late-stage disease.
- Support care (see Chapter 43).
- Splenectomy or splenic irradiation is useful if the spleen is large and causes local symptoms or is a result of hypersplenism.

Variants of chronic lymphocytic leukaemia

Prolymphocytic leukaemia (PLL, Fig. 25c(i)) resembles CLL but usually occurs in older (>70 years) patients, the white cell count is high and responds poorly to treatment.

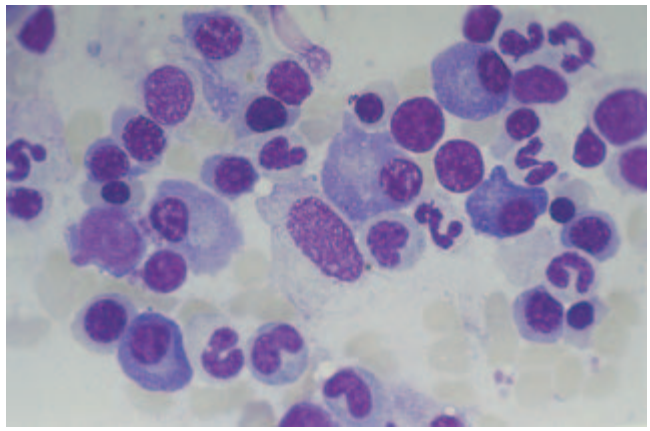
Hairy cell leukaemia (HCL, Fig. 25c(ii)) is rare (male/female ratio of 4 : 1, peak age of 55 years), presents with splenomegaly and pancytopenia. 'Hairy cells' are present in bone marrow and blood. Infections are frequent. They are B cells which stain for tartrate resistant acid phosphatase (TRAP). Effective treatments include 2-chlorodeoxyadenosine (2-CDA) deoxycoformycin, interferon-α and splenectomy. The prognosis is excellent.

T-cell variants of CLL, PLL and HCL are much rarer than B-cell types and are more aggressive.

Leukaemia/lymphoma syndromes. Circulating lymphoma cells may occur in different non-Hodgkin lymphomas, e.g. follicular lymphoma, mantle cell lymphoma, lymphoplasmacytoid lymphoma and adult T-cell leukaemia/lymphoma.

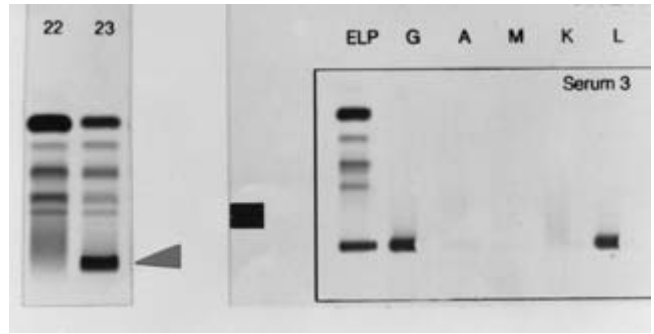
26 Multiple myeloma

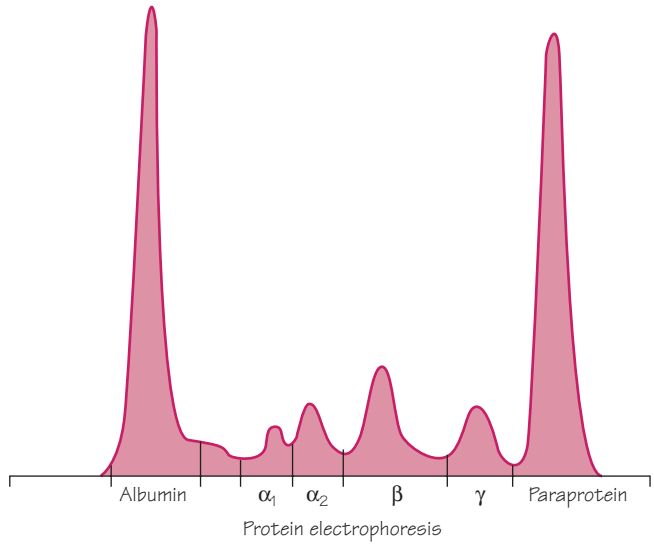
(a) Multiple myeloma: bone marrow showing infiltration by plasma cells.



(b) Multiple myeloma: protein electrophoresis. (i) Lane 22 shows a normal patient. Patient 23 has a paraprotein. The panel on the right shows that this paraprotein reacts with IgG (G) and λ (L) antisera, and is therefore of IgG λ type. (ii) Data from lane 23 presented graphically and numerically.

(i)

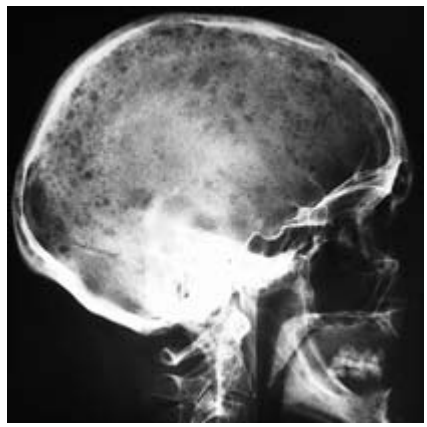




(ii)

Name	%	g/L	Normal (g/L)
Albumin	27.2	24.8	35 – 47
Gammaglobulin	2.1	1.9	25 – 33
Paraprotein	47.6	43.4	

(c) Multiple myeloma: skull X-ray showing multiple lytic lesions.



Multiple myeloma (MM) is a malignant disorder of plasma cells characterized by:

1 a monoclonal paraprotein in serum and/or urine;
2 bone changes leading to pain and pathological fractures
3 excess plasma cells in the bone marrow.

Incidence

Approximately 50 cases/million population; 15% of lymphoid malignancies; 2% of all malignancies; twice as common in black than white people; slightly more common in males than females; median age at diagnosis 71 years.

Aetiology and pathogenesis

The aetiology is unknown. The cell of origin is probably a postgerminal centre B-lymphoid cell. The cells all secrete the same immunoglobulin (Ig) or Ig component, e.g. part of a heavy chain attached to a light chain or light chain (κ or λ) alone. Rarely (<1%) the cells are non-secretory. Interleukin-6 (IL-6) from myeloma cells themselves or accessory cells promotes plasma cell growth. Tumour necrosis factor (TNF) and IL-1 mediate bone resorption. Oncogene mutations (e.g. *ras*, p53, *myc*) and translocations to 14q occur. Chromosome 13q deletions generally imply a poor prognosis.

Clinical features

- Skeletal involvement—bone pain, especially lower backache, or pathological fracture.
- Marrow infiltration—features of bone marrow failure.
- Infection—lack of normal immunoglobulins (immune paresis) and neutropenia.

• Renal failure occurs in up to one-third of patients and is caused by hypercalcaemia, infection, deposition of paraprotein or light chains, uric acid or amyloid.
• Amyloidosis may cause macroglossia, hepatosplenomegaly, cardiac or renal failure, carpal tunnel syndrome and autonomic neuropathy.

Laboratory features

• Anaemia is frequent, often with neutropenia and thrombocytopenia. Erythrocyte sedimentation rate (ESR) often >100 mm/h.
• Blood film shows rouleaux with a bluish background staining, caused by the protein increase. Leucoerythroblastic picture may be present.
• Bone marrow shows >10% plasma cells, often with multinucleate and other abnormal forms (Fig. 26a).
• A paraprotein in serum and/or Bence Jones protein (light chains) in urine with suppression of normal serum immunoglobulins is usual (Fig. 26b).
• The paraprotein is IgG in 70%; IgA in 20%; IgM is uncommon; IgD and IgE are rare.
• Serum β_2 microglobulin (β_2M) often raised and higher levels correlate with worse prognosis.
• X-rays, CT scan or MRI show lytic lesions typically in skull and axial skeleton and/or osteoporosis, often with pathological fractures (Fig. 26c). Occasional patients show localized plasma cell deposits, typically in the axial skeleton (multiple or solitary plasmacytoma).
• Prognostic data include haemoglobin level, serum levels of β_2M, serum creatinine and extent of skeletal disease.

Treatment

• Symptomless patients who are stable with normal blood counts and renal function, no skeletal disease and low levels of paraprotein warrant observation rather than therapy.
• Chemotherapy: initial treatment depends on age. In patients >65 years, induction is usually with melphalan and prednisolone or combination chemotherapy, e.g. Adriamycin, BCNU, cyclophosphamide and melphalan (ABCM) given intermittently every 4–6 weeks.
• Most patients will reach a stable (plateau) phase (clinically well with near normal blood count, <5% plasma cells in bone marrow, stable paraprotein level) after 4–6 cycles of treatment. This lasts 1–3 years. α-Interferon may prolong duration of plateau phase.
• Younger patients (<65 years) benefit from intensive induction with courses of, for example, vincristine, Adriamycin and dexamethasone (VAD) or cyclophosphamide, dexamethazone and thalidomide (CDT), followed by high-dose chemotherapy, for example with high-dose melphalan, followed by autologous peripheral blood stem cell transplant (PBSCT).
• Most patients relapse and median survival is 4–6 years from diagnosis. Relapsed cases may be retreated with initial therapy or with other combinations, e.g. idarubicin and dexamethasone.
• New drug therapies include thalidomide and the proteosome inhibitor bortezimide (Velcade).
• Radiotherapy is helpful in relieving pain from localized skeletal disease; hemi-body radiotherapy may help to control systemic disease.
• Allogeneic SCT may be curative if applied to selected patients early in the course of the disease.
• Supportive care includes hydration to prevent/treat renal failure, allopurinol to prevent hyperuricaemia, hydration, steroids and bisphosphonates for hypercalcaemia, antibiotics, erythropoietin and blood components. Bisphosphonates (e.g. oral sodium clodronate or ibandronate, or intravenous pamidronate or zoledronate) are useful in reducing skeletal complications and may improve survival. Surgery may be required for complications (e.g. pathological fracture, spinal cord compression). Plasma exchange is helpful in reducing the paraprotein level quickly.

Related disorders

Benign monoclonal gammopathy (also termed monoclonal gammopathy of undetermined significance, MGUS) is an indolent disorder, more common than myeloma and characterized by a low (< 20 g/L) and stationary serum level of paraprotein, no reduction in normal immunoglobulins, absence of skeletal abnormalities and of Bence Jones protein and less than 10% plasma cells in the marrow. It may progress slowly to myeloma or lymphoma in approximately 10–30% of patients.

Primary amyloidosis also shows less than 10% marrow plasma cells and no skeletal lesions but Bence Jones protein and low-level serum paraprotein may occur (see p. 87). Treatment as for myeloma may be beneficial.

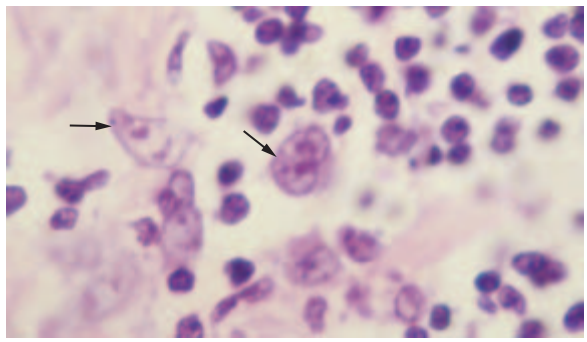
Solitary plasmacytoma may occur in bone or in soft tissues, a low level of serum paraprotein may occur and some cases later develop myeloma.

Waldenström's macroglobulinaemia is a chronic lymphoproliferative disorder with a median age at diagnosis of 72 years, and is associated with an IgM paraprotein. Hyperviscosity is common and may cause visual disturbance, central nervous system changes (confusion, impaired consciousness level) and headache. Cells resembling plasma cells and lymphocytes are present in the marrow and often in the spleen and lymph nodes. Treatment is usually as for a low grade lymphoma.

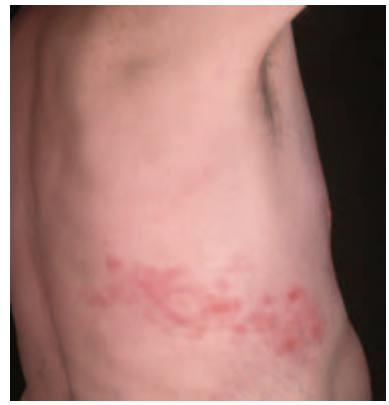
Plasma cell leukaemia is an aggressive disorder in which large numbers of plasma cells circulate. The prognosis is poor.

27 Lymphoma I: Hodgkin lymphoma (Hodgkin's disease)

(a) Hodgkin lymphoma: lymph node biopsy showing a Reed–Sternberg cell (multinucleate cell) (arrows).

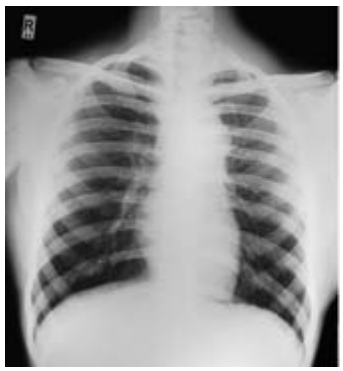


(b) Hodgkin lymphoma: varicella zoster infection.

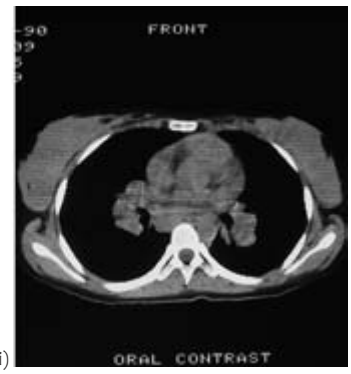


(c) Hodgkin lymphoma: (i) chest X-ray and (ii) CT scan showing hilar lymphadenopathy.

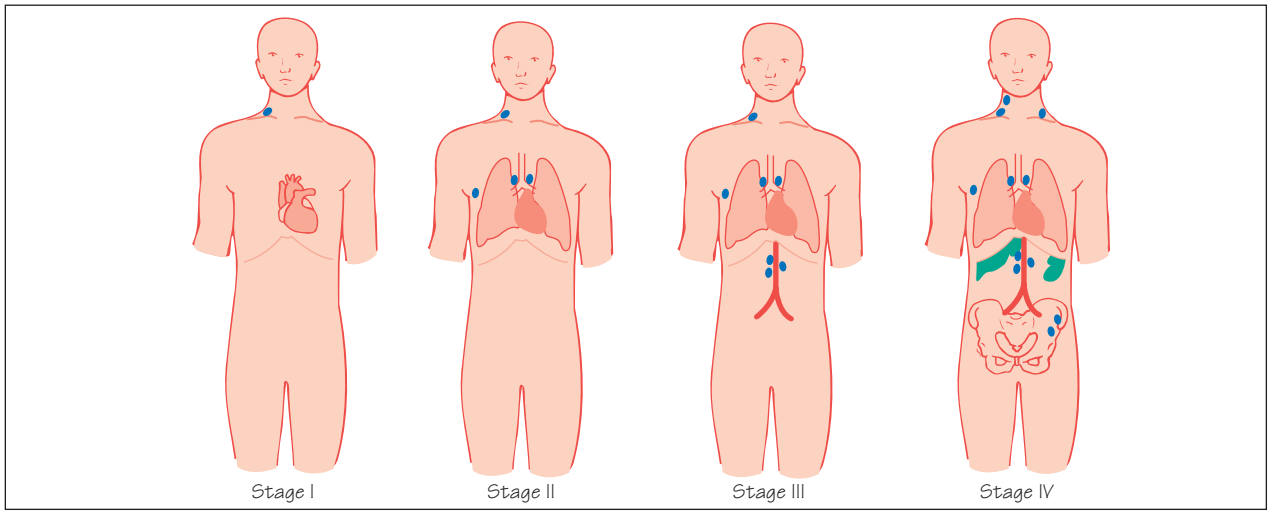
(i)



(ii)



(d) Hodgkin lymphoma: clinical features and staging. Stage I: involvement of a single lymph node region or structure; stage II: involvement of two or more lymph node regions on the same side of the diaphragm; stage III: involvement of lymph node regions or structures on both sides of the diaphragm; stage IV: involvement of other organs, e.g. liver, bone marrow, CNS. A: no symptoms; B: fever, night sweats, weight loss >10% in preceding 6 months; X: bulky disease; >1/3 widening of mediastinum; 10cm max dimension of nodal mass; E: extralymphoid disease (e.g. in lung, skin).



Lymphoma is a clonal neoplastic proliferation of lymphoid cells originating in lymph nodes or other lymphoid tissue. It is a heterogeneous group of disorders, divided into Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). Approximately 200 new cases are diagnosed each year per million population, with a ratio of NHL/HL of approximately 6 : 1; the incidence is rising.

Aetiology and epidemiology

Hodgkin lymphoma is more prevalent in males than females (M/F ratio 1.5–2.0:1) and has two peak incidences in age range of 15–40 and >60 years. The cause is not known, but Epstein–Barr virus (EBV) infection may be a cofactor.

Histological classification

This is well defined and of prognostic significance (see p. 64). Reed–Sternberg (RS) cells are characteristic of HL (Fig. 27a) but are usually outnumbered by a nonmalignant reactive infiltrate of eosinophils, plasma cells, lymphocytes and histiocytes. HL is of B-cell origin.

Clinical features

- Enlarged, painless lymphadenopathy (Fig. 27c) (typically cervical) is the characteristic presentation. The nodes often fluctuate in size, and alcohol ingestion may precipitate pain.
- Hepatic and splenic enlargement may occur.
- Systemic symptoms (fever, weight loss, pruritus and drenching night sweats) occur in 25%.
- Extranodal disease is uncommon but lung, CNS, skin and bone involvement may occur.
- Infection caused by defective cell-mediated/humoral immunity (Fig. 27b).

Laboratory features

- Anaemia (normochromic, normocytic).
- Leucocytosis (occasionally eosinophilia).
- Leucoerythroblastic blood film.
- Raised erythrocyte sedimentation rate (ESR), raised lactate dehydrogenase (LDH)—useful as prognostic marker and for monitoring response—and abnormal liver function tests.

Staging

Staging influences both treatment and prognosis. Clinical staging with careful physical examination is followed by cervical, thoracic, abdominal and pelvic CT or MRI scanning (Fig. 27c). Bone marrow aspirate and trephine are performed to detect marrow involvement. The most commonly used staging system is the Cotswold Classification (Fig. 27d). PET scanning (see p. 64) is particularly useful for detecting residual disease *post* therapy.

Treatment

This depends principally on stage:

- Radiotherapy alone may be used for patients with clinical or pathological stage IA or IIA disease with favourable histology.
- Advanced stages (IB, IIB, III and IV) HD should be treated with combination chemotherapy (CCT) using one of the standard regimes (e.g. six cycles of Adriamycin, Bleomycin, Vinblastine and Dacarbazine, ABVD).
- For bulky mediastinal disease, especially common in young females with nodular sclerosing HL, chemotherapy followed by deep X-ray therapy (DXT) (combined modality therapy) may be given and local DXT may be needed for other sites of bulky or resistant disease.

For complications of treatment see Chapter 43.

Relapsed disease

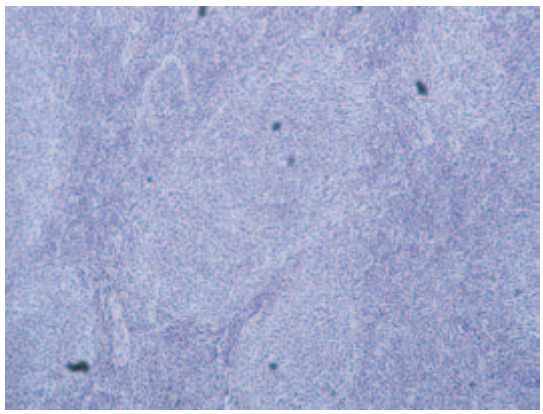
Patients who relapse following DXT alone generally have a very good response to CCT (>80% complete remission (CR) rate). Patients initially treated with chemotherapy who relapse after a remission lasting more than 1 year are likely to achieve CR again, and up to 50% may be cured with CCT. However, patients relapsing within 1 year of initial therapy, or failing to achieve complete remission, have a poorer prognosis and should be considered for high-dose therapy with stem cell rescue (see Chapter 42).

Prognosis

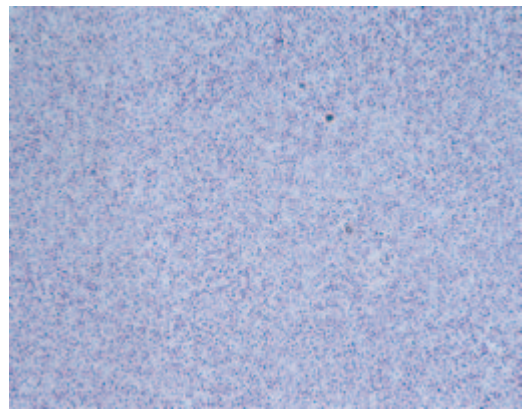
Stage is of paramount importance for HL. While >90% of stage I and II may be cured, the rate falls progressively to 50–70% of stage IV patients. Older patients generally do less well, as do those with lymphocyte-depleted histology.

28 Lymphoma II: non-Hodgkin lymphoma—aetiology and diagnosis

(a) Low-grade (indolent) NHL: lymph node biopsy showing follicular (nodular) structure.

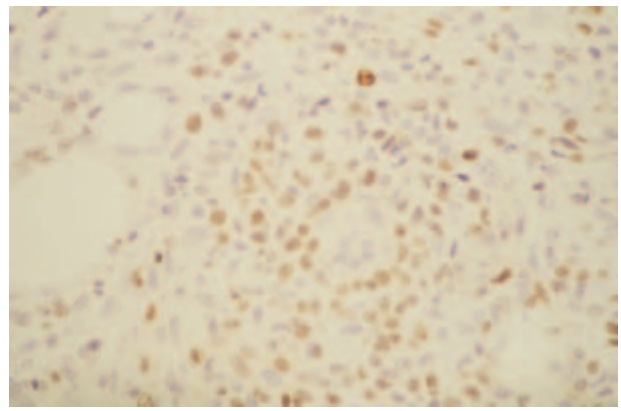


(b) High grade (diffuse) NHL: lymph node biopsy showing diffuse replacement of the node with large cells and destruction of normal nodal architecture.



(c) Low-grade NHL: immunostaining showing presence of BCL-2 protein (brown), which prevents apoptosis.

(d) High-grade NHL: immunostaining showing positive staining for Ki67, indicating high proliferation rate.



The non-Hodgkin lymphomas (NHLs) are a diverse group of disorders, which encompass a range of clinical presentations, histological appearances and prognostic categories.

Aetiology and epidemiology

Non-Hodgkin lymphoma occurs at all ages, with indolent tumours being most common in the elderly. There is clonal expansion from a normal cell which is 'frozen' at a particular level of differentiation. Most NHLs are B-cell disorders. Environmental factors include abnormal response to viral infection, e.g. Epstein–Barr virus (EBV) in Burkitt's lymphoma (BL) and human T-cell leukaemia virus (HTLV-1) in adult T-cell leukaemia lymphoma (ATLL), or to bacterial infection (e.g. chronic *Helicobacter pylori* infection in gastric lymphoma), or to radiation or certain drugs (e.g. phenytoin). Autoimmune disease (e.g. Sjögren's syndrome, rheumatoid arthritis) and immune suppression (e.g. AIDS, post-transplant) also predispose to NHL. Chromosome translocations in NHL involving oncogenes and immunoglobulin genes include t(14;18) (follicular lymphoma, BCL-2 oncogene) and t(8;14) (BL, MYC oncogene). The t(11; 14) translocation in mantle cell lymphoma involves the immunoglobulin heavy chain locus on chromosome 14 and the BCL-1 locus on chromosome 11. The molecular consequence is overexpression of the protein cyclin D1, which plays an important role in cell cycle regulation.

Histological classification

At least six different classifications for NHL have existed but NHL is now classified as disease entities based on clinical, biological, cytogenetic and histological criteria in the WHO system (see p. 64). Membrane marker and molecular studies of NHL show most (>80%) to be derived from B cells (either follicle centre or from other zones in the lymph node), and the remainder are T cell or unclassified. Indolent NHL may evolve into aggressive disease (Figs 28a–d).

Clinical features

General

• Similar to Hodgkin lymphoma (HL), including painless lymphadenopathy, hepatic and splenic enlargement, systemic symptoms and infection.

• Extranodal disease is more common in NHL than in HL. Thus, gastrointestinal, central nervous system, skin, endocrine organ (including testes), pulmonary and ocular lymphomas will all have the characteristic presenting features of tumours affecting these organs.

Clinical features of subtypes of non-Hodgkin lymphoma

NHL is clinically heterogeneous, but various clinical patterns emerge:

• Indolent NHL (small lymphocytic lymphoma (SLL), follicular lymphoma) is usually of gradual onset and is often asymptomatic or presents as painless lymphadenopathy. It is more common in older patients (>60 years). Although the presentation may be with localized (stage 1 or 2) disease, the disease is typically widely disseminated (stage 3 or 4) at first presentation. The disease may only warrant observation, not treatment. Variants include some T-cell NHL (T-NHL) (e.g. mycosis fungoides) which affect the skin, and splenic marginal zone lymphoma predominantly causing splenomegaly.

• Mantle cell lymphoma is a form of NHL which may be indolent or intermediate in its clinical course. Lymphadenopathy, peripheral blood lymphocytosis, anaemia and splenomegaly and gastrointestinal involvement may all occur. The malignant cells typically have the chromosome translocation t(11;14) (see p. 62).

• Paraprotein-associated NHL is characterized by the presence of a paraprotein, lymphadenopathy and often splenomegaly. The paraprotein is typically of IgM type and in Waldenström's macroglobulinaemia (see p. 59) the malignant cells have lymphoplasmacytic histologic appearance. In contrast to myeloma, bone lesions do not usually occur.

• Gastrointestinal lymphomas include mucosa-associated lymphoid tissue (MALT) lymphoma, and T-cell NHL complicating coeliac disease.

• Epidemiology-defined aggressive NHL includes BL (African children, higher incidence in males than females, jaw tumours, EBV related); ATLL (Caribbean and Japan, hypercalcaemia, skin and lymphoid area involvement; HTLV-1 related) and AIDS-related NHL (usually aggressive and frequent central nervous system disease).

• Aggressive NHL often has an acute presentation. Stage 1 or 2 disease is more common at presentation than in indolent lyphomas; advanced stage disease with liver, spleen and extra-nodal disease also occurs, and occasionally presentation is with bone marrow and/or peripheral blood disease, similar to acute leukaemia.

Laboratory features

In addition to the changes seen in HL, NHL may cause:

• pancytopenia as a result of bone marrow involvement leading to bone marrow failure;

• peripheral blood lymphocytosis caused by the presence of lymphoma cells in the blood;

• paraprotein and hypogammaglobulinaemia;

• raised LDH;

• raised β_2 microglobulin.

DNA microarrays are valuable for diagnosis and prognosis.

Investigation

All patients with NHL will require careful clinical assessment followed by investigations leading to biopsy (and histological diagnosis) and staging. Investigations will typically include blood tests, lymph node biopsy, bone marrow biopsy and radiological examinations (CT, MRI and PET scanning).

Staging

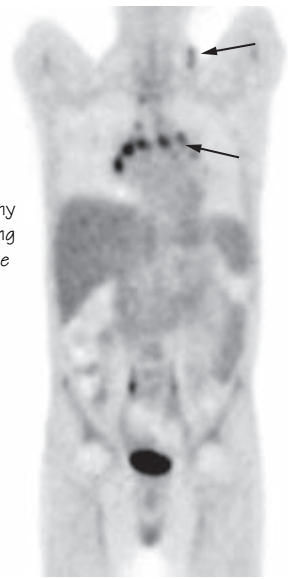
This is of less importance than in HL. More than 50% of patients with indolent NHL have stage IV disease, whereas aggressive NHL is often of early stage.

29 Lymphoma III: non-Hodgkin lymphoma—treatment and prognosis

(a) The WHO classification. CLL, chronic lymphocytic leukaemia; HD, Hodgkin's disease; PLL, prolymphocytic leukaemia; SLL, small lymphocytic lymphoma; SLVL, splenic lymphoma with villous lymphocytes.

B-cell neoplasms		T/NK-cell neoplasms
	Indolent/chronic (untreated survival measured in years)	
	Indolent disseminated lymphoma/leukaemia	
B-cell CLL/PLL/SLL Lymphoplasmacytic lymphoma Hairy cell leukaemia Plasmacytoma/myeloma Splenic marginal zone lymphoma/SLVL		T-cell CLL/PLL Large granular lymphocyte leukaemia (LGL)
	Indolent extranodal lymphomas	
Extranodal marginal zone/MALT Lymphoma		Mycosis fungoides
	Indolent nodal lymphomas	
Follicle centre lymphoma (follicular lymphoma) (grades 1, 2, 3) Mantle cell lymphoma		
	Aggressive (untreated survival measured in months)	
Diffuse large B-cell lymphoma Mediastinal B-cell lymphoma		Anaplastic large-cell lymphoma Peripheral T-cell lymphomas (subtypes)
	Highly aggressive (untreated survival measured in weeks)	
Precursor B-lymphoma leukaemia/lymphoma Burkitt's lymphoma		Precursor T-lymphoblastic leukaemia/lymphoma Adult T-cell lymphoma/leukaemia (HTLV-1)
	Hodgkin lymphoma	
Lymphocyte predominance, nodular ± diffuse	Classic HD Nodular sclerosis Mixed cellularity Lymphocyte depletion Lymphocyte-rich classical HD	
Diffuse large cell lymphoma, Hodgkin's-like/related		

* Small lymphocytic lymphoma



(b) Positron emission tomography (PET) scan showing persisting areas of active disease in the mediastinum and cervical region (arrows).

Treatment

This depends principally on histology. Paradoxically, aggressive tumours respond more dramatically to treatment and are more likely to be cured than indolent tumours; however, they are also more aggressive if untreated, frequently relapse and are associated with higher short- to medium-term mortality.

Aggressive

Localized (stage I or II) aggressive NHL, deep X-ray therapy (DXT) with adjuvant combination chemotherapy (CCT) (e.g. three cycles of CHOP, a 21-day cycle of cyclophosphamide, hydroxydaunorubicin (Adriamycin), vincristine and prednisolone) with anti-CD20 monoclonal antibody (rituximab) if needed. Advanced stage aggressive NHL is treated with CCT (usually CHOP-rituximab, up to complete remission plus at least two cycles) followed by DXT to sites of bulky disease. Patients with lymphoblastic lymphoma are best treated with chemotherapy as for acute lymphoblastic leukaemia; such patients are candidates for allogeneic stem cell transplantation (see Chapter 42). Intrathecal therapy is given for patients with a high risk of CNS disease.

Indolent

Asymptomatic patients may be followed closely without therapy for months or even years. When treatment is required, options include DXT, single-agent chemotherapy (e.g. oral chlorambucil) and CCT, e.g. cyclophosphamide, vincristine, prednisone (CVP) with or without rituximab or DXT. The relapse rate is high. Trials of aggressive chemotherapy followed by allogenic or autologous stem cell transplantation are in progress for younger patients.

Relapsed disease

Over 50% of NHL patients will relapse after initial therapy. A PET scan (Fig. 29b) can be helpful in detecting relapse. Indolent NHL will typically respond to single agent, CCT or radiotherapy. Relapsed aggressive NHL carries a poor prognosis but may respond to second-line CCT regimes followed by autologous or allogeneic stem cell transplantation.

New therapies

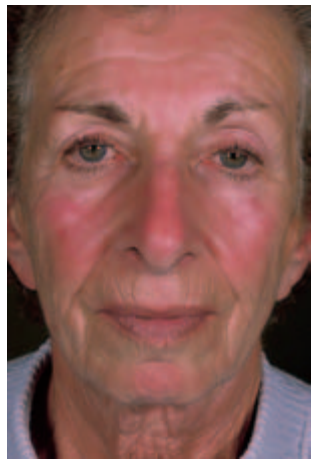
Monoclonal antibodies, some bound to one or other radioactive toxin (see Chapter 43), interleukin-2, interferon have been introduced into different regimes.

Prognosis

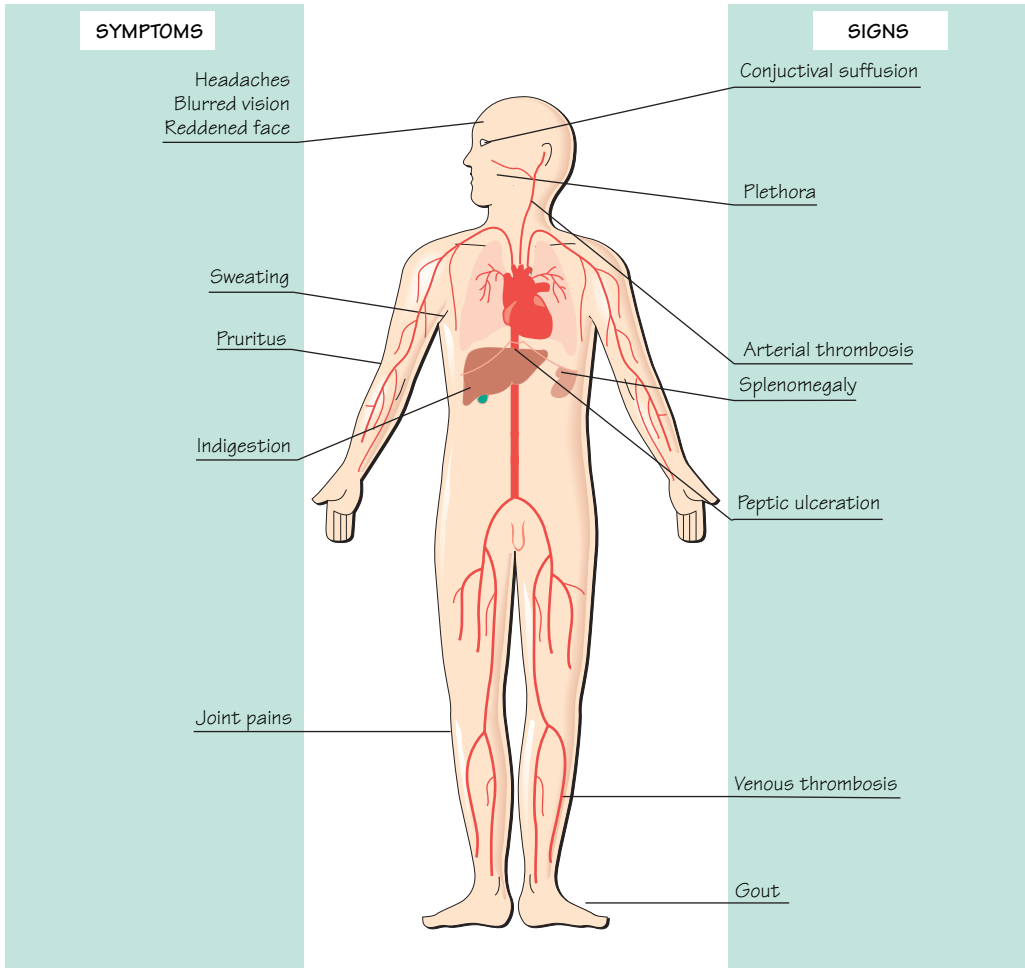
Prognosis in NHL is dependent on histology, laboratory features, stage and the score derived from the International Prognosis Index (IPI). The IPI score is derived from five factors, each scored as 1 mark (age >60, raised LDH, impaired performance status, stage III/IV and two or more sites of extranodal disease). Low, intermediate and high risk groups are derived. Long-term side effects of therapy are considered in Chapter 43.

30 Myeloproliferative disorders I: polycythaemia

(a) Polycythaemia rubra vera: patient with plethora.



(b) Polycythaemia rubra vera: clinical features.



Myeloproliferative disorders (MPD) are chronic diseases caused by clonal proliferation of bone marrow stem cells leading to excess production of one or more haemopoietic lineage. The clinical syndromes include polycythaemia rubra vera (red cells), essential thrombocythaemia (platelets), chronic myeloid leukaemia (white cells) and myelofibrosis in which there is a reactive fibrosis of the marrow and extramedullary haemopoiesis in the liver and spleen. Intermediate forms may occur and the diseases may all transform into acute myeloid leukaemia.

Polycythaemia

Polycythaemia (erythrocytosis) is defined as an increase in haemoglobin concentration above normal (Table 30.1). True polycythaemia exists when the total red cell mass (RCM), measured by dilution of isotopically labelled red cells, is increased above normal. Spurious (pseudo or stress) polycythaemia exists when an elevated haemoglobin concentration is caused by a reduction in plasma volume.

Polycythaemia rubra vera

Aetiology and pathophysiology

Polycythaemia rubra vera (PRV) is a primary neoplastic disorder in which bone marrow erythropoiesis is increased, usually accompanied by increased thrombopoiesis and granulopoiesis. A mutation (Val 617 Phe) of the Janus kinase 2 (JAK 2) gene is present in 65–97% of cases and also in a proportion of patients with essential thrombocythaemia and myelofibrosis.

Clinical features

- PRV occurs equally in males and females, typically over 55 years of age.
- Raised RCM causes a ruddy complexion (Fig. 30a) and conjunctival suffusion; hyperviscosity may lead to headaches and visual disturbance.
- Thrombosis (e.g. deep vein thrombosis (DVT), Budd–Chiari syndrome, stroke) is also caused by hyperviscosity and increased platelets.
- Haemorrhage, especially gastrointestinal, may occur.
- Excess histamine secretion from basophils leads to increased gastric acid and peptic ulcer is frequent.

Table 30.1 Causes of polycythaemia.

True polycythaemia
Primary
Polycythaemia rubra vera (PRV)
Secondary
Erythropoietin appropriately increased
High altitude
Cyanotic congenital heart disease
Chronic lung disease
Haemoglobin variant with increased oxygen affinity
Erythropoietin inappropriately increased
Renal disease: hypernephroma, renal cyst, hydronephrosis
Uterine myoma
Other tumours, e.g. hepatocellular carcinoma, bronchial carcinoma
Relative (spurious) polycythaemia
Plasma volume depletion
Stress ('pseudo-polycythaemia')
Dehydration
Diuretic therapy

- Pruritus, typically after a hot bath, and gout, caused by increased uric acid production, also occur frequently.
- Enlarged spleen is found in 75% of patients and distinguishes PRV from other causes of polycythaemia (Fig. 30b).

Laboratory features

- Raised haematocrit, haemoglobin concentration, red cell count and RCM.
- 75% of patients have raised white cells (neutrophil leucocytosis) and/or platelets.
- Neutrophil alkaline phosphatase (NAP) score, serum B_{12}, serum B_{12} binding capacity and serum uric acid are all usually raised.
- Bone marrow is hypercellular with prominent megakaryocytes, iron stores are depleted because of excessive iron utilization, and the trephine biopsy may show mildly increased reticulin.
- Abdominal ultrasound to exclude renal disease, assess spleen size.
- Culture of peripheral blood cells shows spontaneous formation of erythroid colonies.

Differential diagnosis

Secondary or reactive polycythaemia may occur in conditions where arterial oxygen saturation is reduced, leading to a physiological rise in EPO, or when EPO levels are inappropriately raised (e.g. caused by secretion of EPO by a renal neoplasm). The JAK 2 mutation is absent.

Spurious (*pseudo*) *polycythaemia* arises when plasma volume is reduced by dehydration, vomiting or diuretic therapy. A common form occurs particularly in young male adults, especially smokers, and is associated with stress, increased vasomotor tone and hypertension (Gaisböck's syndrome). The white cell and platelet counts are normal, as is the bone marrow and RCM. If the packed cell volume (PCV) is over 0.50, it is treated by venesections; patients should reduce weight, stop smoking, moderate alcohol intake and avoid diuretics.

The following additional tests are occasionally required:

- Chest X-ray; arterial blood gas analysis to exclude lung disease.
- Haemoglobin oxygen dissociation curve to identify a variant haemoglobin with increased oxygen affinity.
- Serum EPO assay.

Treatment

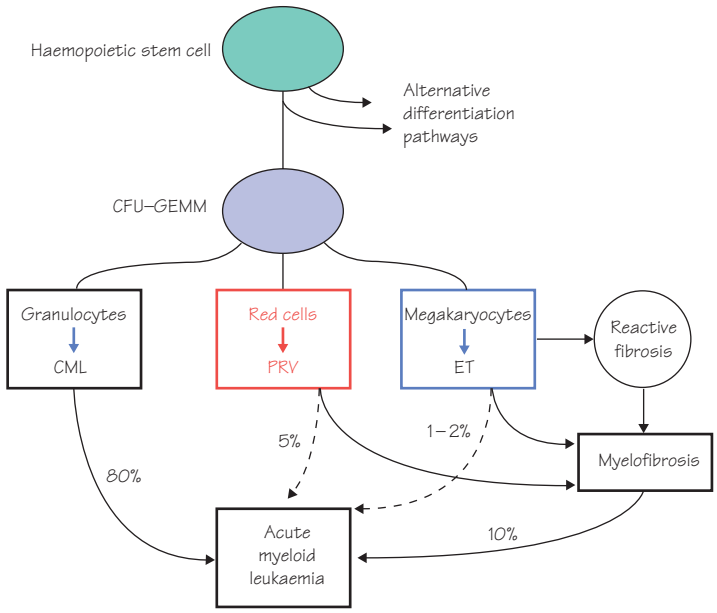
- Thrombosis is the main cause of morbidity and mortality and its incidence can be reduced by maintaining the PCV below 0.45 and platelets below 600×10^9/L. Aspirin (75 mg daily) is often used to inhibit platelet function.
- Regular venesection is used initially to lower the PCV.
- Chemotherapy (e.g. oral hydroxyurea) is also usually required.
- ^{32}P is a β-emitter which is taken up and concentrated by bone and may be used to give prolonged myelosuppression (about 2 years) in older patients.
- Busulfan may be given orally. It has a more prolonged action than hydroxyurea and more side-effects and is now more rarely used.

Prognosis

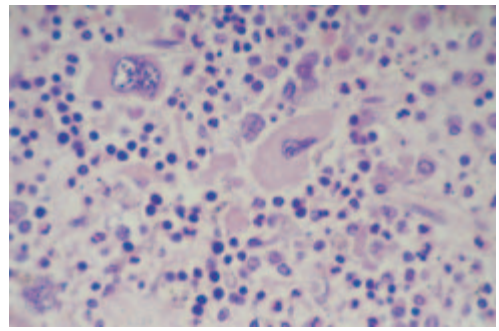
Median survival is about 16 years. Up to 30% of patients develop myelofibrosis (see below). Acute myeloid leukaemia occurs in up to 5% of patients, probably increased in patients treated with a ^{32}P and some types of chemotherapy.

31 Myeloproliferative disorders II: essential thrombocythaemia and myelofibrosis

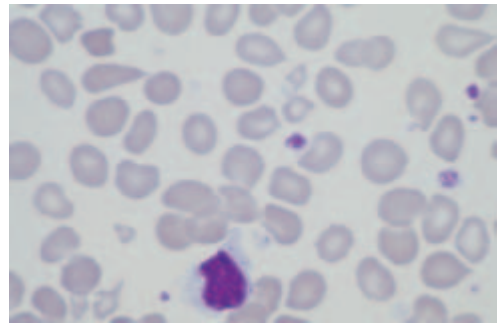
(a) The myeloproliferative disorders.



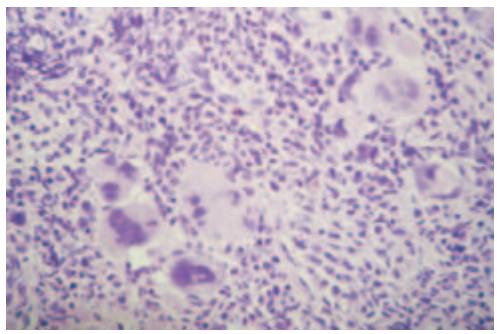
(b) Myelofibrosis: spleen section showing extramedullary haemopoiesis.



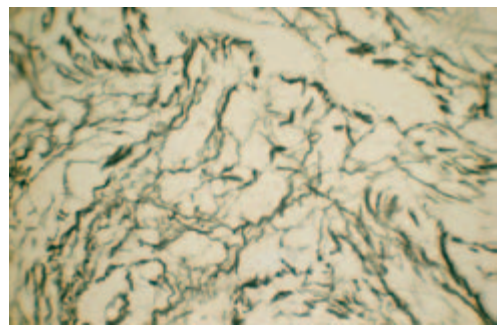
(c) Myelofibrosis: peripheral blood film showing aniso/poikilocytosis, teardrop forms and giant platelets.



(d) Myelofibrosis: bone marrow biopsy showing increased cellularity and large numbers of megakaryocytes.



(e) Myelofibrosis: bone marrow biopsy (reticulin stain) showing increased reticulin.



Essential thrombocythaemia

Essential thrombocythaemia (ET) is defined as persistent elevation of the peripheral blood platelet count as a result of increased marrow production in the absence of a systemic cause for thrombocytosis (Table 31.1, Fig. 31a).

Aetiology and pathophysiology

Similar to PRV, distinction between the two conditions is not exact. In younger adults ET occurs more frequently than PRV.

Clinical features

- Thrombosis, both arterial (peripheral vessels with gangrene of toes, cerebral, coronary and mesenteric arteries) and venous (e.g. Budd–Chiari syndrome, DVT), headaches, visual disturbance and peripheral vascular disease occur.
- At least 20% of patients are asymptomatic and detected as an incidental finding.
- Excessive haemorrhage may occur spontaneously or after trauma or surgery.
- Pruritus and sweating are uncommon.
- Splenomegaly in about 30% of patients; in others the spleen is atrophied because of infarction.

Table 31.1 Causes of an elevated platelet count.

Primary
Essential thrombocythaemia
As part of another myeloproliferative disorder, e.g. PRV, CML, myelofibrosis
Reactive
Iron deficiency
Haemorrhage
Severe haemolysis
Trauma, postoperatively
Infection, inflammation
Malignancy
Hyposplenism

CML, chronic myeloid leukaemia; PRV, polycythaemia rubra vera.

Laboratory features

- Platelet count is persistently raised and often >1000 × 10^9/L, raised red cell and/or white cell count is present in about 30%.
- Blood film shows platelet anisocytosis with circulating megakaryocyte fragments. Autoinfarction of the spleen causes changes in red cells (target cells, Howell–Jolly bodies).
- The JAK 2 mutation is present in 30–50% of cases.
- The NAP score may be raised or normal.
- Serum uric acid is often raised, serum LDH may be raised.
- Bone marrow is hypercellular with increased numbers of megakaryocytes, often in aggregates.
- Defective platelet function, especially defective aggregation in response to adenosine diphosphate (ADP) and adrenaline, may help to distinguish primary from reactive thrombocythaemia.

Treatment

- Chemotherapy, e.g. hydroxyurea, is used to maintain the platelet count below 600 × 10^9/L.
- α-Interferon and oral anagrelide are also effective but have more side-effects.
- Aspirin (75 mg daily), except in those with haemorrhage.

Prognosis

Median survival is more than 20 years; thrombosis and haemorrhage are the main causes of morbidity and mortality. Transformation to AML may occur.

Myelofibrosis

Myelofibrosis (myelosclerosis, agnogenic myeloid metaplasia) is characterized by splenomegaly, extramedullary haemopoiesis, a leucoerythroblastic blood picture and replacement of bone marrow by collagen fibrosis.

Aetiology and pathophysiology

The primary defect is within the haemopoietic stem cell; fibrosis results from a reactive non-neoplastic proliferation of marrow stromal cells. One-third of patients have a preceding history of PRV or ET.

Clinical features

- Sexes affected equally; age of onset rarely below 50 years.
- Massive splenomegaly may lead to left hypochondrial pain and anaemia, leucopenia and thrombocytopenia (hypersplenism) (Fig. 31b).
- Fever, weight loss, pruritus, hepatomegaly and night sweats are frequent; gout, bone and joint pain are less common.
- Abdominal swelling, ascites and bleeding from oesophageal varices occur, caused by portal hypertension, in late stages.

Laboratory features

- Normochromic normocytic anaemia.
- Leucocytosis and thrombocytosis with circulating megakaryocyte fragments occur early; leucopenia and thrombocytopenia occur later.
- Blood film: red cell poikilocytosis with teardrop forms and circulating red cell and white cell precursors (leucoerythroblastic picture) (Fig. 31c).
- The JAK 2 mutation is present in about 50% of cases.
- Serum LDH is raised. Liver function tests are often abnormal because of extramedullary haemopoiesis.
- NAP score is usually raised.
- Bone marrow aspiration is usually unsuccessful ('dry tap'); the trephine biopsy shows increased cellularity, increased megakaryocytes and fibrosis (Figs 31d, e).

Treatment

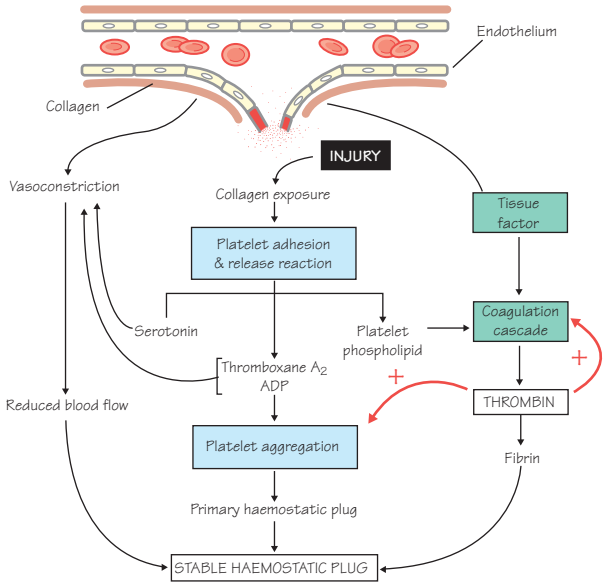
- Chemotherapy (e.g. hydroxyurea) for patients with hypermetabolism and myeloproliferation.
- Thalidomide improves marrow function and reduces spleen size in about a third of cases.
- Supportive therapy with red cell transfusions, folic acid and occasionally platelet transfusions. Iron chelation may be needed.
- Allopurinol to prevent hyperuricaemia and gout.
- Splenectomy or splenic irradiation to reduce symptoms from splenomegaly, anaemia or thrombocytopenia (selected patients only).
- Allogeneic bone marrow transplantation has cured a few younger patients (<50 years).

Prognosis

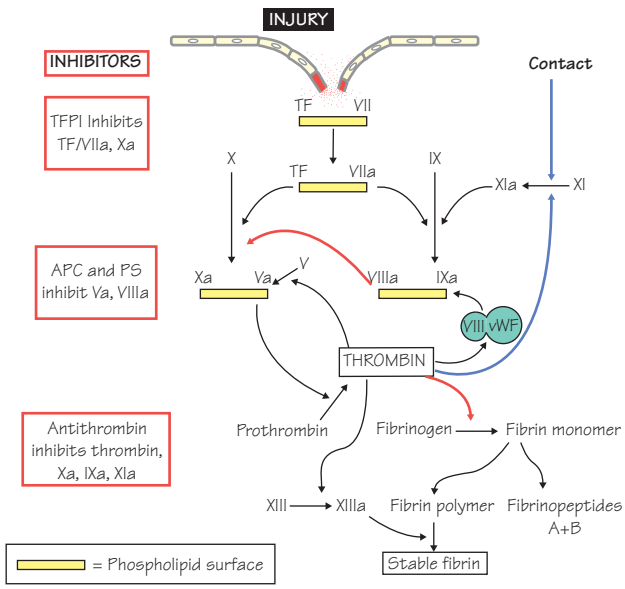
Median survival is about 5 years; acute leukaemia occurs in about 20%.

32 Normal haemostasis

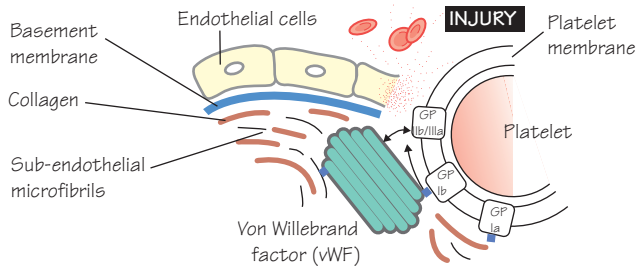
(a) Haemostasis.



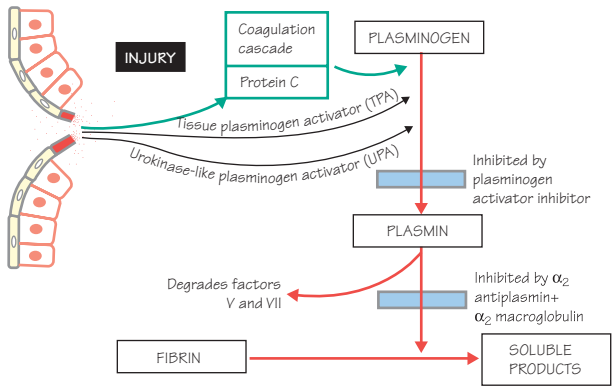
(b) The coagulation pathway. Injury initiates release of tissue factor (TF) which binds and activates factor VII. The TF VIIa complex activates factors X and IX; the activity of the TF VIIa complex is inhibited by TF pathway inhibitor (TFPI). The VIIIa–IXa complex amplifies Xa production from X. Thrombin is generated from prothrombin by the action of Xa–Va complex and this leads to fibrin formation. Thrombin also: (i) activates FXI leading to increased FIXa production; (ii) cleaves FVIII from its carrier protein vWF activating FVIII; (iii) activates FV to FVa; and (iv) activates FXIII to XIIIa, which stabilizes the fibrin clot. Note that: (i) TFPI inhibits TF/VIIa, Xa; (ii) Activated PC (APC) and PS inhibit Va, VIIIa; and (iii) antithrombin inhibits thrombin, Xa, IXa. Extrinsic pathway, Factor VII. Intrinsic pathway, Factors XI, IX, VIII. Common pathway, Factors X, V, II, fibrinogen.



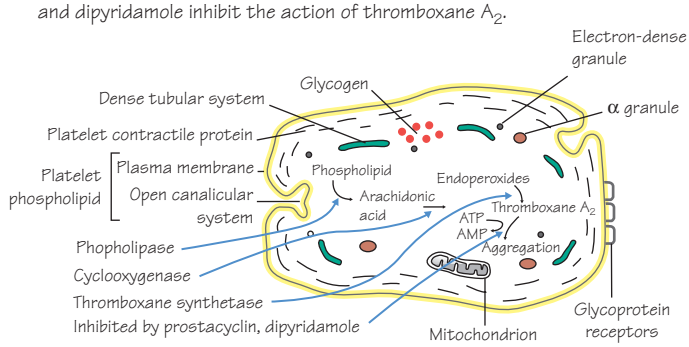
(c) Platelet adhesion. Subendothelial microfibrils bind von Willebrand factor (vWF) which in turn binds platelets at the glycoprotein 1b (GP1b) receptor. This binding exposes the platelet glycoprotein IIb/IIIa (GPIIb/IIIa) receptor which binds further with vWF. The GPIIb/IIIa receptor also binds fibrinogen to allow platelet–platelet aggregation. The platelet glycoprotein 1a (GP1a) receptor binds directly to collagen.



(d) Fibrinolysis. Injury causes release of TPA and UPA which, together with activated components from coagulation pathway and protein C, activate plasminogen to plasmin. Plasmin acts on insoluble fibrin to form a series of soluble products (fragment X, fragments Y+D, fragments E+D). Note that: (i) plasminogen activator inhibitor inhibits activation of plasminogen; and (ii) α_2 antiplasmin and α_2 macroglobulin inhibit action of plasmin.



(e) Diagram of a platelet. Platelets do not have nuclei. Electron dense granules contain platelet nucleotides (ADP), Ca^{2+} and serotonin. α granules contain a heparin antagonist (platelet factor 4), platelet derived growth factor, β thromboglobulin, fibrinogen and other clotting factors. Glycoproteins on the surface, e.g. Ia (adhesion to collagen), Ib (defective in Bernard–Soulier syndrome) and IIb/IIIa (defective in thrombasthenia) are important in adhesion and aggregation. The plasma membrane and canalicular system provides a large reactive surface on which plasma coagulation factors are absorbed and activated. Aspirin and sulphinpyrazone inhibit platelet function by inhibiting cyclo-oxygenase. Prostacyclin from endothelial cells and dipyridamole inhibit the action of thromboxane A_2.



Haemostasis (Fig. 32a) is the process whereby haemorrhage following vascular injury is arrested. It depends on closely linked interaction between:

- the vessel wall;
- platelets;
- coagulation factors.

The fibrinolytic system and inhibitors of coagulation ensure coagulation is limited to the site of injury.

The vessel wall

The intact vessel wall has an important role in preventing haemostasis. Endothelial cells produce:

- prostacyclin, which causes vasodilatation and inhibits platelet aggregation;
- protein C (PC) activator (thrombomodulin), which inhibits coagulation;
- tissue plasminogen activator (TPA) which activates fibrinolysis.

Injury to the vessel wall: (i) activates membrane-bound tissue factor which initiates coagulation (Fig. 32b); and (ii) exposes subendothelial connective tissue allowing binding of platelets to von Willebrand factor (vWF), a large, multimeric protein made by endothelial cells, which mediates platelet adhesion to endothelium and carries clotting factor VIII in plasma.

Platelets

Platelets have a large surface area onto which coagulation factors are adsorbed. Glycoproteins GPIb and IIb/IIIa allow attachment of platelets to vWF (Fig. 32c) and hence to endothelium. Collagen exposure and thrombin promote platelet aggregation and the platelet release reaction whereby platelets release their granule contents. Adenosine diphosphate (ADP) promotes platelet aggregation to form a primary haemostatic plug. Platelet prostaglandin synthesis is activated to form thromboxane A_2 which potentiates the platelet release reaction, promotes platelet aggregation and also has vasoconstrictor activity. Fibrin, produced by blood coagulation, binds to vWF and enmeshes the platelets to form a stable haemostatic plug. Activated platelets promote coagulation, as they have exposed phospholipid binding sites which are involved in activation of factor X and of prothrombin to thrombin in the coagulation cascade.

Thrombopoiesis

Megakaryocytes (see Fig. 1a) are large multinucleated cells derived from haemopoietic stem cells. Platelets break off from the megakaryocyte cytoplasm and enter the peripheral blood. Thrombopoietin is produced mainly in the liver and stimulates megakaryocyte and platelet production by increasing differentiation of stem cells into megakaryocytes, increasing megakaryocyte numbers and also by increasing the number of divisions of megakaryocyte nuclei (ploidy). Platelets (Fig. 32e) are non-nucleated cells required for normal haemostasis. They circulate for 7–10 days. Their lifespan is reduced when there is increased platelet consumption (thrombosis, infection and splenic enlargement). Platelets appear in peripheral blood films as granular basophilic forms with a mean diameter of 1–2 μm. The normal concentration is 140–400 $\times$ 10^9/L; a lower number is found in neonates (100–300 $\times$ 10^9/L) and among certain racial populations, e.g. in Southern Europe or the Middle East.

Coagulation factors

The proteins of the coagulation cascade are proenzymes (serine proteases) and procofactors which are activated sequentially (Fig. 32b). The cascade has been divided on the basis of laboratory tests into intrinsic, extrinsic and common pathways. This division is useful in understanding results of *in vitro* coagulation tests. *In vivo*, however, these pathways are closely interlinked. Coagulation begins when tissue factor activated on the surface of injured cells binds and activates factor VII; the complex activates factor IX which, with cofactor VIII, activates factor X to Xa.

Platelets accelerate the coagulation process by providing membrane phospholipid. The complex of Xa and Va, activated from factor V by thrombin, acts on prothrombin (factor II) to generate thrombin. Thrombin then converts fibrinogen into fibrin monomers, with the release of fibrinopeptides A and B. The monomers combine to form a fibrin polymer clot. Factor XIII crosslinks the polymer to form a more stable clot.

Thrombin has a number of key roles in the coagulation process:

1 It converts plasma fibrinogen into fibrin.

2 It amplifies coagulation by: (i) activating factor XI which increases IXa production; (ii) cleaving factor VIII from its carrier molecule vWF to activate it and augment Xa production; and (iii) activating factor V to factor Va.

3 It also activates factor XIII to factor XIIIa, which stabilizes the fibrin clot.

4 It potentiates platelet aggregation.

5 It binds to thrombomodulin on the endothelial cell surface to form a complex which activates protein C, which is involved in regulating coagulation.

Coagulation inhibitory factors

These inhibit the coagulation cascade and ensure the action of thrombin is limited to the site of injury:

- Antithrombin inactivates serine proteases, principally factor Xa and thrombin. Heparin activates antithrombin.
- α_2 macroglobulins, α_2 antiplasmin, α_2 antitrypsin and heparin cofactor II also inhibit circulating serine proteases.
- Proteins C and S are vitamin K-dependent proteins made in the liver. Protein C is activated via a thrombin–thrombomodulin complex (Figs 32d and Fig. 36b) and, like protein S, inhibits coagulation by inactivating factors Va and VIIIa; it also enhances fibrinolysis by inactivating the tissue plasmogen activator (TPA) inhibitor (Fig. 32d).
- Tissue factor pathway inhibitor (TFPI) inhibits the main *in vivo* coagulation pathway by inhibiting factors VIIa and Xa.

The fibrinolytic pathway (Fig. 32d)

Fibrinolysis is the process whereby fibrin is degraded by plasmin. A circulating pro-enzyme, plasminogen, may be activated to plasmin:

- following injury, by TPA and urokinase-like plasminogen activator (UPA) released from damaged or activated cells, or
- by exogenous agents, e.g. streptokinase, or by therapeutic TPA or UPA.

Plasmin digests fibrin (or fibrinogen) into fibrin degradation products (FDPs) and also degrades factors V and VII. Free plasmin is inactivated by plasma α_2 antiplasmin and α_2 macroglobulin.

Table 32.1 Laboratory tests of coagulation.

Screening test (normal range)	Abnormalities indicated (prolonged abnormal)	Most common cause of disorder
Prothrombin time (PT) (10–14 s)	Extrinsic and common coagulation pathways Deficiency/inhibition of factor VII, factors X, V, II and fibrinogen	Liver disease, Warfarin therapy, DIC
Activated partial thromblastin time (APTT or PTTK) (30–40 s)	Intrinsic and common coagulation pathways Deficiency/inhibition of one or more of factors XII, IX, VIII, X, V, II and fibrinogen	Liver disease, heparin therapy, haemophilia A and B, DIC
Thrombin time (14–16 s)	Deficiency or abnormality of fibrinogen; inhibition of thrombin by heparin or FDPs	DIC, heparin therapy, fibrinolytic therapy
Fibrin degradation products (<10 mg/mL)	Accelerated destruction of fibrinogen	DIC
Platelet aggregation tests	Abnormal platelet function	Drugs (e.g. aspirin), uraemia, von Willebrand's disease.
Euglobulin clot lysis time	Fibrinolytic pathway defect	Smoking

Laboratory tests of coagulation

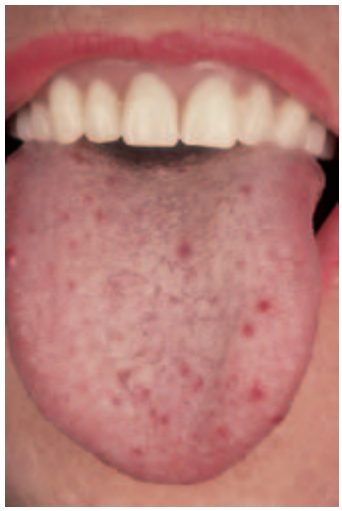
These are listed in Table 32.1.

Specialized tests

Individual coagulation factors can be assayed by functional tests or immunological methods. Platelet function tests include tests of platelet aggregation with different agonists, platelet function analyser-100 (PFA-100) which measures the length of time blood can be passed through a small orifice before the platelets completely occlude it, platelet adhesion and assessment of platelet granule contents. Tests for abnormalities leading to thrombosis (thrombophilia) are described in Chapter 36.

33 Disorders of haemostasis: vessel wall and platelets

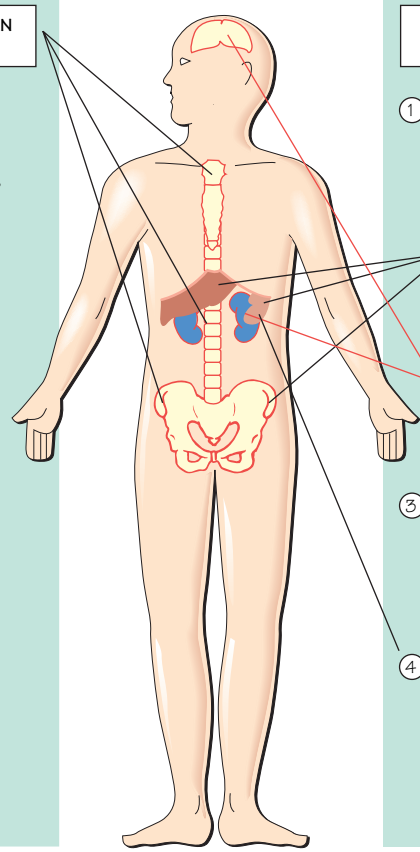
(a) Hereditary haemorrhagic telangiectasia: tongue showing multiple telangiectasia.



(b) Causes of thrombocytopenia.

DECREASED PRODUCTION BY BONE MARROW

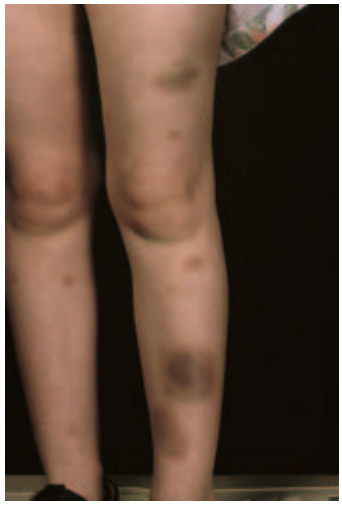
1. As part of generalized bone marrow failure
   - Aplastic anaemia, MDS, cytotoxic therapy, DXT, marrow infiltration, drugs
2. Selective reduction of megakaryocytes
   - Drugs (sulphonamides, thiazides, NSAIDs)
   - Congenital infection (CMV, rubella)
   - Congenital absence of megakaryocytes (rare)
3. Other
   - Megaloblastic anaemia,
   - HIV and other viral infections



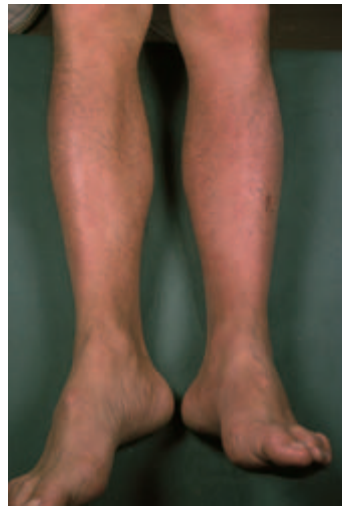
INCREASED PLATELET UTILIZATION

1. Immune
   - Auto-immune and allo-immune Antibodies made in bone marrow and RE system; coated platelets destroyed in spleen, liver and bone marrow
2. Thrombosis in small vessels
   - TTP inc. brain, kidney
   - HUS
   - vasculitis
   - microangiopathy
3. Disseminated coagulation (see DIC, Chapter 31)
4. Platelet-pooling
   - splenomegaly
   - giant haemangioma
   - dilution following massive transfusion

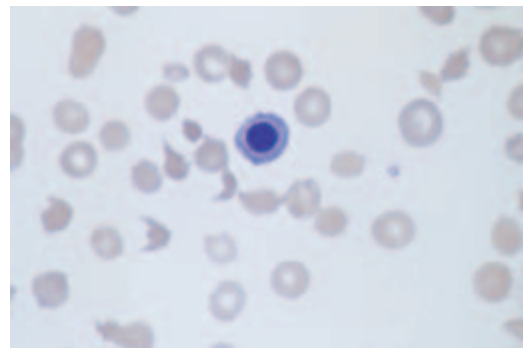
(c) Immune thrombocytopenia: multiple bruises, ecchymoses and purpura.



(d) Thrombocytopenia: petechial rash.



(e) Haemolytic uraemic syndrome (HUS): blood film showing red cell fragmentation with a circulating nucleated red blood cell and low platelet count.



Defective haemostasis with abnormal bleeding may be caused by:
- abnormalities of the vessel wall;
- thrombocytopenia;
- disordered platelet function;
- defective blood coagulation (see Chapters 34 and 35).

Vessel wall abnormalities

These are associated with easy bruising, purpura and ecchymosis and spontaneous bleeding from mucosal surfaces. The bleeding time, prothrombin time (PT), activated partial thromboplastin time (APTT) and platelet count are all normal.

Inherited

- Hereditary haemorrhagic telangiectasia. This is autosomal dominant with multiple dilated microvascular swellings, typically in oropharynx (Fig. 33a) and gastrointestinal tract, which bleed spontaneously or following minor trauma. Local treatment (e.g. nasal packing) may control bleeding; tranexamic acid helps to reduce bleeding. Chronic iron deficiency is frequent.
- Ehlers–Danlos syndrome, Marfan's syndrome and other rare connective tissue disorders.

Acquired

Causes include vitamin C deficiency (scurvy), steroid therapy, normal ageing (senile purpura), amyloid in blood vessels, cryoglobulinanaemia and immune complex deposition (e.g. purpura fulminans in septicaemia). Henoch–Schönlein purpura is an allergic vasculitis which follows an acute infection, usually in childhood, and may be associated with arthropathy, haematuria and gastrointestinal symptoms.

Platelets

Excessive bleeding caused by thrombocytopenia or disordered platelet function is mucosal (e.g. epistaxis, gastrointestinal bleeding or menorrhagia) or affects the skin (purpura, petechiae and ecchymoses). Symptoms usually occur when the platelet count is $<10 \times 10^9$/L but this may be higher when there is impaired platelet function.

Thrombocytopenia (platelets $<140 \times 10^9$/L) (Fig. 33b)

Congenital

This is rare: causes include congenital aplastic anaemia, thrombocytopenia with absent radii (TAR) syndrome or Wiskott–Aldrich syndrome (thrombocytopenia with eczema and hypogammaglobulinaemia). Congenital infection (e.g. rubella, cytomegalovirus) frequently leads to thrombocytopenia.

Acquired

This is a result of deficient platelet production or accelerated platelet destruction.

Autoimmune thrombocytopenia

The platelets are coated with autoantibody (immunoglobulin) and are removed by the macrophages of the reticuloendothelial system. Their lifespan is therefore reduced from 7–10 days to a few hours.

Acute

- Usually presents in childhood (2–7 years).
- Often follows a viral infection.
- Purpuric rash or epistaxis frequent (Figs 33c, d).
- Typically resolves spontaneously. A minority develop mucosal bleeding and should be treated with prednisolone or intravenous immunoglobulin. Up to 20% develop chronic immune thrombocytopenia.

Chronic

Immune thrombocytopenia in adults is less likely to resolve without therapy and is usually chronic. It is more common in females (M/F ratio 1 : 4). Autoantibody is present on the platelet surface and may also be present as free antibody in serum.

Laboratory findings

- Normal haemoglobin and white cell count; platelets low, often $<20 \times 10^9$/L.
- Bone marrow is normal or there are increased numbers of megakaryocytes.
- PT and APTT are normal, fibrinogen is normal.

Immune thrombocytopenia also occurs in association with some malignancies (e.g. chronic lymphocytic leukaemia, non-Hodgkin lymphoma, myelodysplasia), infections (e.g. Epstein–Barr virus, HIV, malaria) and connective tissue disease (e.g. systemic lupus erythematosus). Patients should be tested for ANF and anticardiolipin antibodies.

Treatment

Treatment, if necessary, is with the following:
- Prednisolone (1 mg/kg/day, reducing over 4–6 weeks).
- Intravenous immunoglobulin is valuable for obtaining a temporary rise in platelet count.
- Splenectomy is required for non-responders with continuing symptoms and/or very low platelet counts.
- Additional immunosuppressive therapy (e.g. azathioprine, cyclophosphamide, cyclosporin A, rhesus anti-D, vincristine) or combination chemotherapy have been used. Rituximab (anti-CD20) and danazol are also of value in some cases.

Alloimmune thrombocytopenia

Transplacental passage of maternal antibody in immune thrombocytopenia can lead to neonatal thrombocytopenia, which typically resolves spontaneously over a few weeks. Mothers who have been sensitized (e.g. by blood transfusion or previous pregnancy) to platelet antigens may develop antibodies which cross the placenta and coat fetal and neonatal platelets, which are then removed in the reticuloendothelial system (RES). Individuals with such platelet alloantibodies can also become thrombocytopenic after blood transfusion (post-transfusion purpura). The antibody is then directed against the HPA1-a antigen on platelets.

Other causes of thrombocytopenia

Drugs

Drugs cause thrombocytopenia by inhibiting marrow production or by an immune mechanism. The most common immune mechanism (e.g. quinine, heparin) is when the drug forms an antigen with a

plasma protein, an antibody is formed to it, and circulating antigen–antibody complexes are absorbed on the platelet surface. Heparin-induced thrombocytopenia is associated with thrombosis. A 'heparinoid' drug can be used to continue anticoagulation.

Disseminated intravascular coagulation

This is discussed in Chapter 36.

Thrombotic thrombocytopenic purpura and haemolytic uraemic syndrome

Thrombotic thrombocytopenic purpura (TTP) and haemolytic uraemic syndrome (HUS) are characterized by thrombosis in small vessels, red cell fragmentation, haemolytic anaemia (Fig. 33e) and thrombocytopenia. Renal failure often occurs in HUS and fever, neurological changes and liver dysfunction in TTP. The PT and APTT are normal. TTP is caused by a deficiency—either congenital or acquired from an antibody—of a plasma protease ADAMTS-13 which normally cleaves von Willebrand factor (vWF). Abnormally high molecular weight vWF complexes are present in plasma. Haemolytic uraemic syndrome occurs in childhood and follows infection with verotoxin-producing strains of *E. coli*; it is also associated with *Shigella*, *Salmonella* and streptococcal infection, pregnancy, autoimmune diseases and drugs (e.g. ciclosporin A). The ADAMTS-13 levels are normal. TTP is more frequently fatal, occurs in adults and may be associated with autoimmune conditions (e.g. SLE), pregnancy and infection.

Treatment of TTP is with plasma exchange using fresh frozen plasma (FFP) as the replacement fluid. Fresh frozen plasma depleted of cryoprecipitate may be more effective, while FFP which has been solvent treated is less likely to transmit viral infection. Antiplatelet drugs (aspirin or dipyridamole), corticosteroids, splenectomy and vincristine have all been used. Response to treatment may be monitored by haemoglobin level, reticulocytes, lactate dehydrogenase, platelet count, plasma bilirubin and presence of vWF multimers in plasma. In HUS, treatment for fits, hypertension and renal failure may be needed.

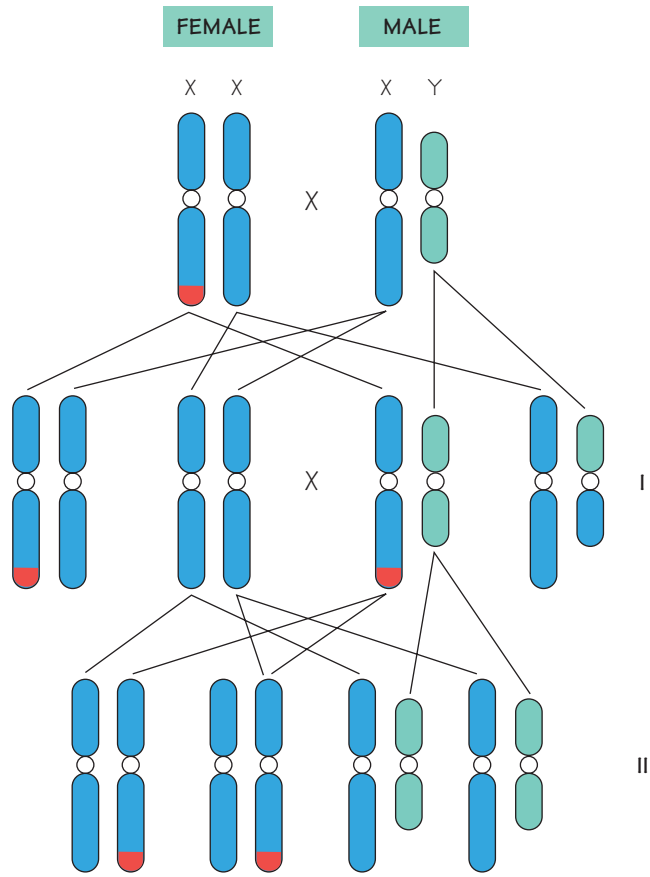
Table 33.1 Disorders of platelet function.

Inherited
Bernard–Soulier syndrome(defective glycoprotein 1b, giant platelets)
Glanzmann's thrombasthaenia (defective glycoproteins IIb, IIIa)
Storage pool diseases, von Willebrand's disease (see Chapter 34)
Acquired
Drugs: aspirin, other non-steroidal anti-inflammatory agents, clopidrogel, dextran, antibiotic therapy (e.g. cephalosporins)
Myeloproliferative disorders: polycythaemia rubra vera, essential thrombocythaemia, myelofibrosis
Uraemia
Paraproteinaemia, e.g. myeloma or Waldenström's macroglobulinaemia

Disorders of platelet function (Table 33.1)

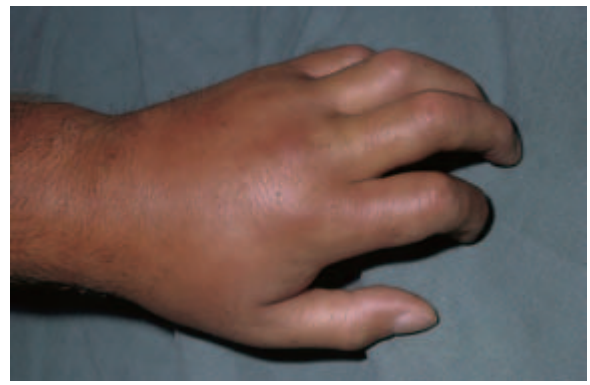
These are characterized by a prolonged bleeding time with normal platelet count, abnormal PFA-100 test and disordered platelet aggregation. **Inherited disorders** are rare and present with bruising/excessive bleeding after surgery or injury in childhood. The most common **acquired** cause is aspirin and other non-steroidal anti-inflammatory drugs.

34 Disorders of coagulation I: inherited



(a) Inheritance of haemophilia. A female carrier mating with a normal male will produce 50% of daughters as carriers and 50% of sons will have haemophilia (generation I). A haemophiliac male mating with a normal female will produce 100% carrier females and normal males (generation II).

(b) Factor VIII deficiency: bleeding into the hand causing haematoma formation, following trauma in a patient with factor VIII deficiency.



Excessive bleeding may occur as a result of an inherited defect of one or other protein involved in coagulation. Inherited deficiency of each of the coagulation factors has been described.

Factor VIII deficiency (haemophilia A)

Factor VIII deficiency (haemophilia A) is the most common inherited disorder. The factor VIII gene is on the X chromosome so inheritance is sex-linked (Fig. 34a). A wide range of genetic changes is described including deletions, insertions, point mutations and a common intragene inversion.

Clinical features

- Range from severe spontaneous bleeding, especially into joints (haemarthroses) and muscles, to mild symptoms (Fig. 34b).
- Onset in early childhood (e.g. postcircumcision).
- Increased risk of postoperative or post-traumatic haemorrhage.
- Chronic debilitating joint disease caused by repeated bleeds.

Laboratory features (Table 34.1)

- Prolonged activated partial thromboplastin time (APTT), normal prothrombin time (PT), normal bleeding time, plasma factor VIII reduced (<1% of normal in severe cases, but up to 10% in mild cases).
- Carriers have factor VIII approximately 50% of normal. DNA analysis is helpful in carrier detection and counselling.
- Von Willebrand factor level is normal.

Table 34.1 Laboratory features of major inherited coagulation disorders.

Condition	PT	APTT	Bleeding time (PFA-100)	Other
Haemophilia A	N	↑	N	Factor VIII ↓
Haemophilia B	N	↑	N	Factor IX ↓
von Willebrand's disease	N	↑	↑	von Willebrand factor ↓ Factor VIII ↓ Abnormal platelet aggregation with ristocetin

Treatment (see also Chapter 41)

- Infusions of factor VIII concentrate to elevate the patient's level to 20–50% of normal for severe bleeding.

- Level is raised to and maintained at 80–100% for elective surgery.
- Desmopressin, an analogue of vasopressin, leads to a modest rise in endogenous factor VIII which is useful in mild cases.
- Avoid aspirin, other antiplatelet drugs and intramuscular injections.
- Patients should be registered with a recognized haemophilia centre and should carry a card with details of their condition.
- Patients may need to have continuing or prophylactic treatment at home.

Complications of treatment

- HIV and hepatitis C from impure preparations (prior to the early 1980s), subsequent AIDS, hepatitis and cirrhosis.
- Neutralizing antibodies to factor VIII in 15% of severe patients may require immunosuppressive therapy, treatment with porcine factor VIII or plasma exchange.

Factor IX deficiency (haemophilia B, Christmas disease)

Factor IX deficiency (haemophilia B, Christmas disease) has similar clinical features to haemophilia A. Also sex-linked, it is four times less common and usually milder than haemophilia A. Diagnosis and treatment are similar to haemophilia A, except that factor IX concentrate is used for treatment and desmopressin is not effective.

Von Willebrand's disease

Von Willebrand's disease is usually autosomal dominant, and results from mutations in the von Willebrand factor (vWF) gene. Von Willebrand factor is a large multimeric protein produced by endothelial cells, which carries factor VIII in plasma and mediates platelet adhesion to endothelium (see Chapter 32). The disease is more frequent than haemophilia A; males and females are affected equally.

Clinical features

- Bleeding, typically from mucous membranes (mouth, epistaxes, menorrhagia).
- Excess blood loss following trauma or surgery.
- Haemarthroses and muscle bleeding are rare.

Diagnosis

- APTT is prolonged, PT normal.
- Factor VIII and vWF levels are reduced.
- Bleeding time is prolonged.
- Defective platelet function, reduced aggregation with ristocetin.
- Mild thrombocytopenia may occur.
- The disease is divided into subtypes depending on whether there is a reduction in vWF or different types of functional defect.

Treatment

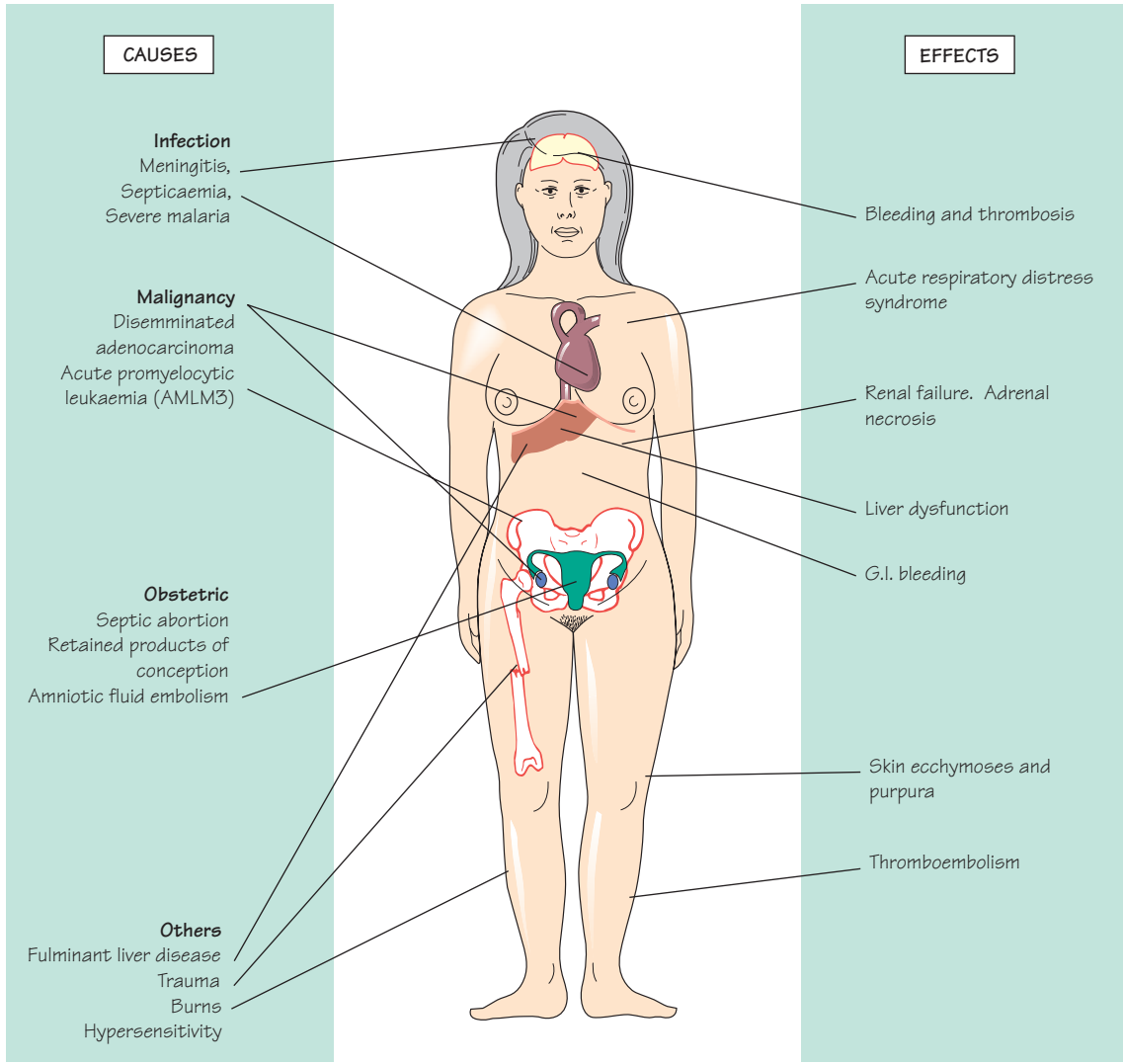
- Intermediate purity factor VIII concentrate (contains both vWF and factor VIII) for bleeding.
- Desmopressin is helpful for mild bleeding.
- Fibrinolytic inhibitors (e.g. tranexamic acid) are helpful.
- Carrier detection and antenatal diagnosis based on fetal DNA analysis is now available.

Other conditions

Factor XI deficiency is less frequent than haemophilia A, has higher incidence among Ashkenazi Jews, and is autosomal recessive. There is poor correlation between factor XI levels and symptoms. It is generally mild, but severe spontaneous and postsurgical bleeding may occur. Congenital deficiencies of factors II, V, VII, X and XIII are rare and usually cause mild bleeding disorders. Factor XII deficiency prolongs the APTT but does not cause clinical symptoms. Fibrinogen deficiency occurs as a moderately severe autosomal recessive disorder. Dysfibrinogenaemia (presence of a functionally abnormal molecule) is both a rare autosomal dominant disorder and a more common acquired disorder (liver disease, malignancy and systemic lupus erythematosus).

35 Disorders of coagulation II: acquired

(a) Causes and effects of disseminated intravascular coagulation.



Liver disease

Liver disease leads to defects of coagulation, platelets and fibrinolysis.

- Reduced synthesis of vitamin K dependent factors (II, VII, IX, X, proteins C and S) caused by impaired vitamin K absorption (biliary obstruction).
- Impaired synthesis of other coagulation proteins (factor I and V).
- Thrombocytopenia (hypersplenism) and abnormal platelet function (cirrhosis).
- Fibrinolysis impaired.
- Reduced levels of proteins C and S, antithrombin and α_2 antiplasmin lead to susceptibility to disseminated intravascular coagulation (DIC).
- Dysfibrinogenaemia may lead to haemorrhage or thrombosis.

Disseminated intravascular coagulation

(Fig. 35a)

Release of procoagulant material into the circulation or endothelial cell damage causes generalized activation of the coagulation and fibrinolytic pathways leading to widespread fibrin deposition in the circulation.

Clinical features

- Both bleeding and thrombosis may occur.
- Tissue damage caused by thrombosis leads to necrosis and further activation of coagulation and fibrinolysis.
- Purpura, ecchymoses, gastrointestinal bleeding, bleeding from

Table 35.1 Coagulation changes in acquired disorders of coagulation.

	PT	APTT	TT	Platelets	Other
Liver disease	↑	↑	N/↑	↓	Dysfibrinogenaemia
DIC	↑	↑	↑	↓	FDP ↑ ±RBC fragments on blood film
Vitamin K deficiency	↑	↑ or N	N	N	
Massive transfusion	↑	↑	N	↓	
Oral anticoagulants	↑	↑	N	N	
Heparin	↑	↑	↑	N (rarely ↓)	Anti-Xa ↓

APTT, activated partial thromboplastin time; DIC, disseminated intravascular coagulation; FDP, fibrin degradation products; N, normal; PT, prothrombin time; RBC, red blood cell; TT, thrombin time.

intravenous sites and following venepuncture may occur as a result of low levels of coagulation factors and platelets resulting from increased consumption.

- Renal function may be impaired due to microvascular thrombosis.
- Other manifestations include acute respiratory distress syndrome (both a cause and complication of DIC), adrenal necrosis, shock and thromboembolism.

Laboratory features (Table 35.1)

- Thrombocytopenia.
- Nearly all tests of coagulation and fibrinolysis are abnormal with low levels of fibrinogen.
- Fibrin degradation products (e.g. X-DP or FDP) are present in plasma (X = clotting factor).
- Blood film: microangiopathic haemolytic anaemia (see Chapter 16) may occur.

Treatment

- Treat the cause, e.g. antibiotics for septicaemia, removal of the procoagulant stimulus (e.g. a dead fetus).
- Supportive therapy with fresh frozen plasma, platelet concentrates and cryoprecipitate if bleeding is dominant.
- Anticoagulant therapy (e.g. heparin) if thrombosis is dominant.
- Protein C and antithrombin in selected patients.

Other acquired disorders of coagulation

Drugs

- Anticoagulants and drugs affecting anticoagulation (see Chapter 37) are the most common drugs to disturb coagulation.
- Chemotherapy (e.g. l-asparaginase may lead to thrombosis).

Acquired coagulation inhibitors

These antibodies to coagulation factors are idiopathic, commoner in the elderly, or occur in malignancy (e.g. lymphoma), connective tissue disease (e.g. SLE) and with paraproteins (e.g. myeloma). They lead to excessive bleeding, both spontaneously and following injury.

Vitamin K deficiency

Vitamin K is required to activate factors II, VII, IX and X and protein C and S by γ carboxylation (see Chapter 37). It is fat-soluble and derived from vegetables in food and intestinal flora. Deficiency occurs in patients on poor diets, those taking broad-spectrum antibiotics which reduce the gut flora, in biliary tract disease and with intestinal malabsorption.

Massive post-trauma/-surgery uncontrollable bleeding

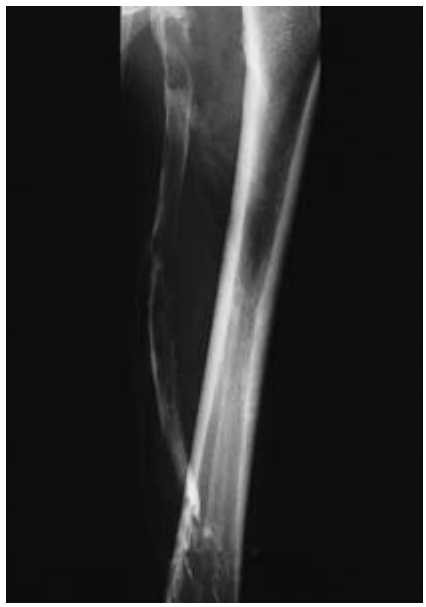
This can be due to multiple factors, DIC with consumption of platelets and clotting factors and excess fibrinolysis. If bleeding persists despite replacement of platelets and clotting factors, recombinant human factor VIIa may be life-saving.

Haemorrhagic disease of the newborn

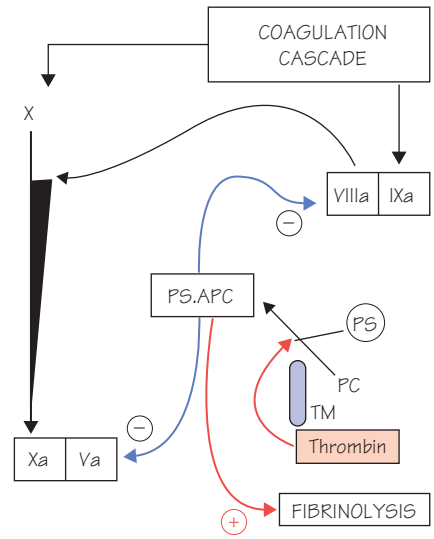
Newborn infants are at an increased risk of bleeding because of hepatic immaturity and low levels of vitamin K. It is customary to give an injection of vitamin K (1 mg) to all newborn infants in the UK. Fears that this may lead to an increased risk of cancer have not been substantiated.

36 Thrombosis and thrombophilia

(a) Thrombosis: venogram showing filling defects due to a deep vein thrombus in a patient with polycythaemia.



(b) The protein C pathway. Thrombin bound to thrombomodulin (TM) on intact endothelium activates protein C to activated protein C (APC). This combines with protein S (PS) and inactivates Va and VIIIa. Factor V Leiden is resistant to APC.



Thrombosis

Thrombosis is the pathological process whereby platelets and fibrin interact with the vessel wall to form a haemostatic plug to cause vascular obstruction. It may be arterial, causing ischaemia, or venous leading to stasis (Fig. 36a). The thrombus may be subsequently lysed by fibrinolysis, alternatively it may organize, recanalize or embolize. Thrombosis underlies ischaemic heart, cerebrovascular and peripheral vascular disease; venous occlusion and pulmonary embolism; and it plays an important part in pre-eclampsia.

Arterial thrombosis (Table 36.1)

This occurs in relation to damaged endothelium, e.g. atherosclerotic plaques. Exposed collagen and released tissue factor cause platelet aggregation and fibrin formation.

Venous thrombosis (Table 36.2)

Factors affecting blood flow (e.g. stasis, obesity), alterations in blood constituents and damage to vascular endothelium (e.g. caused

Table 36.1 Risk factors for arterial thrombosis.

Hypertension
Smoking
Diabetes*
Hyperlipidaemia*
↑ Homocysteine*
Polycythaemia/thrombocythaemia
↑ Factor VIII
↑ Fibrinogen
Lupus anticoagulant

*May be related to an inherited abnormality.

Table 36.2 Risk factors for venous thrombosis.

Conditions causing stasis
Advanced age
Cardiac failure, oedema, nephrotic syndrome
Postoperative
Immobility, bed rest, obesity, limb paresis
Trauma
Pelvic obstruction
Myocardial infarct, long distance travel, central venous catheter
Altered blood constituents
Coagulation factors
Hereditary
Factor V Leiden
Protein C deficiency
Protein S deficiency
Antithrombin deficiency
Prothrombin mutation
Acquired
Oestrogen therapy, contraceptive pill
Malignancy
Pregnancy and puerperium
Lupus anticoagulant/antiphospholipid syndrome
Raised plasma homocysteine (may also be inherited)
Raised factors VIII, IX or XI
Blood cells
Polycythaemia
Thrombocythaemia

by sepsis, surgery or indwelling catheters) are important risk factors. Diagnosis can be confirmed by imaging, e.g. venography (Fig. 36a) or Doppler ultrasound. Blood tests, e.g. detection of elevated levels of D-dimers, which are derived from fibrinogen, can also be helpful.

Thrombophilia

Thrombophilia is a congenital or acquired predisposition to thrombosis. It should be suspected and screened for in patients with thrombosis who are young, have a positive family history, have a thrombosis in an unusual site, spontaneous recurrence and in females with recurrent fetal loss.

Inherited thrombophilia

This has been increasingly recognized recently (Tables 36.1, 36.2; Fig. 36b). Presentation may be during early childhood or in adulthood, e.g. at commencement of oral contraceptives or during pregnancy/puerperium. Inheritance of a variant form of factor V (factor V Leiden) is the most common (up to 5% of the population). Activated factor V Leiden is relatively resistant to inactivation by protein C. The risk of thrombosis is increased 5- to 10-fold in heterozygotes, and 50- to 100-fold in homozygotes. Rarer causes include protein C, protein S or antithrombin deficiency or functional abnormality, defective fibrinolysis (e.g. TPAI deficiency, see Chapter 32), mutant prothrombin and hyperhomocysteinaemia. The combination of two abnormalities often underlies severe cases.

Acquired thrombophilia

Acquired hypercoagulable states are listed in Tables 36.1 and 36.2. Pathogenesis, e.g. in pregnancy, oral contraceptive pill therapy and malignancy, is multifactorial and relates to elevated levels of procoagulant factors, depressed levels of inhibitor proteins and physical factors (e.g. stasis, surgery).

Lupus anticoagulant syndrome

Despite its name, this syndrome usually presents with arterial or venous thrombosis or recurrent miscarriages. It may be associated with systemic lupus erythematosus or other connective tissue disorders, with malignancy or infections, or be idiopathic. Patients may show a spectrum of antibodies which interfere with phospholipid-dependent coagulation tests *in vitro* and/or react with cardiolipin. The APTT is prolonged and not corrected by a 50 : 50 mix of normal plasma in patient plasma. Anticoagulant therapy is needed for patients with thrombosis.

Antiplatelet therapy

The use of heparin and warfarin is discussed in Chapter 37. Antiplatelet drugs (see Fig. 32e) and fibrinolytic drugs are discussed here.

- Aspirin (75 mg daily and 300 mg post myocardial infarction) is most widely used. It inhibits platelet function by inhibiting cyclo-oxygenase thus reducing thromboxane A_2 production.
- Others, clopidrogel and monoclonal antibodies directed to platelet glycoproteins (e.g. Abciximab, which is directed against glycoprotein IIb/IIIa) or small molecule inhibitors of GpIIb/IIIa eptifibatide or tirofiban, are used, for example, post angioplasty or stent insertion. The combination of aspirin and clopidrogel is used in high risk cases.

Indications

Prevention of thrombosis in patients with:
- previous myocardial infarction, transient ischaemic attacks and stroke or high risk of first myocardial infarct in males;
- thrombocytosis, e.g. myeloproliferative disorders, postsplenectomy;
- prosthetic valves and post coronary artery surgery or angioplasty;
- pre-eclampsia;
- severe peripheral vascular disease.

Fibrinolytic therapy

This is used to enhance conversion of plasminogen to plasmin (see Chapter 32, Fig. 32d), which degrades fibrin. It must be used within 5–7 days for venous thrombi and 5–7 hours for arterial thrombi:
- Streptokinase directly activates plasminogen. Most individuals have antistreptococcal antibodies; a loading dose is therefore required and treatment becomes ineffective after 4–10 days.
- Urokinase has a similar action but may be used if there are high levels of antistreptococcal antibodies. Single chain urokinase-type plasminogen activator (SCU-PA) has also been developed.
- Acylated plasminogen streptokinase activator complex (APSAC) activates streptokinase bound to plasminogen.
- Recombinant tissue plasminogen activation (TPA) causes activation of fibrin-bound plasminogen only, and is associated with less systemic activation of fibrinolysis.

Indications

- Acute myocardial infarction: streptokinase is usually given with 300 mg aspirin and heparin intravenously.
- Treatment of arterial and venous thrombosis, e.g. pulmonary embolism, peripheral arterial or venous thrombosis.

Contraindications

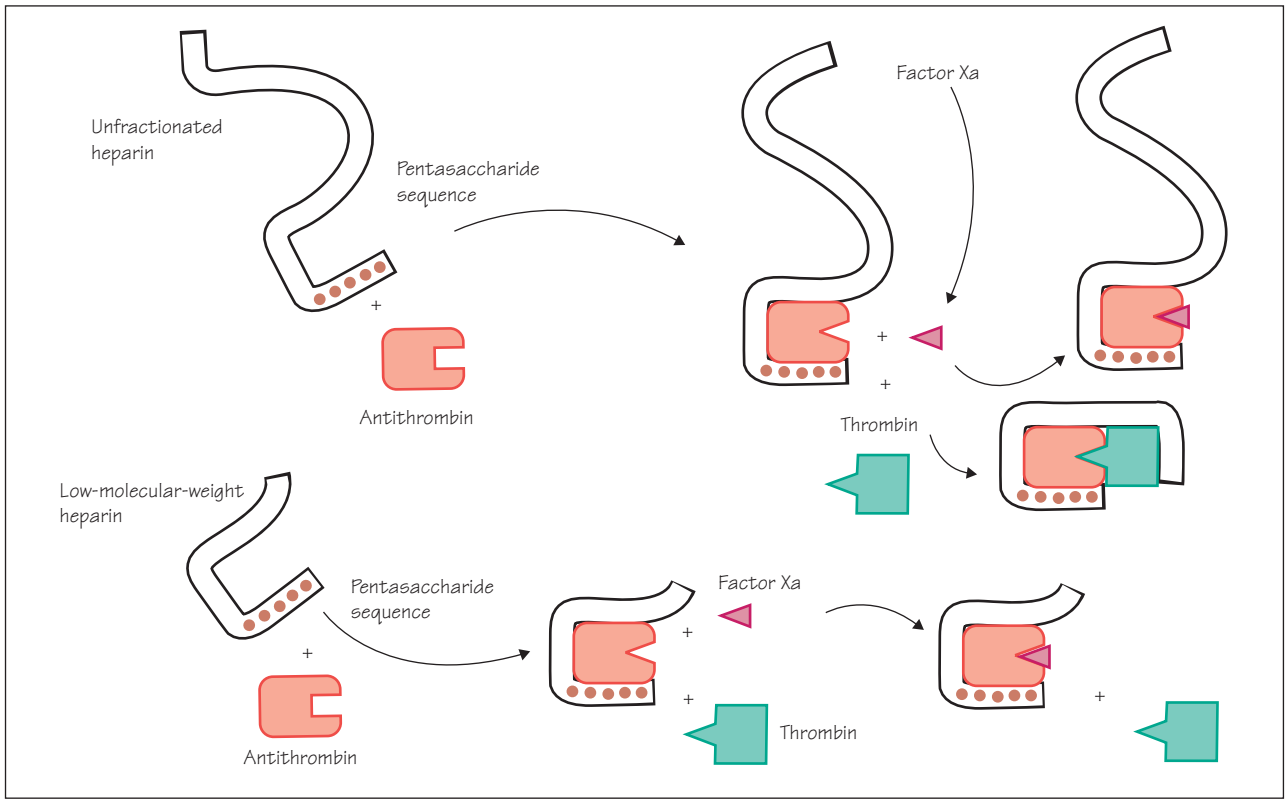
- Patients with active gastrointestinal bleeding, aortic dissection, head injury or recent (<2 months) neurosurgery, and bleeding diathesis.

Side effects

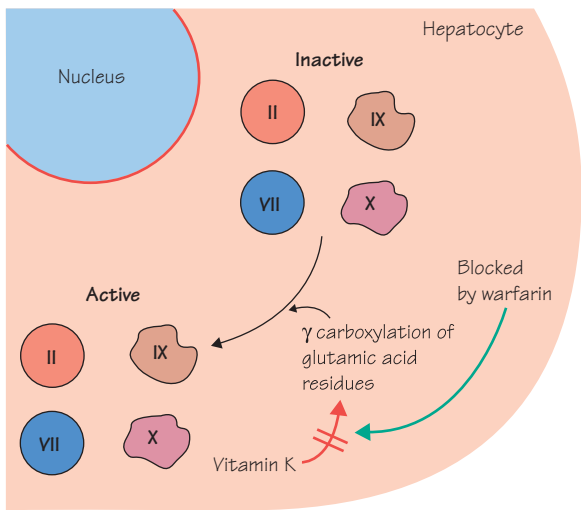
- Bleeding, especially in patients taking anticoagulants or antiplatelet drugs.
- Anaphylactic reactions may occur with streptokinase.

37 Anticoagulation

(a) Heparin binds to antithrombin via a pentasaccharide sequence, and induces a conformational change which allows antithrombin to bind Xa and thrombin. The shorter chain length of low molecular weight heparin allows binding to only Xa, while unfractionated heparin will bind both Xa and thrombin. Thus, LMW heparin allows a selective inhibition of factor Xa. Modified from Weitz J.I. (1997) Low molecular-weight heparin. *New England Journal of Medicine*, **337**: 688–98.



(b) Action of warfarin.



Heparin

Heparin is a mucopolysaccharide; it is not absorbed orally and is therefore given subcutaneously (s.c) or intravenously (i.v.). It activates antithrombin (AT) which irreversibly inactivates prothrombin, Xa, IXa and XIa. It also impairs platelet function. Unfractionated heparin (UFH) is a heterogeneous mixture of polysaccharide chains. Low-molecular-weight (LMW) heparin preparations (MW <5000) have a greater ability to inactivate Xa and less effect on thrombin (Fig. 37a) and platelet function, and therefore have a lesser tendency to cause bleeding. They have a longer plasma half-life allowing once daily s.c. administration. They also interact less than UFH with endothelium, plasma proteins, macrophages and platelets, making their action more predictable.

Indications

- Acute venous thrombosis, e.g. deep vein thrombosis (DVT) and pulmonary embolism (PE). Continuous i.v. infusion of UFH for 5–7 days until warfarinized; s.c. LMW heparin is equally effective. Warfarin is usually started 1–2 days after heparin.
- Unstable angina, post myocardial infarction.

• Disseminated intravascular coagulation complicated by thrombosis.
• Acute peripheral arterial occlusion.
• Prophylaxis of DVT in surgical patients (LMW heparin 2000–5000 units once daily).
• Thrombosis prophylaxis in patients undergoing cardiac surgery or renal dialysis.
• Pregnancy. As warfarin is teratogenic, heparin is used in the first trimester of pregnancy when anticoagulation is needed.
• Maintaining patency of indwelling lines and catheters.

Monitoring

For continuous i.v. infusion, the APPT should be maintained at 1.5–2 × normal. LMW heparin therapy is not normally monitored; if necessary, e.g. in renal failure or in those of very low (<50 kg) or high (>80 kg) body weight, by factor Xa assay.

Side effects

• Haemorrhage, particularly if combined with antiplatelet therapy, overdosage or, rarely, platelet function defect. Heparin has a short half-life (1 h); levels fall rapidly when infusion is stopped. Protamine sulfate will reverse heparin immediately, but must be used with caution as it can cause haemorrhage at high dosage.
• Long-term therapy (>2 months) can lead to osteoporosis.
• Thrombocytopenia, which is antibody-mediated, may lead to platelet clumping with arterial thrombosis.
• LMW heparin is less likely to cause all these side effects.

Warfarin

Vitamin K promotes the γ carboxylation of glutamic acid residues of factors II, VII, IX and X; warfarin prevents this to cause a 50% drop of factor VII levels within 24 hours and of factor II in 4 days. Full anticoagulation occurs 48–72 hours after starting warfarin therapy. Non-carboxylated factors II, VII, IX and X (proteins formed in vitamin K absence, PIVKAs) appear in plasma (Fig. 37b). Protein C and S levels also fall and this initially (first 2–3 days) leads to an increased risk of thrombosis and may lead to skin necrosis.

Monitoring

The PT is measured and expressed as an international normalized ratio (INR) against the mean normal PT using a calibrated thromboplastin. Treatment is monitored by maintaining the INR at 2.0–3.5, the precise level depending on indication.

Indications

• Treatment of DVT, pulmonary embolism, systemic embolism (3–6 months therapy).
• Prophylaxis against thrombosis in patients with atrial fibrillation, prosthetic valves, arterial grafts, repeated pulmonary embolism and in patients with inherited or acquired predisposition and previous DVT.
• Low doses (to maintain INR 1.5) of value in prevention of myocardial infarct in high-risk groups.
• Warfarin crosses the placenta and is teratogenic in early pregnancy; heparin is given in pregnancy.

Side effects and reversal of action

• Haemorrhage—especially in patients taking other anticoagulants, antiplatelet drugs or thrombolytic therapy, and in those with liver disease.
• Raised INR with haemorrhage is managed with vitamin K (1–5 mg i.v.), prothrombin concentrates (of factors II, VII, IX and X) and fresh frozen plasma.
• Raised INR without haemorrhage is managed by withholding therapy for 1–2 days and repeating the INR. Vitamin K 1–2 mg orally should be given if other risk factors exist.

Drug interactions

Warfarin is highly bound to albumin and is metabolized by the liver. The minor unbound fraction is active. Action is increased by drugs which:
• reduce its binding to albumin, e.g. aspirin, sulfonamides;
• inhibit hepatic metabolism, e.g. allopurinol, tricyclic antidepressants, sulfonamides;
• decrease absorption of vitamin K, e.g. antibiotics, laxatives;
• decrease synthesis of vitamin K factors, e.g. high-dose salicylates.
Action is decreased by drugs which:
• accelerate its metabolism, e.g. barbiturates, rifampicin, and
• enhance synthesis of coagulation factors, e.g. oral contraceptives, hormone replacement therapy.

Other anticoagulant drugs

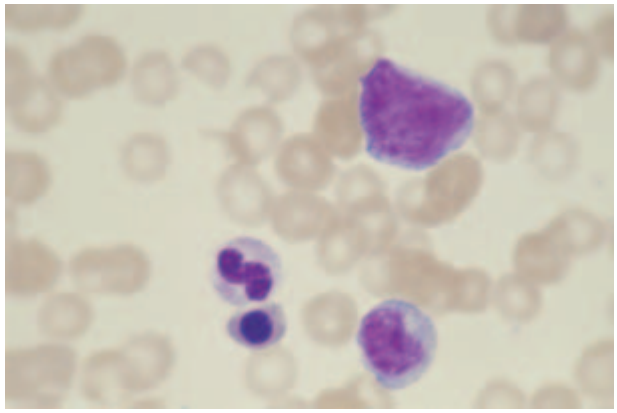
Hirudin, available as the recombinant product lepirudin, is a specific direct inhibitor of thrombin licensed for i.v. use in adults who cannot receive heparin (e.g. because of heparin-induced thrombocytopenia). Ximelagatran is an orally active direct thrombin inhibitor, which may be equivalent to warfarin and has similar indications but does not needing monitoring. It is pre-registration and not yet licensed at the time of writing. Fondaparinux is a direct inhibitor of factor X used parenterally in prophylaxis of DVT in orthopaedic patients.

Other treatments

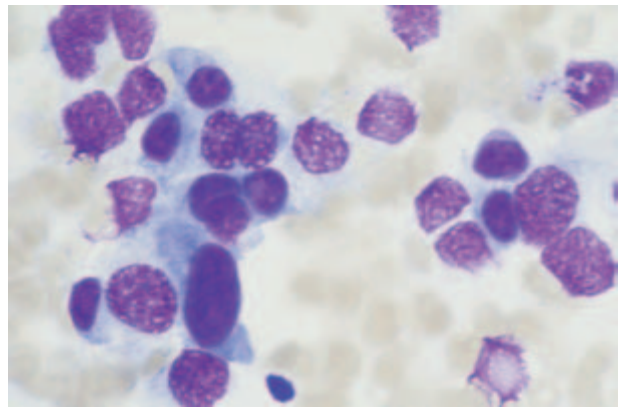
Graduated elastic compression stockings help to prevent DVT postoperatively and to prevent a postphlebitic syndrome occurring after DVT. An inferior vena cava filter may be inserted to reduce the risk of pulmonary embolus.

38 Haematological aspects of systemic disease I: inflammation—malignancy

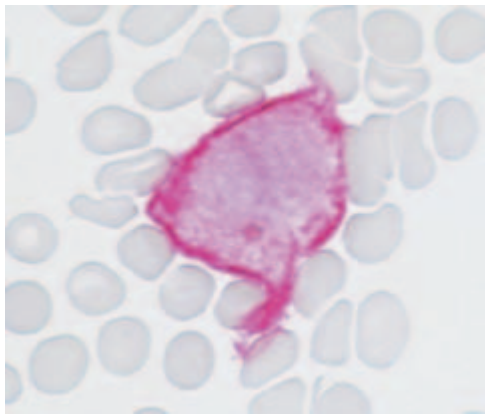
(a) Leucoerythroblastic blood film showing circulating immature granulocytes and nucleated red blood cells indicating in this case marrow infiltration.



(b) Secondary carcinoma: bone marrow aspirate showing infiltration by breast carcinoma.



(c) Secondary carcinoma: bone marrow aspirate immunocytochemistry showing positive staining for cytokeratin in breast carcinoma cells.



A wide range of abnormalities affecting red cells, white cells, platelets and coagulation factors occur in association with systemic illness.

Anaemia of chronic disease

- Anaemia of chronic disease (ACD) is a common normochromic or mildly hypochromic anaemia, occurring in patients with a systemic disease (Table 38.1). Moderate anaemia occurs (haemoglobin level >9.0 g/dL, the severity of anaemia correlating with the severity of underlying disease).
- Reduced serum iron and total iron binding capacity.
- Normal or raised serum ferritin with adequate iron stores in the bone marrow but stainable iron absent from erythroblasts.

Pathogenesis

Hepcidin, released by the liver in response to inflammatory

Table 38.1 Conditions associated with anaemia of chronic disorders.

Chronic infections	Especially osteomyelitis, bacterial endocarditis, tuberculosis, chronic abscesses, bronchiectasis, chronic urinary tract infections, HIV, AIDS, malaria
Other chronic inflammatory disorders	Rheumatoid arthritis, polymyalgia rheumatica, systemic lupus erythematosus, scleroderma, inflammatory bowel disease, thrombophlebitis
Malignant diseases	Carcinoma, especially metastatic or associated with infection, lymphoma
Others	Congestive heart failure, ischaemic heart disease

cytokines, reduces iron absorption and iron release by macrophages into plasma. Increased levels of cytokines, especially IL-1, IL-6, tumour necrosis factor and interferon-γ, interact with accessory marrow stromal cells, macrophages and erythroid progenitors to reduce erythropoiesis, iron utilization and response to erythropoietin (EPO).

Treatment

- Therapy of the chronic disease gradually reduces levels of mediator cytokines.
- Recombinant EPO may improve anaemia in patients with, for example, rheumatoid arthritis (RA), cancer and myeloma.

Malignancy

Anaemia

- Anaemia of chronic disease affects almost all cancer patients at some stage.
- Blood loss in gastrointestinal and gynaecological malignancies.
- Autoimmune haemolytic anaemia (AIHA), especially in lymphoma.
- Microangiopathic haemolytic anaemia (see Chapter 16) may occur with disseminated mucin-secreting adenocarcinoma.
- Leucoerythroblastic anaemia indicates marrow infiltration by tumour (Fig. 38a).
- Red cell aplasia is associated with thymoma, lymphoma and chronic lymphocytic leukaemia.

Other causes

Chemotherapy or radiotherapy-induced inhibition of bone marrow. Folate deficiency as a result of poor diet and widespread disease.

Polycythaemia

Tumour cells may produce EPO or EPO-like peptides in renal cell carcinoma, hepatoma and uterine myoma (see Chapter 31).

White cell changes

Cancer patients frequently have opportunistic infections or bleed, which raises white cells (usually neutrophils), or receive chemotherapy or radiotherapy, which lowers them.

Platelets

Thrombocytopenia may be due to decreased production (e.g. extensive marrow infiltration, chemotherapy or radiotherapy), accelerated peripheral destruction (e.g. disseminated intravascular coagulation, DIC) and/or hypersplenism (Figs 38b, c). Immune thrombocytopenia may occur especially with lymphoma.

Thrombocytosis is a frequent reactive phenomenon in malignancy.

Coagulation changes

Activation of both coagulation and fibrinolysis may predispose to either haemorrhage or thrombosis. Chronic DIC (e.g. with pancreatic carcinoma) causes thrombosis, including migratory thrombophlebitis (Trousseau's syndrome). Circulating anticoagulants (e.g. acquired von Willebrand factor inhibitors) and specific coagulation factor inhibitors may occur.

Connective tissue disorders

Anaemia

- Anaemia of chronic disease is common. Iron deficiency may coexist in patients with gastrointestinal haemorrhage caused by non-steroidal anti-inflammatory agents.
- Autoimmune haemolytic anaemia occurs in systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and mixed connective tissue disorders (CTD).
- Red cell aplasia occurs in SLE.

White cells

Inflammation leads to neutrophilia. Neutropenia with splenomegaly occurs in patients with RA (Felty's syndrome). Antibody and immune-complex-mediated neutrophil destruction and decreased neutrophil production in the marrow may also occur in SLE. Eosinophilia may occur in SLE, RA and polyarteritis nodosa.

Platelets

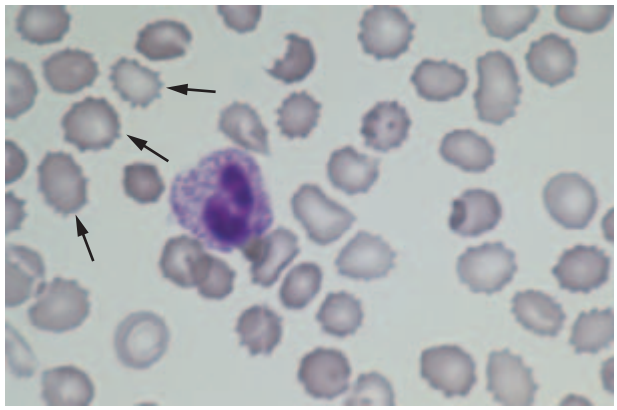
Thrombocytopenia may be immune (SLE and RA). Thrombocytosis is a non-specific reactive phenomenon to inflammation in CTD.

Coagulation changes

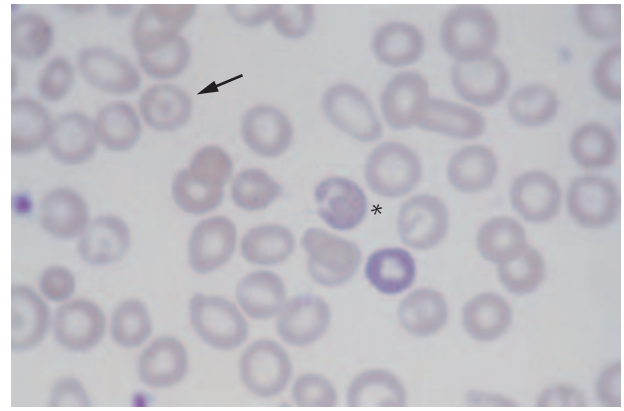
These may be caused by associated renal disease, drug therapy, DIC and specific coagulation factor inhibitors. The lupus anticoagulant occurs in approximately 10% of patients with SLE (see Chapter 36).

39 Haematological aspects of systemic disease II: renal, liver, endocrine, amyloid

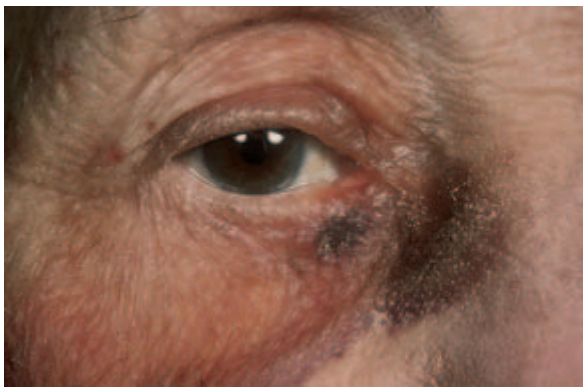
(a) Renal failure: peripheral blood film showing irregular red cells ('burr'cells) (arrows), fragmented red cells and a neutrophil showing toxic granulation and vacuolation.



(b) Liver disease: peripheral blood film showing target cells (arrow), macrocytes and basophilic stippling*.



(c) Amyloidosis: characteristic waxy deposits around the eye.



Renal disease

Anaemia

Acute or chronic renal failure causes a normochromic normocytic anaemia, with reduced erythropoietin (EPO) levels—the main cause of anaemia—and ecchinocytes (burr cells) in the blood film (Fig. 39a). Iron deficiency (blood loss) and haemolysis in haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura are other causes. Erythropoietin corrects anaemia up to a haemoglobin level of 12 g/dL. A poor response to EPO occurs with iron or folate deficiency, haemolysis, infection, occult malignancy, aluminium toxicity, hyperparathyroidism and inadequate dialysis. Hypertension and thrombosis of an arteriovenous fistula may occur with EPO therapy.

Polycythaemia

Polycythaemia may occur with renal tumours or cysts (see Chapter 30).

Haemostatic abnormalities

Coagulation factors II, XI or XIII may be reduced and platelet function is impaired (predispose to bleeding), whereas low levels of protein C, AT or plasminogen may lead to thrombosis.

Endocrine disease

Anaemia

Both hyper- and hypothyroidism cause mild anaemia (MCV raised in hypothyroidism, low in thyrotoxicosis). Deficiencies of iron, as a result of menorrhagia or achlorhydria, or B_{12} (increased incidence of PA in hypothyroidism, hypoadrenalism and hypoparathyroidism), may complicate the anaemia. Antithyroid drugs (carbimazole and propylthiouracil) can cause aplastic anaemia or agranulocytosis.

Liver disease

Anaemia

This may be caused by anaemia of chronic disease, haemodilution (increased plasma volume), pooling of red cells (splenomegaly) and haemorrhage (e.g. caused by oesophageal varices). The MCV is raised, particularly in alcoholics, and target cells, ecchinocytes and acanthocytes occur in the blood film (Fig. 39b). Haemolysis and hypertriglyceridaemia with alcoholic liver disease (Zieve's syndrome) is rare. Direct toxicity of copper for red cells causes haemolysis in Wilson's disease. Viral hepatitis, including hepatitis A, B and C and hepatitis viruses yet to be characterized, may lead to aplastic anaemia.

Platelets and haemostasis

Platelets may be low (hypersplenism or DIC). Coagulation abnormalities are discussed in Chapter 35.

Amyloid

Amyloidosis is the tissue deposition of a fibrillary homogeneous eosinophilic protein material which is birefringent and stains with Congo red. It is classified into:

- amyloid derived from clonal lymphocyte or plasma cell proliferation (AL) (e.g. myeloma, primary amyloidosis) when immunoglobulin light chains or components of them are deposited (see Chapter 26); and
- reactive amyloidosis (AA) which occurs when serum amyloid A protein, an apolipoprotein, is deposited in chronic inflammatory disease (e.g. rheumatoid arthritis, inflammatory bowel disease) or chronic infection (tuberculosis, leprosy, osteomyelitis and bronchiectasis). Familial Mediterranean fever is a chronic inflammatory disease often affecting the kidneys and joints in which amyloidosis is a frequent complication. It is due to mutation of the pyrinin gene. The protein affects complement activation and neutrophil function.

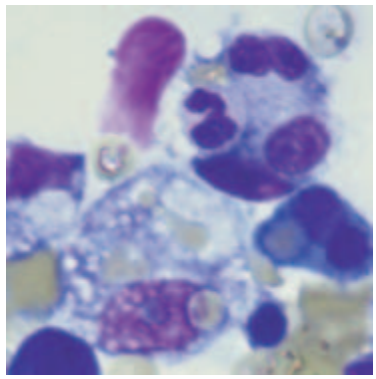
Localized amyloid occurs in, for example, endocrine organs or skin in old age (Fig. 39c), with deposition of protein A, hormones and other constituents.

There are also a number of rare inherited forms of amyloid due to genetic abnormalities in various proteins.

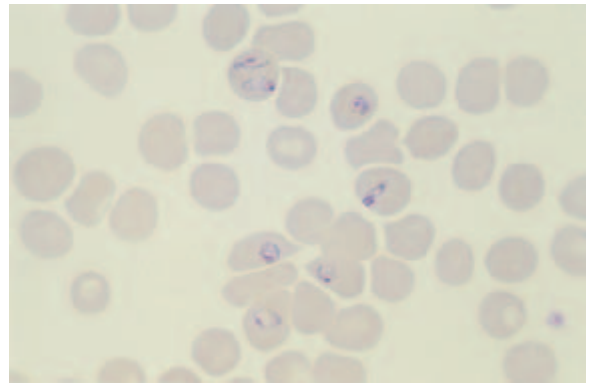
Amyloid P protein is a serum protein related to C-reactive protein which is deposited in both AL or AA types of amyloid. Amyloid deposition leads to organ enlargement and dysfunction. Tissues involved include kidneys, heart, skin, tongue, endocrine organs, liver, spleen, gastrointestinal and respiratory tracts and the autonomic nervous system. Diagnosis is made by biopsy of tongue, gums or rectum with special staining.

40 Haematological aspects of systemic disease III: infection

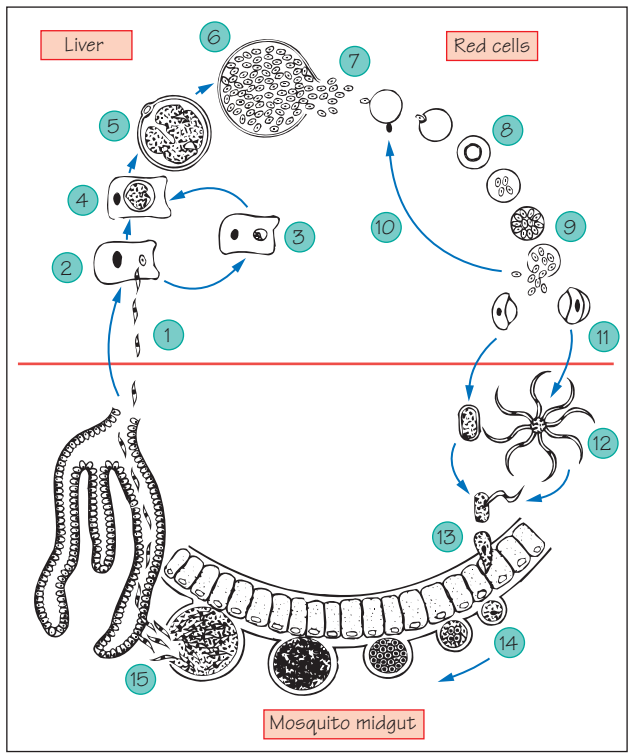
(a) Haemophagocytic syndrome: bone marrow aspirate showing a macrophage laden with cellular debris.



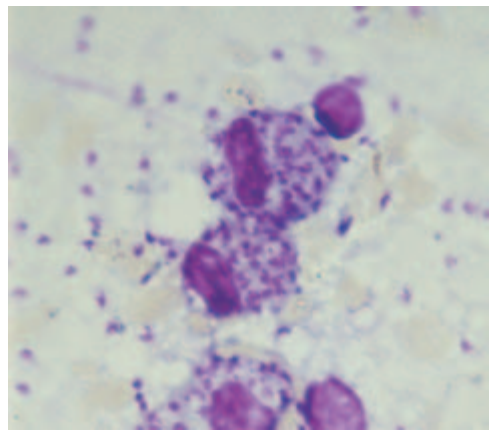
(b) Malaria: peripheral blood film showing red cells invaded by ring forms of *Plasmodium falciparum*.



(c) Malaria life cycle. 1, Sporozoites, injected through the skin by female anopheline mosquito; 2, sporozoites infect hepatocytes; 3, some sporozoites develop into 'hypnozoites' (*Plasmodium vivax* and *P. ovale* only); 4, liver-stage parasite develops; 5–6, tissue schizogony; 7, merozoites are released into the circulation; 8, ring-stage trophozoites in red cells; 9, erythrocytic schizogony; 10, merozoites invade other red cells; 11, some parasites develop into female (macro-) or male (micro gametocytes, taken up by mosquito; 12, mature macrogametocyte and exflagellating microgametes; 13, ookinete penetrated gut wall; 14, development of oocyst; 15, sporozoites penetrate salivary glands.



(d) Leishmaniasis: bone marrow aspirate showing a macrophage containing Leishman–Donovan bodies.



Infections

Viruses

Anaemia

Autoimmune haemolytic anaemia may occur, especially in infectious mononucleosis, usually of cold type. B19 parvovirus causes erythema variegata or fifth disease in children and leads to transient red cell aplasia in patients with haemolytic anaemia (aplastic crisis). Anaemia occurs with pancytopenia in virus-associated bone marrow aplasia (hepatitis viruses, HIV and cytomegalovirus in organ transplant recipients). Microangiopathic haemolytic anaemia (MAHA) with thrombotic thrombocytopenic purpura (TTP) may occur.

White cells

Typically neutropenia with lymphopenia or lymphocytosis (see Chapter 8) occurs.

Platelets

Thrombocytopenia may be immune (e.g. infectious mononucleosis, HIV), caused by bone marrow aplasia, or by increased consumption—disseminated intravascular coagulation (DIC), haemophagocytosis (Fig. 40a), haemolytic uraemic syndrome (HUS) and TTP. Reactive thrombocytosis can also occur.

Bacterial, fungal and protozoal infection

Anaemia

Anaemia of chronic disease is frequent. Haemolytic anaemia may be immune (e.g. cold antibodies with anti-I specificity in mycoplasma infection) or non-immune (e.g. direct red cell invasion, *Bartonella bacilliformis*; or toxin-mediated, *Clostridium perfringens* and *Staphylococcus aureus*). Disseminated intravascular coagulation and MAHA may occur. Haemolytic uraemic syndrome may follow infection by verotoxin-producing strains of *Escherichia coli*, *Salmonella*, *Shigella* and *Campylobacter*. Blood loss can occur with *Helicobacter pylori* and ankylostoma infections.

White cells

Neutrophilia is most common (see Chapter 8).

Platelets

Thrombocytosis is frequently reactive. Thrombocytopenia may also occur, caused by immune destruction, circulating immune complexes, decreased platelet production and DIC in severe bacterial, fungal and rickettsial infection.

Haemostasis

Disseminated intravascular coagulation may dominate the clinical picture in certain infections, e.g. bacterial meningitis.

Malaria (Figs 40b, c)

Anaemia is caused by haemolysis (cellular disruption and haemoglobin digestion), splenic sequestration, haemodilution (raised plasma volume) and ineffective erythropoiesis. Malarial antigens attached to red cells may cause immune haemolysis. Acute intravascular haemolysis with haemoglobinuria and renal failure (blackwater fever) occurs rarely in *Plasmodium falciparum* infection. Anaemia of chronic disease (ACD) may also occur. Eosinophilia is variable. Thrombocytopenia (in up to 70% of *P. falciparum* infections) may be caused by immune destruction, splenic sequestration and DIC.

Leishmaniasis

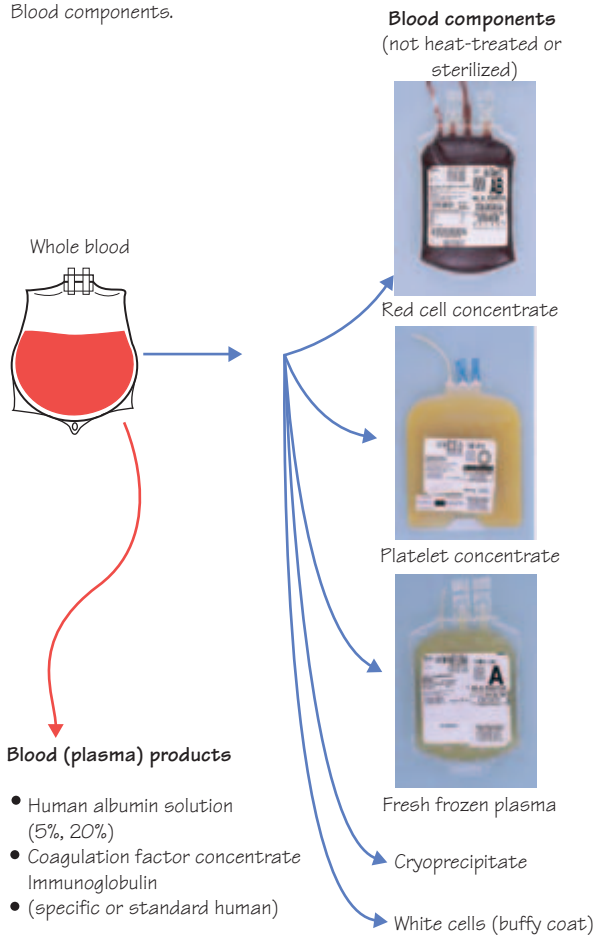
Visceral leishmaniasis is a protozoal infection caused by *Leishmania donovani*. Hepatosplenomegaly, hypergammaglobulinaemia, normochromic anaemia and a raised ESR occur. Bone marrow aspirate shows macrophages containing Leishman–Donovan bodies (Fig. 40d)

HIV infection and AIDS

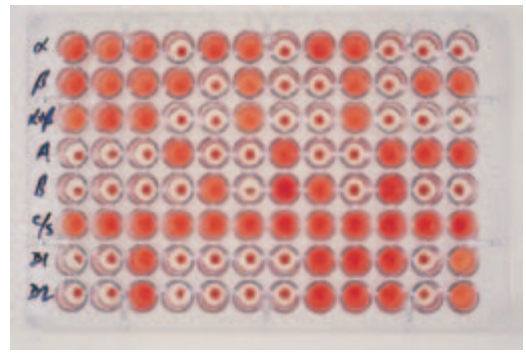
HIV-1 is a retrovirus transmitted by semen, blood and other body fluids, which infects and kills $CD4^+$ T lymphocytes to cause immune suppression. A non-specific febrile illness with lymphadenopathy often marks initial infection. A proportion of patients progress to AIDS with a CD4 count $<0.2 \times 10^9$/L. Clinical manifestations include recurring infections, anaemia and lymphadenopathy. There is an increased risk of non-Hodgkin lymphoma (especially high grade and involving the CNS) and of Kaposi's sarcoma. Treatment may also induce anaemia; e.g. azidothymidine (AZT) and co-trimoxazole both cause megaloblastic change. Thrombocytopenia, lymphopenia and neutropenia (immune or caused by marrow failure or drug therapy) are also frequent. The bone marrow is usually normo- or hyper-cellular, with dysplastic features and an increase in plasma cells. A serum paraprotein occurs in 10–15% of cases.

41 Blood transfusion

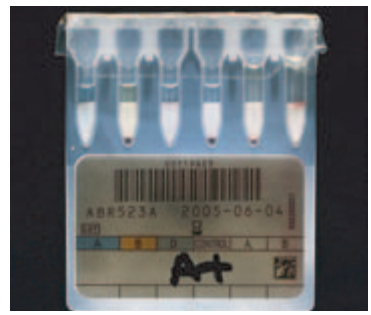
(a) Blood components.



(b) Blood grouping of 12 subjects using microtitre plates. Agglutinated cells form a dense 'button' leaving the plasma clear. Reagents placed in the rows 1–3 are for red cell group (α = anti-A, β = anti-B, α + β = anti A + B), serum group (rows 4/5, A cells, B cells) and row 6 is a negative control (patient cells in patient serum). Rows 7 and 8 are patients' red cells with two different anti-D reagents to determine the Rh (D) group.



(c) Blood group using a gel system within an automated analyser. Reading left to right: lane 1 has anti-A, lane 2 has anti-B and lane 3 has anti-Rhesus D anti-serum. Lane 4 is a negative control, lane 5 has A cells and lane 6 has B cells. This individual is therefore group A Rh (D) positive.



Whole blood or plasma is collected from volunteer donors. Over 90% of the donated blood is separated to allow use of individual cell components and plasma from which specific blood products can be manufactured (Fig. 41a).

In the UK blood donors are healthy volunteers, aged 17–70 years, who are not on medication, have had no serious previous illnesses and are at low risk for transmitting infectious agents. Those who have received blood product transfusion, drug abusers, haemophiliacs, those who have recently travelled outside Europe or lived in Africa—where malaria or AIDS may be endemic—and their sexual partners, are excluded. Donors are screened for anaemia and they donate two or three times each year.

Donated blood is routinely tested:

- for hepatitis B and C, HIV 1 and 2, *Treponema pallidum*;
- serologically to determine the blood group (A, B or O) and Rh C, D and E type;
- selectively as antibodies to cytomegalovirus (CMV) are used to identify donations which are CMV-negative and thus suitable for certain patients.

Blood grouping and compatibility testing

Red cells

Red cells have surface antigens which are glycoproteins or glycolipids (Table 41.1). Individuals lacking a red cell antigen may make antibodies if exposed to it by transfusion or by transfer of fetal red cells across the placenta in pregnancy. Antibodies to ABO antigens occur naturally, are IgM and complete (detectable by incubation of red cells with antibody in saline at room temperature). Antibodies to other red cell antigens appear only after sensitization. They are usually IgG and incomplete, detected by special techniques, e.g. enzyme-treated red cells, addition of albumin to the reaction mixture or the indirect antiglobulin (Coombs') reaction (see Fig. 16b). Antibodies may cause:

- intravascular (e.g. ABO incompatibility) or extravascular (e.g. Rh incompatibility) haemolysis of donor red cells in the recipient; and
- haemolytic disease of the fetus and newborn because of transplacental passage.

Table 41.1 Red cell antigens and antibodies. Incidence in UK individuals given in brackets.

Cell antigens	Naturally occurring antibodies (usually IgM)	Antibodies only occurring after sensitization ('atypical' or immune (usually IgG))
A (40%)	Anti-B	
B (8%)	Anti-A	
AB (3%)	—	
O (45%)	Anti-A and Anti-B	
Rhesus (D) (85%)	—	
Rhesus cde/cde (i.e. Rh-negative) (15%)	—	Anti-D (Anti-C, Anti-c, Anti-E less common)
Kell (K) (9%)	—	Anti-Kell
Duffy (Fya, Fyb) (60%)	—	Anti-Duffy
Kidd (JKa, JKb) (75%)	—	Anti-Kidd

N.B. Red cells with antigens AA or AO group as A, BB or BO group as B.

Blood grouping

An individual's red cell group is determined by suspending washed red cells with diluted anti-A, anti-B, anti A+B and anti Rh (D). This is usually carried out in microtitre plates (Fig. 41b) or gels (Fig. 41c) but automated machines are increasingly used. Agglutination indicates a positive test. Serum is simultaneously incubated with group A, B and O cells to confirm the presence of the expected naturally occurring ABO antibodies. The recipient's serum is also incubated against a pool of group O cells which together express the most common antigens against which 'atypical' antibodies occur. If such an antibody is found, it is characterized. If it is clinically significant, donor blood negative for the corresponding antigen is used for transfusion.

Compatibility testing

Compatibility testing (cross-matching) entails suspension of red cells from a donor pack with recipient serum, incubation (at room temperature and 37°C) to allow reactions to occur, and examination for agglutination, including indirect antiglobulin test (see Fig. 16b) to ensure that no reaction has occurred.

Red cell transfusion (Fig. 41a)

Indications

- Haemorrhage, severe anaemia refractory to other therapy or needing rapid correction.
- If repeated transfusions likely, phenotyped ABO and Rh (D) compatible red cells which correspond as closely as possible to the minor red cell antigens of the recipient are used to minimize sensitization.

Types of red cells

- Whole blood—for treatment of acute haemorrhage with hypovolaemia. Fresh whole blood (<5 days post collection) is preferable for neonates.
- Red cells in optimal additive solution (OAS) ('packed'), e.g. containing sodium chloride, adenine, glucose and mannitol (SAG-M) which gives red cells a shelf life of 30–35 days. These are generally used for patients with anaemia requiring red cell transfusion.
- Leucocyte-depleted red cells have been passed through a leucocyte filter either at the bedside or in the laboratory. They are given to reduce reactions to leucocytes in patients sensitized to HLA antigens (e.g. multiply transfused patients), to reduce incidence of sensitization to HLA antigens and in patients requiring CMV-negative components (e.g. CMV-negative transplant recipients, neonates/premature babies, pregnant females) when CMV status of donor is unknown. Leucodepletion reduces the theoretical risk of transmission of new variant Creutzfeldt–Jacob disease (nvCJD, see below) and all red cells for transfusion in the UK are now leucodepleted.

Autologous donation

Autologous donation of red cells is suitable for some patients awaiting elective surgery. Patients donate their own red cells preoperatively on several occasions and receive iron. Donated units are screened for infectious agents in the usual way and stored at 4°C. Directed donations, e.g. within families, are not considered ethical within the UK.

Platelet transfusion (Fig. 41a)

A single donor unit is prepared from a unit of whole blood by centrifugation within hours of collection. It contains approximately 5×10^{10} platelets in 50–60 mL fresh plasma; shelf life of 4–6 days. Standard adult dose is five pooled units and group ABO and Rh compatible, but not crossmatched, units are given. Patients with HLA antibodies may require platelets from HLA-compatible donors who have donated platelets by platelet pheresis.

Indications

Indications for platelet transfusion are:

- Thrombocytopenia $<50 \times 10^9$/L—in presence of significant bleeding or prior to an invasive procedure.
- Thrombocytopenia $<10 \times 10^9$/L—prophylactic transfusions are required in patients post chemotherapy or stem cell transplant (SCT) or with failure of marrow production.
- Platelet function defects (in presence of bleeding or prior to surgery), DIC (see Chapter 35) and dilutional thrombocytopenia following massive transfusion.

White cell transfusion

White cell (buffy coat) transfusions are now rarely used in neutropenic patients as there are few data demonstrating clinical efficacy.

Fresh frozen plasma (Fig. 41a)

Fresh frozen plasma (FFP) is a source of all coagulation and other plasma proteins. Compatibility testing is not required, but blood group compatible units are used. Fresh frozen plasma from group AB donors may be used if the recipient blood group is unknown. As FFP is not heat sterilized and may transmit infection, solvent-treated FFP may be safer in this regard.

Indications

- Coagulation factor replacement. Perform coagulation tests and platelet count prior to use. Patients with DIC or massive transfusion at risk of bleeding and with coagulation abnormalities may benefit. Single factor deficiencies are best treated with a specific factor concentrate.
- Liver disease—in the presence of bleeding or prior to invasive procedures, e.g. liver biopsy; combined with vitamin K.
- Haemolytic uraemic syndrome or thrombotic thrombocytopenic purpura often with plasma exchange. Cryoprecipitate-poor FFP is preferred.
- Reversal of oral anticoagulation or thrombolytic therapy.

Cryoprecipitate

Cyroprecipitate is prepared from the precipitate formed from FFP during controlled thawing, resuspended in 20 mL plasma. It is rich in fibrinogen, fibronectin and factor VIII. Group compatible units are used. It may be useful in patients with DIC, liver disease, following massive transfusion and, rarely, in von Willebrand's disease.

Other blood products

These are derived from pooled human plasma which has undergone a manufacturing process designed to concentrate and sterilize the component. They carry a theoretical risk of transmitting diseases caused by prions (e.g. nvCJD). Current UK practice is to use products made by recombinant DNA or plasma from non-UK donors wherever possible.

Coagulation factor concentrates

Coagulation factor concentrates are available as freeze-dried powder of high purity. Factor VIII concentrate is used for the treatment of haemophilia A and von Willebrand's disease; recombinant factor VIII is now available. Factor IX concentrate, also available as recombinant, is used in patients with haemophilia B. Factor IX complex (prothrombin complex) also contains factors II, VII and X and is of value in patients with specific disorders involving factors II and X, oral anticoagulant overdose, in severe liver failure and to overcome inhibitors to factor VIII in patients with haemophilia A who have developed inhibitors. Its use carries a risk of provoking thrombosis and DIC.

Other concentrates include protein C, antithrombin, factors VII, XI and XIII, and are used in the corresponding congenital deficiencies. Recombinant human factor VIIa may be used for patients with massive uncontrollable haemorrhage post trauma and surgery.

Albumin solution

Albumin solution is available as 5%, 20% and 20% salt-poor formulations. It contains no coagulation factors. It is used in the treatment of hypovolaemia, particularly when caused by burns, and shock associated with multiple organ failure. Synthetic plasma volume expanders (e.g. dextrans, gelatin and hydroxyethyl starch) are of equal value in initial management. These 'colloids' remain longer within the intravascular space than 'crystalloid' solutions (e.g. 0.9% NaCl), exert a colloid osmotic effect and may elevate blood pressure. Resistant oedema in patients with renal and hepatic disease requires 20% albumin.

Immunoglobulins

Immunoglobulins (Igs) are prepared from pooled donor plasma by fractionation and sterile filtration. Specific Igs include hepatitis B and herpes zoster which provide passive immune protection. Standard human Ig for intramuscular injection is used for prophylaxis against hepatitis A, rubella and measles, whereas hyperimmune globulin is prepared from donors with high titres of the relevant antibodies for prophylaxis of tetanus, hepatitis A, diphtheria, rabies, mumps, measles, rubella, CMV and *Pseudomonas* infections. Intravenous Ig may be used to protect against infections in patients with

Table 41.2 Complications of blood transfusion.

1 Administrative and clerical errors leading to incompatibility between donor and recipient
2 Circulatory overload
Congestive heart failure
3 Immunological reactions
Haemolytic transfusion reactions
Immediate, e.g. ABO compatibility
Delayed, e.g. Rh incompatibility
Non-haemolytic transfusion reactions
Caused by HLA antibodies
Caused by hypersensitivity to plasma components
4 Transmission of microbial disease
Bacterial, e.g. *Yersinia*
Protozoal, e.g. malaria
Viral
Plasma-borne viruses
Hepatitis B + variants
Hepatitis A (rarely)
Other unidentified hepatitis viruses
HIV-1 and HIV-2 (also cellular)
Parvovirus
Cell associated viruses
Cytomegalovirus
Epstein–Barr virus
HTLV-I and HTLV-II
Prions
5 Iron overload
6 Other complications
Immune deficiency, graft vs. host disease
7 Complications of massive transfusion
Hypothermia, disseminated intravascular coagulation, thrombocytopenia, electrolyte disturbance
Transfusion-associated lung injury

Hepatitis B vaccine should be given to HBV-negative recipients of pooled plasma products or patients requiring repeated red cell transfusion.

congenital or acquired immune deficiency and is of value in some autoimmune disorders, e.g. immune thrombocytopenic purpura.

Complications of transfusion

(See Table 41.2)

- **Administrative and clerical errors** must be avoided by rigorous adherence to procedures for checking and documentation when ordering, prescribing, issuing and administering blood components. **These are by far the most common cause of serious and 'near miss' incidents.**
- **Congestive heart failure** caused by circulatory overload.
- **Immunological reactions** may occur with transfusion of cellular and plasma derived blood components. ABO incompatible red cell transfusions may lead to life-threatening intravascular haemolysis of transfused cells with fever, rigors, haemoglobinuria, hypotension and renal failure. Atypical antibodies arising from previous transfusions or pregnancy may cause intravascular or, more commonly, delayed extravascular haemolysis with anaemia, jaundice, splenomegaly and fever.
- **Hypersensitivity reactions** to plasma components may cause urticaria, wheezing, facial oedema and pyrexia but can cause anaphylactic shock, especially in IgA-deficient subjects.

Treatment

The transfusion must be stopped immediately. For severe reactions, the clerical details must be checked and samples from the donor unit and recipient are analysed for compatibility and haemolysis. Recipient serum is analysed for the presence of atypical red cell, leucocyte, HLA and plasma protein antibodies. Support care to maintain blood pressure and renal function, to promote diuresis and treat shock (intravenous steroids, antihistamines, adrenaline (epinephrine) in severe cases) may be necessary.

Transmission of infection

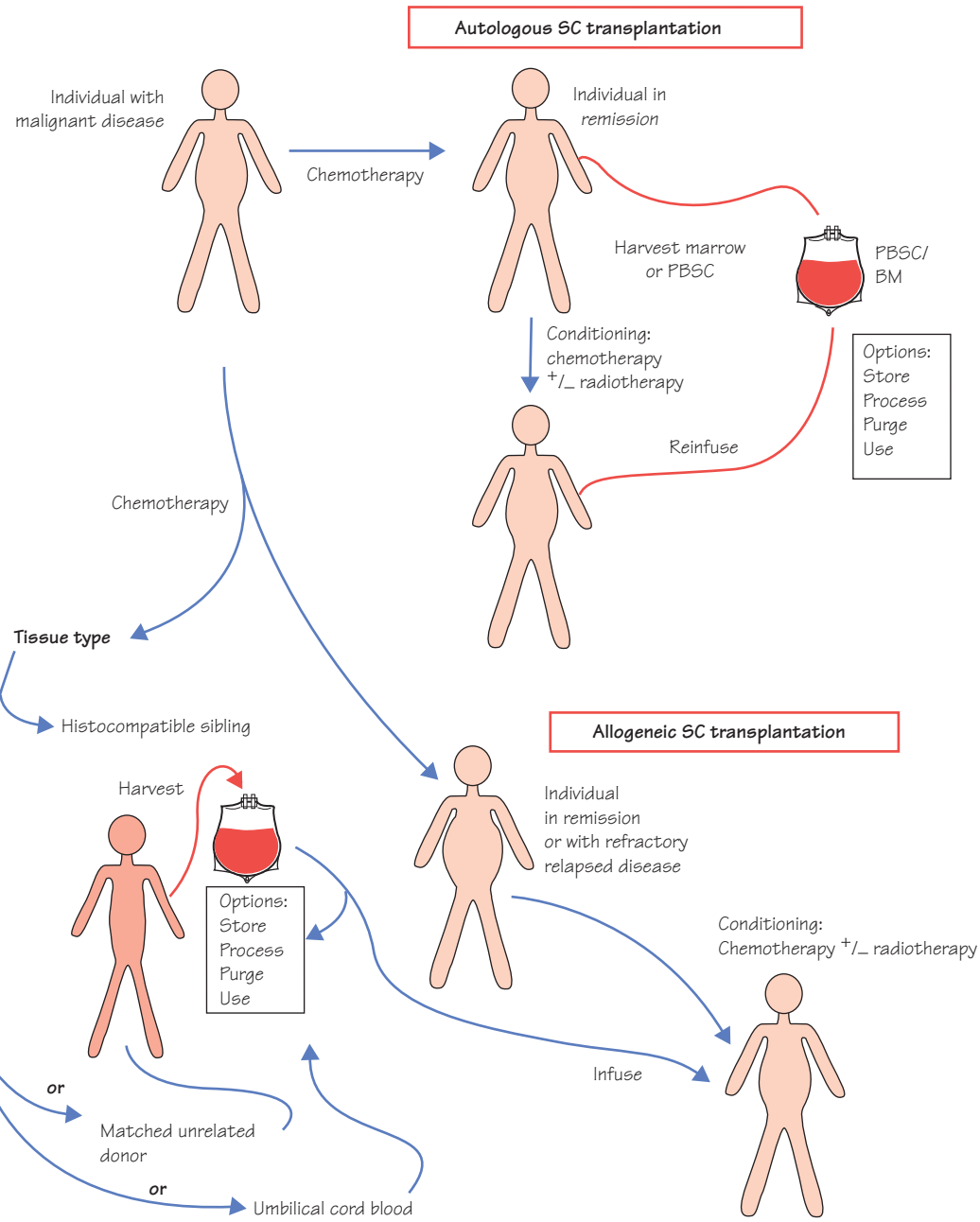
Bacterial infections can occur through failure of sterile technique at the time of collection or because of bacteraemia in the donor. **Protozoal infections** (e.g. malaria) can be transmitted and at-risk donors are not eligible to donate. **Viral infection** can be transmitted in spite of mandatory screening, as seroconversion may not have occurred in an infected donor, the virus may not have been identified, or the most sensitive serological tests may not be routinely performed (e.g. testing for antihepatitis B core antibodies). The risk of transmission is much lower for those blood products which have undergone a manufacturing and sterilization process. There is a risk of transmitting prion diseases, e.g. nvCJD, by plasma products (see above).

Other complications

Iron overload occurs in multiply transfused patients (see Chapter 11). Graft vs. host disease may be caused by transfusion of viable T lymphocytes into severely immunosuppressed hosts so cellular components should be irradiated prior to transfusion to fetuses, premature neonates, SCT recipients and other severely immunocompromised patients.

42 Stem cell transplantation

(a) Stem cell transplantation. Harvested stem cells may be frozen and stored indefinitely. They may be 'processed', for example to concentrate CD34 cells or remove T cells. Procedures are available to 'purge' them of residual malignant cells (e.g. by use of monoclonal antibodies).



Stem cell transplantation (SCT) (Fig. 42a) is the use of haemopoietic stem cells (HSC) from a donor harvested from peripheral blood (peripheral blood stem cells, PBSC) or bone marrow, to repopulate recipient bone marrow.

- **Allogeneic** SCT involves transplantation of HSC from one individual to another. This is usually between two HLA matching individuals, most frequently siblings but, in their absence, volunteer and HLA matched unrelated donors (MUD) are increasingly being

Table 42.1 Indications for stem cell transplantation.

Allogeneic	Autologous
Acute leukaemia	**Selected patients**
Standard/poor risk AML in first remission	Multiple myeloma
AML in second remission	Lymphoma
Poor risk childhood or adult ALL in first remission	Acute leukaemia
ALL in second remission	Autoimmune disease e.g. scleroderma
Chronic or accelerated phase CML	
Severe aplastic anaemia	
Selected patients	
Myelodysplasia	
Lymphoma	
Myeloma	
Chronic lymphocytic leukaemia	
Thalassaemia major, sickle cell disease	
Severe inherited metabolic diseases, e.g. adenosine deaminase deficiency, Hurler's syndrome	

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia.

used. HLA matching includes class I (A,B tested serologically) and class II (DR tested serologically or by molecular typing). If the donor is an identical twin, the transplant is termed 'syngeneic'. Allogeneic SCT with 'full' conditioning is rarely performed in individuals >55 years of age, as it carries a risk of treatment-related morbidity and even mortality (up to 5–10%) which increases with age. 'Mini' transplants in which the recipient receives immunosuppressive but not myeloablative therapy are currently being explored. Pre-transplant conditioning is less intensive and the procedure appears to be safer in older patients.

- **Autologous** SCT utilizes the patient's own stem cells. These are harvested from the patient then used to repopulate the marrow after further high-dose chemotherapy and/or radiotherapy. Autologous SCT may be performed more safely in older patients, up to 70 years.
- **Cord** blood transplantation utilizes fetal stem cells harvested at the time of birth from the umbilical cord.

Indications (Table 42.1)

Stem cell transplantation is used to 'rescue' the patient from bone marrow failure or following intensive chemotherapy (± radiotherapy). For allogeneic SCT the recipient requires 'conditioning' therapy (chemotherapy ± radiotherapy) pretransplant to cause immunosuppression, thereby reducing the risk of marrow rejection, and to eradicate malignant disease in bone marrow and elsewhere. The transplanted immune system in an allogeneic SCT itself has an antitumour, e.g. graft vs. leukaemia (GVL) effect.

Procedure

Treatment with a haemopoietic growth factor (e.g. G-CSF), combined in the case of autologous SCT with chemotherapy, e.g. high-dose cyclophosphamide, is used to mobilize HSC from bone marrow into peripheral blood, where they are collected by leucopheresis (see Figs. 43a and 44d). Alternatively, HSC may be harvested from marrow by multiple bone marrow aspirations, performed under general anaesthesia. Approximately 2×10^8/kg nucleated cells or 2×10^6/kg CD34 cells are needed (CD34 is a surface marker of early haemopoietic stem and progenitor cells). The recipient of an allogeneic or MUD transplant then receives immunosuppressive drugs to reduce the risk of graft vs. host disease (GVHD) (see below).

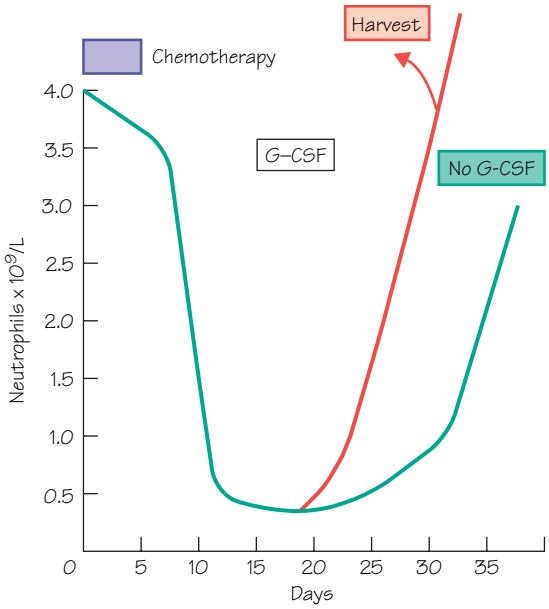
Complications

Complications of SCT include the following:

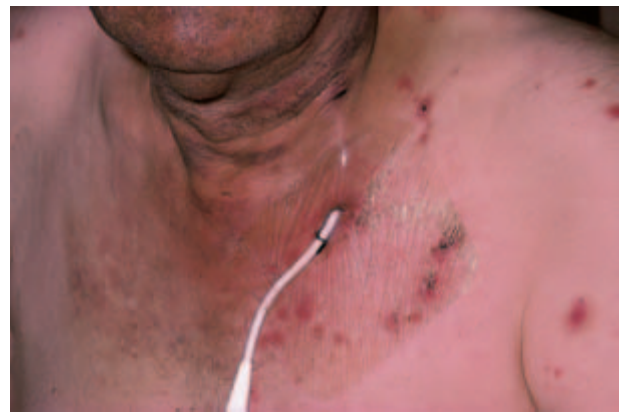
- Side effects of conditioning chemotherapy/radiotherapy, e.g. bone marrow failure, nausea, alopecia, skin burns, pulmonary toxicity, hepatic veno-occlusive disease, toxicity to endocrine organs and growth retardation.
- Rejection of transplanted HSC.
- Relapse of original disease. This is sometimes treated by infusion of lymphocytes from the allogeneic donor, which will have a GVL effect.
- Infection following SCT occurs because patients are severely immunosuppressed. Infection may be bacterial, viral, protozoal or fungal. Prophylactic antibiotics, antifungal and antiviral therapy is given. CMV-negative recipients should receive blood components which are leucodepleted or from CMV-negative donors. CMV infection may cause pneumonitis, diarrhoea, liver dysfunction, skin rash and graft failure, and is a major cause of transplant-related mortality. Ganciclovir and foscarnet are useful in the treatment of CMV infection. Prophylaxis against *Pneumocystis carinii* infection is with oral co-trimoxazole and/or nebulized pentamidine.
- Metabolic problems, often caused by multiple intravenous drugs (antibiotics, antivirals, antifungals), renal failure, blood component therapy, intravenous feeding, etc.
- Graft vs. host disease (GVHD) (allogeneic SCT). Transplanted lymphocytes may recognize the recipient as 'foreign' and mount an immunological onslaught, to cause skin rash, liver disease and diarrhoea. The incidence of GVHD is higher in older patients. Acute GVHD (<100 days after SCT) typically begins 7–10 days after transplantation and is graded according to severity. Chronic GVHD (>100 days) presents as a scleroderma-like syndrome with liver, lung, gastrointestinal or joint abnormalities. The incidence and severity of GVHD may be decreased by depleting T cells from donor marrow and immune suppression of the recipient, e.g. with ciclosporin and methotrexate.

43 General aspects of treatment

(a) The use of the growth factor G-CSF accelerates recovery of the white cell count after chemotherapy. It also affects granulocyte function.



(b) Acute leukaemia: Hickman line infection, which has rapidly evolved to septicaemia, and bloodborne skin lesions caused by coagulase-negative staphylococci.



Chemotherapy

Chemotherapy is the use of pharmacological agents (Table 43.1) to treat malignant or other proliferative diseases. It may be given orally, by bolus, prolonged subcutaneous or intravenous injection/infusion or intrathecally. It may be a single agent or combination chemotherapy utilizing drugs with different, preferably synergistic, modes of action, with limited or no overlapping toxicity, and aimed at delaying emergence of drug resistance. Chemotherapy drugs are often given as a cycle of a few days' treatment every 3–6 weeks to allow normal cells, especially of the bone marrow and gastrointestinal tract, to recover from toxicity. Extravasation into tissues can cause severe local reactions. Intravenous chemotherapy is usually given through a central line or through a tunnelled intravenous catheter (e.g. Hickman's) or indwelling chamber (e.g. Porta-Cath).

Side effects of chemotherapy (Fig. 46a)

Most chemotherapeutic agents are toxic to normal dividing cells (gastrointestinal tract, haemopoietic cells, hair, skin) as well as to malignant cells. Common side effects include the following:

- Bone marrow failure (anaemia, thrombocytopenia, leucopenia) with increased susceptibility to bleeding and infection, which may require therapy with antimicrobials, blood components and recombinant growth factors (G-CSF, GM-CSF, erythropoietin).
- Nausea and vomiting, requiring antiemetic therapy—metoclopramide, dexamethazone and 5-HT antagonists (e.g. ondansetron, granisetron).
- Mucositis (sore mouth and throat), abdominal pain and diarrhoea. An epidermal growth factor is in trial.
- Infertility—sperm storage considered *before* chemotherapy.
- Tumour lysis syndrome—prevented by good hydration, alkalinization of urine, allopurinol.
- Hyperuricaemia—prevented by allopurinol, rasburicase.
- Specific side effects of chemotherapy drugs are shown in Fig. 46a.

Mechanism of action

Chemotherapy drugs generally affect DNA synthesis or repair and promote cellular apoptosis. Cycle-specific agents prevent DNA synthesis and act on the S phase of the cell cycle (Table 43.1). Non-cycle-specific agents act at all phases of the cell cycle and include alkylating agents, which bind to DNA, and anthracyclines, which cause DNA strand breaks. Inhibition of the DNA repair enzyme, topoisomerase II, is an important component of the action of anthracyclines and etoposide. Bortezomib is a proteosome inhibitor and modulates cytokine/growth factor levels.

Biological therapies

Growth factors in clinical use (see Chapter 1) include G-CSF (Fig. 43a), and erythropoietin. The interferons are naturally occurring agents which have both antineoplastic and anti-infective properties. Interferon-α is used in the treatment of chronic myeloid leukaemia, multiple myeloma, non-Hodgkin lymphoma and myeloproliferative disorders. Monoclonal antibodies, e.g. rituximab (anti CD20), Alemtuzimab (anti CD52) and Mylotarg (anti CD33), alone or bound to toxins or radioactive isotopes, may be used to kill specific cells or target drug therapy. Thalidomide and its derivatives are used to treat myeloma, myelodysplasia and myelofibrosis.

Table 43.1 Chemotherapy agents.

DNA binding	Thioguanine	
Anthracyclines	Cytosine arabinoside	
Daunorubucin	Hydroxyurea	
Hydroxydaunorubicin	Azacytidine	
Idarubicin		
Other	**Inhibitors of DNA repair enzymes**	
Mitoxantrone	Epipodophyllotoxins	
Bleomycin		
	Antipurines	
Alkylating agents	Fludarabine	
Cyclophosphamide	Deoxycoformycin	
Ifosphamide	2-Chlorodeoxyadenosine	
Chlorambucil		
Melphalan	**Others**	
Nitrosoureas (BCNU, CCNU)	Corticosteroids	
Busulfan	L-Asparaginase	
	Biological agents: interferon,	
Mitotic inhibitors	thalidomide and derivatives	
Vincristine	Bortezomib	
Vindesine		
Vinblastine	**Monoclonal antibodies**	
	Rituximab	anti CD20
Antimetabolites	Alemtuzimab	anti CD52
Methotrexate	Mylotarg	anti CD33
Mercaptopurine	Zevalin	anti CD20

Infection

The main risk factors are:

- neutropenia (particularly if $<0.5 \times 10^9$/L) for bacterial and fungal infections;
- organisms which are normal commensals may be pathogenic for immunocompromised patients;
- defective cell-mediated or humoral immunity for viral, bacterial and atypical infections (e.g. tuberculosis);
- others include indwelling catheters (intravenous, urethral), corticosteroid therapy, mucositis and impaired splenic function.

Organisms

These include the following:

- bacterial—Gram-positive (coagulase-negative and -positive staphylococci, streptococci, enterococci); Gram-negative (klebsiella, pseudomonas, *Escherichia coli*, proteus); others, e.g. tuberculosis, atypical mycobacteria (Figs 43b);
- fungal—*Candida*, aspergillus;
- viral—cytomegalovirus, herpesviruses, adenoviruses;
- protozoal—*Toxoplasma*, *Pneumocystis*, *Leishmania*, *Histoplasma*.

Prevention

- Good hygiene on the part of the patient and staff, regular hand cleaning and avoidance of contact with infected individuals.
- Barrier nursing in isolation is preferred. Staff wear gowns and gloves when in contact with severely neutropenic patients.
- Filtered air at positive pressure reduces risk from fungal spores.
- Food should ideally be cooked. Foods frequently contaminated with bacteria (soft cheeses, uncooked eggs and meat, salads, live yoghurt) are avoided and only peeled fruits are allowed.
- Oral non-absorbable antibiotics (e.g. neomycin, colistin) will reduce colonization of the gastrointestinal tract, oral systemic antibiotics (ciprofloxacin, co-trimoxazole) reduce the incidence of bacteraemia and oral antifungals (fluconazole/amphotericin/itraconazole) and/or oral antiviral (aciclovir) prophylaxis are routinely given in some units.

Diagnosis

- Fever is the cardinal sign of infection: tachycardia, tachypnoea, fall in blood pressure, cough, dysuria, altered mental state may also occur.
- Physical signs include reddened throat, inflamed intravenous catheter site, skin rash, chest signs, mouth signs and perineal inflammation. Pus is absent in neutropenic patients.
- Special tests to identify the responsible organism include microbial culture (sputum, urine, throat and perineal swab, blood cultures from peripheral blood and indwelling catheter, lumbar puncture—if neurological symptoms—skin swabs, faecal culture). Bronchoalveolar lavage may be necessary. Serological tests for specific organisms, e.g. aspergillus, may be of value. Imaging tests may include chest X-ray, CT scan, especially of chest if fungal infection is suspected, and sinus X-rays.

Treatment

- Supportive care for renal failure/hypotension/respiratory failure.
- Empirical antibacterial therapy should be commenced in patients who are neutropenic ($<0.5 \times 10^9$/L) or otherwise severely immunocompromised and develop fever (temperature of 38°C or greater lasting for more than 2 hours). Cultures should be taken and intravenous antibiotics should be commenced, with either a single, potent broad-spectrum agent (e.g. a fourth generation cephalosporin or ureidopenicillin) or a combination of agents with activity against Gram-negative and Gram-positive (including coagulase negative staphylococci) organisms.

Radiotherapy

Ionizing radiation, usually derived from an external source, is used to treat disease by causing DNA damage in malignant cells. It is commonly used in the treatment of haematological malignancies (e.g. lymphoid leukaemias, lymphoma, myeloma) and as part of conditioning therapy for stem cell transplant for malignant disease. Side effects include nausea, vomiting, alopecia, bone marrow failure, damage to normal tissues (e.g. skin burns), growth retardation and induction of second malignancies.

Counselling

Counselling is valuable for various groups of haematological patients:

- Patients and families with malignant disease need emotional support during treatment and/or bereavement. Practical help with housing, transport, welfare benefits, etc. There should be good liaison with GPs, terminal care support teams in the community and hospices.
- Genetic counselling is needed in families with haemophilia, thalassaemia, sickle cell disease and related conditions.

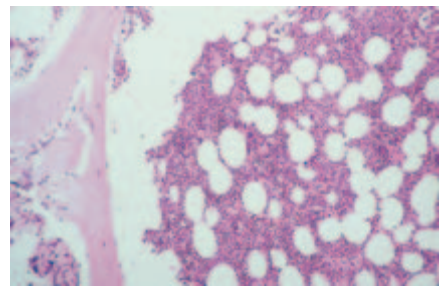
44 Practical procedures

(a) (i) Bone marrow trephine biopsy needle. (ii) Bone marrow aspirate needle.

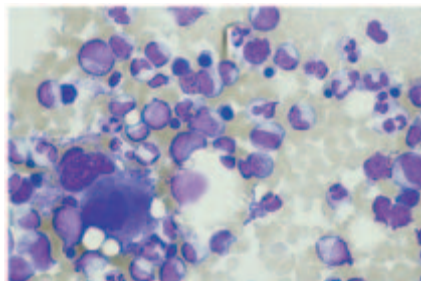
(i)

(ii)

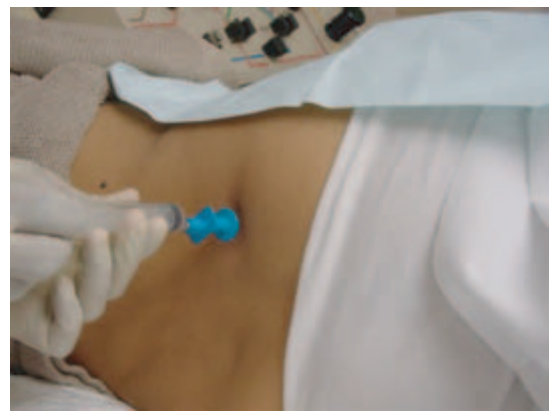
(b) Low power microscopic view of bone marrow trephine biopsy.



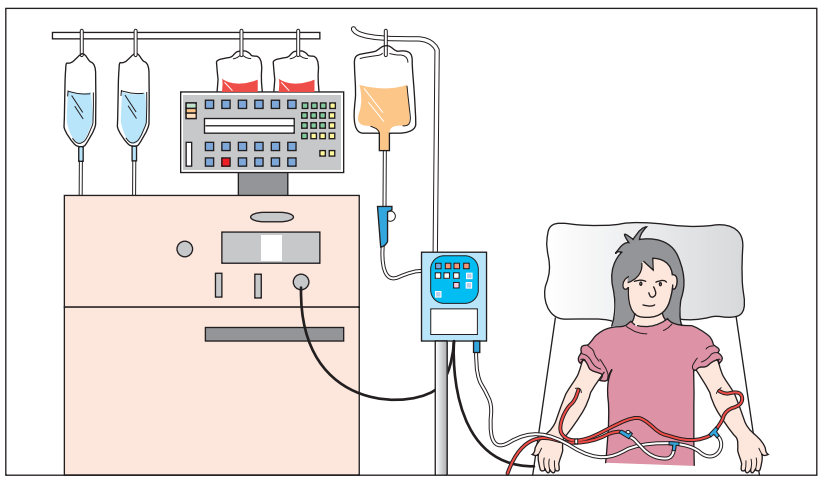
(c) Low power microscopic view of bone marrow aspirate.



(d) Bone marrow aspiration.



(e) Apheresis.



Apheresis

This is a technique whereby whole blood is removed from the body and processed by centrifugation into its cellular components and plasma (Fig. 44e). In plasma exchange, the plasma is removed and replaced by albumin (or fresh frozen plasma in thrombotic thrombocytopenia purpura, TTP, see p. 75). In leucapheresis, the white cells are removed. The process can also be used to perform a red cell exchange (e.g. in sickle cell disease); to remove platelets (platelet

Table 44.1 Indications for bone marrow aspiration (and trephine).*

Unexplained cytopenia:*
Anaemia, leucopenia, thrombocytopenia
Suspected marrow infiltrate:*
Leukaemia, myelodysplasia, lymphoma, myeloproliferative disease, myeloma, carcinoma, storage disorders
Suspected infection:
Leishmaniasis, tuberculosis

*Bone marrow trephine is required for pancytopenia or suspected marrow infiltration.

pheresis); to isolate lymphocytes for donation (donor lymphocyte infusion, DLI, e.g. for the treatment of relapse following a stem cell transplant); or for harvesting of haemopoietic stem cells from peripheral blood.

Bone marrow aspiration and trephine biopsy

Bone marrow is aspirated from the posterior iliac crest. Alternative sites for aspiration of marrow are the sternum and the medial part of the tibia (children). The procedure is under local anaesthesia with or without intravenous sedation (Fig. 44d). Indications for marrow aspiration are listed in Table 44.1. Aspirated cells and particles of marrow are spread on slides (Fig. 44c), stained by Romanowsky's stain and Perls' stain (for iron; see Fig. 11b or Figs 44b, c). Specialized tests may also be performed (Table 44.2).

Bone marrow trephine biopsy

This is a more invasive procedure, using a larger needle (Fig. 44a(i)), whereby a core of bone is biopsied from the iliac crest. This is then fixed in formalin and sections are cut. A trephine biopsy is a good way of assessing the overall architecture and cellularity of the marrow and can identify foci of infiltration by malignant or non-haemopoietic cells.

Lumbar puncture

Cerebrospinal fluid is sampled from patients with haematological diseases for:

Table 44.2 Special tests on bone marrow cells.

Chromosomes:
Conventional cytogenetics, e.g. diagnosis and classification of leukaemia, myelodysplasia
Fluorescent in situ hybridization (FISH) for translocations, location of oncogenes
Molecular tests:
DNA/RNA analysis/polymerase chain reaction (PCR) eg. diagnosis and classification of leukaemia
Detection of minimal residual disease
Immune phenotype analysis:
Diagnosis and classification of leukaemia, lymphoproliferative diseases
Detection of residual disease
Microbiological cultures, e.g. tuberculosis
Cytochemistry:
Diagnosis of acute leukaemias

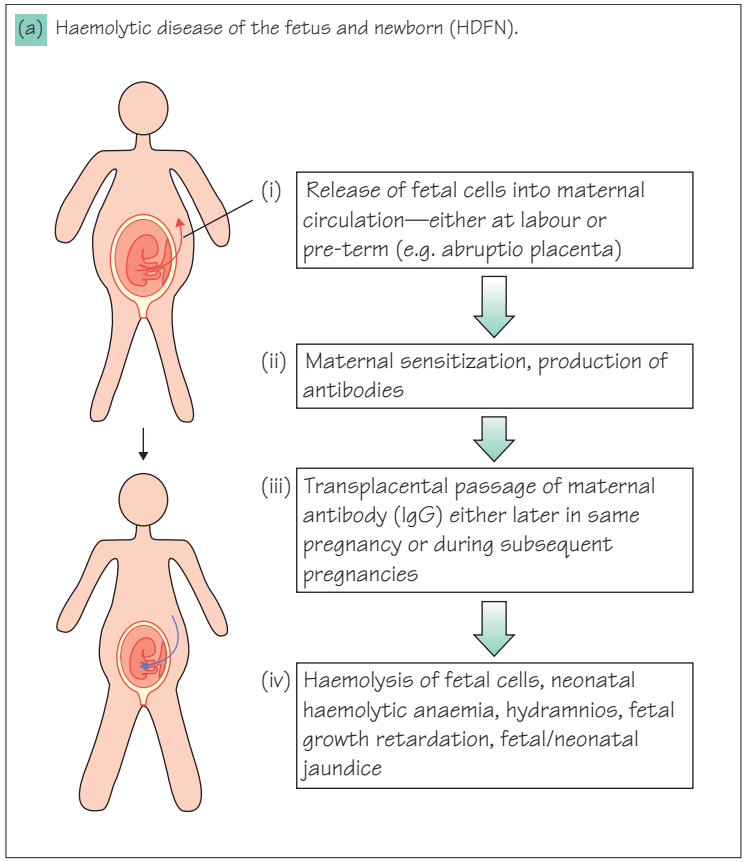
- Diagnosis—e.g. in case of infection; or infiltration by malignant cells.
- Treatment—e.g. malignancy (acute leukaemia, lymphoma) with intrathecal chemotherapy.

Lumbar puncture should only by undertaken in patients who have a platelet count >50 × 10^9/L and after correction of coagulation abnormalities. Intrathecal chemotherapy should only be prescribed and administered by those who have appropriate training and experience.

Hickman line care

Indwelling lines which have been tunnelled under the skin and then into the subclavian vein (see Chapter 43) allow easy venous access for blood sampling, administration of intravenous drugs, e.g. chemotherapy, antibiotics and feeding. They must be kept patent by instillation of heparin when not in use. They should be sampled wearing gloves and sterile technique. The first 5–10 mL of blood must be discarded before samples are sent for testing. If the tube is blocked, the installation of urokinase or alteplase (tissue plasminogen activator) is used to dissolve the thrombus.

45 Haematology of pregnancy and infancy



Anaemia

Plasma volume increases by up to 50% the during first and second trimesters, whereas red cell mass (RCM) increases by only 20–30%. Haemodilution results (haemoglobin falls to a mean 10.5 g/dL between 16 and 40 weeks). A physiological rise in MCV of 5–10 fL occurs. Increase in RCM, iron transfer to the fetus and blood loss during labour together require about 1000 mg of iron, so that iron deficiency is frequent. Folate requirements rise because of increased catabolism. Early supplementation (e.g. 400 µg daily) reduces the risk of megaloblastic anaemia and of fetal neural tube defects (see Chapter 13). The serum B_{12} level falls below normal in 20–30% of pregnant woman, to rise again spontaneously post delivery. Autoimmune haemolytic anaemia in pregnancy is typically severe and refractory to therapy. Haemolytic anaemia with elevated liver enzymes and low platelets (HELLP syndrome) and epigastric pain may occur in the last trimester. Disseminated intravascular coagulation may accompany HELLP syndrome and induction of labour or caesarean section is often necessary.

White cells

Mid neutrophil leucocytosis with a left shift.

Platelets

Gestational thrombocytopenia complicates 8–10% of pregnancies, is mild (platelets 80–150 × 10^9/L) and is not associated with neonatal thrombocytopenia or significant bleeding. Maternal immune thrombocytopenic purpura may antedate or present in pregnancy and is associated with increased levels of platelet-associated IgG or serum platelet autoantibodies. Management includes no therapy (absence of bleeding, platelets >50 × 10^9/L), corticosteroids or intravenous immunoglobulin, which also crosses placenta to elevate the fetal platelet count. Thrombocytopenia occurs in pre-eclampsia (mechanism unknown); low-dose aspirin therapy may reduce platelet consumption.

Coagulation changes

Coagulation changes (Table 45.1) combine to give an increased risk of thrombosis and disseminated intravascular coagulation (DIC). This occurs in up to 40% of cases of abruptio placenta, leading to haemorrhage and shock. Retention of a dead fetus usually leads to chronic low-grade DIC with onset over 1–2 weeks. Venous stasis resulting from the gravid uterus combines with these changes to make pregnancy a hypercoagulable state; operative delivery imposes an additional risk.

Table 45.1 Haemostatic changes during pregnancy.

Coagulation factors:
Vitamin K dependent factors II, VII, IX, X ↑
Factor VII ↑, von Willebrand factor ↑
Fibrinogen ↑
Coagulation inhibitors:
Protein C ↑ or NC
Antithrombin ↑ or NC
Fibrinolytic activity:
Reduced
Thrombocytopenia:
Gestational
Immune
Pre-eclampsia
Thrombotic thrombocytopenic purpura (typically mid trimester)
Haemolytic uraemic syndrome (typically post delivery)
Haemolytic anaemia with elevated liver enzymes and low platelets (HELLP) syndrome

Haemolytic disease of the fetus and newborn

Haemolytic disease of the fetus and newborn (HDFN) is the haemolysis of fetal or neonatal red cells caused by transplacental passage of maternal red cell antibodies (Fig. 45a).

HDFN caused by ABO antibodies

Although ABO compatibility between mother and fetus is common, this type of HDFN is rarely severe. Most ABO antibodies are IgM and therefore cannot cross the placenta. Fetal A and B antigens are not fully developed at birth and the maternal antibodies can be partially neutralized by A and B antigens present on other cells in the plasma and in the tissue fluids.

HDFN caused by other antibodies

The most important cause is anti-D, produced in Rh (D)-negative woman as a result of sensitization during a previous pregnancy or blood transfusion and causing haemolysis in Rh (D)-positive infants. Other important causes are other antibodies within the Rh system (eg. anti-C), anti-Kell, anti-Duffy and anti-JKa antibodies.

Haemolysis of fetal cells can lead to hydrops fetalis, though nowadays it more commonly leads to neonatal haemolytic anaemia. All women must have their blood group determined at booking and atypical red cell antibodies in plasma should be detected. If present, their titre is monitored throughout pregnancy. Ultrasound for fetal growth, fetal blood sampling and measurements of bilirubin levels in amniotic fluid are used to monitor fetal well-being.

Treatment

Fetuses may be given transfusion of red cells (CMV negative and irradiated) which are compatible with maternal serum. Maternal antibody levels can be lowered by plasma exchange. Phototherapy and exchange transfusion of the neonate allows removal of unconjugated bilirubin which may otherwise deposit in the basal ganglia to cause neurological sequelae (kernicterus).

Prevention

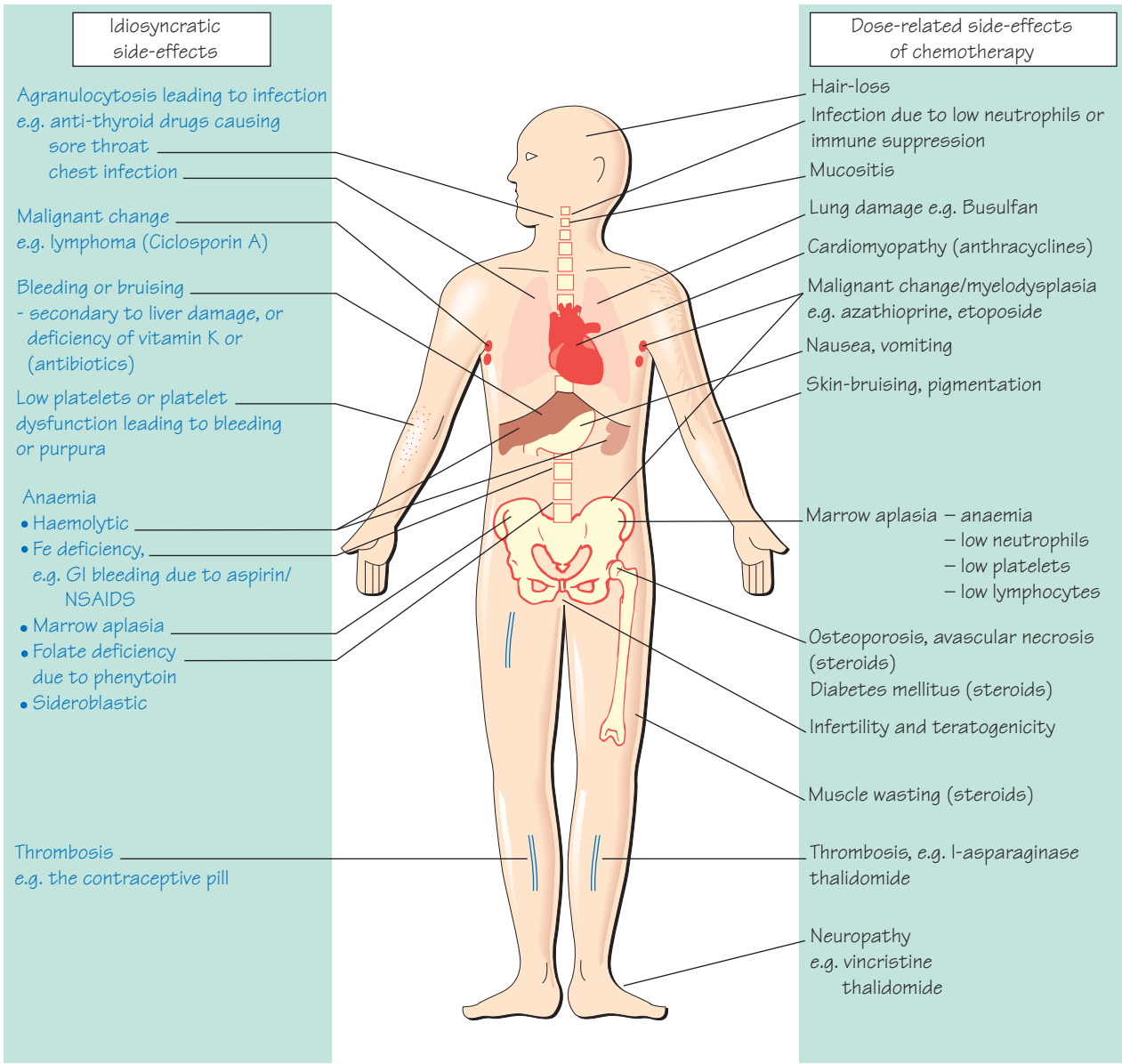
This is by administration of anti-D to unsensitized Rh(D) negative women within 72 hours of a potentially sensitizing event (e.g. birth of an Rh(D) positive fetus, abortion or ante-partum haemorrhage). The anti-D will coat fetal cells which are then removed from the maternal circulation before sensitization occurs. The dose of anti-D is adjusted according to the number of fetal cells detected in the maternal circulation (Kleihauer test).

Neonatal haematology

Anaemia—normal neonates have a raised Hb (16–18.5 g/dL^{-1}). Premature infants are typically anaemic, and this worsens over the course of the first 6 weeks. Deficiency of iron, vitamin E and folic acid may contribute. Neonates (particularly premature) have an increased susceptibility to *infection*, through typically have normal leucocyte counts. The normal range for the *platelet count* is lower in neonates (300–400; particularly in the premature). Important causes of neonatal thrombocytopenia are congenital infection (e.g. maternal CMV, rubella, toxoplasmosis and herpes simplex) and neonatal alloimmune thrombocytopenia (due to transplacental passage of anti-PLA antibodies**).** The incidence of haemorrhagic disease of the newborn can be reduced by giving an injection of vitamin K (1 mg) to newborn infants (see p. 79).

46 Haematological effects of drugs

(a) Drugs may have a wide variety of idiosyncratic side-effects on the haemopoietic system (left-hand panel). Side-effects of chemotherapy are also given on the right-hand panel and are related to the dose/duration of therapy.



Drugs may cause a wide variety of haematological changes (Fig. 46a). Two broad categories of effect occur:

1 Idiosyncratic—i.e. effects which only occur in certain individuals and are independent of the dose.

2 Dose-dependent and predictable effects.

Genetic mechanisms may underlie individual susceptibility to side-effects. Genetic traits may also influence drug metabolism—e.g. some individuals metabolize purines such that certain drugs (azathioprine) are more likely to cause bone marrow suppression.

Recognizing, monitoring and reporting haematologic toxicity is an important part of the marketing and postmarketing surveillance and assessment of new drugs. Mechanisms of haematologic toxicity include:

- direct toxicity of the drug or its metabolites to haemopoietic stem cells or more mature cells;
- induction of immune-mediated damage to haematologic stem cells;
- effects on intermediary metabolism of haematinics or vitamins;
- indirect effects via damage to other organs, e.g. the liver;
- predisposition to malignant change.

Stem cell damage

Pancytopenia occurs in a *predictable* dose-dependent fashion following chemotherapy or radiotherapy. Chemotherapeutic agents which particularly induce marrow hypocellularity include anthracyclines, epipodophyllotoxins, alkylating agents and antimetabolities. Pancytopenia typically occurs 5–8 days after commencement of treatment, its degree is dose dependent, and recovery occurs 10–20 days after commencement of therapy. Growth factors (eg. G-CSF, see p. 9) may be used to accelerate recovery of blood counts.

Idiosynchratic aplastic anaemia occurs rarely (e.g. 1 in 20 000–100 000 individuals exposed to the same drug) and is independent of the dose. Drugs with this potential side effect include antibiotics (e.g. chloramphenicol, sulfonamides), antirheumatic drugs (e.g. gold, indometacin (indomethacin)) and chlorpromazine. It is typically severe, up to 50% of patients do not recover their blood counts, and may require treatment for bone marrow failure (see p. 44).

Anaemia

The commonest form of drug-induced anaemia is iron deficiency due to blood loss. Aspirin, non-steroidal anti-inflammatory drugs (NSAIDs) and steroids can all cause bleeding from the upper GI tract. Iron absorption is impaired by tetracyclines. Megaloblastic anaemia due to folate deficiency may complicate treatment with anti-epilepsy (e.g. phenytoin) and other drugs. Some drugs, e.g. isoniazid, antagonize vitamin B_6 and cause sideroblastic anaemia and peripheral neuropathy by acting as competitive antagonists; vitamin B_6 must therefore be given with isoniazid.

Drug-induced haemolytic anaemias

These may be immune or non-immune. Immune mechanisms include the following:

- antibody directed against the drug (e.g. penicillin)–red cell membrane complex, the drug acting as a hapten;
- antibody against a drug (e.g. quinidine) with subsequent deposition of the immune complex on red cells;
- stimulation of autoantibody (warm type) production against the red cell, e.g. methyldopa, fludarabine.

Non-immune mechanisms include:

- haemolysis in G6PD deficient individuals (many drugs, see p. 37);
- haemolysis in normal individuals, e.g. dapsone.

White cells

Agranulocytosis may occur as part of aplastic anaemia or in isolation. Idiosynchratic agranulocytosis is seen with antithyroid drugs (e.g. carbimazole), deferiprone (up to 1% of all recipients), antipsychotic drugs (e.g. clozaril), antibiotics (sulfonamide, tetracycline) and anti-inflammatory drugs (e.g. some NSAIDs). Eosinophilia may be seen as part of an allergic reaction to virtually any drug.

Platelets

Drugs can cause an increased risk of bruising and bleeding by interfering with platelet function (e.g. aspirin and NSAID, which inhibit prostaglandin synthesis). Thrombocytopenia may occur as an immune phenomenon (e.g. due to sulfonamides, thiazide diuretics, quinine) or due to direct toxicity, alone or as part of aplastic anaemia (e.g. thiazides, sulfonamides).

Coagulation factors

Alterations may lead to increased risk of bleeding (e.g. aspirin-induced hypofibrinogenaemia) or prolonged antibiotic therapy causing impaired vitamin K absorption. Alternatively, an increased risk of thrombosis may occur, e.g. the contraceptive pill, or hormone replacement therapy may cause an increase in coagulation factors and a reduction in circulating levels of coagulation inhibitors (e.g. protein S).

Drug-induced malignant change

Myelodysplasia may occur following prolonged use of alkylating agents or combination chemotherapy for acute leukaemia or lymphoma. MDS or non-Hodgkin lymphoma may occur following immunosuppressive therapy (e.g. ciclosporin A or azathioprine). Immunosuppressive therapy can cause Epstein–Barr virus-associated lymphoproliferative disorders, particularly after transplantation. Lymphoma has also been reported following phenytoin therapy.

Appendix I: normal values

Normal peripheral blood count.

Cell	Normal concentration
Haemoglobin	11.5–15.5 g/dL (female)
	13.5–17.5 g/dL (male)
Red cell	$3.9–5.6 \times 10^{12}$/L (female)
	$4.5–6.5 \times 10^{12}$/L (male)
Reticulocyte	0.5–3.5%
	approx. $25–95 \times 10^{9}$/L
White cells	$4.0–11.0 \times 10^{9}$/L
Neutrophils	$2.5–7.5 \times 10^{3}$/L
	($1.5–7.5 \times 10^{9}$/L in black people)
Eosinophils	$0.04–0.4 \times 10^{9}$/L
Basophils	$0.01–0.1 \times 10^{9}$/L
Monocytes	$0.2–0.8 \times 10^{9}$/L
Lymphocytes	$1.5–3.0 \times 10^{9}$/L
Haematocrit	0.38–0.54
Mean cell volume	80–100 fL
Mean cell haemoglobin	27–33
Haematinics:	
Serum iron	10–30 µmol/L
Total iron binding capacity	40–75 µmol/L (2–4 g/L as transferrin)
Serum ferritin	40–340 µg/L (males)
	15–150 µg/L (females)
Serum folate	3.0–15.0 µg/L (4–30 nmol/L)
Red cell folate	160–640 µg/L (360–1460 nmol/L)
Serum vitamin B_{12}	160–925 µg/L (120–682 pmol/L)

Appendix II: cluster of differentiation nomenclature system

Cell surface markers are molecules in the cell membrane that can be recognized by reactivity with specific monoclonal antibodies. Their presence gives information about the lineage, function or stage of development of a particular cell population. The cluster of differentiation (CD) nomenclature system groups together antibodies recognizing the same surface molecule (antigen).

T-cell markers

CD no.	Remarks
1a, b, c	Thymocytes, Langerhans' cells (CD1a)
2	E-rosette receptor. All T cells
3	T-cell receptor. Mature T cells
4	T helper/inducer subset
5	T cells (aberrantly expressed in B-CLL, mantle cell lymphoma)
7	T cells (aberrantly expressed in some AML)
8	T cytotoxic/suppressor

B-cell markers.

CD no.	Remarks
19	B cells, including early B cells
20	Mature B cells
21	Mature B cells. C3d receptor, EBV receptor
22	B cells
23	Activated B cells
79	B cell antigen receptor
103	Hairy cells
138	Plasma cells

Myeloid and other markers.

CD no.	Remarks
Myeloid markers	
11a, 11b, 11c	Adhesion molecule ligand. Also expressed on some B and T cells and monocytes
13	All mature myeloid cells
33	Myelin-associated protein. Early myeloid cells
61	Early myeloid cells
117	Early myeloid cells
Others	
14	Monocytes, macrophages
25	IL-2 receptor-activated B cells
34	Stem cells
45	Leucocyte common antigen: all haemopoietic cells
56	Natural killer cells
9, 29, 31, 41, 42	Platelet markers
38	Plasma cell marker
71	Red cell precursors
TdT	Terminal deoxynucleotidyl transferase—early B- and T-cell precursors

Appendix III: further reading

Bain B.J. (1995) *Blood Cells: A Practical Guide* (2nd edn). Blackwell Science, Oxford.

Hoffbrand A.V., Pettit J.E. & Moss P.A.H. (2001) *Essential Haematology* (4th edn). Blackwell Science, Oxford.

Hoffbrand A.V., Catovsky D. & Tuddenham E.G.D. (eds) (2005) *Postgraduate Haematology* (5th edn). Blackwell Publishing, Oxford.

Hoffman R., Benz E.J., Shattil S.J., Furie B., Cohen M.J., Silberstein L.E. & McGlove P. (2005) *Hematology: Basic Principles and Practice* (4th edn). Churchill Livingstone, Philadelphia.

Beutler E., Lichtman M.A., Coller B.S., Kipps T.J. & Seligsohn U. (2001) *Williams Hematology* (6th edn). McGraw Hill, New York.

Appendix IV: sample questions and case histories

Questions

1 Iron deficiency anaemia
- (a) Is usually associated with a raised MCV.
- (b) The MCH is usually low.
- (c) Is most commonly due to dietary deficiency.
- (d) Is associated with a low serum ferritin.
- (e) Responds much more quickly to parenteral than oral therapy.

2 Macrocytic anaemia
- (a) Occurs in renal failure.
- (b) May result from vitamin B_{12} deficiency.
- (c) Occurs in the context of chronic inflammatory disease.
- (d) May be associated with myxoedema.
- (e) May be associated with thalassaemia.

3 Chronic myeloid leukaemia
- (a) Is the commonest form of leukaemia worldwide.
- (b) Usually presents with bone marrow failure.
- (c) Is usually associated with the presence of the Philadelphia chromosome.
- (d) May respond to treatment with interferon.
- (e) Usually transforms to an acute leukaemia.

4 Chronic lymphocytic leukaemia
- (a) Is a cause of hypogammaglobulinaemia.
- (b) Is commonly treated with intensive combination chemotherapy.
- (c) Is associated with a median survival of <2 years.
- (d) Often presents asymptomatically.
- (e) Is more commonly derived from B cells than T cells.

5 The myelodysplastic syndrome (MDS)
- (a) May occur as a result of prior chemotherapy.
- (b) Is thought to have a viral aetiology.
- (c) May be associated with the presence of sideroblastic anaemia.
- (d) May be associated with pancytopenia.
- (e) Is characterized by a reduction in the circulating monocyte count.

6 With regard to anticoagulant therapy
- (a) Warfarin is safer than heparin in pregnancy.
- (b) The INR is used to control heparin therapy.
- (c) Low molecular weight heparin can be given orally.
- (d) Vitamin K is used to reverse the action of warfarin.
- (e) Should be undertaken lifelong after a single pulmonary embolus.

7 Fresh frozen plasma
- (a) Is recommended in the treatment of haemophilia A.
- (b) Is heat treated and therefore free from risk of transmission of viral disease.
- (c) Is useful in the treatment of immune thrombocytopenia.
- (d) Is useful in the treatment of thrombotic thrombocytopenic pupura.
- (e) Must be prepared from whole blood within a few hours of donation.

8 Neonatal thrombocytopenia
- (a) Can occur in infants of mothers with immune thrombocytopenia.
- (b) Is often due to intrauterine viral infection.
- (c) May be due to transplacental passage of anti-platelet antibodies from the mother.
- (d) Often improves spontaneously.
- (e) May be associated with absent radii.

9 Haemolytic anaemia
- (a) Occurs whenever red cell survival is reduced.
- (b) Is often accompanied by an increase in serum unconjugated bilirubin.
- (c) Is usually accompanied by increased urinary bilirubin.
- (d) Is predominantly extravascular in hereditary spherocytosis.
- (e) Can lead to kernicterus in the neonate.

10 An increase in peripheral blood eosinophils (eosinophilia)
- (a) Is commonly seen in bacterial infection.
- (b) May be an indicator of drug hypersensitivity.
- (c) Is commonly seen in myeloproliferative disorders.
- (d) Can lead to cardiomyopathy.
- (e) Can occur in connective tissue disorders.

11 Haematological changes during normal pregancy include
- (a) An increase in MCV.
- (b) An increased incidence of thalassaemia trait.
- (c) Increased circulating levels of factor VIII.
- (d) Neutrophilia.
- (e) Increased platelet count.

12 Polycythaemia rubra vera
- (a) Occurs more frequently in smokers.
- (b) May present as gout.
- (c) Many transform to acute leukaemia.
- (d) Is frequently associated with raised white cell and platelet counts.
- (e) Is associated with an enlarged spleen.

13 Platelets
- (a) Are an important source of thrombin.
- (b) Are often multinucleated.
- (c) Are often increased in number in pateints with iron deficiency.
- (d) Will aggregate in response to ADP.
- (e) Are sometimes reduced in number in von Willebrand's disease.

14 Haemopoietic stem cells
(a) Are derived from the thymus.
(b) Circulate in peripheral blood.
(c) Are progenitors for plasma cells.
(d) Do not express the CD34 antigen.
(e) Decline in number with increasing age.

15 With regard to autosomal recessive conditions
(a) Glucose-6-phosphate dehydrogenase deficiency is an example.
(b) Hereditary spherocytosis is an example.
(c) There is a 1 : 2 chance that the offspring of two carriers will be homozygous.
(d) The carrier state may be associated with a small survival advantage.
(e) There is usually a disease-related mutation within a single gene.

16 The following are known to cause aplastic anaemia
(a) Chloramphenicol therapy.
(b) Malaria.
(c) Amyloidosis.
(d) Viral hepatitis.
(e) Renal cysts.

17 With regard to stem cell transplantation (SCT)
(a) Allogeneic SCT is indicated for all patients with AML in first remission who have an HLA-identical sbiling.
(b) Matched unrelated donor (MUD) transplantation is contraindicated in children.
(c) The incidence of graft versus host disease (GVHD) is reduced by depletion of T cells from the graft.
(d) Donor stem cells are irradiated to reduce GVHD.
(e) EBV infection is a major cause of post-transplant mortality.

18 The non-Hodgkin lymphomas
(a) Are more likely to be T-cell than B-cell lineage.
(b) Occur more frequently in patients with HIV infection.
(c) Are more likely to be disseminated (stage IV) when the histology is of indolent disease than when the histology shows aggressive disease.
(d) Are commoner than Hodgkin lymphoma.
(e) Are declining in incidence.

19 The following are risk factors for thrombosis
(a) Haemophilia B.
(b) Resistance is activated protein C.
(c) Nephrotic syndrome.
(d) Raised levels of plasma homocysteine.
(e) Paroxysmal nocturnal haemoglobinuria.

20 Important causes of humoral immunodeficency include
(a) Pyruvate kinase deficiency.
(b) Multiple myeloma.
(c) Indolent non-Hodgkin lymphoma.
(d) Lymphadenopathy.
(e) Presence of factor V Leiden.

21 Disseminated intravascular coagulation
(a) Is commonly seen as a presenting feature of acute promyelocytic leukaemia.
(b) Is usually associated with a raised platelet count.
(c) Is usually associated with reduced fibrinogen levels.
(d) Is usually associated with a prolonged APTT.
(e) Is usually associated with a normal TT.

22 Features suggesting a population of haemopoietic cells are monoclonal include:
(a) Reactive proliferation in response to infection.
(b) Uniform presence of an oncogene mutation.
(c) Demonstration of a common chromosomal abnormality.
(d) Positive staining for CD13 antigen.
(e) Presence of Howell Jolly bodies.

23 Thrombin
(a) Is activated by heparin.
(b) Promotes platelet aggregation.
(c) Causes deficient platelet aggregation in von Willebrand's disease.
(d) Is crosslinked by factor XIII.
(e) Is cleaved by plasmin.

24 Protein C
(a) Levels are reduced in vitamin K deficiency.
(b) Deficiency predisposes to skin necrosis after commencing oral anticoagulant therapy.
(c) Levels are inversely related to protein S levels.
(d) Levels are reduced in liver disease.
(e) Deficiency is a risk factor for thrombosis.

25 Acute leukaemia in children
(a) Is more likely to be lymphoid than myeloid.
(b) Has a remission rate following chemotherapy of <50%.
(c) Is more common in children with Down syndrome.
(d) Carries a worse prognosis if presenting WBC is $>50 \times 10^9$/L.
(e) May present with lytic bone lesions.

Answers

1 (a) False. MCV is usually low.
(b) True.
(c) False. Is most commonly due to bleeding.
(d) True.
(e) False. The rate of response is similar and related to time taken for haemopoiesis to occur (5–7 days).

2 (a) False. Renal failure is usually associated with a normochromic normocytic anaemia.
(b) True. For example, pernicious anaemia, vegetarianism, postgastrectomy.
(c) False. The anaemia of chronic disease is normocytic or mildly microcytic.
(d) True.
(e) False.

3 (a) False.
(b) False. Bone marrow failure = anaemia, leucopenia and thrombocytopenia. CML presents with leucocytosis, splenomegaly.
(c) True. >95% of patients have the Philadelphia chromosome t(9;22).
(d) True.
(e) True.

4 (a) True.
(b) False.
(c) False. Median survival is 7–10 years.
(d) True. Up to 30% of patients.
(e) True. >95% are B cell.

5 (a) True.
(b) False.
(c) True.
(d) True.
(e) False. Monocytes often raised >1000 $\times$ 10^9/L (chronic myelomonocytic leukaemia).

6 (a) False. Heparin is preferable during pregnancy. Warfarin is teratogenic.
(b) False. The INR is used to monitor warfarin therapy.
(c) False.
(d) True. Protamine is used to reverse heparin.
(e) False. Three months, unless there are any other thrombosis risk factors.

7 (a) False. Factor VIII concentrate or recombinant factor VIII is used.
(b) False. It is not heat treated. Viral transmission can occur, although the risk is low.
(c) False. Corticosteroids, immunosuppressives, splenectomy and intravenous gammaglobulin.
(d) True. Especially in conjunction with plasma exchange and if first depleted of cryoprecipitate.
(e) True.

8 (a) True. Due to transplacental passage of maternal IgG antibodies.
(b) True. For example, congenital rubella, CMV.
(c) True. For example, anti-HPA 1a antibodies.
(d) True. Due to half-life of maternally derived antibodies.
(e) True. Thrombocytopenia with absent radii (TAR).

9 (a) False. Anaemia only occurs when marrow compensation fails.
(b) True.
(c) False. The anaemia is usually acholuric.
(d) True. Haemolysis occurs within the marrow and RES.
(e) True. This is due to deposition of unconjugated bilirubin in the neonatal brain.

10 (a) False. It is commonly seen in parasitic diseases.
(b) True.
(c) False. Basophilia is much more common.
(d) True.
(e) True.

11 (a) True.
(b) False. Thalassaemia trait occurs independently of pregnancy.
(c) True.
(d) True.
(e) False. Platelet count often lowered in pregnancy.

12 (a) False. Smokers can develop secondary or spurious polycythaemia.
(b) True. This is due to hyperuricaemia.
(c) True. Approximately 5% of cases.
(d) True. In two-thirds of cases.
(e) True.

13 (a) False. They are a source of thromboxane.
(b) False. They do not have nuclei.
(c) True.
(d) True.
(e) True.

14 (a) False.
(b) True.
(c) True.
(d) False.
(e) True.

15 (a) False. It is sex-linked.
(b) False. It is autosomal dominant.
(c) False. There is a 1 : 4 chance.
(d) True.
(e) True.

16 (a) True.
(b) False.
(c) False.
(d) True.
(e) False.

17 (a) False. Selected, poor risk patients only.
(b) False. Children generally tolerate the procedure better than adults.
(c) True.
(d) False. Blood products used in supportive care are irradiated.
(e) False. CMV infection is important, however.

18 (a) False. More commonly B cell.
(b) True.
(c) True.
(d) True.
(e) False. They are increasing.

19 (a) False.
(b) True.

(c) True.
(d) True.
(e) True.

20 (a) False. This is a red cell enzymopathy.
(b) True.
(c) True. As is chronic lymphocytic leukaemia.
(d) False.
(e) False. This is a risk factor for thrombosis.

21 (a) True.
(b) False. The platelet count is usually low.
(c) True.
(d) True.
(e) False. Usually prolonged.

22 (a) False. Reactive proliferations are usually polyclonal.
(b) True.
(c) True.
(d) False.
(e) False. These are found postsplenectomy in red cells.

23 (a) False. Heparin activates antithrombin.
(b) True.
(c) False.
(d) False.
(e) False.

24 (a) True.
(b) True.
(c) False.
(d) True.
(e) True.

25 (a) True.
(b) False. Remission rates for ALL and AML >90%.
(c) True.
(d) True.
(e) True.

Case history 1

A 66-year-old caucasian man gives a history of increasing tiredness and lethargy over the preceding 2–4 months. He has recently lost his wife and has been drinking more alcohol than usual. His appetite is poor and he has lost 1 stone (6.5 kg) in weight over the past 3 months. He eats a mixed diet. His bowels are regular and he does not report any blood loss. He is not on any medication. He has not had any illnesses in the past.

On examination he is pale but not jaundiced. His blood pressure is 135/80. Abdominal examination, including rectal, is normal and there are no other abnormalities.

His FBC shows:
Hb = 7.6 g/dL
MCV = 68 fL
MCH = 26
WBC = 8.6×10^9/L
Platelets = 490×10^9/L
ESR = 83 mm/h.

Questions

What is the differential diagnosis?
How would you manage him?

Answers

A history of anorexia and weight loss suggest occult malignancy. A microcytic anaemia suggests iron deficiency, and the elevated platelet count suggests bleeding as the cause. The raised ESR supports a diagnosis of underlying malignancy.

An accurate dietary and alcohol history should be taken, although alcohol usually causes a macrocytic anaemia. A poor diet may also cause folate or B_{12} deficiency, which would also cause a macrocytic anaemia.

Management: diagnosis and treatment

Further diagnostic tests should include serum ferritin, serum B_{12} and serum folate. Urea and electrolytes and liver function tests should also be done. A search for a cause of bleeding is mandatory, even though physical examination does not offer clues to the source of blood loss. Once iron deficiency is confirmed, endoscopic and/or radiological investigation of the GI tract is indicated.

The serum ferritin was reduced at 5 µg/L confirming iron deficiency. Upper GI endoscopy revealed a malignant gastric ulcer which was successfully resected.

Case history 2

A 71-year-old man has back pain. This has been present for over 3 months and is worse in the lower back. He has also developed upper abdominal pain and constipation over the last month. He has had no serious illnesses in the past. His appetite is poor and he has lost 1 stone (6.5 kg) in weight over the previous month. His medication includes pain killers (paracetamol and ibuprofen).

On examination he is pale. His blood pressure is slightly elevated (160/100). Urine examination shows 2+ proteinuria.

Investigations show:
Hb = 8.6 g/dL
WBC = 9.5×10^9/L
Platelets = 65×10^9/L
ESR = 110 mm/h
Blood film report: Rouleaux
Leucoerythroblastic changes present.

Questions

What is the differential diagnosis?
What further tests are indicated?

Answers

A history of recent onset of back pain, with poor appetite and weight loss, suggests malignant infiltration of the skeleton. Abdominal pain and constipation are suggestive of hypercalcaemia.

Proteinuria on urine testing suggests renal disease. There is no history of prostatic obstruction. The very high ESR suggests myeloma or carcinoma with bony metastases. Further tests should

include urea and electrolytes, creatinine clearance, calcium level and serum alkaline phosphatase. X-rays of his back are required. Serum and urinary protein electrophoresis are needed to exclude myeloma.

A prostate specific antigen test to exclude prostatic carcinoma is required. This patient's calcium level was raised at 3.6 mmol/L and he was in renal failure (serum creatinine 860 mmol/L).

The serum alkaline phosphatase was normal, which is in keeping with multiple myeloma rather than secondary deposits. Skeletal survey, bone marrow, serum paraprotein level, urine Bence Jones protein, serum β_2 microglobulin are indicated (see Chapter 24).

Case history 3

A 64-year-old caucasian lady complains of gradually increasing tiredness. She feels the cold more than she used to. She also has a sore tongue. Over the past 2 months she has complained of numbness of her feet. Her sister suffers from hypothyroidism. She eats a normal diet.

On examination she is pale and slightly jaundiced. Her tongue is reddened and enlarged; she has grey hair. The thyroid gland is clinically normal. She has reduced touch and joint position sense in the toes and the ankle jerks are absent. Neurological examination is otherwise normal.

Investigations show:
Hb = 6.4 g/dL
MCV = 131 fL
WBC = 3.1×10^9/L
Platelets = 63×10^9/L.

Questions

What is the diagnosis?
Which further investigations are required?
How would you treat her?

Answer

This lady presents a classic clinical picture of pernicious anaemia (see Chapter 12).

The further investigations required are listed on p. 33.

Treatment is with hydroxocobalamin (1 mg intramuscular) immediately followed by further B_{12} injections. It is important not to give folic acid before giving B_{12}, as it may precipitate neuropathy.

Index

Page numbers in *italic* refer to figures; those in **bold** refer to tables.