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In early fetal life, blood is produced in the mesoderm of the yolk sac. During the second to seventh months the liver and spleen take over. Only in the last 2 months of fetal development does the bone marrow become the predominant site of blood formation. During childhood, marrow in the more peripheral bones becomes gradually replaced by fat, so that in adult life over 70% is located in the pelvis, vertebrae and sternum (Fig. 1). This explains the sites used for bone marrow sampling.

**Fig. 1** Sites of blood production in the fetus and after birth.

**THE STRUCTURE OF THE BONE MARROW**

A trephine biopsy allows a two-dimensional view of the bone marrow down the light microscope (Fig. 2). Haematopoietic cells of varying lineage and maturity are packed between fat spaces and bony trabeculae. Ultra-structural studies reveal clusters of haematopoietic cells surrounding vascular sinuses which allow eventual discharge of mature cells into the blood. Different lineages are compartmentalised; for example, the most immature myeloid precursors lie deep in the marrow parenchyma whilst more mature forms migrate towards the sinus wall. Lymphocytes tend to surround small radial arteries whilst erythrocytes form islands around the sinus walls.
Blood precursor cells in the marrow exist in close proximity to stromal cells. Stromal cells are those cells which do not mature into the three main types of peripheral blood cells thus they include macrophages, fat cells, endothelial cells and reticulum cells.

Immature blood cells are attached to these stromal cells by multiple cellular adhesion molecules (e.g. fibronectin and collagen). Adhesive molecules have specific receptors on stromal and haematopoietic cells. As blood cells mature, the receptors down-regulate and the cells become less adherent and commence the journey through the sinus wall and into the blood stream. The regulation of receptors is under the control of growth factors described below.

Fig. 2 Normal bone marrow

HAEMATOPOIESIS: THE STEM CELL HIERARCHY

Haematopoiesis means the formation of blood. The punctual release of blood cells from the marrow described above is the culminating event in a process simple in concept but complex in terminology.

Figures show how the cells recognisable in blood are ultimately all derived from pluripotential stem cells. Stem cells are not detectable by microscopic techniques but their existence can be inferred from cell cultures. Culture of these early cells on agar generates groups of more mature and thus recognisable progenitor cells known as colony forming units (CFUs). For myeloid development the earliest detectable precursor cell creates granulocytes, erythrocytes, monocytes and megakaryocytes and is thus called CFUGEMM. If we focus on neutrophil development it can be seen that CFUGEMM engenders the more committed precursor cells CFUGM and CFUG prior to the development of the myeloblast, the first cell in the sequence to be recognisable by light microscopy.

Pluripotential cells have the capacity for self-renewal as well as differentiation and the system allows enormous amplification. A lifetime of human haematopoiesis with the generation of incalculable numbers of mature cells may rely on only a few thousand stem cells present at birth.

GROWTH FACTORS

The events described above require regulation. This control is mediated via a group of haematopoietic growth factors. Growth factors are generally glycoproteins produced by stroma and differentiated blood cells. They may act on more than one cell lineage and frequently show additive and synergistic interactions with each other. Their actions are multiple, including the promotion of proliferation, differentiation and maturation, as well as changing functional activity. Growth factors alter the behaviour of cells by interacting with specific receptors on the cell surface (Fig. 3).

Receptors for several growth and differentiation factors have been molecularly cloned and shown to be related in structure (the 'haematopoietic growth receptor family'). The combination of factor and membrane receptor leads to a structural change in the receptor and the triggering of a complex sequence of biochemical events (signal transduction). Activation of tyrosine kinase
domains in the intracellular part of the receptor is a common mechanism (e.g. receptors for M-CSF and c-kit ligand), but there are others. The end result is the generation of intracellular regulators in the cell cytoplasm (e.g. protein kinase C, calcium ions) which have the capacity to activate genes, which in turn encode proteins essential in cell activation. Receptors are themselves highly regulated with changing numbers during cell differentiation and modulation by their own and other growth factors. Several growth factors have common receptor sub-units and mechanisms for signalling. Under normal circumstances growth factors circulate in the plasma at virtually unidentifiable levels. The activities of many factors are likely to be localised and transient so that systemic levels are of limited significance. For instance, in the marrow, factors acting at the earliest stages of haematopoiesis (e.g. c-kit ligand) are released from stromal cells in close proximity to haematopoietic precursor cells. Major haematopoietic growth factors and their key actions are illustrated. Again, the nomenclature is confusing. The colony stimulating factors (CSFS) were originally defined by their ability to stimulate blood progenitor cells while the interleukins (ILs) were defined by their effects on mature lymphocytes. Subsequent discoveries have rendered this dual nomenclature unhelpful - thus IL-3 is a key stem cell growth factor and is more logically grouped with the CSFS. The term cytokine incorporates all growth factors.

Fig. 3 Schematic view of action of growth factor on haematopoietic cell.

**The bone marrow**

The bone marrow is the site of blood formation (haematopoiesis) after birth. The cells recognisable in the blood are ultimately all derived from pluripotent stem cells in the marrow.

Immature blood cells in the marrow are attached to stromal cells by multiple cellular adhesion molecules. Maturing blood cells are eventually released through vascular sinus walls into the blood stream.

Regulation of haematopoiesis is mediated via a group of haematopoietic growth factors - these interact with specific receptors on the surface of haematopoietic cells.
The mature red cells of the blood transport the respiratory gases, oxygen and carbon dioxide (CO₂). Oxygen is carried from the lungs to the tissues where it is exchanged for CO₂. Red cells are equipped to perform this function for 120 days during which they make a 300 mile journey around the microcirculation. Prior to discharge from marrow sinuses into the peripheral blood, red cells shed their nuclei. This gives the advantages of reduced weight and transformation into a biconcave disc with increased deformability compared with the more rigid spheroidal nucleated precursor (Fig. 1).

The blood volume is comprised of the mass of red cells and the plasma. Plasma volume is regulated by stretch receptors in the heart and kidney which influence secretion of antidiuretic hormone (ADH) and aldosterone. Erythropoiesis is regulated chiefly by the growth factor erythropoietin.

Fig. 1 Scanning electron microscope picture of mature red cells showing clearly the characteristic biconcave shape,

ERYTHROPOIETIN

Unlike other growth factors, erythropoietin is mainly synthesised by the peritubular endothelial cells of the kidney. Production is triggered by tissue hypoxia (lack of oxygen), although the precise mechanism is unclear. Erythropoietin molecules bind to specific membrane receptors on primitive erythroid cells in the bone marrow and induce maturation. The increase in red cells released into the blood stops when normal oxygen transport is restored - this feedback circuit is illustrated in Figure 2.

Fig. 2 Feedback circuit in production of erythropoietin.

STRUCTURE

The mature red cell is around 7.8 µm across and 1.7 µm thick. Its biconcave shape allows maximum flexibility and an umbrella shape is adopted to traverse the smallest capillaries which have diameters of only 5 µm. The ability of red cells to recover from the recurrent stresses of the turbulent circulation hinges on the design of the membrane.

The red cell membrane is composed of a collapsible lattice of specialised proteins (the 'cytoskeleton') and an outer lipid bilayer (Fig. 3). The protein skeleton is responsible for maintaining red cell shape whilst the lipid bilayer provides a hydrophobic skin. The four skeletal proteins are spectrin, actin, protein 4.1 and ankyrin. Spectrin is the most abundant and
consists of alpha and beta chains wound around each other. Spectrin heterodimers can align at the ends to form tetramers (i.e. four chains). Spectrin tetramers are joined together by actin in association with protein 4.1. This flexible skeleton is attached to the rest of the membrane by ankyrin which links the spectrin beta chain to the cytoplasmic end of the transmembrane protein Band 3. The lipid bilayer consists mainly of a mixture of phospholipids and cholesterol. Cholesterol molecules are inserted between phospholipid molecules in such a way that they stiffen the membrane whilst still allowing a degree of fluidity between the bilayers. Defects of both the red cell membrane proteins and lipids may lead to changes in red cell shape and premature destruction.

**METABOLISM**

Red cells require an energy source to maintain their structure and also a mechanism for detoxification of oxidants. Energy is provided by the *Embden-Meyerhof pathway*, a sequence of biochemical reactions in which glucose is metabolised to lactate with the generation of two molecules of ATP. ATP maintains the osmotic pressure of the cell by driving sodium and calcium pumps in the membrane. It also provides energy for the cytoskeletal changes needed for recovery of cell shape. The Embden-Meyerhof pathway does not require oxygen as a substrate but a small amount of oxidative glycolysis occurs by the *hexose monophosphate shunt* in which glucose-6-phosphate is metabolised to generate NADPH. The hexose monophosphate shunt plays a vital role in oxygen detoxification and when oxidised substrates accumulate in the cell it increases activity several fold. Inherited deficiencies of red cell enzymes in either the Embden-Meyerhof pathway (e.g. pyruvate kinase) or the hexose monophosphate shunt (e.g. glucose-6-phosphate dehydrogenase) can lead to shortened red cell survival and haemolytic anaemia.

**HAEMOGLOBIN AND OXYGEN TRANSPORT**

*Fig. 3 The essential elements of the haemoglobin molecule.*

The key function of red cells, to carry oxygen to the tissues and return CO$_2$ from the tissues to the lungs, depends on the specialised protein haemoglobin which is present in large amounts in mature cells. The normal adult haemoglobin molecule (HbA) contains four polypeptide chains ('globin' chains): the two alpha chains and two beta chains are often notated as $\alpha_2\beta_2$. Combined with each of the polypeptide chains is a 'haem' molecule which contains ferrous iron (Fe$^{2+}$) and protoporphyrin (Fig. 3). The iron combines reversibly with oxygen and thus haem forms the oxygen carrying part of the molecule. Other globin chains are formed by the fetus and the change from fetal to adult haemoglobin occurs in the first 3 to 6 months of life. However the subunits designated $\gamma$ and $\delta$ persist into later life and small amounts of fetal haemoglobin (HbF; $\alpha_2\gamma_2$) and HbA$_2$ ($\alpha_2\delta_2$) are found in adults. Haemoglobin is more than an inert carrier molecule. The individual globin chains interact with each other to facilitate the off-loading of oxygen at lower oxygen saturations. The metabolite 2,3-Diphosphoglyceride (2,3-DPG) generated in a side-arm of the Embden-Meyerhof pathway has an important role in the process which results in a sigmoid shaped oxygen dissociation curve.
In anatomical terms haemoglobin has a high affinity for oxygen in the lungs and a much lower affinity in the tissues. The oxygen dissociation curve moves to the left when oxygen affinity increases; this occurs when $\text{H}^+$ ion concentration is reduced or haemoglobin F (which cannot bind 2,3-DPG) raised. The curve moves to the right when oxygen affinity decreases; for instance when 2,3-DPG concentration rises or the abnormal sickle haemoglobin (HbS) is present. The $P_{50}$ level is defined as the partial pressure of oxygen at which haemoglobin is half saturated.

**AGEING AND DEATH**

Beyond 100 days red cells start to show features of ageing including a declining rate of glycolysis, reduced levels of ATP and membrane lipid, and a loss of flexibility. The terminal event is unclear but effete cells are removed from the circulation by the macrophages of the liver and spleen. Most of the catabolised haemoglobin, particularly the iron, is reused. The protoporphyrin of haem is metabolised to the yellow pigment bilirubin which is bound to albumin in the plasma. Bilirubin is conjugated in the liver to a water soluble diglucuronide that is converted to stercobilin and stercobilinogen and excreted in the faeces. Some stercobilin and stercobilinogen are reabsorbed from the intestine and excreted in the urine as urobilin and urobilinogen.

![Fig. 4 The red cell membrane.](image)

**Red cells**

Erythropoiesis (the formation of red cells) is regulated by the growth factor erythropoietin. Mature red cells have a biconcave disc shape and no nucleus. The red cell membrane consists of a lattice of specialised proteins and an outer lipid bilayer. Red cells derive energy principally from the metabolism of glucose to lactate (Embden-Meyerhof pathway). Red cells contain a specialised protein, haemoglobin, which allows carriage of oxygen to the tissue and return of $\text{CO}_2$ from the tissues to the lungs.
NEUTROPHILS, EOSINOPHILS, BASOPHILS AND MONOCYTES

The term 'white cells' or 'leucocytes' refers to the nucleated cells of the blood - the neutrophils, lymphocytes, monocytes, eosinophils and basophils. All these cells play a role in defending the host against infection and other insults. Neutrophils, monocytes, eosinophils and basophils are phagocytes. They engulf and destroy foreign material and damaged cells. The term 'granulocytes' may be used to particularly describe neutrophils, eosinophils and basophils.

NEUTROPHILS

The blood neutrophil (Fig. 1a) is the end-product of an orchestrated sequence of differentiation in the myeloid cells of the bone marrow. The mature cell has a multi-lobed nucleus and small granules ('secondary' or 'specific') in the cytoplasm. Neutrophils have a limited lifespan of around 10 hours in the blood. Approximately half the cells are included in a normal blood count (the circulating pool) the remainder being in the, marginal pool'. The essential function of all these cells is to enter the tissues and combat infection. This requires both migration to the site of infection or tissue injury (chemotaxis) and the destruction of foreign material (phagocytosis). Normal chemotaxis is dependent on the release of chemotactic factors generated by bacteria and leucocytes already present at the infection site. Such factors provide the stimulus for neutrophils to leave the circulation and enter the extravascular space.

Neutrophil mobility is imbued both by the presence of adhesion molecules on the cell surface and an actin-myosin assembly in the cell membrane, the latter mediating the movement necessary for locomotion and phagocytosis. Once the cell is at the target site the foreign antigen or particle is engulfed within a phagocytic vacuole. There are various methods of killing; key mechanisms are reduction of pH within the vacuole, the release of digestive enzymes and oxidative metabolism in which antimicrobial oxidants are formed (the 'respiratory burst'). Cytokines such as G-CSF and GM-CSF not only increase neutrophil production but also promote chemotaxis and phagocytosis.

In clinical practice an increase in neutrophils in the blood ('neutrophil leucocytosis' or 'neutrophilia') is a common accompaniment to infection and tissue injury (Table 1). The strain on the neutrophil compartment often leads to younger 'band forms' being discharged from the marrow into the blood stream and the appearance of toxic changes, including coarsened granulation and vacuolation. Occasionally, phagocytosed bacteria are visible.

Reduced neutrophils in the blood (neutropenia) is seen in a wide range of inherited and acquired disorders. Serious infection is not seen regularly until the count falls below 0.5 x 10^9/l. Neutropenia may be an isolated abnormality or associated with a pancytopenia. Some common causes of an isolated neutropenia are listed in Table 2. In general, neutropenia may be caused by underproduction from the marrow (e.g. leukaemia), reduced neutrophil lifespan (e.g. immune
neutropenia), or pooling of neutrophils in a large spleen. It is important to remember that drugs may be responsible. The term benign idiopathic neutropenia is used to describe a moderate neutropenia caused by an increased fraction of cells in the marginal pool with a resultant reduction in the circulating pool. The disorder is familial and is not associated with an increased risk of infection. A similar mechanism explains the lower neutrophil normal reference range in black people compared with that in white people. In the genetic disorder, cyclical neutropenia, the neutrophil count falls every 14-21 days and recurrent infections occur.

In addition to quantitative abnormalities, neutrophils can be functionally abnormal. There are several rare inherited diseases characterised by impaired neutrophil adherence, chemotaxis or bactericidal activity. In chronic granulomatous disease, neutrophils are able to phagocytose but not kill catalase-positive microorganisms. Inheritance is autosomal or X-linked and patients suffer recurrent purulent infections and associated granuloma formation. Diagnosis is made in the nitroblue tetrazolium test where the patients neutrophils fail to reduce the dye.

Table 1 Common causes of a neutrophil leucocytosis

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physiological (e.g. pregnancy)</td>
<td></td>
</tr>
<tr>
<td>Bacterial infections</td>
<td></td>
</tr>
<tr>
<td>Inflammatory diseases (e.g. vasculitis, inflammatory bowel disease)</td>
<td></td>
</tr>
<tr>
<td>Trauma/surgery</td>
<td></td>
</tr>
<tr>
<td>Malignancy</td>
<td></td>
</tr>
<tr>
<td>Acute haemorrhage</td>
<td></td>
</tr>
<tr>
<td>Severe metabolic disorders (e.g. diabetic ketoacidosis)</td>
<td></td>
</tr>
<tr>
<td>Myeloproliferative diseases (e.g. chronic myeloid leukaemia)</td>
<td></td>
</tr>
<tr>
<td>Latrogenic (e.g. prednisolone, growth factors)</td>
<td></td>
</tr>
</tbody>
</table>

EOSINOPHILS
Eosinophils (Fig. le) are characterised by their two-lobed nucleus and red-orange staining granules. Their role is not entirely clear but like neutrophils they are attracted chemotactically and are capable of phagocytosis. They may particularly target foreign material too large for normal phagocytosis, inflicting damage by the secretion of cytotoxic enzymes. The most common causes of eosinophilia in the Western World are allergic disorders such as asthma, eczema and hay fever. In developing countries, parasitic infections are frequently implicated. Other relatively common aetiologies are drug hypersensitivity, various skin diseases and connective tissue disorders. A marked eosinophilia is occasionally seen in association with Hodgkin's disease.

**BASOPHILS**

Basophils are the least numerous of the blood leucocytes. They are easily recognised by their abundant dark purple cytoplasmic granules (Fig. 1d). The granules contain mediators of acute inflammation, including heparin and histamine. Basophils and their tissue equivalent, mast cells, have receptors for the Fc portion of IgE. They play a central role in immediate hypersensitivity reactions. Basophilia is usually associated with myeloproliferative disorders (e.g. chronic myeloid leukaemia). However, it may be reactive to a range of systemic diseases including inflammatory bowel disease and hypothyroidism. It sometimes occurs during the recovery phase from acute infection.

**MONOCYTES**

Monocytes (Fig. le) circulate in the blood before entering the tissues where they undergo transformation into macrophages. The 'mononuclear phagocyte' system consisting of monocytes and macrophages is a potentially confusing concept as macrophages subserve different functions and adopt discrete nomenclature in different tissues (e.g. osteoclasts in bone, Kupffer cells in liver). Macrophages are phagocytic cells but unlike neutrophils are able to survive the phagocytic event. They also act as accessory cells in the immune response by presenting antigens to T-lymphocytes (see p. 8) and secreting a wide range of cytokines involved in inflammation, immunity and haematopoiesis. A monocytosis in the blood occurs in chronic bacterial infections such as tuberculosis and may accompany a wide range of infective, inflammatory and malignant disorders. Monocytopenia is less frequently noted but can be severe in patients receiving corticosteroid treatment.

Table 2 **Common causes of an isolated neutropenia**

<table>
<thead>
<tr>
<th>Category</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drugs</td>
<td></td>
</tr>
<tr>
<td>Idiopathic/benign/constitutional</td>
<td></td>
</tr>
<tr>
<td>Autoimmune (sometimes with a connective tissue disorder)</td>
<td></td>
</tr>
<tr>
<td>Infections (e.g. viral, typhoid, tuberculosis)</td>
<td></td>
</tr>
</tbody>
</table>

**Neutrophils, eosinophils, basophils and monocytes**
The white cells of the blood (leucocytes) play a key role in defending the host against infection and other insults.
Neutrophils, monocytes, eosinophils and basophils are phagocytes.
These phagocytic cells may perform other functions; monocytes act as accessory cells presenting antigens to T-lymphocytes.
Each cell has a characteristic morphological appearance in the blood film.
Changes in leucocyte numbers (e.g. neutrophil leucocytosis) are common accompaniments of various disease states.
LYMPHOCYTES

Lymphocytes are essential for immunity. B-lymphocytes produce antibody against a specific antigen (humoral immunity) whilst T-lymphocytes are the cells of the cell-mediated response. T-lymphocytes require antigens to be presented by other cells including transformed monocytes termed macrophages. This is just one example of many interactions between leucocytes in the fight against foreign invasion. Most mature lymphocytes appear under the light microscope as cells with round nuclei and a thin rim of agranular cytoplasm (Fig. 1). Although B- and T-cells are not distinguishable by their morphology, there are major differences in their mode of maturation and function.

Fig. 1 Three mature lymphocytes in the blood

T-LYMPHOCYTES

T-cells make up 75% of the lymphocytes of the blood and form the basis of cell-mediated immunity. They are less autonomous than their B-cell companions, needing the cooperation of antigen-presenting cells expressing self histocompatibility molecules (human leucocyte antigens [HLA]) for the recognition of the antigen by the T-cell receptor (TCR). T-cells originate in the marrow but many are destroyed in subsequent processing by the thymus, the objective being to select the minority of cells which will recognise self-HLA but not react with self-tissue antigens. The maturation sequence is characterised by changing patterns of cell surface molecules. Mature T-cells are divisible into three basic types. Around two-thirds of blood T-cells are 'helper' cells expressing the surface marker CD4, whilst the remainder express CD8 and are of 'suppressor' or cytotoxic, type.

It appears that helper cells recognise the combination of antigen and self-HLA class 11 molecules on the antigen-presenting cell, and cytotoxic cells bind with antigen in conjunction with HLA class I molecules on the target cell. TCR genes, like immunoglobulin genes, are subject to rearrangement of germ-line DNA. Following triggering of T-cells by specific antigen reacting with the TCR, the clonal proliferation of activated T-cells is sustained by the secretion of cytokines. Interleukin-2 is the main T-cell growth factor.

B-LYMPHOCYTES

B-lymphocytes are responsible for humoral immunity. Following an appropriate antigenic stimulus they transform into plasma cells and secrete antibody specific to that antigen. B-cells are derived from the stem cells of the bone marrow. Unlike T-cells it is not clear whether they are subject to further processing at a site outside the marrow in man. Within the lymphoid tissues, such as the lymph nodes and spleen, B-cells can be stimulated by antigen to undergo a morphological transformation into immunoblasts and, ultimately, plasma cells. Stimulation of a single B-cell by antigen combining with its cell surface immunoglobulin variable region leads to a sequence of proliferation and differentiation resulting in a clone of
immunoglobulin-secreting plasma cells. Helper T-cells and cytokine-secreting macrophages facilitate this response. Memories of particular antigens are immortalised by 'memory' B-cells, allowing a prompt response to re-infection. The immunoglobulins secreted by lymphocytes and plasma cells are heterogeneous proteins, each designed to interact with a specific antigen in the defence of the body against infection (Fig. 4). There are five subclasses of immunoglobulin (Ig), dependent on the type of heavy chain (IgG, IgA, IgM, IgD and IgE) with some further division of subclasses (e.g. IgG1-a) IgM is generally produced as the initial response to infection, followed by a more prolonged production of IgG. IgA is found in secretions, whilst IgE plays a role in delayed hypersensitivity reactions. The genes encoding the heavy and light chains of immunoglobulin are rearranged from their germ line configuration during early B-cell maturation. The variable (V), diverse (D), joining (J) and constant (C) region exons undergo a complex sequence of DNA splicing, deletions and juxtapositions. The rationale of this frenetic activity prior to transcription is to allow the totality of B-cells to produce an enormously diverse population of immunoglobulins (antibodies) targeting a vast number of potential antigens. Mistakes in gene rearrangement can lead to chromosomal translocations implicated in lymphoid malignancy.

NATURAL KILLER (NK) CELLS

NK cells are a poorly defined subset of lymphocytes which share many of the characteristics of cytotoxic T-cells. However, NK cells are not restricted by the need for HLA identity and do not rearrange TCR genes. Killing is mediated either by direct adhesion to the target cell or by antibody dependent cell-mediated cytotoxicity (ADCC) in which the NK cell attacks the target cell via the Fe portion of antibody bound to antigen on the target cell surface. In the blood film, NK cells appear as large lymphocytes with abundant cytoplasmic granules.

CHANGES IN DISEASE

As can be seen from Table 1 an increase in lymphocytes in the blood (lymphocytosis) is generally a reaction to infection or is part of a malignancy. A polyclonal T-cell lymphocytosis is a common response to viral infection, particularly in childhood. Lymphocytes may be morphologically abnormal with variable changes including increased size and cytoplasmic basophilia. These heterogeneous atypical lymphocytes are seen in numerous viral infections but they are a particular feature of infectious mononucleosis. Natural killer cells play a role in the response to viral and other infections, and this may manifest as an increase in large granular lymphocytes in the blood. A number of lymphoid malignancies are associated with lymphocytosis. In acute lymphoblastic leukaemia and 'spillover' of non-Hodgkin's lymphoma cells into the blood, the malignant lymphocytes are usually morphologically distinctive and confusion with a reactive lymphocytosis rarely occurs. In chronic lymphatic leukaemia (CLL), the lymphocytes (usually B-cells) often appear unremarkable although the presence of disrupted forms, termed 'smear cells', is characteristic. CLL is the commonest form of leukaemia in many parts of the world and is frequently the cause of an otherwise unexplained lymphocytosis in an elderly person. Further tests for distinguishing between a reactive lymphocytosis and CLL are discussed on page 46.
Lymphocyte counts are often transiently low after surgery and trauma. A more chronic lymphopenia is a feature of ongoing cytotoxic drug treatment and late HIV infection when CD4 counts fall to low levels.

Table 1 **Common causes of a lymphocytosis**

**Infections**  
Acute infections (e.g. penussis, infectious mononucleosis, rubella)  
Chronic infections (e.g. tuberculosis, toxoplasmosis)

**Malignancy**  
Chronic lymphatic leukaemia and variants  
Non-Hodgkin's lymphoma (minority)  
Acute lymphoblastic leukaemia

**Lymphocytes**

Lymphocytes are essential for normal immunity.  
B-lymphocytes respond to an appropriate antigen by transforming into plasma cells and secreting specific antibody (humoral immunity).  
T-lymphocytes cooperate with antigen-presenting cells in the recognition of antigen; recognition triggers a clonal proliferation of activated T-celis (cell-mediated immunity).  
The genes encoding immunoglobulin chains and the T-cell receptor are subject to rearrangement of germ-line DNA.  
Various disease states lead to an increase in blood lymphocyte numbers (lymphocytosis): in those over 50 years, chronic lymphatic leukaemia is the usual cause.
THE SPLEEN

Although the spleen has been known of since ancient times its function has remained obscure until relatively recently. Hippocrates thought it was the source of 'black bile'. Galen suggested it may be a filter in view of its spongy consistency. Our current understanding of the spleen is dependent on a detailed appreciation of its vascular supply and the organisation of its main component parts: the white pulp, the red pulp and the intervening marginal zone.

Fig. 1 Light microscopy of the spleen clearly showing the distribution of red and white pulp.

STRUCTURE

The spleen is derived from condensation of the mesoderm in the dorsal mesogastrium of the embryo. It plays a modest haematopoietic role in the middle part of fetal life, but in the adult haematopoiesis is usually only seen in pathological states. An average adult spleen weighs about 150g and it has to become enlarged to at least three times its normal size before becoming palpable on routine clinical examination.

The splenic artery penetrates the thick capsule which invests the organ. Branches of the splenic artery are surrounded by a highly organised aggregate of lymphoid tissue which is termed the 'white pulp'. Intimate to the central arteriole is the 'periarteriolar lymphatic sheath' - an area mainly populated by T-lymphocytes. Amongst these T-lymphocytes are nonphagocytic, antigen-presenting cells known as 'interdigitating cells'. Spaced at intervals in the periarteriolar lymphatic sheath are lymphoid follicles ('Malpighian bodies'). In an inactive state these follicles are composed of recirculating B-lymphocytes intertwined with cytoplasmic processes of follicular dendritic cells. The latter cells may play a role in long-term antibody production. When contact with antigen stimulates B-cell activation a germinal centre of rapidly dividing cells forms in the follicle. This is a key area in the normal B-lymphocyte proliferative response and development of B-cell memory.

The periarteriolar lymphatic sheath and B-lymphocyte follicles are separated from the red pulp by a 'marginal zone' constituted mainly of non-circulating B-cells. The marginal zone also contains specialised macrophages able to take up carbohydrate antigens. The red pulp is composed of two alternating structures: the splenic sinuses and the splenic cords (the 'cords of Billroth'). The cords are a reticular meshwork packed with macrophages and antibody secreting plasma cells. The sinuses are broad channels lined with fusiform endothelial cells. Most of the central arterioles open into the marginal zone. As alluded to already, circulating T-lymphocytes move into the periarteriolar lymphatic sheath and B-lymphocytes migrate to the follicles. Other blood cells move slowly through the complex meshwork of the red pulp and cells which are sufficiently deformable and compliant squeeze between the endothelial cells in the sinus wall into the lumen of the sinus and back into the circulation. A small component of the splenic blood flow (the 'fast component') bypasses this slow filtration through the red pulp and passes directly into the splenic sinuses.
FUNCTION

The spleen has two major general functions:
- it removes unwanted material from the blood as well as from within red cells
- it has an important role in the immune system.

Fig. 1 The blood film in hyposplenism. There are target cells and acanthocytes

The spleen removes unwanted red cells and particles from the blood in three ways. Firstly, they can be removed by phagocytes. Bacteria, particularly encapsulated organisms that are not opsonised by antibodies and complement, are cleared from the circulation. The spleen is probably the site of the initial immune response to these organisms. Phagocytic cells in the spleen also remove red cells coated with IgG antibody.

The second mechanism at work is the removal of red cells which are not sufficiently deformable to pass through the sinus wall. Pathological states where red cells lose deformability and are destroyed prematurely in the spleen include sickle cell anaemia, hereditary spherocytosis and malaria.

Finally, the spleen can remove debris or organisms from within cells. Howell-Jolly bodies (fragments of nucleus) and malarial parasites are removed when most of the cell passes through the inter-endothelial slit with the intracellular particle abandoned on the cord side.

In addition to its filtration function the spleen has the capacity to produce antibodies. The splenic marginal zone Blymphocytes may be a source of antibodies to polysaccharide antigens.

ABNORMAL SPLENIC STATES

The syndromes arising out of splenic hypofunction and enlargement give additional insights into the normal role of the spleen.

Aspenism and Hyposplenism

Surgical removal of the spleen (spienectomy) may be indicated in a variety of haematological disorders and following trauma. The spleen may also be absent as a congenital anomaly, often associated with transpositions or malformations of the great vessels and viscera ('asplenia syndrome'). Reduced splenic function can result from splenic atrophy in disorders such as sickle cell anaemia, adult coeliac disease and essential thrombocythaemia (Table 1).

Table 1 Causes of hyposplenism

Congenital absence of spleen
Splenectomy
Sickle cell anaemia
Hyposplenism leads to characteristic changes in the blood film. Changes in red cell appearance include the presence of Howell-Jolly bodies, Pappenheimer (siderotic) granules and target cells. Other less regular red cell features are lipid-rich acanthocytes and circulating nucleated cells. There is often a moderate rise in the lymphocyte, monocyte and platelet count. Approximately one-third of circulating platelets are pooled in the normal spleen. The increase in platelets post-splenectomy is frequently impressive (greater than 1000 x 10^9/l) but the count usually falls to a lower level in the longer term.

The haematological changes are a useful guide to the presence of hyposplenism but the clinical significance of an absent spleen is the associated increased risk of life-threatening infection. The risk is greatest in children under five years of age and where there is a serious underlying medical disorder such as Hodgkin's disease or thalassaemia. Most infections occur within 2 years of splenectomy but fulminating infection can strike at any stage. In most cases infection is with encapsulated bacteria, notably Streptococcus pneumoniae, Haemophilus influenzae, and Neisseria meningitidis. In temperate regions more than half of serious infections are caused by the pneumococcus, with high mortality. Splenectomised patients have an increased susceptibility to severe malaria. Prophylaxis against such infections is evidently the best approach and recommendations for the management of asplenic patients are shown in Table 2.

**Hypersplenism**

Hypersplenism is usually defined as a depression of one or more of the cell counts in the blood which can be wholly attributed to splenic enlargement. Other criteria such as the presence of a normal bone marrow, or correction of cytopenia by splenectomy may be appended. Although the definition only requires an isolated anaemia, leucopenia or thrombocytopenia, there is frequently a moderate pancytopenia.

Splenomegaly is not always associated with hypersplenism, and hypersplenism can occur irrespective of the degree of splenic enlargement. Thus, it may be seen in the modest splenomegaly of liver cirrhosis.

The pancytopenia of hypersplenism is probably induced by three contributory mechanisms:

- Hypervolaemia consequent upon a disproportionately expanded plasma volume filling the vascular space of the enlarged spleen and the splanchnic bed.
- Intraspnic pooling of red cells which is increased from the normal 5-15% to 40% in moderate splenomegaly. This is accompanied by pooling of neutrophils and platelets.
- Premature destruction of circulating blood cells.

**Table 2 Management recommendations in the asplenic patient**

<table>
<thead>
<tr>
<th>Immunisation</th>
<th>Against Pneumococcus, Haemophilus and Meningococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotic prophylaxis</td>
<td>Oral penicillin V 250 mg bd</td>
</tr>
</tbody>
</table>
**Prompt treatment of infection**
Patient keeps course of antibiotics to avoid possible delay in treatment

**Medicalert disc or card**
Detailing asplenic state and medical contacts

**Avoid travel to high risk malarial areas**

Where possible at least two weeks prior to splenectomy. Reimmunisation is usually required, the timing determined by measurement of specific antibody levels.  
The duration of antibiotic prophylaxis is controversial: probably at least up to 18 years in children and at least 5 years in adults.  
Amoxycillin is first choice in children less than 5 years old and erythromycin at any age where there is penicillin allergy.

**The spleen**

The spleen is organised into three main components: the white pulp, the red pulp and the intervening marginal zone.  
The spleen acts as a filter, removing unwanted red cells and particles from the blood. The spleen plays a role in the immune response producing antibodies.  
An absent or poorly functioning spleen leads to characteristic blood changes and an increased risk of overwhelming infection, including fulminating malaria.  
An enlarged spleen (splenomegaly) may cause 'hypersplenism' with reduced cell counts in the blood.
HAEMOSTASIS

The clotting of blood is a critical defence mechanism which, in conjunction with inflammatory and general repair responses, helps protect the integrity of the vascular system after injury. The complex sequence of events described in detail below is activated within seconds of tissue damage. Both cells (particularly platelets) and plasma proteins play essential roles in the haemostatic mechanism. It is easiest to divide the description of normal haemostasis into a platelet component, with formation of a loose platelet plug at the site of injury, and a coagulation component where there is generation of a more robust fibrin scaffold (thrombus) around the platelets. This approach facilitates understanding but in practice the two are inextricably linked.

THE ROLE OF PLATELETS

Following damage to a blood vessel there is immediate vasoconstriction to slow blood flow and reduce the risk of exsanguination. The break in the endothelial cell barrier leads to the recruitment of platelets from the circulation to form an occlusive plug. Platelets interact both with the vessel subendothelial matrix (platelet 'adhesion') and with each other (platelet aggregation). The first step in this process, adhesion, does not require platelet metabolic activity. It does, however, lead to the activation of platelets. Platelets are small disc-shaped particles produced in megakaryocyte cytoplasm. They have no nucleus and no capacity for DNA biosynthesis but do have a complex infrastructure. Pores in the trilaminar platelet membrane connect with an open canalicular system allowing transport of agonists in and discharge of secretions out. The membrane receptors for agonists include:

- the glycoprotein (gp) Ia/IIa complex which is a receptor for collagen
- the gpIb/IX complex, a receptor for vessel wall von Willebrand's factor (vWF) and thrombin
- the gpIIb/IIIa complex which is an agonist-induced receptor for fibrinogen and vWF.

In the platelet cytoplasm are organelles including alpha granules (containing fibrinogen, vWF, thrombospondin and other proteins) and dense granules (containing small molecules such as ADP and calcium).

Platelet activation follows stimulation by agonists such as ADP and thrombin interacting with surface receptors, or by direct contact with the vessel wall subendothelial matrix. The changes occurring during platelet activation are shown schematically in Figure 1. Platelets convert from a compact disc to a sphere, surface receptors become activated, and cytoplasmic granules secrete their contents. The net effect is the mediation and reinforcement of aggregation and adhesion, and the promotion of further activation. Other circulating platelets adhere to the initial layer and a loose platelet plug is formed. In addition to the formation of a physical barrier at the site of injury platelets have a procoagulant action. The coagulation sequence described below completes much more rapidly in the presence of platelets. Following activation, platelets rearrange their membrane phospholipids and shed vesicles from their surface. The platelet surface and vesicles reveal binding sites for coagulation proteins leading to the creation of coagulation complexes (e.g. the 'prothrombinase complex') which accelerate formation of Factor X, and thrombin.
COAGULATION

Although often loosely used to encompass all aspects of clot formation, the term 'coagulation' more specifically refers to the mechanism directly leading to the conversion of the soluble plasma protein fibrinogen to the insoluble rigid polymer fibrin. The formation of the stable haemostatic plug composed of enmeshed fibrin and platelets is the culmination of a complex biochemical cascade involving circulating coagulation factors. This system allows extreme amplification with a robust thrombus arising from the initial stimulus of tissue injury. Most activated coagulation factors are proteolytic enzymes (serine proteases) which in the presence of cofactors cleave other factors in an ordered sequence. Thus, prothrombin (Factor 11), Factor VII, Factor IX and Factor X are proenzymes which are converted to their active enzyme form (denoted by the subscript 'a') by cleavage of one or two peptide bonds. Factors V and VIII are procofactors which are converted to the active cofactors (V. and VIII.) also by cleavage of peptide bonds. The blood clotting proenzymes prothrombin and Factors VII, IX and X require vitamin K for their activation.

The coagulation cascade, leading to the generation of thrombin and the formation of a fibrin thrombus, is classically divided into two parts: the intrinsic and extrinsic pathways (Table 1). In the intrinsic pathway Factor XII is activated by exposed collagen and other negatively charged components of the subendothelium. Activation of Factor XII leads to the sequential activation of Factors XI, IX, VIII (as cofactor), X and prothrombin. In the extrinsic pathway tissue factor complexes with Factor VII with sequential activation of Factors VII, X and prothrombin. Both intrinsic and extrinsic pathways terminate in the final common pathway where activated Factor X, in association with the cofactor Factor Va, in the presence of phospholipid and calcium, converts prothrombin into thrombin. Thrombin in turn converts fibrinogen to fibrin by splitting the fibrinopeptides A and B from the centre domain to form fibrin monomers. These monomers combine spontaneously into dimers which assemble to form the fibrin polymer. Factor XIII crosslinks the fibrin polymer to consolidate the thrombus. The final fibrin thrombus forms a meshwork which reinforces the platelet plug. It is fully strengthened by other adhesive proteins including thrombospondin, fibrinonectin and platelet fibrinogen.

The conventional division into two pathways is useful in the interpretation of in vitro laboratory tests of haemostasis. The prothrombin time (PT) is a simple measure of the function of the extrinsic pathway and the activated partial thromboplastin time (APTT) monitors the intrinsic pathway. However, the physiological pathways at work in vivo are not so simply defined. It seems that the intrinsic pathway is rarely relevant to coagulation in vivo - patients with a hereditary deficiency of Factor XII have a prolonged APTT but no bleeding disorder. The crucial protein in the initiation of blood coagulation is tissue factor. Tissue factor is an integral membrane protein expressed on non-vascular cells. The tissue factor-Factor VII, complex activates not only Factor X (the extrinsic pathway) but also Factor IX (the alternative pathway).

Regulation of coagulation
Blood clotting is a vital defence mechanism. However, it is important that coagulation is not allowed to become generalised with occlusion of vessels. Several regulatory mechanisms are in place. There are inhibitors of coagulation circulating in the plasma:
**Anti-thrombin III.** This is the most important inhibitor of the terminal proteins of the cascade, particularly Factor X₉ and thrombin. Its activity is greatly increased by interaction with heparin in the microvasculature and on the surface of endothelial cells. **Proteins C and S.** Protein C is a vitamin K dependent plasma protein which inactivates the cofactors V and VIII and stimulates fibrinolysis. Protein C is converted to its active enzymic form by interaction with thrombin. Protein S acts as a cofactor for Protein C.

In addition to these naturally occurring anticoagulants there is routine system for thrombus digestion - the fibrinolytic system.

**Fibrinolysis**

Once damaged endothelium is repaired the fibrin thrombus must be removed to restore normal blood flow. Thrombus removal is facilitated by a fibrin-splitting serine protease, plasmin. The fibrinolytic system is shown schematically in Figure 3. Release of tissue plasminogen activator (t-PA) from endothelial cells leads to conversion of the proenzyme plasminogen into plasmin. t-PA is most active when bound to fibrin thus maximising its action at the site of the thrombus. Plasmin has the capacity to digest fibrin in addition to fibrinogen and a number of other proteins. Digestion of a cross-linked thrombus by plasmin leads to the formation of 'degradation products' which themselves act as anticoagulants. Fibrinolysis is under strict control; circulating plasmin is inactivated by the protease inhibitor α₂-antiplasmin.

**Haemostasis**

The clotting of blood is a critical defence mechanism for integrity of the vascular system after injury.

Platelets form an occlusive plug at the site of tissue injury. They also have procoagulant action.

The term 'coagulation' describes the process by which fibrinogen is converted to the insoluble rigid polymer fibrin; the final thrombus is formed of enmeshed fibrin and platelets.

The term 'coagulation cascade' describes the sequential activation of coagulation factors; in vivo the major initiator of coagulation is tissue factor.

Fibrin generation is regulated by naturally occurring anticoagulants and fibrin is ultimately removed by the 'fibrinolytic system'.
INTRODUCTION AND CLASSIFICATION

DEFINITION
The term 'anaemia' refers to a reduction of haemoglobin or red cell concentration in the blood. With the widespread introduction of automated equipment into haematology laboratories the haemoglobin concentration has replaced the haematocrit (or 'packed cell volume') as the key measurement. Haemoglobin concentration can be determined accurately and reproducibly and is probably the laboratory value most closely correlated with the pathophysiological consequences of anaemia. Thus, anaemia is simply defined as a haemoglobin concentration below the accepted normal range.

The normal range for haemoglobin concentration varies in men and women and in different age groups (Table 1). The definition of normality requires accurate haemoglobin estimation in a carefully selected reference population. Subjects with iron deficiency (up to 30% in some unselected populations) and pregnant women must be excluded or the lower level of normality will be misleadingly low. Normal haemoglobin ranges may vary between ethnic groups and between populations living at different altitudes.

PREVALENCE
The prevalence of anaemia and the aetiologies vary in different populations. In developed countries where most studies have been performed, anaemia is more common in women than in men. Particularly susceptible groups include pregnant women, children under 5 years and those on low income. The majority of cases are caused by iron deficiency. In developing countries, factors influencing the prevalence of anaemia include climate, socio-economic conditions and, most importantly, the incidence of co-existent diseases.

GENERAL FEATURES
In anaemia the blood's reduced oxygen carrying capacity can lead to tissue hypoxia. The clinical manifestations of significant anaemia are to a large extent due to the compensatory mechanisms mobilised to counteract this hypoxia. Cardiac overactivity causes palpitations, tachycardia and heart murmurs. The dyspnoea of severe anaemia may be a sign of incipient cardiorespiratory failure. Pallor is due primarily to skin vasoconstriction with redistribution of blood flow to tissues with higher oxygen dependency such as the brain and myocardium. Anaemia is one of the most common clinical problems presenting in general practice, hospitals and in medical examinations. Usually characteristic symptoms and signs prompt a blood count to confirm the diagnosis but on occasion an unexpectedly low haemoglobin estimation in a 'routine' blood count precedes the clinical consultation. Whatever the sequence of events, anaemia is not in itself an adequate diagnosis; further enquiry to establish the underlying cause is essential.

A logical approach to anaemia demands a clear understanding of both its possible causes and its clinical and laboratory features. There are two major classifications - both have advantages and they are best used together.

Table 1 Normal haemoglobin concentrations at different ages
## CLASSIFICATION

### Morphological classification

As already discussed, modern electronic laboratory equipment can provide estimations of red cell indices in addition to haemoglobin concentration. Abnormal red cell indices should be confirmed by microscopic examination of blood films. The 'morphological' classification is based on a correlation between red cell indices and the underlying cause of anaemia. The most important measurements are of red cell size (mean cell volume or MCV) and red cell haemoglobin concentration (mean cell haemoglobin [MCH] or mean cell haemoglobin concentration [MCHC]).

Anaemias with raised, normal and reduced red cell size (MCV) are termed macrocytic, normocytic and microcytic respectively. Anaemias associated with a reduced haemoglobin concentration within red cells are termed hypochromic and those with a normal MCH are termed normochromic. Characteristic combinations are of microcytosis and hypochromia, and normocytosis and normochromia. As can be seen in Figure 1 this terminology is helpful in narrowing the differential diagnosis of anaemia. It is perhaps least helpful in normocytic anaemia as the possible causes are numerous and diverse.

The value of the blood film in diagnosis should not be underestimated. For instance, combined iron deficiency (a cause of microcytosis) and folate deficiency (a cause of macrocytosis) may
cause an anaemia with a normal MCV. However, inspection of the film will reveal a dual population of microcytic hypochromic red cells and macrocytic red cells.

**Aetiological classification**

Figure 2 illustrates a classification of anaemia based on cause. It is less immediately helpful than the morphological classification in forming a differential diagnosis but it does illuminate the pathogenesis of anaemia. The fundamental division is between excessive loss or destruction of mature red cells, and inadequate production of red cells by the marrow. Loss of red cells occurs in haemorrhage and excessive destruction in haemolysis. A normal bone marrow will respond by increasing red cell production with accelerated discharge of young red cells (reticulocytes) into the blood. Inadequate red cell production may result from insufficient erythropoiesis (i.e. a quantitative lack of red cell precursors) or ineffective erythropoiesis (i.e. defective erythrocytes destroyed in the marrow). Examples of insufficient erythropoiesis include bone marrow hypoplasia as in aplastic anaemia, and infiltration of the marrow by a leukaemia or other malignancy. Inefficient erythropoiesis is seen in disorders such as megaloblastic anaemia, thalassaemia and myelodysplastic syndromes. The above provides a useful framework for thinking about anaemia. In reality different mechanisms can operate simultaneously, e.g. the anaemia of thalassaemia is caused by both ineffective erythropoiesis and haemolysis.

**MANAGEMENT**

The treatment of specific types of anaemia is discussed in subsequent sections. However, some general statements can be made. Whenever possible, the cause of anaemia should be determined before treatment is instituted. Blood transfusion should only be used where the haemoglobin is dangerously low, where there is risk of a further dangerous fall in haemoglobin (e.g. rapid bleeding), or where no other effective treatment of anaemia is available. Prompt blood transfusion can be life-saving in a profoundly anaemic patient but it should be undertaken with great caution as heart failure can be exacerbated.

**Introduction and classification**

Anaemia is defined as a haemoglobin concentration below the accepted normal range. The normal range for haemoglobin is affected by sex, age, ethnic group and altitude. The clinical features of anaemia are largely caused by compensatory measures mobilised to counteract hypoxia. Anaemia can be classified according to red cell morphology or aetiology. Red cell indices and morphology correlate with the underlying cause of anaemia. Wherever possible the cause of anaemia should be determined before treatment is started. Blood transfusion is only required in a minority of cases.
IRON DEFICIENCY ANAEMIA

IRON

Iron is a constituent of haemoglobin and rate limiting for erythropoiesis. The metabolism of iron in the body is dominated by its role in haemoglobin synthesis (Fig. 1). Normally, the total iron content of the body remains within narrow limits: absorption of iron from food must replace any iron losses. Iron is not excreted as such but is lost in desquamated cells, particularly epithelial cells from the gastrointestinal tract. Menstruating women will lose an additional highly variable amount of iron, and in pregnancy the rate of iron loss is about 3.5 times greater than in normal men. The storage forms of iron, ferritin and haemosiderin, constitute about 30% of body iron stores.

IRON DEFICIENCY

Clinically significant iron deficiency is characterised by an anaemia which can usually be confidently diagnosed on the basis of the clinical history and blood count. It cannot be overstressed that the diagnosis of iron deficiency is not adequate in itself - a cause for the deficiency must always be sought.

CAUSES

The likely cause will vary with the age, sex and geographic location of the patient (Table 1). Iron deficiency is usually caused by long-term blood loss, generally due to gastrointestinal or uterine bleeding and less commonly to bleeding in the urinary tract or elsewhere. Particularly in elderly patients, deficiency may be the presenting feature of gastrointestinal malignancy. Hookworm infection is the commonest cause of iron deficiency worldwide. Malabsorption and increased demand for iron as in pregnancy are other possible causes. Poor diet may exacerbate iron deficiency but is rarely the sole cause outside the growth spurts of infancy and teenage years.

CLINICAL FEATURES

These can be conveniently grouped into three categories:

General symptoms and signs of anaemia

Symptoms and signs specific to iron deficiency. Iron is required by many tissues in the body, shortage particularly affecting endothelial cells. Patients with long-standing deficiency may develop nail flattening and koilonychia (concave nails), sore tongues and papillary atrophy, angular stomatitis, dysphagia due to an oesophageal web (Plummer-Vinson syndrome) and a gastritis which is usually symptomless. Many patients show none of these features and their absence is thus of little significance. Iron deficiency in young children can contribute to psychomotor delay and behavioural problems.

Symptoms and signs due to the underlying cause of iron deficiency. Patients may spontaneously complain of heavy periods, indigestion or a change in bowel habit. Once the diagnosis of iron deficiency is known, it is often useful to retake the history and re-examine the
patient with a view to detecting any clue of an underlying disorder. Rectal examination should be routine.

Table 1 Causes of iron deficiency

<table>
<thead>
<tr>
<th>Very Common</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding from the gastrointestinal tract</td>
</tr>
<tr>
<td>(e.g. benign ulcer, malignancy)</td>
</tr>
<tr>
<td>Menorrhagia</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy</td>
</tr>
<tr>
<td>Malabsorption (e.g. coeliac disease)</td>
</tr>
<tr>
<td>Malnutrition</td>
</tr>
<tr>
<td>Bleeding from urinary tract</td>
</tr>
<tr>
<td>Pulmonary haemosiderosis</td>
</tr>
</tbody>
</table>

DIAGNOSIS

The diagnosis may be suspected on the basis of the history and examination but laboratory investigations are required for confirmation.

The blood count
Iron deficiency causes a hypochromic microcytic anaemia. The automated red cell analyser generates a report with haemoglobin, MCV and MCH values below the normal range. There is a variation in red cell size (anisocytosis) reflected by a high red cell distribution width (RDW). A blood film will show characteristic features.

Confirmatory tests
Further tests are helpful in confirming the diagnosis (Table 2) and excluding other causes of a hypochromic microcytic anaemia. Measurement of serum ferritin is probably the most useful of these tests: a low level always indicates iron deficiency but a normal level does not guarantee normal stores as ferritin is increased in chronic inflammation and liver disease. In occasional difficult cases (e.g. where the patient has recently been transfused) a bone marrow aspirate is helpful in showing absence of iron stores. In practice the most likely confusion is with the anaemia of chronic disorders.

MANAGEMENT

This is divisible into investigations of the underlying cause and the correction of iron deficiency.

Investigation of underlying cause
Where the likely cause is apparent, further investigations can be highly selective. Thus in a young woman with severe menorrhagia and no other symptoms it can be assumed that uterine bleeding is the cause of iron deficiency, and investigation of the gastrointestinal tract is not necessary. A gynaecological referral would be adequate. Complaints of indigestion or a change in bowel habit should prompt an endoscopy or a colonoscopy or barium enema as first
investigations. However, often there are no symptoms suggesting a site of blood loss. As the gastrointestinal tract is the most common site in men and postmenopausal women, stool samples can be taken to check for occult blood (faecal occult bloods or FOBs). Persistent positivity is a good indicator of bleeding but negative results do not entirely exclude it. A reasonable approach to this common problem is to commence with colonoscopy and, if normal, to proceed to upper GI endoscopy. If upper GI endoscopy is performed first in an elderly patient and shows a benign ulcerative lesion then assessment of the lower GI tract should probably still be performed as coexistent colonic neoplasms are found in a significant minority of cases. If the GI tract is normal, the urine can be screened for haematuria and a chest X-ray checked to exclude the rare diagnosis, pulmonary haemosiderosis. In 20% of cases of iron deficiency no cause is found.

Table 2 **Tests to confirm iron deficiency**

<table>
<thead>
<tr>
<th>Test</th>
<th>Result in iron deficiency</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin</td>
<td>Low</td>
<td>Level increased in chronic inflammation/liver disease</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>Low</td>
<td>Low levels also in elderly and chronic disease</td>
</tr>
<tr>
<td>Serum iron</td>
<td>Low</td>
<td>Levels fluctuate significantly and low in chronic disease</td>
</tr>
<tr>
<td>TIBC</td>
<td>High</td>
<td>Useful test as low in anaemia of chronic disease</td>
</tr>
<tr>
<td>Zinc protoporphyrin</td>
<td>High</td>
<td>Late finding only</td>
</tr>
<tr>
<td>BM iron</td>
<td>Low</td>
<td>Informative but invasive investigation</td>
</tr>
<tr>
<td>Serum transferrin receptor level</td>
<td>High</td>
<td>Also high in haemolysis</td>
</tr>
</tbody>
</table>

TIBC, total iron binding capacity; BM, bone marrow

**Correction**

Oral iron is given to correct the anaemia. The normal regimen is ferrous sulphate 200 mg three times a day (providing 180 mg elemental iron daily). Side-effects, including nausea, epigastric
pain, diarrhoea and constipation, are best managed by reducing the dosage rather than changing the preparation. An adequate response to oral iron is an increase in haemoglobin of 20 g/l every 3 weeks. Iron is given for at least 6 months to replete body stores. There are several possible causes of a failure to respond to oral iron (Table 3). Intramuscular iron may occasionally be used in patients who are intolerant of oral iron or malabsorb it, or where it is necessary to rapidly replenish body stores (e.g. in late pregnancy). Intravenous infusion of iron is rarely indicated. It must be given under close supervision as anaphylaxis can occur.

Table 3 Failure to respond to oral iron possible causes
Wrong diagnosis (i.e., other cause of anaemia)
Non-compliance
Malabsorption
Continued bleeding

Iron deficiency anaemia

Iron is a constituent of haemoglobin and is essential for erythropoiesis.

Iron deficiency is most often caused by long-term blood loss.

Iron deficiency causes a hypochromic microcytic anaemia.

The anaemia is usually easily corrected with oral iron supplements.

It is important to establish the cause of iron deficiency - it may be the presenting feature of gastrointestinal malignancy.
MEGALOBLASTIC ANAEMIA

The megaloblastic anaemias are characterised by delayed maturation of the nucleus of red cells in the bone marrow due to defective synthesis of DNA. Red cells either die in the marrow ('ineffective haematopoiesis') or enter the bloodstream as enlarged, misshapen cells with a reduced survival time. In clinical practice megaloblastic anaemia is almost always caused by deficiency of vitamin B<sub>12</sub> (cobalamin) or folate (pteroylmono-glutamate). It is one of the most common causes of a macrocytic anaemia.

VITAMIN B<sub>12</sub> AND FOLATE

Key characteristics of these essential vitamins are summarised in Table 1. Vitamin B<sub>12</sub>, deficiency most commonly arises from malabsorption whilst folate deficiency is more often due to frank dietary deficiency or increased dietary requirements, as in pregnancy.

WHY DOES DEFICIENCY OF VITAMIN B<sub>12</sub>, OR FOLATE LEAD TO MEGALOBLASTIC ANAEMIA?

Both folate and vitamin B<sub>12</sub> are necessary for the synthesis of DNA. Folate is needed in its tetrahydrofolate form (FH<sub>4</sub>) as a cofactor in DNA synthesis. Deficiency of B<sub>12</sub> leads to impaired conversion of homocysteine to methionine causing folate to be 'trapped' in the methyl form. The resultant deficiency in methylene FH<sub>4</sub> deprives the cell of the coenzyme necessary for DNA formation. All dividing cells in the body suffer from the impaired DNA synthesis of B<sub>12</sub> and folate deficiency. However, the actively proliferating cells of the bone marrow are particularly affected. As RNA synthesis progresses unhindered in the cytoplasm the erythroid cells develop nuclear-cytoplasmic imbalance with abundant basophilic cytoplasm and enlarged nuclei. The chromatin pattern in the nucleus is characteristically abnormal; one author has described it as resembling 'fine scroll work', another as 'sliced salami'. The slow-down in synthesis of DNA leads to prolonged cell cycling and the cells being discharged into the blood without the normal quota of divisions. Thus the red cells are enlarged and egg shaped and the neutrophils hypersegmented due to retention of surplus nuclear material.

Table 1 Vitamin B<sub>12</sub> and folate

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Vitamin B&lt;sub&gt;12&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average dietary intake/day (mcg)</td>
<td>20</td>
</tr>
<tr>
<td>Minimum adequate intake/day (mcg)</td>
<td>1-2</td>
</tr>
<tr>
<td>Major food sources</td>
<td>Animal produce</td>
</tr>
<tr>
<td>Normal body stores</td>
<td>Sufficient for se</td>
</tr>
<tr>
<td>Mode of absorption</td>
<td>Combined with</td>
</tr>
<tr>
<td></td>
<td>secreted by gas</td>
</tr>
<tr>
<td></td>
<td>absorbed through</td>
</tr>
<tr>
<td></td>
<td>ileum via special</td>
</tr>
</tbody>
</table>
VITAMIN B₁₂ DEFICIENCY
Pernicious anaemia
This classical cause of vitamin B₁₂ deficiency is an autoimmune disorder. The majority of patients have IgG autoantibodies targeted against gastric parietal cells and the B₁₂ transport protein intrinsic factor. The precise pathogenesis, and particularly the role of the autoantibodies, is incompletely understood but B₁₂ deficiency ultimately arises from reduced secretion of intrinsic factor (IF) by parietal cells and, hence, reduced availability of the B₁₂-IF complex which is absorbed in the terminal ileum. The clinical hallmarks of pernicious anaemia are gastric parietal cell atrophy and achlorhydria, a more generalised epithelial cell atrophy and megaloblastic anaemia. The disease is most common in Northern Europe in women greater than 50 years of age and is familial. Affected patients classically have premature greying of the hair and blue eyes and may develop other autoimmune disorders including vitiligo, thyroid disease and Addison's disease. Slight jaundice is caused by the haemolysis of ineffective erythropoiesis. Patients usually have symptoms of anaemia and the generalised epithelial abnormality can manifest as glossitis and angular stomatitis. The archetypal neurological syndrome of pernicious anaemia - 'subacute combined degeneration' - arises from demyelination of the dorsal and lateral columns of the spinal cord. Patients most commonly complain of an unsteady gait, and if B₁₂ deficiency is not corrected there can be progression to irreversible damage of the central nervous system and bladder disturbance. There is an increased incidence of carcinoma of the stomach in mates in pernicious anaemia.

Diagnosis. The normal sequence of investigations is as follows:
1. Blood count and film. There is a macrocytic anaemia with the typical film appearance of megaloblastic anaemia. There may be leucopenia and thrombocytopenia.
2. Bone arrow aspirate. This is not always necessary. It will confirm megaloblastic anaemia but will not illuminate the underlying cause.
3. Estimation of vitamin B₁₂ and folate, levels. Normal methodologies include immunoassay techniques. In pernicious anaemia the serum vitamin B₁₂ level is normally very low. The serum folate may be elevated and the red cell folate reduced (folate is trapped in its extracellular methyl FH₄ form).
4. Autoantibodies. Parietal cell antibodies are found more commonly in the serum than IF antibodies (90% vs 50%) but whereas IF antibodies are almost diagnostic of pernicious anaemia, parietal cell antibodies occur in about 15% of healthy elderly people. The antibodies may also be detected in gastric juice.
5. Tests for vitamin B₁₂ absorption. Patients swallow B₁₂ labeled with radioactive cobalt and absorption is usually measured indirectly by quantifying urinary excretion (Schilling test). If malabsorption is corrected by adding IF to the oral dose, pernicious anaemia is the likely cause.
Treatment. Vitamin B\textsubscript{12} levels are replenished by intramuscular injection of the vitamin. Several injections of 1 mg hydroxycobalamin are given over the first few weeks and then one injection every 3 months for life. The increase in reticulocytes in the blood peaks 6 to 7 days after the start of treatment.

In practice patients with megaloblastic anaemia are often started on both B\textsubscript{12} and folate supplements after a blood sample has been taken for assay of the vitamins. When the results are known the unnecessary vitamin can be stopped. Blood transfusion is best avoided as it may lead to circulatory overload where judged necessary to correct hypoxia it is undertaken with extreme caution. Hypekalaemia occasionally requires correction.

Other causes of Vitamin B\textsubscript{12} deficiency As B\textsubscript{12} absorption depends on IF secretion by gastric parietal cells and a normal ileum it follows that abnormalities of the stomach or ileum may cause deficiency. Dietary deficiency is rare and usually restricted to vegans. As normal body stores are sufficient for 2 years, clinically apparent deficiency from any cause will develop slowly.

FOLATE DEFICIENCY

Folate deficiency is caused by dietary insufficiency, malabsorption, excessive utilisation or a combination of these. Patients may complain of symptoms of anaemia or of an underlying disease. Initial investigations are as for pernicious anaemia with a macrocytic anaemia and a megaloblastic bone marrow. Detection of folate deficiency is slightly complicated by the availability of assays for both serum and red cell levels. In significant deficiency both are usually low but the red cell folate is the better measure of tissue folate stores. In addition to a thorough dietary history patients may need investigations for malabsorption (e.g. jejeunal biopsy).

Folate deficiency is treated with oral folic acid 5mg once daily. This is given for several months at least, the precise duration of therapy depending on the underlying cause. Folate is prescribed prophylactically in pregnancy (400 mcg daily) and in groups of patients at high risk of deficiency (Table 2). Before folate is prescribed, vitamin B\textsubscript{12} deficiency must be excluded (or corrected) as subacute combined degeneration of the cord can be precipitated.

Megaloblastic anaemia

Megaloblastic anaemia is a common cause of a macrocytic anaemia.

In clinical practice it is almost always caused by deficiency of vitamin B\textsubscript{12} or folate.

Vitamin B\textsubscript{12} deficiency normally arises from malabsorption – the classical clinical syndrome is the autoimmune disorder pernicious anaemia.

Folate deficiency is more often due to frank dietary deficiency or increased dietary requirements as in pregnancy.

Vitamin B\textsubscript{12} deficiency should be excluded or corrected before folate is administered as subacute combined degeneration of the cord can be precipitated.
Table 2  The megaloblastic anaemias

**Vitamin B\textsubscript{12} deficiency**

<table>
<thead>
<tr>
<th>Cause</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficiency of gastric intrinsic factor</td>
<td>Pernicious anaemia</td>
</tr>
<tr>
<td>Intestinal malabsorption</td>
<td>Ileal resection/Crohn's disease</td>
</tr>
<tr>
<td></td>
<td>Stagnant loop syndrome</td>
</tr>
<tr>
<td></td>
<td>Tropical sprue</td>
</tr>
<tr>
<td></td>
<td>Fish tapeworm</td>
</tr>
<tr>
<td></td>
<td>Congenital malabsorption</td>
</tr>
<tr>
<td>Dietary deficiency</td>
<td>Vegans</td>
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</table>

**Folate deficiency**

<table>
<thead>
<tr>
<th>Cause</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary deficiency</td>
<td>Coeliac disease</td>
</tr>
<tr>
<td>Malabsorption</td>
<td>Tropical sprue</td>
</tr>
<tr>
<td>Increased requirement</td>
<td>Pregnancy</td>
</tr>
<tr>
<td></td>
<td>Haemolytic anaemia</td>
</tr>
<tr>
<td></td>
<td>Myeloproliferative / malignant / inflammatory disorders</td>
</tr>
</tbody>
</table>

**Other causes**

<table>
<thead>
<tr>
<th>Cause</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug induced suppression of DNA synthesis</td>
<td>Folate antagonists</td>
</tr>
<tr>
<td></td>
<td>Metabolic inhibitors</td>
</tr>
<tr>
<td></td>
<td>Nitrous oxide (prolonged use)</td>
</tr>
<tr>
<td>Inborn errors</td>
<td>Hereditary orotic aciduria</td>
</tr>
</tbody>
</table>
HAEMOLYTIC ANAEMIA I –
General features and inherited disorders

GENERAL FEATURES OF HAEMOLYSIS

The term 'haemolytic anaemia' describes a group of anaemias of differing aetiology that are all characterised by abnormal destruction of red cells. The hallmark of these disorders is reduced life span of the red cells rather than underproduction by the bone marrow. In classification of the haemolytic anaemias there are three main considerations:

The mode of acquisition of the disease: is it an inherited disorder or a disorder acquired in later life?
The location of the abnormality: is the abnormality within the red cell (idtrinsc) or outside it (extrinsic)?
The site of red cell destruction: red cells may be prematurely destroyed in the blood stream (intravascular haemolysis) or outside it in the spleen, and liver (extravascular haemolysis).

The simple classification in Table 1 relies upon division of the main clinical disorders into inherited and acquired types. In general, it can be seen that inherited disorders are intrinsic to the red cell and acquired disorders extrinsic. The inherited disorders can be subdivided depending on the site of the defect within the cell - in the membrane, in haemoglobin, or in metabolic pathways. Acquired disorders (discussed in the next section) are broadly divided depending on whether the aetiology has an immune basis.

DIAGNOSIS OF A HAEMOLYTIC ANAEMIA

Recognition of the general clinical and laboratory features of haemolysis usually precedes diagnosis of a particular clinical syndrome. Where haemolysis leads to significant anaemia the resultant symptoms are as for other causes of anaemia. However, the increased red cell breakdown of the haemolytic anaemias causes an additional set of problems. Accelerated catabolism of haemoglobin releases increased amounts of bilirubin into the plasma such that patients may present with jaundice. Where the spleen is a major site of red cell destruction there may be palpable spienomegaly. Severe prolonged haemolytic anaemia in childhood can lead to expansion of the mattro cavity and associated skeletal abnormalities including frontal bossing of the skull.

Table 1 Classification of the haemolytic anaemias

Inherited disorders

<table>
<thead>
<tr>
<th>Red cell membrane</th>
<th>Hereditary spherocytosis and hereditary elliptocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>Thalassaemia syndromes and sickling disorders</td>
</tr>
</tbody>
</table>
Metabolic pathways

Glucose-6-phosphate dehydrogenase and pyruvate kinase deficiency

Acquired disorders

Immune
Warm and cold autoimmune haemolytic anaemia

Isoimmune
Rhesus or ABO incompatibility (e.g. haemolytic disease of newborn, haemolytic transfusion reaction)

Non-immune and trauma
Valve prostheses, microanglopathy, infection, drugs or chemicals, hypersplenism

Initial laboratory investigations of haemoysis will include an automated blood count, a blood film and a reticulecyte count. The blood count will show low haemoglobin. Many cases of haemolysis have 'normochromic normocytic' red cell indices although some are moderately macrocytic. The latter observation is caused by the increased number of large immature red cells (reticulocytes) in the peripheral blood following a compensatory increase in red cell production by the bone marrow. Reticulocytes have a characteristic blue tinge with Romanovsky stains and their presence in the film causes 'polychromasias'. A count of reticulocytes is performed either manually on a blood film stained with a supravital stain or by the automated cell counter.

Simple laboratory tests to detect increased breakdown of red cells are also useful indicators of haemolysis. In addition to moderately raised serum bilirubin (often 30-50 mol/l), there may be raised levels of urine urobilinogen and faecal stercobilinogen. Bilirubin itself is unconjugated and therefore does not appear in the urine. Haptoglobin, a glycoprotein bound to free haemoglobin in the plasma, is depleted in haemolysis. In intravascular haemolysis, haemoglobin and haemosidetin can be detected in the urine. Haemosiderin is present for several weeks after a haemolytic episode and is simply demonstrated by staining urine sediment for iron. Examination of the bone marrow is not usually necessary in the work-up of haemolysis but, where performed, will show an increased number of immature erythroid cells. Formal demonstration of reduced red cell survival by tagging of cells with radioactive chromium ($^{51}$Cr) and in vivo surface counting of radioactivity to identify the site of red cell destruction are other possible investigations infrequently performed in practice.

INHERITED DISORDERS
DISORDERS OF THE RED CELL MEMBRANE

Hereditary spherocytosis
This autosomal dominant disease is the most common cause of inherited haemolytic disease in Northern Europeans. The defect in the red cell is a deficiency of spectrin, the major skeletal protein in the lattice-like structure which supports the membrane. Spectrin deficiency causes instability of the cell's lipid bilayer and a loss of surface area. In a blood film the red cells are spheroidal ('spherocytes') with a reduced diameter and more intense staining than normal red cells. These abnormal red cells are prone to premature destruction in the microvasculature of the spleen.
The severity of haemolysis is variable and the disease may present at any age. Fluctuating levels of jaundice and palpable splenomegaly are common features. Occasionally, patients develop severe anaemia associated with the transient marrow suppression of a viral infection; this so-called 'aplastic crisis', which may intervene in any form of chronic haemolysis, is nearly always caused by the parvovirus. Prolonged haemolysis may lead to bilirubin gallstones. Diagnosis is facilitated by the presence of a family history. The combination of general features of haemolysis and spherocytosis in the blood is suggestive of hereditary spherocytosis but not diagnostic as spherocytosis may also be seen in autoimmune haemolysis. The two haemolytic disorders are distinguished by the direct antiglobin test which is negative in hereditary spherocytosis and nearly always positive in immune haemolysis.

Spherocytes have increased 'osmotic fragility' - they lyse at higher saline concentrations than normal red cells (Fig. 3). They also show an increased rate of haemolysis when incubated in their own plasma (the 'autohaemolysis test'). No treatment is required in patients with mild disease. In more serious cases the spleen is removed as this is the main site of destruction of the abnormal red cells.

Hereditary elliptocytosis
This disease has many similarities to hereditary spherocytosis but the cells are elliptical in shape and the clinical course is usually milder. Splenectomy helps in the rare severe cases. There are various subtypes with the most common structural change being a defective spectrin molecule.

ABNORMALITIES OF HAEMOGLOBIN
These disorders are referred to collectively as the 'haemoglobinopathies'. Thalassaemia and sickle cell syndromes are discussed in later sections.

ABNORMALITIES OF RED CELL METABOLISM
The red cell has metabolic pathways to generate energy and also to protect it from oxidant stress (Fig. 4). Loss of activity of key enzymes may lead to premature destruction; there are two common examples.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency
G6PD is a necessary enzyme in the generation of reduced glutathione which protects the red cell from oxidant stress. Deficiency is sex-linked affecting males; female carriers show half normal G6PD levels. The disorder is most common in West Africa, Southern Europe, the Middle East and South-East Asia. Patients are usually asymptomatic until increased oxidant stress leads to a severe haemolytic anaemia, often with intravascular destruction of red cells. Common triggers include fava beans, drugs (many including antimalarials and analgesics) and infections. The disease can alternatively present as jaundice in the neonate. Diagnosis requires demonstration of the enzyme deficiency by direct assay - this should not be done during acute haemolysis as reticulocytes have higher enzyme levels than mature red cells and a 'false normal' level may result. Treatment is to stop any offending drug and to support the patient. Blood transfusion may be necessary.
Pyruvate kinase (PK) deficiency

In this autosomal recessive disorder patients lack an enzyme in the Embden-Meyerhof pathway. Red cells are unable to generate adequate ATP and become rigid. All general features of haemolysis can be present, but clinical symptoms are often surprisingly mild for the degree of anaemia as the block in metabolism leads to increased intracellular 2,3DPG levels facilitating release of oxygen by haemoglobin. Splenectomy may help in reducing transfusion requirements.

Haemolytic anaemia I - general features and inherited disorders

'Haemolytic anaemias' are caused by abnormal destruction of red cells.

Most inherited haemolytic disorders have a defect within the red cell whilst most acquired disorders have the defect outside the cell. Haemolysis causes characteristic clinical features and laboratory abnormalities. It may be intra- or extravascular. Hereditary spherocytosis and hereditary elliptocytosis are haemolytic disorders caused by a deficiency in the red cell membrane.

Glucose-6-phosphate dehydrogenase and pyruvate kinase are key enzymes in red cell metabolism; inherited deficiency leads to haemolysis.
AUTOIMMUNE HAEMOLYTIC ANAEMIAS

Autoimmune haemolytic anaemia (AIHA) is an example of an acquired form of haemolysis with a defect occurring outside the red cell. The bone marrow produces structurally normal red cells and premature destruction is caused by the production of an aberrant autoantibody targeted against one or more antigens on the cell membrane. Once an antibody has attached itself to the red cell, the exact nature of the haemolysis is determined by the class of antibody and the density and distribution of surface antigens. IgM autoantibodies cause destruction by agglutination or by direct activation of serum complement. IgG class antibodies generally mediate destruction by binding of the Fc portion of the cell-bound immunoglobulin molecule by macrophages in the spleen and liver. The disparate behaviour of different types of autoantibody provides the explanation for a number of different clinical syndromes.

Classification

Table I shows a simple approach to the classification of autoimmune haemolytic anaemia. The disease can be divided into warm' and 'cold' types depending on whether the antibody reacts better with red cells at 37°C or 4°C. For each of these two basic types of autoimmune haemolysis there are a number of possible causes and these can be incorporated into the classification. A diagnosis of autoimmune haemolysis may precede diagnosis of the causative underlying disease.

Clinical presentation and management

Warm autoimmune haemolytic anaemia Warm AIHA (Figs 1 & 2) is the most common form of the disease. The red cells are coated with either IgG alone, IgG and complement, or complement alone. Premature destruction of these cells usually takes place in the reticuloendothelial system. Approximately half of all cases are idiopathic but in the other half there is an apparent underlying cause (Table 1). The autoantibody is usually non-specific with reactivity against basic membrane constituents present on virtually all red cells. Patients present with the clinical and laboratory features of haemolysis discussed in the last section. Splenomegaly is a frequent examination finding. The most characteristic laboratory abnormality in warm AIHA is a positive direct antiglobulin test (DAT) sometimes known as the Coombs’ Test.

A major priority in management is the identification and treatment of any causative disorder. It is particularly important to stop an offending drug - commonly implicated agents include methyldopa and penicillin. Where the haemolysis itself requires treatment steroids are normally used (e.g. prednisolone 40-60 mg daily). In idiopathic AIHA most patients will respond to steroids with a significant rise in haemoglobin and diminished clinical symptoms. However, the disease is usually controlled rather than cured and relapses often occur when steroids are reduced in dose or stopped. Where refractoriness to steroids develops, splenectomy is usually indicated. Other immunosuppressive drugs (e.g. azathioprine) or even cytotoxic agents may be helpful in supplementing the immunesuppressive effect of prednisolone.

Table 1 Classification of the autoimmune haemolytic anaemias
Warm AIHA (usually IgG)
Primary (Idiopathic)
Secondary       Lymphoproliferative disorders
                Other neoplasms
                Connective tissue disorders
                Drugs
                Infections

Cold       AIHA (usually IgM)
Primary (Cold haemagglutinin disease)
Secondary       Lymphoproliferative disorders
                Infections (e.g. mycoplasma)
                Paroxysmal cold haemoglobinura

Cold autoimmune haemolytic anaemia
In cold AIHA the antibody is generally of IgM type with specificity for the I red cell antigen. It
attaches best to red cells in the peripheral circulation where the blood temperature is lower. As
is seen in Table I this kind of haemolysis can occur in the context of a monoclonal (i.e.
malignant) proliferation of B-lymphocytes in the so called 'Idiopathic cold haemagglutinin
syndrome' or in a variety of lymphomas. The other major cause is infection.
The severity of haemolysis varies and agglutination (clumping) of red cells) may cause
circulatory problems such as acrocyanosis, Raynaud's phenomenon and ulceration. The
haemolysis, where longstanding, is often worse in the winter. On occasion red cell destruction
is intravascular due to direct lysis by activated complement. Where this occurs free
haemoglobin is released into the plasma (haemoglobinaemia) and may appear in the urine
(haemoglobinuria) giving it a dark colour. Cold AIHA arising from infection is usually
self-limiting. Where it is chronic the mainstay of treatment is keeping the patient warm,
particularly in the extremities. In forms associated with lymphoproliferative disorders,
cytotoxic drugs such as chlorambucil and cyclophosphamide can be helpful.

ISOIMMUNE HAEMOLYTIC ANAEMIA

Here alloantibodies (isoantibodies) cause haemolysis as a result of transfusion or transfer across
the placenta. These antibodies are conventional antibodies specific for foreign antigens on
incompatible red cells.

MICROANGIOPATHIC HAEMOLYTIC ANAEMIA

Collectively, microangiopathic haemolytic anaemia (MAHA) is one of the most frequent causes
of haemolysis. The term describes intravascular destruction of red cells in the presence of an
abnormal microcirculation. There are many causes of MAHA (Table 2) but common triggers
are the presence of disseminated intravascular coagulation (DIC), abnormal platelet aggregation and vasculitis. Characteristic laboratory findings include red cell fragmentation in the blood film (Fig. 4) and the coagulation changes seen in DIC. Two specific syndromes merit brief description.

**Thrombotic thrombocytopenic purpura (TTP)**

This rare disorder often affects young adults and is characterised by MAHA, thrombocytopenia, fluctuating neurological symptoms, fever and renal failure. The pathology appears to be platelet clumping in small vessels. Mortality is high but patients can be rescued with intensive supportive care including plasma exchange with infusion of fresh frozen plasma (FFP).

**OTHER ACQUIRED HAEMOLYTIC ANAEMIAS**

Haemolysis associated with red cell fragmentation may also occur due to the mechanical effects of defective heart valves or in long distance runners who effectively stamp repeatedly on a hard surface ('march haemoglobinuria'). Certain drugs (e.g. dapsone and sulphasalazine) can cause oxidative intravascular haemolysis in normal people if taken in sufficient dosage. Many infections can cause haemolysis, either by direct invasion of red cells or via the circulatory changes already discussed. The anaemia of malaria often has a haemolytic component. *Paroxysmal nocturnal haemoglobinuria* (PNH) (Fig. 5) is a rare example of acquired haemolysis caused by an intrinsic red cell defect. In this clonal disorder arising from a somatic mutation in a stem cell, the mature blood cells have faulty anchoring of several proteins to membraneglycophosphilipids containing phosphatidylinisotol. Clinical features are highly variable and include intravascular haemolysis, pancytopenia and recurrent thrombotic episodes, including portal vein thrombosis. There is coexistent marrow damage and PNH is often associated with aplastic anaemia and may even terminate in acute leukaemia. The traditional diagnostic test exploits the cell's unusual sensitivity to complement lysis (Ham Test) but the cell's characteristic lack of certain proteins (e.g. decay accelerating factor) can also be demonstrated by flow cytometry. Treatment is generally supportive with blood transfusion and anticoagulation as required. In young patients with severe disease allogeneic bone marrow transplantation can be curative.

Table 2 *Causes of microanglopathic haemolytic anaemia*

- Haemolytic uraemic syndrome
- Thrombotic thrombocytopenic purpura
- Carcinomatosis
- Vasculitis
- Severe infections
- Pre-eclampsia
- Glomerulonephritis
- Malignant hypertension

**Haemolytic anaemia II - acquired disorders**
Autoimmune haemolytic anaemia (AIHA) can be divided into 'warm' and 'cold' types dependent on the temperature at which the antibody reacts optimally with red cells. For each type of AIHA there are possible underlying causes which must be identified and treated. The term 'microangiopathic haemolytic anaemia' (MAHA) describes the intravascular destruction of red cells in the presence of an abnormal microenvironment. Clinical syndromes associated with MAHA include haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura. Paroxysmal nocturnal haemoglobinuria (PNH) is a rare example of acquired haemolysis caused by an intrinsic red cell defect.

**Haemolytic uraemic syndrome (HUS)**

HUS mainly affects infants and children. The three main features are MAHA, renal failure and thrombocytopenia. The disease can occur as seasonal epidemics caused by *Escherichia coli* producing verotoxin; it is then preceded by bloody diarrhoea. Treatment is essentially supportive with dialysis for renal failure. Oratility range from 5 to 50%
THE THALASSAEMIAS

The thalassaemias are a heterogeneous group of inherited disorders of haemoglobin synthesis. They are characterised by a reduction in the rate of synthesis of either alpha or beta chains and are classified accordingly (i.e. cc-thalassaemia, P-thalassaemia). The basic haematological abnormality in the thalassaemias is a hypochromic microcytic anaemia of variable severity. Unbalanced synthesis of (x-and 0-globin chains can damage red cells in two ways. Firstly, failure of (x- and P-chains to combine leads to diminished haemoglobinisation of red cells to levels incompatible with survival ('ineffective erythropoiesis'). Even those hypochromic cells released into the circulation transport oxygen poorly. The second mechanism for red cell damage is the aggregation of unmatched globin chains - the inclusion bodies lead to red cell destruction either in the marrow or in the spleen (i.e. haemolysis). In general tens-ns, the clinical severity of any case of thalassaemia is proportionate to the degree of imbalance of α- and β-globin chain synthesis.

Thalassaemias are amongst the most common inherited disorders. Cases occur sporadically in most populations but the highest thalassaemia gene frequency is in a broad geographical region extending from the Mediterranean through the Middle East and India to South-East Asia.

CLASSIFICATION

The classification illustrated in Table 1 is based on the mode of inheritance of thalassaemia. As the α-globin chain gene is duplicated on each chromosome there may be total loss of α-globin chain production (termed α⁰ or -/- haplotype) or partial loss of α chain production resulting from loss of only one gene (termed α⁺ or α⁻/ haplotype). The most important clinical syndromes are haemoglobin (Hb)-Barts hydrops syndrome (-/-/-) which is incompatible with life and Hb H Disease (-α/-/-).At the molecular level the majority of cases of (α-thalassaemia result from large deletions in the α-globin gene complex; occasionally mutations can depress expression of the gene.

β-thalassaemias are autosomal recessive disorders characterised by reduced (β⁺) or absent (β⁰) production of β-chains. The heterozygous ('trait' or 'minor') form of the disease is usually symptomless whilst homozygosity is associated with the clinical disease β-thalassaemia 'major'. Homozygous mild β⁺-thalassaemia may, however, lead to a less severe clinical syndrome termed 'thalassaemia intermedia'. The β-thalassaemias are very heterogenous at the molecular level - the large majority of defects are single nucleotide substitutions affecting critical areas for the function of the β-globin gene.

Although precise classification of a thalassaemia syndrome may require sophisticated molecular analysis, diagnosis of the major clinical syndromes is normally possible from a careful consideration of the clinical features and simple laboratory tests. The latter must include a blood count and blood film, and haemoglobin electrophoresis with quantification of the different types of haemoglobin (i.e. HbA, HbA₂, HbF).

Other structural Hb variants may coexist with thalassaemias giving rise to a wide range of clinical disorders. Only the more common thalassaemia syndromes are discussed here.

CLINICAL SYNDROMES
α-thalassaemias

Hb-Barts hydrops syndrome (-/-/-/-)

Here deletion of all four genes leads to complete absence of α-chain synthesis. As α-globin chain is needed for fetal haemoglobin (HbF) as well as adult haemoglobin (HbA) the disorder is incompatible with life and death occurs in utero (hydrops fetalis).

HbH disease (-α/-/-)

This disorder arises from deletion of three of the four α globin genes and is found most commonly in South-East Asia. The clinical features are variable but there is often a moderate chronic haemolytic anaemia (Hb 70-110 g/1) with splenomegaly and sometimes hepatomegaly. The blood film shows hypochromic microcytic red cells with poikilocytosis, polychromasia and target cells. The HbH molecule is formed of unstable tetramers of unpaired β chains (β₄). It is best detected by electrophoresis (at pH 6-7) but may be demonstrated as red cell inclusion bodies in reticulocyte preparations. Special studies of globin chain synthesis show α / β chain ratios varying between 0.2 and 0.4 (normally 1).

Table 1 Classification of thalassaemia

<table>
<thead>
<tr>
<th>Type of thalassaemia</th>
<th>Heterozygote</th>
<th>Homozygote</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-thalassaemia*</td>
<td>Hydrops fetals</td>
<td></td>
</tr>
<tr>
<td>Thai. minor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a, (- (X</td>
<td>Thal. minor</td>
<td>Thal. minor</td>
</tr>
<tr>
<td>P-thalassaemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00Thal. minor</td>
<td>Thal. major</td>
<td></td>
</tr>
<tr>
<td>PIThal. minor</td>
<td>Thal. major or intermedia</td>
<td></td>
</tr>
</tbody>
</table>

Compound heterozygos4 u.) leads to HbH disease.

α-thalassaemia traits

Deletion of a single α -globin chain leads only to a slight lowering of red cell mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) and even deletion of two genes usually only minimally lowers the haemoglobin with a raised red cell count and hypochromia and microcytosis. These carrier states can be difficult to identify in the routine laboratory as haemoglobin electrophoresis is normal. Occasional HbH bodies may be detected in reticulocyte preparations. Definitive diagnosis requires globin chain synthesis studies and/or DNA analysis.

β-thalassaemias
**β-thalassaemia major**

β-thalassaemia major is characterised by an anaemia caused by α chain excess leading to ineffective erythropoiesis and haemolysis. The anaemia is usually severe (Hb less than 70 g/l) and becomes apparent between 3-6 months when production of HbF declines. The child fails to thrive and develops hepatosplenomegaly. Compensatory expansion of the marrow space causes the characteristic thalassaemia facies with skull bossing and maxillary enlargement (Fig. 1a). The 'hair-on-end' radiological appearance of the skull (Fig. 1b) is due to expansion of bone marrow into cortical bone. If left untreated further complications can include repeated infections, bone fractures and leg ulcers.

Usually laboratory diagnosis is straightforward. Testing should precede blood transfusion. There is a severe hypochromic microcytic anaemia with a characteristic blood film (Fig. 2) and Hb electrophoresis demonstrates absence or near absence of HbA with small amounts of HbA₂ and the remainder HbF.

Recently, the objectives of management of thalassaemia major have subtly changed. With intense supportive therapy, increasing numbers of patients survive into adulthood and there is greater emphasis on the quality of life. Interventions are frequently directed at therapy related complications (e.g. iron overload, hepatitis C infection) and clinical problems which are not life threatening.

Blood transfusion remains the mainstay of management. Raising the haemoglobin concentration has two goals: to reduce tissue hypoxia and suppress endogenous haematopoiesis which is largely ineffective. Transfusion is generally given at 2-4 week intervals to maintain a mean haemoglobin level of around 120 g/l. Splenectomy can reduce the transfusion frequency. With such regular transfusion iron chelation is necessary to minimise iron overload. Without chelation, accumulation of iron damages the liver, endocrine organs and heart with death in the second or third decades. The most commonly used regimen is subcutaneous Desferrioxamine given for 5-7 days per week. Compliance may be problematic (especially in teenagers) but where good there is a considerably improved life expectancy. Oral iron chelators are available but their use to date has been limited by side-effects. Unfortunately, patients with thalassaemia are also at risk for the infective complications of blood transfusion - hepatitis C is a potential cause of liver disease in many adults.

*Other aspects of management.* Folate should be prescribed wherever the diet is poor. Endocrine disturbances related to iron overload may require replacement therapy. Bone marrow transplantation is now a serious option in β-thalassaemia major. In 'best risk' patients the probability of survival exceeds 90%. Gene therapy may eventually be the definitive treatment.

**Thalassaemia intermedia**

Thalassaemia intermedia is a clinical syndrome which may result from a variety of genetic abnormalities (see Table 2). The clinical features are less severe than in β-thalassaemia major as the α/β-globin chain imbalance is less pronounced possible mechanisms include mild β-thalassaemia alleles, the coexistence of β-thalassaemia and α-thalassaemia, and enhancement of γ-chain production. Patients usually present later than is the case for β-thalassaemia major (often at 2-5 years), and have relatively high haemoglobin levels (80-100 g/l), moderate bone changes and normal growth. Patients do not require regular transfusion.

**β-thalassaemia trait (minor)**

Heterozygotes for β⁰ or β⁺ are usually asymptomatic with hypochromic microcytic red cells and slightly reduced haemoglobin levels. The red cell count is elevated (greater than 5.5 x 100g/l).
The key diagnostic feature is a raised HbA, level (4-7%). As in α-thalassaemia trait the disorder is symptomless but not irrelevant. Firstly, it may be confused with iron deficiency leading to unnecessary investigations. Secondly, if both parents have β-thalassaemia trait there is a 25% chance of a child having β-thalassaemia major.

**PRENATAL DIAGNOSIS**

Prenatal diagnosis and carrier screening has led to a dramatic reduction in the incidence of thalassaemia in several Mediterranean populations. Prenatal detection is generally performed by molecular methods on amplified trophoblast DNA.

**Table 2 Possible causes of thalassaemia intermedia**

Mild defects of β-globin chain production, e.g. Homozygous mild β+-thalassaemia
Homozygosity or compound heterozygosity for severe β-thalassaemia with co-inheritance of u.-thalassaemia or genetic factors enhancing y chain production.
β-thalassaemia with co-inheritance of "ddltio", "IX", "Jolin" ene,
δβ-thalassaemia and hereditary persistence of fetal haemoglobin
HbH disease

**The thalassaemias**
The thalassaemias are a heterogeneous group of inherited disorders where there is a reduction in the rate of synthesis of haemoglobin α chains (α-thalassaemia) or β chains (β-thalassaemia).

There may be both ineffective erythropoiesis and haemolysis. The basic haematological abnormality is a hypochromic microcytic anaemia.

There are several clinical syndromes. In general the severity is proportionate to the degree of imbalance of α and β globin chains.

β-thalassaemia major leads to severe anaemia requiring regular blood transfusion and iron chelation.

Thalassaemia trait is a symptomless clinical disorder which should not be confused with iron deficiency. Genetic counseling is required in selected cases.
SICKLE CELL SYNDROMES

The sickle cell syndromes are a group of haemoglobinopathies which primarily affect the Afro-Caribbean population. The common feature of these diseases is inheritance of an abnormal haemoglobin β chain gene - the gene is designated βS. Inheritance of two βS genes leads to a serious disorder termed sickle cell anaemia. A similar syndrome can result from inheritance of the βs gene with another abnormal β gene such as the haemoglobin C gene or β thalassaemia gene. Inheritance of the βs gene with a normal β chain gene (βA) causes the innocuous sickle cell trait.

PATHOPHYSIOLOGY

The abnormal βs gene has a high, incidence in tropical and subtropical regions as the abnormal haemoglobin produced (HbS) gives some protection against falciparum malaria. HbS differs from normal haemoglobin (HbA) in that glutamic acid has been replaced by valine at the sixth amino acid from the N-terminus of the β globin chain. The clinical features of sickle cell anaemia arise from the propensity of red cells containing haemoglobin S to undergo 'sickling'. In the deoxygenated state the HbS molecules aggregate into long polymers which then align to form liquid crystals (tactoids). The red cell loses its normal deformability and becomes characteristically sickle shaped (Fig. 2). Damage to the membrane leads to increased rigidity and the ultimate sequestration of the red cell in the reticuloendothelial system causing haemolytic anaemia. The inflexible sickle cells also become lodged in the microcirculation causing stasis and obstruction.

CLINICAL SYNDROMES

SICKLE CELL ANAEMIA (HBSS)

This classical form of sickle cell syndrome is enormously variable in severity.

Haemolytic anaemia
The haemoglobin is generally in the range 60-100 g/l. Because HbS releases oxygen more readily than HbA, the symptoms of anaemia are often surprisingly mild. Intercurrent infection with parvovirus or folate deficiency can block erythropoiesis and cause a sudden fall in haemoglobin - the 'aplastic crisis'.

Vascular-occlusive crises
Acute, episodic, painful crises are a potentially disabling feature of sickle cell anaemia. They may be triggered by infection or cold. Patients complain of musculoskeletal pain which may be severe and require hospital admission. Hips, shoulders and vertebrae are most affected. Attacks are generally self-limiting but infarction of bone can occur and must be distinguished from salmonella osteomyelitis. Avascular necrosis of the femoral head is a crippling complication. Other organs are vulnerable to infarction; most serious is neurological damage
which may manifest as seizures, transient ischaemic attacks (TIAS) and strokes.
Vaso-occlusion in infancy is responsible for the 'hand-foot syndrome', a type of dactylitis
damaging the small bones of hands and feet.

**Sequestration crises**
These arise from sickling and infarction within particular organs. Specific syndromes include
'acute chest syndrome' with occlusion of the pulmonary vasculature, 'girdle sequestration' caused
by occlusion of the mesenteric blood supply, and hepatic and splenic sequestration.

**Other complications**
These are multiple, usually caused by vascular stasis and local ischaemia.

**Genitourinary.** Papillary necrosis with haematuria; loss of ability to concentrate urine;
nephrotic syndrome; priapism.

**Skin.** Lower limb ulceration.

**Eyes.** Proliferative retinopathy; glaucoma.

**Hepatobiliary.** Liver damage; pigment gallstones.

**Diagnosis**
Diagnosis depends on the following:

**Blood film appearance**

**Screening tests for sickling.** The blood sample is deoxygenated (e.g. with sodium
metabisulphate) to induce sickling.

**Haemoglobin electrophoresis.**
In sickle cell anaemia (HBSS) there is no HbA detectable.

**Management**

**General.** Patients need support in the community and easy access to centres experienced in the
management of sickle cell anaemia. Prophylaxis is important. Thus, patients should avoid
factors known to precipitate crises, take folate supplements (because of chronic haemolysis) and
be prescribed penicillin and pneumococcal vaccine (because of hyposplenism caused by

**Painful vascular-occlusive crises** First line treatment is rest, increased fluids and adequate oral
analgesia. Constitutional upset or pain not relieved by oral analgesia necessitates hospital
admission with continued rest, warmth, intravenous fluids and opiate analgesia.

**Blood transfusion.** Blood transfusion should be considered where severe anaemia (Hb less
than 40 g/l) accompanies an acute crisis (haemolytic, aplastic or sequestration). Exchange
transfusion can benefit patients with neurological symptoms, chest or girdle syndrome, priapism,
or an unusually severe painful crisis. Longer term transfusion may be indicated where there are
neurological complications, growth problems in childhood and, occasionally, to reduce the
frequency of painful crises. The usual aim in these cases is to maintain the haemoglobin
between 100 and 150 g/l with a HbS level of less than 25%.

**Pregnancy and surgery.** In pregnancy, women with problematic sickle cell anaemia (e.g.
recent major complications or painful crises) or previous obstetric problems are likely to benefit
from regular exchange transfusion. During surgery it is important to avoid hypoxia and
dehydration. More severely affected patients can be given a course of transfusions
preoperatively to reduce the HbS level.
Newer treatments

Stimulating HbF production. Increasing the level of fetal haemoglobin in red cells may reduce the severity of the disease. Recent studies of the antimetabolite hydroxyurea have been encouraging with a significant reduction in painful crises, major complications, blood transfusion and hospital admissions.

Bone marrow transplantation. This offers the possibility of a cure in selected patients but it will not be widely applicable until the toxicity is reduced.

Gene therapy. This has the potential to provide a cure without the risks of bone marrow transplantation.

Prognosis
With good management 80-90% of patients will survive to 20 years of age. The most common causes of death are infection in infancy, cerebrovascular accidents in adolescence and respiratory complications in adult life.

DOUBLY HETEROZYGOUS SICKLING DISORDERS

Here patients inherit the $\beta^S$ gene and another abnormal $\beta$ gene - usually HbC or $\beta$-thalassaemia. HbSC disease is similar to HbSS but there is a tendency for fewer painful crises and a higher incidence of proliferative retinopathy and avascular necrosis. HbSp-thal is often severe with the entire range of sickling disabilities.

SICKLE CELL TRAIT (HbAS)

Sickle cell trait normally causes no clinical problems as there is enough HbA in red cells (approximately 60%) to prevent sickling. However, haematuria occasionally occurs as a result of renal papillary necrosis and additional care is required during pregnancy and anaesthesia. Diagnosis is by a sickling test and Hb electrophoresis.

COUNSELLING AND PRENATAL DIAGNOSIS

Genetic counseling is needed by those affected with either the homozygous disease, compound heterozygosity or the trait. Prenatal diagnosis is possible at 8 weeks from a chorionic villous sample. Various molecular methods are available for detecting the $\beta^S$ gene but most laboratories currently rely on the polymerase chain reaction (PCR) for DNA amplification followed by direct detection by restriction enzyme analysis. The amplification refractory mutation system (ARMS) is used in ambiguous cases. Fetal blood analysis is still a useful backup where DNA analysis is not technically possible or where previously unstudied couples are referred late in pregnancy.

Sickle cell syndromes

The sickle cell syndromes are a group of haemoglobinopathies which primarily affect people of African origin.
Inheritance of two $\beta^S$ genes leads to the serious clinical disorder, sickle cell anaemia (HbSS). Clinical problems in sickle cell anaemia include chronic haemolytic anaemia, vascular-occlusive crises, sequestration crises and susceptibility to infection. Routine management of sickle cell anaemia entails prophylactic measures, supportive care during vascular-occlusive crises and the selective use of blood transfusion. Sickle cell trait (HBAS) is an innocuous clinical disorder but genetic counselling is often needed.
ANAEOMIA OF CHRONIC DISORDERS

Anaemia of chronic disorders (ACD) is a term used to describe a type of anaemia seen in a wide range of chronic inflammatory, infective and malignant diseases (Table 1). The anaemia often becomes apparent during the first few months of illness and then remains fairly constant (Fig. 1). It is rarely severe (haemoglobin $\geq 90$ g/l; packed cell volume (PCV) $\geq 0.30$) but there is some correlation with the intensity of the underlying illness. For instance, in infection the anaemia is often more marked where there is a persistent fever and in malignancy where there is widespread dissemination. Patients often suffer no symptoms from their anaemia or have only slight fatigue. The importance of this type of anaemia arises not from its severity but from its ubiquity. It is widely misunderstood (for such a common disorder) and ill patients are frequently subjected to excessive haematological investigation and unnecessary treatment with haematinics. The term ACD should not be used to describe other causes of anaemia such as haemolysis or bleeding which may also complicate chronic disorders. It has been argued that the designation ACD is inappropriate but other suggested terms appear even less satisfactory.

INCIDENCE

Because its causes are common, ACD is probably only second to iron deficiency as a cause of anaemia. It has been estimated to account for approximately half of all hospital cases of anaemia not explained by blood loss.

PATHOPHYSIOLOGY

The causation of the anaemia of chronic disorders has been extensively studied but questions remain. It appears to arise from a combination of decreased red cell production and shortened red cell survival. The reason for shortened red cell survival is unclear. Patients with severe rheumatoid arthritis have mean red cell survivals of 80-90 days compared to 100-120 days in normal people. One hypothesis is that red cells are prematurely consumed due to overactivity of a reticuloendothelial system hypertrophied in the presence of chronic inflammation.

Table 1 Common causes of the anaemia of chronic disorders
Malignancy
Rheumatoid arthritis
Various connective tissue disorders
Chronic infection
Extensive trauma

Probably reduced red cell survival is less important than red cell underproduction by the bone marrow. Abnormalities of iron metabolism are well documented in ACD. These include:

- reduced iron absorption from the gastrointestinal tract
- decreased plasma iron concentration
excessive retention of iron in reticuloendothelial cells (macrophages) with diminished release to erythroid cells. The cause of these changes is contentious. It is possible that lactoferrin, a protein released from neutrophils during inflammation, binds serum iron and transfers it to macrophages thereby withholding it from red cell precursors. Another possibility is that increased levels of apoferrin, an acute phase reactant produced during inflammation, leads to the diversion of iron away from the bone marrow to be stored as ferritin. There could well be other factors impairing marrow function in ACD. Erythropoietin levels in some patients are lower than would be expected for the degree of anaemia. The response to erythropoietin is unimpaired, but there is no good evidence of an inhibitor to erythropoietin. It has recently been suggested that many of the features of ACD can be explained by the release of interleukin-1, a cytokine produced by macrophages and present in inflammatory exudates. Its biological actions include:

generation of fever
increased neutrophil production
stimulation of the release of acute phase reactants from the liver
lymphocyte proliferation.

Notably it also appears to promote release of lactoferrin from neutrophils and, at least experimentally, can cause a fall in serum iron concentration. Chronically increased production of interleukin-1, or other cytokines such as tumour necrosis factor (TNF), may be the mechanism for ACD.

DIAGNOSIS

Fig. 1. Bone marrow aspirate stained with Perls stain showing increased reticuloendothelial iron stores in ACD

Most patients will have a documented chronic disorder and a moderate anaemia. On occasion the anaemia is a more dominant feature and the underlying cause is not immediately apparent. The anaemia is usually of normochromic normocytic type although it can be slightly hypochromic microcytic. The blood film appearance is often unremarkable but there may be changes 'reactive' to the underlying disorder such as a neutrophil leucocytosis, thrombocytosis and rouleaux formation. There is a reticulocytopenia. Serum iron concentration and total iron binding capacity (TLBC) are both low. The serum ferritin level is normal or high (as an acute phase reactant). In practice ACD is most commonly confused with mild iron deficiency anaemia, particularly if the MCV and MCH are reduced. However, the two forms of anaemia should be distinguishable as in uncomplicated iron deficiency the TIBC is elevated and the ferritin level is low (Table 2). Bone marrow examination is not routinely required in ACD but
where performed will show normal or increased marrow iron stores with decreased marrow sideroblasts. It should be remembered that anaemia in a patient with a chronic medical disorder may be of multifactorial origin. It is important not to misdiagnose ACD as something else but equally it cannot be assumed that every patient with long-standing disease and a low haemoglobin has only ACD. In rheumatoid arthritis there is frequently co-existence of ACD and iron deficiency anaemia resulting from gastrointestinal bleeding due to drug therapy.

MANAGEMENT

As the anaemia is usually non-severe and not progressive, the management is essentially that of the underlying disorder. Occasionally, patients cannot adequately compensate for the anaemia and require transfusion with plasma reduced red cells. In the absence of any proven deficiency replacement treatment with iron, vitamin B₁₂ or folate is worthless. Erythropoietin can be effective in relieving anaemia, particularly in rheumatoid arthritis and malignancy. However, there is not necessarily any symptomatic improvement. Erythropoietin is probably best reserved for patients with unusually severe ACD which is unlikely to respond rapidly to treatment of the chronic disorder.

**Anaemia of chronic disorders (ACD)**

ACD is seen in a wide range of chronic malignant, inflammatory and infective disorders. The pathogenesis of ACD is complex. There is a reduction in both red cell production and survival. Possible factors include abnormal iron metabolism, low erythropoietin levels and release of inflammatory cytokines. The anaemia is usually of normochromic, normocytic type, nonprogressive and is rarely severe. Treatment is that of the underlying disorder. Transfusion and erythropoietin may help in selected cases. In the absence of proven deficiency haematinic therapy is worthless.
LEUKAEMIA

INTRODUCTION

The leukaemias are a heterogeneous group of malignant blood disorders. In this introductory section general characteristics such as definitions, aetiology and classification are discussed. Each of the more common types of leukaemia is subsequently described in more detail.

DEFINITION

Leukaemia is a type of cancer caused by the unregulated proliferation of a clone of immature blood cells derived from mutant haematopoietic stem cells. The malignant cells arise out of the arrest of normal blood cell maturation - they are effectively trapped at an early stage of differentiation. It is tempting to think of these leukaemic cells as fast-growing and aggressive. In fact, even in acute leukaemia the malignant cells cycle much more slowly than normal cells. If this was not the case, normal haematopoietic cells would not repopulate the bone marrow after the ablation that follows cytotoxic chemotherapy. However, leukaemic cells are indifferent to normal feedback signals and proliferate relentlessly, eventually squeezing out normal cells from the bone marrow and causing marrow failure and death.

Fig. 1 Trisomy 8 in acute myeloid leukaemia

INCIDENCE

Leukaemia is not a rare disorder but it is less common than some malignancies of solid organs. There is a male preponderance in most types of leukaemia. Geographic variations exist; for instance, chronic lymphatic leukaemia is the most common form of leukaemia in the western world but is much less frequent in Japan, South America and Africa.

AETIOLOGY

As for other malignancies the evolution of leukaemia is likely to be a multistep process. It is easiest to think about the aetiology in terms of acquired cytogenetic abnormalities and other predisposing factors.

Chromosomal abnormalities
Recent advances in cytogenetic analysis and particularly in molecular cytogenetic techniques have revealed various acquired chromosomal derangements which play a fundamental role in leukaemogenesis. There are a number of different types of possible chromosomal change.
Chromosomal Translations

One chromosome breaks and donates a fragment to another chromosome which reciprocates by
returning a fragment of its own. Such translocations can result in the movement of
protooncogenes to new sites where they have the capacity to cause leukaemic transformations.
The classical example of a translocation is the 'Philadelphia chromosome' which is found in 95%
of cases of chronic myeloid leukaemia (CML) where breakages in chromosomes 9 and 22 result
in the creation of a new fusion gene (bcr-abl) which encodes a novel protein with intense
tyrosine kinase activity. In a manner incompletely understood, this protein causes deregulated
myeloid cell growth.

Chromosome deletions and additions
A chromosome may be completely or partly deleted, for example monosomy 7 in acute myeloid
leukaemia (AML). Here a normal gene may be lost allowing expression of a recessive cancer
gene. Conversely, an additional chromosome may be gained (e.g. Trisomy 8 in AML).

Point mutations
A change in the base sequence of certain oncogenes may predispose to leukaemia. The c-ras
oncogene which encodes a protein vital in signal transduction is mutated in 50% of cases of
AML.

Gene amplification
Certain proto-oncogenes may be amplified in leukaemia (e.g. C-MYC in AML).
Particular chromosome changes are often associated with specific types of leukaemia (e.g. the
Philadelphia chromosome in CML). However, few abnormalities are entirely specific - the
Philadelphia chromosome can be found in cases of acute leukaemia. It should also be noted that
not all cases of leukaemia have a detectable cytogenetic abnormality. The incidence of
abnormality is partly dependent on the laboratory expertise available.

Table 1 Factors predisposing to leukaemia

Radiation exposure
Previous chemotherapy (particularly alkylating agents)
Occupational chemical exposure (e.g. benzene)
Some genetically determined disorders (e.g. Down's syndrome)
Viral infection (only HTLV-1 proven as a causative factor)
Other possible (e.g. cigarette smoking)

Predisposing factors
In a small subpopulation of leukaemic patients there is another obvious predisposing factor - the
more common of these are listed in Table 1.
There is no doubt that higher doses of radiation can cause leukaemia. The incidence of acute
leukaemia and chronic myeloid leukaemia increases with dose exposure for all age groups.
Classic studies have included people exposed to the atomic bombs in Japan and patients receiving radiotherapy for ankylosing spondylitis in the middle years of this century. Of greater current concern is the estimate that approximately 1% of all leukaemias may be attributed to diagnostic radiation. The risk of leukaemia increases with an increasing number of X-rays but it is difficult to recommend a safe' upper limit. Paternal preconception X-ray exposure has been associated with an increased incidence of acute leukaemia in offspring.

Cytotoxic chemotherapy, particularly with alkylating agents, leads to an increased risk of leukaemia. The risk appears to be greatest in older patients also treated with radiotherapy. The best established occupational leukaemogenic exposure is undoubtedly to benzene. A number of genetically determined diseases also predispose to leukaemia. Here the liability to leukaemia is probably caused by factors such as increased chromosomal breakage (e.g. Fanconi's anaemia) and immunosuppression (e.g. ataxia telangiectasia).

Viruses are known to be the main cause of leukaemia in many animals but in man the only well-proven association is of the HTLV-1 virus with the rare disorder T-cell leukaemia lymphoma.

Fig. 2        Fig. 3

Fig. 2 Peripheral blood film in a young woman with acute myeloid leukaemia.

Fig. 3 Bone marrow trephine appearance in human T-cell leukaemia lymphoma.

CLASSIFICATION

In such a potentially complex group of disorders it is helpful to use a relatively simple classification. The leukaemias can most broadly be divided into acute and chronic types depending on their clinical course. The classification illustrated here further divides leukaemias into their cell of origin (i.e. myeloid or lymphoid) and refers to the microscopic appearance (morphology) of the leukaemic cells. The standard classification of the acute leukaemias is that of the FAB group - the abbreviation being for the French, American and British nationalities of the terminologists.

In the following pages are discussed acute myeloid leukaemia, acute lymphoblastic leukaemia, chronic myeloid leukaemia and chronic lymphatic leukaemia. Together these four diseases
constitute the overwhelming majority of leukaemias in clinical practice. A few rarer types of leukaemia are discussed separately.

Table 2 Classification of leukaemia

**Acute leukaemia**  
*Acute myeloid leukaemia*  
Subdivided into eight types designated FAB M0-7 depending on the morphology of leukaemic cells (e.g. FAB M5 is acute monocytic leukaemia)

*Acute lymphoblastic leukaemia* Subdivided either on the basis of morphology (FAB L1, L2 and L3) or on the basis of leukaemic cell expression of surface antigens. This latter 'immunologic' classification includes common, null, B and T types.

**Chronic leukaemia**  
*Chronic myeloid leukaemia*  
*Chronic lymphatic leukaemia*

**Other types**  
Hairy cell leukaemia  
Prolymphocytic leukaemia  
T-cell leukaemia lymphoma

**Leukaemia: introduction**

Leukaemia is a type of cancer caused by the unregulated proliferation of a clone of immature blood cells.

Leukaemia is a heterogeneous group of clinical disorders classified on the basis of their clinical course (acute or chronic) and their cell of origin (myeloid or lymphoid).

The aetiology of leukaemia is likely to be multifactorial with known predisposing factors such as radiation exposure present in only a minority of cases. Acquired chromosomal abnormalities play a fundamental role in leukaemogenesis with certain changes associated with particular types of leukaemia.
ACUTE MYELOID LEUKAEMIA

INTRODUCTION

Acute myeloid leukaemia (AML) arises out of the malignant transformation of a myeloid precursor cell. Usually this occurs at a very early stage of myeloid development, although acute promyelocytic leukaemia, a subtype of AML, involves proliferation of a more mature cell. AML is rare in childhood and the incidence increases with age. Cases may occur de novo or secondary to well-defined predisposing factors such as previous chemotherapy or a myelodysplastic syndrome - such cases are referred to as, secondary AML.

Fig. 1 Gum infiltration in acute monocytic leukaemia.

CLASSIFICATION

The classification of AML is based upon the appearance of the leukaemic cells in a bone marrow aspirate. The French American-British (FAB) group have described eight different variants of AML. An experienced haematologist can often identify the subtype on microscopy, but certain types (e.g. AML MO) routinely require other tests to establish a final diagnosis. In all cases, but particularly in the elderly, the presence of dysplastic features suggests evolution from a myelodysplastic syndrome. Where dysplasia is prominent, the disease is arbitrarily termed leukaemia when the leukaemia cell ('blast') number in the marrow equals or exceeds 30% of all nucleated cells. Occasional cases of acute leukaemia show megakaryocytic or erythroid differentiation but are included in the AML classification.

CLINICAL FEATURES

In practice there is little uniformity in presentation. Some patients are remarkably asymptomatic whilst others are seriously ill.

General
Bone marrow infiltration by leukaemic blast cells usually leads to anaemia, neutropenia and thrombocytopenia. Thus, patients often have symptoms of anaemia, infection and haemorrhage. Tissue infiltration by leukaemic cells and clotting problems may occur in any case of AML but are characteristic of specific subtypes.

Table 1 Classification of acute myeloid leukaemia

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>MO</td>
<td>Undifferentiated</td>
</tr>
<tr>
<td>M1</td>
<td>Without maturation</td>
</tr>
<tr>
<td>M2</td>
<td>With maturation</td>
</tr>
<tr>
<td>M3</td>
<td>Promyelocytic</td>
</tr>
<tr>
<td>M4</td>
<td>Myelomonocytic</td>
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</table>
M5 Monocytic
M6 Erythroleukaemia
M7 Megakaryoblastic

Particular subtypes
AML FAB M3 (acute promyelocytic leukaemia). AML always requires prompt attention but this subtype is a true medical emergency. Patients are likely to develop disseminated intravascular coagulation (DIC) with disordered clotting tests and a high risk of spontaneous bleeding into vital organs.

AML FAB M5 (acute monocytic leukaemia). This subtype has a particular propensity to invade soft tissues. Patients often present with gum infiltration, lymphadenopathy, skin deposits and hepatosplenomegaly. Central nervous system disease is rare in AML but most frequent in monocytic leukaemia.

AML FAB M7 (acute megakaryoblastic leukaemia). This type is often associated with pancytopenia and marrow fibrosis. It is sometimes called 'acute myelofibrosis'.

DIAGNOSIS
Diagnosis depends on a logical sequence of tests.

1. **Blood count.** The white cell count (WCC) is usually elevated (up to 200 x 10^9/l) but may be normal or low. There is often anaemia and thrombocytopenia.
2. **Blood film.** Usually there are leukaemic blast cells although occasionally these are absent. There may be dysplastic changes in other cells.
3. **Bone marrow aspirate and trephine.** The bone marrow is infiltrated by leukaemic blast cells. In more immature forms of AML (e.g. M0, M1), differentiation from acute lymphoblastic leukaemia (ALL) can be difficult.
4. **Cytochemistry.** Special stains are used on bone marrow and blood smears to help differentiate myeloid and lymphoid blast cells. In AML there is positivity with Sudan black and myeloperoxidase - these stains are negative in ALL. AML with monocytic features (i.e. M4 or M5) will stain positively with a non-specific esterase stain.
5. **Immunophenotyping.** The basic concept has been described. In AML the leukaemic cells have characteristic myeloid antigens on the cell surface which have been allotted 'cluster differentiation' (CD) numbers to ease identification. Thus CD 13 and 33 are general myeloid markers and usually positive. CD 11 and 14 expression indicate monocytic differentiation, whilst CD 34 indicates a particularly immature cell of origin.
6. **Cytogenetics.** A bone marrow sample is sent for analysis. Chromosomal abnormalities in the leukaemic cells may suggest a particular subtype and also give prognostic information.
7. **Molecular biology.** As the molecular abnormalities in AML are better understood the role of molecular biology techniques will expand. Currently the best characterised abnormality is the t(15, 17) translocation of acute promyelocytic leukaemia in which the retinoic acid receptor (RAR) gene on chromosome 17 is brought into alignment with the PML gene on chromosome 15.

MANAGEMENT

Supportive care
This includes red cell transfusion for anaemia, platelet concentrates for thrombocytopenia and broad spectrum intravenous antibiotics for infection. An indwelling central venous catheter facilitates support during and after chemotherapy.

Chemotherapy and bone marrow transplantation

The first objective of treatment with cytotoxic drugs is to achieve a 'complete remission' (CR) - defined as less than 5% blast cells in a normocellular bone marrow. Initial cytotoxic drug treatment is termed 'induction'. A CR is followed by a second sequence of drugs termed 'consolidation'. Induction and consolidation take at least several months, but longer term 'maintenance' treatment is rarely given in AML. Regimens are ever changing but drugs commonly used in induction are combinations of an anthracycline (e.g. Daunorubicin), cytosine arabinoside and 6-thioguanine. Other agents such as amsacrine or etoposide may be included in induction or added to consolidation regimens. Acute promyelocytic leukaemia is treated with the differentiating agent all trans retinoic acid (ATRA) which reduces the risk of early death from bleeding and may improve long-term survival compared with chemotherapy alone. Autologous bone marrow transplantation (BMT) can be used to intensify chemotherapy but the benefit has proved difficult to quantify. The precise role of allogeneic BMT is not clearcut - most clinicians would transplant in a younger patient (less than 40 years) in first CR where an HLA identical sibling was available.

Fig. 2 Bone marrow appearance in different subtypes of AML. a: the leukaemic blast cell shows some granulocytic differentiation, b: myelomonocytic

Table 2 Common cytogenetic abnormalities in AML

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Associated subtype</th>
<th>Prognosis*</th>
</tr>
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<tbody>
<tr>
<td>t (8,21)</td>
<td>AML M2</td>
<td>Good</td>
</tr>
<tr>
<td>t (15,17)</td>
<td>AML M3</td>
<td>Good</td>
</tr>
<tr>
<td>inv 16</td>
<td>AML M4</td>
<td>Good</td>
</tr>
<tr>
<td>5 and 7 (various)</td>
<td>Secondary AML</td>
<td>Poor</td>
</tr>
</tbody>
</table>

Compared with AML with normal karyotype
PROGNOSIS

Approximately 80-90% of younger patients with AML will achieve a CR with conventional chemotherapy. Unfortunately, most will relapse and 'cure' rates are around 30%. Allogeneic BMT may increase this figure to around 50% but is generally limited to young patients with a matched sibling donor. Older patients tolerate chemotherapy less well and CR and cure rates are much lower. Indeed, it may be kinder not to use chemotherapy in some elderly patients. In children, intensive chemotherapy gives 3-year survival rates of around 50%. Apart from advancing age, other poor prognostic factors include secondary AML, certain cytogenetic abnormalities (Table 2), central nervous system disease and a high presentation WCC.

Acute myeloid leukaemia

AML arises out of the malignant transformation of a myeloid precursor cell. The disease is divided into eight subtypes (FAB MO-M7) determined by the malignant cells' morphology and immunophenotype. Symptoms result from anaemia, neutropenia and thrombocytopenia. Tissue infiltration often complicates the monocytic subtype (FAB M5) and coagulopathy the promyelocytic subtype (FAB M3). Chemotherapy leads to CR rates of 80-90% in younger patients but cure rates are lower, around 30%. Bone marrow transplantation can cure around 50% of selected patients. Older patients tolerate chemotherapy less well and cure is rarely achievable.
ACUTE LYMPHOBLASTIC LEUKAEMIA

Acute lymphoblastic leukaemia (ALL) is a clonal malignancy of lymphoid precursor cells. In approximately 80% of cases the malignant cells are primitive precursors of B lymphocytes and the remainder are T-cell leukemias. The abnormal cell may arise at various stages of early lymphocyte differentiation.

ALL has a peak incidence in childhood with a gradual rise in incidence in later years. The disease has distinct characteristics in children and adults. Childhood ALL is often curable by chemotherapy whereas cure is elusive in adult ALL. This is probably because most cases of adult disease involve a more immature precursor cell (a multipotential stem cell) whereas the malignant cell of childhood ALL is a committed lymphoid precursor cell.

CLASSIFICATION

Both morphological and immunological features are used in classification. The French-American-British (FAB) morphological classification is based on characteristics of the blast cells including cell size, nuclear-cytoplasmic ratio, number and size of nucleoli and the degree of cytoplasmic basophilia. Most childhood cases have L1 morphology whereas the L2 type most often occurs in adults.

Immunological subtypes of ALL include common, null, B and T phenotypes. Definition depends on the presence or absence of various cell surface antigens. The most frequent immunological subtype is common ALL. Null cell ALL is thought to arise from a very primitive cell and is more common in adults. B-ALL is a rare disease with L3 morphology which often behaves as an aggressive lymphoma (Burkitt's variant'). In a significant minority of cases ALL the blasts express cell surface antigens usually found on myeloid cells (e.g. CD13, CD33) and a few of these are probably true 'biphenotypic' leukaemias with clinical presentations typical of neither ALL or AML.

Fig. 1 a, b, c Morphology of ALL blast cells, L1 type, L2 type, L3 type

CLINICAL FEATURES

These can be very variable. Accumulation of malignant lymphoblasts in the marrow leads to a scarcity of normal cells in the peripheral blood and symptoms may include those associated with anaemia, infection and haemorrhage. Other common complaints are anorexia and back or joint pain. T-cell ALL is associated with a large mediastinal nodal mass and pleural effusions which result in dyspnoea. Central nervous system (CNS) involvement is more often seen in ALL than in AML and patients can present with symptoms of raised intracranial pressure (headache, vomiting) or cranial nerve palsies (particularly VI and VII). Examination findings may include
pallor, haemorrhage into the skin and mucosae, lymphadenopathy and moderate hepatosplenomegaly. In males the testes can be involved and should be routinely examined.

Table 1 Classification of ALL

<table>
<thead>
<tr>
<th>Morphological classification*</th>
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<tbody>
<tr>
<td>L1 Small uniform blast cells with scanty cytoplasm</td>
</tr>
<tr>
<td>L2 Large heterogeneous blast cells with nucleoli and low nuclear cytoplasmic ratio</td>
</tr>
<tr>
<td>L3 Basophilic vacuolated blast cells</td>
</tr>
</tbody>
</table>

Immunological classification

- Precursor B-ALL
- _common ALL
- _null ALL
- _pre-B ALL
- T-ALL
- B-ALL

**DIAGNOSIS**

1. **Blood count**
The white cell count may be raised, normal or low. Only 20% have white cell counts greater than 50x10⁹/l. Anaemia and thrombocytopenia are common.

2. **Blood film**
The proportion of blast cells in the white cell count varies from 0 to 100%. In some cases there is a leucoerythroblastic picture and teardrop shaped red cells.

3. **Bone marrow aspirate and trephine**
This is essential to confirm the diagnosis and for classification. A trephine biopsy is particularly helpful where the marrow is difficult to aspirate.

4. **Cytochemistry**
Stains which classically show positivity in AML-Sudan black and myeloperoxidase-are negative in ALL. Cytochemistry is useful in distinguishing precursor B and B-ALL from T-ALL. Reactivity with the acid phosphatase stain is seen in malignant T lymphocytes but not in B cells which may show periodic acid Schiff (PAS) block positivity.

5. **Immunophenotyping**
This technique plays a key role in classification. Useful reagents for establishing the diagnosis and identifying the immunological subtype include antibodies to CD19 and CD22 (found in most B-lineage ALLS), CDIO (the 'common ALL antigen'), CD3 and CD7 (found in T-lineage ALLS). CD3 may be detected in the cytoplasm but not on the membrane and there are other helpful cytoplasmic markers.
The accumulation of heavy chains in the cytoplasm is characteristic of a subtype of B-lineage ALL termed pre-B. The intracellular marker, terminal deoxynucleotidyl transferase (TdT), is found in most B- and T-lineage ALLs but can also be found in AML.

6. Cytogenetics
Cytogenetic analysis is doubly useful as structural abnormalities correlate with particular subtypes of ALL and both structural and numerical abnormalities give prognostic information. Varying patterns of cytogenetic abnormality may partly explain the different prognosis in children and adults. The Philadelphia chromosome, regarded as a marker of 'incurability' by chemotherapy, is found in 20-30% of adult cases but in only 5% of children.

7. Molecular biology
In B-lineage ALL, rearrangement of the immunoglobulin genes is frozen and monoclonal; the same is true of the T-cell antigen receptor genes in T-ALL. However, interpretation requires caution as cross-lineage rearrangements occur in around 10% of cases and immunoglobulin and T-cell receptor rearrangements may even be found in AML. Where routine cytogenetic analysis fails, molecular techniques should be used to search for the bcr-abl rearrangement as this predicts a poor response to chemotherapy.

MANAGEMENT AND OUTCOME

General principles
Patients with ALL require supportive care. Chemotherapy is the mainstay of treatment. Drug schedules vary but remission induction classically relies on three agents: vincristine, prednisolone and L-asparaginase. The anthracycline daunorubicin may be included in the induction regimen and other drugs, notably methotrexate, etoposide and cytosine arabinoside, then added in 'intensification' and 'consolidation'. The rationale for early intensification of treatment is to reduce the leukaemic cell population quickly and reduce the likelihood of drug resistance. Therapy is completed with a period of 'maintenance' using methotrexate and mercaptopurine. The higher risk of CNS disease in ALL (than in AML) necessitates prophylactic treatment to prevent CNS relapse. The usual method is a combination of intrathecal injections of methotrexate and cranial irradiation.

The ultimate choice of management is influenced by a number of prognostic factors. Where clinical and laboratory features predict a poor response to chemotherapy alone, transplantation (BMT) are considered. Of all the prognostic more intensive treatments such as allogeneic bone marrow indices the most influential is age.

ALL in children
The majority of children are curable with current chemotherapy regimens. The standard strategy is intensive induction therapy, CNS prophylaxis, and maintenance treatment for 2 years. In children receiving the most intensive protocols, 5-year disease free survivals of 70% have been achieved. Autologous and allogenic BMT is best reserved for relapse after chemotherapy or for cases with poor prognostic features at presentation. With improved cure rates the long-term side-effects of the drugs, including endocrine problems, secondary leukaemia and cardiotoxicity, are becoming increasingly relevant. Wherever feasible, the use of agents with the safest profiles is desirable.
**ALL in adults**

The majority of adult patients are not curable with chemotherapy alone and only 20% will become long-term survivors. Most chemocurable patients are aged between 15 and 20 years with other good prognostic features. This 'good risk' subgroup resembles childhood ALL and chemotherapy alone is a reasonable initial policy. For the remainder of adults the hope of cure is likely to depend on even more intensive therapy either with autologous or allogeneic BMT. Allogeneic BMT from an HLA matched family donor performed in first remission gives longterm survival of around 40%. BMT using an unrelated HLA 'matched' donor is more risky but can be successful.

Optimum management of adult ALL has yet to be defined and there is a need for careful consideration of all the known prognostic factors in each case. The choice of drugs for treatina relapsed patients is arbitrary but the increasing availability of laboratory techniques for detecting drug resistance may allow more rational selection in the future. Elderly patients (over 60 years) tolerate chemotherapy less well and cure rates are very low. In these cases it is often kinder to concentrate on palliation of symptoms and provision of a short period of good quality life rather than undertaking aggressive chemotherapy with a negligible chance of success.

**Acute lymphoblastic leukaemia**

ALL is a clonal malignancy of lymphoid precursor cells. There is a peak incidence in childhood and a gradual rise in later years. Accumulation of lymphoblasts in the bone marrow often leads to anaemia, infection and haemorrhage. CNS involvement is more common than in acute myeloid leukaemia. The majority of children are curable with standard chemotherapy regimens and CNS prophylaxis. In adults, cure by chemotherapy alone is much less frequent. Autologous or allogeneic bone marrow transplantation may be considered in 'poor risk' cases.
CHRONIC MYELOID LEUKAEMIA

Chronic myeloid leukaemia (CML) is a clonal myeloproliferative disorder which is thought to result from an acquired genetic change in a pluripotential stem cell. The disease is characterised by a gross overproduction of neutrophils and their precursors. It is unusual in having three clinical phases. A relatively benign 'chronic phase' is followed by an ominous 'accelerated phase' and, finally, an almost invariably fatal acute leukaemic phase termed 'blast crisis'.

The annual incidence of CML is around one per 100 000 with presentation most common in the fifth and sixth decades of life. The diagnosis is increasingly made in asymptomatic patients having routine blood tests.

PATHOGENESIS

The hallmark of CML cells is the presence of a Philadelphia (Ph) chromosome - the (9, 22)(q34, q11) chromosomal translocation. Over 95% of classical CML cases are Ph positive. The Ph translocation causes the fusion of the c-abl proto-oncogene from chromosome 9 to the interrupted end of the breakpoint cluster region (her) of chromosome 22. The normal functions of the abl and her genes are unclear. The chimeric bcr-abl gene created on the Ph chromosome (22q-) encodes a protein with considerably greater tyrosine kinase activity than the normal counterpart. This protein presumably acts as an oncogenic growth factor, although the mechanism by which it stimulates the overproduction of myeloid cells characteristic of CML is unclear. It is probable that alterations in other proto-oncogenes dictate the eventual transformation of chronic phase CML to blast crisis.

The Ph chromosome also occurs in a minority of cases of acute leukaemia; the molecular changes are subtly different from those in CML.

Fig. 1 Blood sample (right) from a patient with CML. The greatly increased white cell component compared with the normal sample.

CLINICAL FEATURES

Patients usually present in chronic phase. Typical symptoms are of anaemia, anorexia and weight loss. Splenomegaly is the most common physical finding and is often marked causing pain, bloating and satiety. The occasional patient presents with gout or hyperviscosity associated with a very high white cell count. Neutropenia and thrombocytopenia are not normally features of chronic phase and infection and haemorrhage are rare.

After a period of stability in chronic phase, patients develop blast crisis with symptoms typical of acute leukaemia. Between chronic phase (CP) and blast crisis is an intervening period of 'acceleration'. The accelerated phase is poorly defined but clinically is usually associated with an insidious deterioration in the patient's health and the need for more intense treatment to control splenic size and white cell count.
DIAGNOSIS

The major laboratory abnormality in CP-CML is an elevated white cell count; this often exceeds $100 \times 10^9/l$. The blood film shows an increase in morphologically normal myeloid cells at all stages of differentiation but with greatest numbers of myelocytes and neutrophils. There is usually an absolute basophilia. Thrombocytosis and nucleated red cells may be present.

The bone marrow appearance is less informative than the blood film; pronounced hypercellularity and abnormal myelopoiesis is characteristic but not specific for CML. The key diagnostic abnormality is the presence of the Ph chromosome. In a few patients with apparent CML the Ph chromosome is absent. Such cases need careful review as they may represent an atypical myeloproliferative disorder or even chronic myelomonocytic leukaemia (a variant of myelodysplastic syndrome not to be confused with CML). The term chronic granulocytic leukaemia (CGL) is sometimes used to distinguish classical Ph positive CML from less typical forms of the disease.

The accelerated phase is characterised by an increase in the number of immature cells in the peripheral blood and in blast crisis the blood appearance is dominated by the presence of myeloblasts (65% of cases) or lymphoblasts (35%). In the rare patients who present in blast crisis, the detection of the Ph chromosome may be the only clue as to the antecedent disease. Attempts at staging have been less successful than in some other haematological malignancies. The most widely used system devised by Sokal is based on patient age, spleen size, blood blast cell count and platelet count. The best predictor of survival is probably the response to initial therapy.

Fig. 2 Blood film in CML showing myeloid cells of varying maturity

MANAGEMENT

Recent advances have rendered the management of CML in chronic phase complex. Unfortunately, there has been less progress in the management of advanced disease.

Chronic phase

Cytotoxic drugs. The two conventional drugs are hydroxyurea and busulphan. These agents depress the white cell count, diminish splenic size and limit hypermetabolic symptoms. Hydroxyurea is the safer of the two drugs and most studies have suggested an improved survival compared with busulphan. Patients receiving cytotoxic drugs have survived an average of 3 to 7 years, although survivals exceeding 20 years are reported.
Alpha interferon. For patients not eligible for marrow transplantation, alpha interferon has now replaced oral cytotoxic drugs as first line treatment for CML. Its mode of action is poorly understood but clinical trials have shown a median survival advantage of 1 to 2 years for patients receiving regular subcutaneous injections of interferon compared with those receiving hydroxyurea or busulphan. Patients on interferon who achieve a good cytogenetic response (i.e. a fall in the percentage of Ph+ cells) or good control of the white cell count derive the greatest benefit. In good responders treatment should be continued indefinitely. Dosage regimens vary but dose escalation is often limited by side-effects including fever and other flu-like symptoms.

Bone marrow transplantation (BMT). Allogeneic BMT performed in chronic phase is the only proven curative treatment for CML. Patients have survived for more than 10 years after BMT with no detectable bcr-abl transcripts in blood or bone marrow studied with the polymerase chain reaction (PCR). The 5 year leukaemia free survival after HLA identical sibling BMT is between 50 and 60%. Results are best when BMT is performed within 1 year of diagnosis. Few clinicians perform allogeneic transplants in patients over 50 years old and only 30% of patients will have a matched sibling donor - thus only around 15% of CML patients are eligible for allogeneic BMT. In younger patients the use of an unrelated donor matched for HLA is possible but results are poorer than for sibling donor BMT with a higher incidence of graft versus host disease (GVHD). Autologous bone marrow or peripheral blood stem cell transplantation can induce Ph negative haematopoiesis and studies are underway to assess the impact on survival.

Choice of treatment in chronic phase. For patients less than 40 years old with an HLA identical sibling, allogeneic BMT remains the treatment of choice. For patients over 40 years or younger patients lacking a family donor, alpha interferon is first line treatment. In younger patients both lacking a family donor and failing on interferon, a search for an HLA matched unrelated donor should be considered. Other strategies in patients failing first line treatment are hydroxyurea and autologous transplantation.

Advanced disease
In the accelerated phase and blast crisis options are limited. Patients may be helped by allogeneic BMT but results are much inferior to those achieved in CP. Blast crisis can be treated with the combination chemotherapy regimens used in acute leukaemia, and some patients (particularly those with lymphoblastic transformation) will initially respond and return to chronic phase. Unfortunately, such ‘remissions’ are usually short-lived.

Chronic myeloid leukaemia

SiblingCML is a clonal myeloproliferative disorder arising from an acquired genetic change in a pluripotential stem cell.

The hallmark of CML cells is the Philadelphia chromosome (t(9,22)) and the resultant chimeric bcr-abl gene.
There is gross overproduction of neutrophils and their precursors.

CML has an indolent chronic phase followed by a period of acceleration and a final, generally fatal, acute leukaemic phase.
CHRONIC LYMPHATIC LEUKAEMIA

Chronic lymphatic (or lymphocytic) leukaemia (CLL) is a disease characterised by a clonal proliferation of B-lymphocytes. Although the malignant cells appear mature morphologically, they are actually arrested at an early stage of B-cell development. CLL is the most frequent form of leukaemia in the Western world and is a disease of the elderly; almost all patients are over 50 years old at diagnosis.

CLINICAL FEATURES

CLL is a highly variable disorder with many patients surviving long periods with minimal symptoms, whilst others have a rapid demise with bone marrow failure and bulky lymphadenopathy and hepatosplenomegaly. Fortunately, the former group are in the majority. Indeed, in up to three-quarters of cases the diagnosis is made by chance on a routine blood count. Elderly patients with CLL often die from other causes.

Where problems do arise, patients most commonly complain of symptoms of anaemia, lymphadenopathy, unusually persistent or severe infections, and weight loss. The most frequent findings on examination are lymphadenopathy (60%) and splenomegaly (25%). In more advanced cases other tissues such as skin, the gastrointestinal tract, the central nervous system, lungs, kidneys and bone may be infiltrated by leukaemic cells. Occasionally there is transformation into a poorly differentiated large cell lymphoma which carries a poor prognosis (Richter syndrome). The immunodeficiency in CLL is caused mainly by hypogammaglobulinaemia, which not only predisposes to infections but also accounts for an increased incidence of other malignancies.

Fig. 1 a, b CLL is a cause of acquired immuno-suppression. A: oral candidiasis, b: severe chickenpox

DIAGNOSIS
The diagnosis is suggested by a high lymphocyte count confirmed by the blood film appearance. Lymphocyte counts in CLL exceed 5 x 10^9/l and may reach levels of 500 x 10^9/l or more. The cells resemble normal mature lymphocytes but are often slightly larger with a tendency to burst during preparation of blood films, resulting in 'smear cells'. Unexplained lymphocytosis in an elderly person should always suggest the possibility of CLL. The diagnosis is made by proving that the lymphocytosis is a proliferation of clonal B-cells; this is most simply demonstrated by using in situ or flow cytometry techniques to show that the cells have characteristic B-lymphocyte antigens and that a single immunoglobulin light chain (kappa or lambda) exists on the cell surface (i.e. it is a monoclonal population). The bone marrow aspirate shows increased numbers of small lymphocytes and a trephine biopsy is worthwhile as the pattern of lymphocyte infiltration gives prognostic information. If there is confusion with a low-grade non-Hodgkin's lymphoma, a lymph node biopsy can give useful histological information.

The blood film appearance may suggest autoimmune haemolysis or autoimmune thrombocytopenia. Immunoglobulin levels should be checked to assess the degree of immunosuppression.

STAGING

Staging is important in CLL as it helps in making a rational decision as to whether to commence treatment, and it also gives useful prognostic information. The easiest method is the Binet adaptation of the previous Rai system; this is simple to apply and correlates closely with survival. Other variables can give additional prognostic information. A diffuse pattern of lymphocyte infiltration in the bone marrow, a very high number of lymphocytes in the blood, a rapid lymphocyte doubling time, a significant number of prolymphocytes in the blood, and an abnormal karyotype are all poor risk factors.

**Fig. 2** Blood film in CLL.

<table>
<thead>
<tr>
<th>Table 1 Binet staging system for CLL</th>
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<tr>
<td><strong>Stage A</strong></td>
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<tr>
<td>Less than 3 lymphoid areas* enlarged</td>
</tr>
<tr>
<td><strong>Stage B</strong></td>
</tr>
<tr>
<td>Three or more lymphoid areas enlarged</td>
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<td><strong>Stage C</strong></td>
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MANAGEMENT
**When to start treatment**
There has to be a reason to start treatment in CLL—many patients with early stage disease are completely well. Early treatment may slow progress but does not improve survival and can lead to significant side-effects including other neoplasms, and the emergence of resistant disease. Patients deserve a full explanation of the disorder. When the disease is early, they particularly need reassurance of its relatively benign nature.

**Choice of treatment**
In general, treatment should be commenced when the patient develops significant symptoms, when the disease is progressing rapidly or when it is already at an advanced clinical stage. The best single agent for initial treatment of CLL is chlorambucil. This is given orally and is probably better tolerated given intermittently than continuously. Prednisolone may be added but, in view of the multiple side-effects of steroids, it is better reserved for patients with pancytopenia, autoimmune haemolysis or thrombocytopenia. Combination chemotherapy (e.g. CHOP - cyclophosphamide, doxorubicin, vincristine, prednisolone) achieves a greater initial response rate than chlorambucil but this does not translate to an improved survival and it is best kept as second-line treatment.

A new class of drugs, the purine analogues, are already the treatment of choice where conventional treatment fails. The optimum agent appears to be fludarabine, which gives complete or partial remission in approximately half of previously treated patients. Radiotherapy can be used as palliation, particularly where enlarged lymph nodes or spleen cause compressive problems. Splenectomy can be beneficial for painful splenomegaly or autoimmune cytopenia. In patients with hypogammaglobulinaemia and recurrent infections, regular intravenous immunoglobulin has been shown to be well tolerated and quality of life is often improved.

None of the above drug regimens or other treatment modalities will cure CLL; the emphasis is on control of symptoms and possible prolongation of life. In the rare younger patient with CLL a more aggressive approach to treatment may be justified to try and eradicate disease. Bone marrow transplantation from an allogeneic donor is potentially curative but experience is currently limited.

**T-CLL**
A small minority of cases of CLL (less T-rather than B-lymphocyte malignancies. The condition is usually associated with the appearance of large granular lymphocytes in the peripheral blood, neutropenia and splenomegaly. It is a relatively benign disorder although a few cases transform to a more malignant form.

**Chronic lymphatic leukaemia**
CLL is the commonest form of leukaemia in the Western world. It is a disease of the elderly. In the usual form of the disorder, there is a clonal proliferation of B-lymphocytes (B-CLL) Signs include anaemia, recurrent infections, weight loss, lymphadenopathy and hepatosplenomegaly. The clinical course is often indolent but it can be more aggressive in advanced stages. Chemotherapy is often not immediately needed in early CLL.
Chlorambucil is the initial drug of choice in most cases. Purine analogues such as fludarabine are increasingly used in resistant and relapsing disease.
HAIRY CELL LEUKAEMIA

Originally called 'Jeukaemic reticuloendotheliosis', this rare lymphoproliferative disorder is now more compellingly known as hairy cell leukaemia, a reference to the characteristic appearance of the malignant cell (Figs I & 2). The disease constitutes 2% of all leukaemias, occurs worldwide, and typically affects middle-aged men.

Hairy cell leukaemia (HCL) is usually a malignant proliferation of B-cells at a pre-plasma cell stage of differentiation. Most cases have clonally rearranged immunoglobulin genes and express relatively mature B-cell antigens. However, occasional cases express antigens typical of T-lymphocytes either alone or expressed with B-cell markers.

Fig. 1 ‘Hairy cells’ in the blood

Clinical features

Patients often have non-specific symptoms including fatigue and weight loss. Infection, the main cause of morbidity and mortality, and bleeding are other possible presentations. The spleen is the probable site of origin of the malignant clone and splenomegaly is found in over 80% of cases. This may be massive and is usually not accompanied by lymphadenopathy. The liver is enlarged in 50% of patients.

Diagnosis

Most cases of HCL have a pancytopenia and there may be circulating hairy cells in the blood film. Neutropenia is often particularly marked accounting for the frequency of infection. The key cytochemical test is the demonstration of tartrate-resistant acid phosphatase (TRAP) activity in hairy cells. Morphology and cytochemistry are usually sufficient for diagnosis but testing with monoclonal antibodies may be useful - strong activity is seen with FMC7, CD25, CD11c and CD22. The bone marrow is normally difficult to aspirate because of increased fibrosis; the trephine will show a variable number of infiltrating hairy cells. Where splenectomy is performed, the sinuses and cords are seen to be infiltrated by a uniform population of lymphoid cells with blood-filled spaces lined by hairy cells (the pathognomonic 'pseudosinuses' of HCL).

Fig. 2 Hairy cells seen with electron microscope

Management

HCL is an unusual haematological malignancy in that there are a number of successful treatments available. The main challenge is in deciding which is most appropriate in an individual patient. A minority of patients (perhaps 10%) are asymptomatic and in the first instance may require no intervention. Treatment options are as follows.
Splenectomy
This is most helpful in patients with significant splenomegaly and limited bone marrow involvement. The procedure provides rapid palliation and improves survival. In around half of all cases there is a complete remission measured as normalisation of the blood count. Here additional therapy may be unnecessary.

Alpha interferon
Given subcutaneously daily or three times weekly, this agent reduces the hairy cell population in the bone marrow, diminishes blood cytopenia and improves quality of life and survival. Where tolerated, treatment should be continued for at least a year. Major side-effects include initial worsening of neutropenia and systemic symptoms such as pyrexia, lethargy and depression. Following cessation, the disease normally slowly relapses but it will often respond to reintroduction of the drug.

2-deoxycoformycin and 2-chlorodeoxyadenosine These recently introduced agents have greater toxicity than interferon but are more effective in eradicating disease. Results so far suggest that limited courses of treatment give rapid clinical responses with a reduced relapse rate compared with interferon. One of these drugs is likely to become the treatment of choice for most patients. Recent progress makes it difficult to give an accurate prognosis for HCL. Most patients can expect long-term survival.

PROLYMPHOCYTIC LEUKAEMIA
Prolymphocytic leukaemia (PLL) may arise out of chronic lymphatic leukaemia but it more often presents de novo and is best regarded as a distinct disease. The malignant cell is usually of B-lineage and is more mature than the B-CLL cell. Thus, in addition to characteristic B-cell antigens, the cells show a high density of surface immunoglobulin and clonal rearrangements of both heavy and light chain immunoglobulin genes. Approximately 20% of cases are of T-cell lineage with T-cell receptor gene rearrangements and either a 'helper' or 'suppressor' cell phenotype.

Clinical features and diagnosis
PLL is very much a disease of the elderly with a maximum incidence in the eighth decade of life. The most common clinical presentation of B-PLL is massive splenomegaly. Lymphadenopathy is usually not conspicuous. In T-PLL, involvement of lymph nodes and other tissues including liver and skin is more common. The characteristic blood abnormality is a marked lymphocytosis (normally greater than 100 x 10^9/l). Anaemia is normal but platelet numbers are often well preserved. Prolymphocytes are large cells recognised by their condensed nucleus with a single prominent nucleolus surrounded by abundant cytoplasm. B- and T-cell types are not distinguishable by routine microscopy but T-cells can be highlighted by acid phosphatase staining.

Management
PLL has a poorer prognosis than chronic lymphatic leukaemia - the median survival is 2 years with an even bleaker outlook in the T-cell variant. Most patients are elderly and the disease is
frequently refractory to chemotherapy. Palliation of symptoms is the usual priority. Options include splenic irradiation, splenectomy and leuapheresis to control the high white cell count. Younger patients can respond to chemotherapy - a combination of cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP) is most used.

**ADULT T-CELL LEUKAEMIA**

**LYMPHOMA**

Adult T-cell leukaemia lymphoma (ATLL) is a malignant disorder of relatively mature T-lymphocytes. It is rare but of great interest as it is conclusively caused by a virus. The majority of patients with ATLL have antibodies to HTLV-1 and definitive evidence for the aetiological role of this retrovirus has come from studies showing monoclonal integration of proviral DNA in the leukaemic cells. Unsurprisingly, the disease is mostly seen in areas endemic for HTLV-1, notably in parts of Japan and in the islands of the Caribbean. There is a long latent period from infection to overt disease with a cumulative risk of ATLL of around 40% in people infected before 20 years of age. Patients most commonly present in the fifth decade and, as its name suggests, ATLL may behave as leukaemia or a lymphoma. In the most acute form presentation is with a frank leukaemia. The malignant cells in the blood are pleomorphic but often have very irregular polylobulated nuclei. Even within the leukaemic group there is great heterogeneity with chronic and smouldering forms. In 25% of cases the disease is better described as a lymphoma as there is no demonstrable blood involvement. Despite the variability of the pathology there are well-defined clinical and laboratory features which should prompt consideration of the diagnosis, particularly in a person from an HTLV-1 endemic area. In practice lymphoma type ATLL may be confused with other forms of T-non-Hodgkin’s lymphoma. Leukaemic ATLL must be distinguished from Sézary syndrome, a lymphoproliferative disorder with circulating T-cells and skin changes including erythroderma and exfoliative dermatitis.

**Diagnosis**

Diagnosis requires morphological examination and immunophenotyping of blood lymphocytes or a lymph node biopsy. HTLV-1 positivity is established by serological testing and by DNA analysis of affected tissue where available. Chromosome abnormalities are found in up to 90% of cases but are not specific for - ATLL.

**Management**

Treatment has been unsatisfactory and the median survival less than one year. The lymphoma type of ATLL has a slightly better outlook than the leukaemia type. Acute forms are frequently resistant to conventional high grade lymphoma chemotherapy protocols (e.g. CHOP). The combination of u. interferon and the anti-viral agent zidovudine is a novel treatment which may give responses where chemotherapy has failed. The chronic and smouldering - leukaemia forms can run a protracted course but eventually transform to an acute phase. Skin lesions may be helped by extracorporeal photochemotherapy.

Other leukaemias
Hairy cell leukaemia (HCL) is a malignant proliferation of B-cells with a characteristic hairy appearance. Pancytopenia and splenomegaly are common.

Possible treatments for symptomatic HCL include splenectomy, interferon and the new drugs 2-deoxycoformycin and 2-chlorodeoxyadenosine. The prognosis is usually good.

Prolymphocytic leukaemia is a B-cell (or less often a T-cell) malignancy typified by presentation in the elderly, a high white cell count, splenomegaly and a poor prognosis. Adult T-cell leukaemia lymphoma is malignant disorder of T-lymphocytes caused at least in part by infection with the HTLV-1 virus. It may present as leukaemia or lymphoma.
The myelodysplastic syndromes (MDS) are a group of clonal disorders of the bone marrow. Their common feature is that the clone of cells derived from an abnormal stem cell maintains the capacity to differentiate, but this differentiation is ineffective and leads to a hypercellular bone marrow and peripheral blood cytopenia. Red cells, neutrophils and platelets may all be depleted. The ineffective haematopoiesis also causes characteristic morphological abnormalities in the marrow and blood which form the basis for diagnosis. During the course of MDS the abnormal clone is prone to lose its ability to differentiate and acute myeloid leukaemia (AML) intervenes. The disease was previously referred to as 'preleukaemia'. MDS is predominantly a disease of the elderly, although it may affect all ages. It may arise de novo or follow previous chemotherapy or radiotherapy for another malignancy. It seems to be increasing in incidence.

**Fig. 1** Purpuric rash in myelodysplastic syndrome

**CLASSIFICATION**

MDS is enormously heterogeneous. At the two extremes are a relatively benign anaemia and a disease resembling AML. The classification relies chiefly on the number of blast cells in the bone marrow and peripheral blood (Table 1). The divisions are inevitably rather arbitrary. Thus, the MDS subtype *refractory anaemia with excess blasts in transformation* (RAEB-t) is deemed to have evolved to AML when the marrow blast cell count reaches 30% of all nucleated cells. Division of the more benign subtypes *refractory anaemia* (RA) and *refractory anaemia with ring sideroblasts* (RARS) is made according to the number of ring sideroblasts in the marrow aspirate. RARS is a different disorder to the very rare congenital sideroblastic anaemia which usually produces a hypochromic microcytic anaemia. Occasionally, acquired sideroblastic anaemia is not primary (i.e. MDS) but secondary to alcohol misuse or drug treatment (e.g. antituberculous drugs). *Chronic myelomonocytic- anaemia* (CMML) fits awkwardly into the classification as its definition is independent of the blast cell count and depends entirely on the presence of a peripheral blood monocytosis.

**CLINICAL FEATURES**

The diagnosis may be made on a routine blood count in an asymptomatic patient. Where symptoms do occur they range from a mild anaemia to the consequences of severe marrow failure with profound anaemia, leucopenia and thrombocytopenia. Abnormal haematopoiesis can cause functional abnormalities of cells and infection and haemorrhage may be more severe
than would be predicted from the degree of cytopenia. Pronounced symptoms are predictably more common in the RAEB and RAEB-T subtypes. CMML, like the acute monocytic leukaemias, has specific features including splenomegaly (rare in other forms of MDS), skin infiltration and serous effusions.

**DIAGNOSIS**

**Morphology**
The diagnosis of MDS depends on careful morphological examination of the blood film and bone marrow aspirate and trephine specimens. Common abnormalities are as follows:


**Bone marrow.** Erythroid cells - multinuclearity, nuclear budding, ring sideroblasts. Myeloid cells - hypogranularity, increased blast cells. Megakaryocytes - giant forms or micromegakaryocytes.

Where there are changes in all three - lines the term 'trilineage dysplasia' is used. The bone marrow trephine biopsy usually confirms marrow hypercellularity, although fibrosis and even hypocellularity may occur.

**Chromosomes**
Up to 70% of cases of MDS show cytogenetic abnormalities. Common changes include monosomy 7 or 7q-, trisomy 8, monosomy 5 or 5q-, and loss of the Y chromosome. The 5q- abnormality is associated with a particular syndrome characterised by anaemia, macrocytosis, thrombocytosis and a relatively good prognosis. It is of interest that a number of genes important for normal haematopoiesis (e.g. GM-CSF, IL-3) are located on the long arm of chromosome 5. Monosomy 7 in children and young adults may represent a specific preleukaeinic disorder with defective neutrophil function.

The most frequent molecular abnormality in MDS is a mutation of the N-ras gene which is seen in up to 40% of cases.

<table>
<thead>
<tr>
<th>Type</th>
<th>Relative incidence (%)</th>
<th>Median survival (yrs)</th>
<th>Progress to AML (%)</th>
</tr>
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<tbody>
<tr>
<td>RA</td>
<td>30</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>RARS</td>
<td>25</td>
<td>4</td>
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<tr>
<td>RAEB-T</td>
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<td>0.5</td>
<td>60</td>
</tr>
<tr>
<td>CMML</td>
<td>15</td>
<td>2</td>
<td>15</td>
</tr>
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</table>

**PROGNOSTIC FACTORS**
The outcome is closely linked to the classification and the risk of leukaemic transformation (Table 2). Prognostic scoring systems have been devised which include severe blood cytopenia as an adverse factor. CMML is often an indolent disorder with mild anaemia but it may progress to acute monocytic leukaemia.

Fig. 2 a, b, c Myelodisplastic syndrome. (a): Pseudo-Pelger neutrophil with bilobed nucleus, (b): Dysplastic megakaryocyte in the bone marrow, (c): Iron stain of the bone marrow

**TREATMENT**

**Supportive care**
In patients with significant marrow failure, supportive care is crucial to ameliorate symptoms and prolong life. Regular blood transfusion is necessary to control symptoms of anaemia, and haemorrhage is managed with platelet transfusions. Infections require swift intervention with broad-spectrum antibiotics.

**Specific treatments**
The only treatment currently offering the chance of prolonged remission and possibly cure is bone marrow transplantation from an allogeneic donor. Unfortunately, the majority of patients with MDS are elderly and this intensive procedure is not feasible.

**The elderly (over 60 years)**
In most elderly patients the use of supportive care alone is reasonable. More specific treatments are designed to reduce cytopenia and slow progression to leukaemia, but real successes are rare. Corticosteroids may be worth a short trial and patients with the RARS subtype occasionally respond to pyridoxine. For more aggressive disease where a short survival is predicted (e.g. RAEB/RAEB-t) options include subcutaneous low dose cytosine arabinoside or single agent oral
chemotherapy. Low dose cytosine may partly work by inducing differentiation of the malignant clone, but its major action is probably as a cytotoxic agent. Complete remissions are unusual but up to a third of patients will derive some benefit. Oral cytotoxic drugs including hydroxyurea, busulphan and etoposide have been used as single agents but with limited impact. Growth factors such as erythropoeitin and G-CSF may reduce the degree of cytopenia and clinical complications and trials are currently underway.

**Children and younger adults**
In children and younger adults with poor prognosis MDS, intensive combination chemotherapy is often justifiable. Initial response rates are high but remissions are usually short-lived. It may be that in MDS the residual normal stem cell pool is so much reduced that repopulation of the marrow with normal cells, and sustained remission, is impossible. The best chance of a prolonged remission follows an allogeneic bone marrow transplant. It has been suggested that in patients aged less than 40 years this procedure should be performed early before the development of a high leukaemic blast cell count or life threatening blood cytopenia. Where a suitable family donor is lacking, a search for an HLA matched unrelated donor may be undertaken.

**The myelodysplastic syndromes**
MDS is a heterogeneous group of clonal disorders of the bone marrow; the abnormal clone differentiates ineffectively leading to a hypercellular marrow and blood cytopenia. MDS may affect all ages but is predominantly a disease of the elderly. Diagnosis depends on the presence of characteristic morphological changes in the blood and marrow. Classification into subtypes relies on quantitation of blast cells in the blood and marrow. Prognosis is highly variable dependent on the sub-type. Elderly patients often receive only supportive care. In younger patients chemotherapy and allogeneic bone marrow transplantation may be justified.
APLASTIC ANAEMIA

The term aplastic anaemia is a misnomer in that the disorder so described is characterised by a *pancytopenia* arising from failure of production of all the normal cells of peripheral blood. The underlying cause is a reduction in the number of pluripotent stem cells. This deficit may be exacerbated by an abnormality in the marrow microenvironment or an autoimmune reaction against the abnormal haematopoietic tissue.

Aplastic anaemia is uncommon (approximately 2-5 cases/ million/year worldwide), has a slight male predominance and affects all ages. The long-term survival of patient-, with severe disease was only 20% in the early 1970s but this has improved with the introduction of both immunosuppressive treatment and bone marrow transplantation.

It must be emphasised that aplastic anaemia is not a subtype of leukaemia. However, the disease's presenting clinical characteristics, the management problems of marrow failure (including fulminating septicaemia and haemorrhage) and the possible evolution to a clonal marrow disorder dictate its inclusion in this section of the book.

![Fig. 1 Fanconi’s anaemia, digital abnormalities](image)

**CLASSIFICATION**

Aplastic anaemia (AA) may be part of a congenital syndrome, be secondary to well-defined insults to the bone marrow, or arise apparently spontaneously with no identifiable cause. A simple classification is shown here. The most common congenital disorder is Fanconi's anaemia. Affected children suffer from defective DNA repair and the aplasia often coexists with skeletal deformities, skin pigmentation and renal abnormalities. Dyskeratosis congenital another form of constitutional aplasia, is distinguished by a later onset, nail dystrophy, leukoplakia of mucosal surfaces and a high incidence of epithelial tumours. Infections known to predispose to AA include viral hepatitis and parvovirus infection. Drugs and radiation can damage stem cells. Drugs may depress haematopoiesis idiosyncratically (e.g. chloram - phenicol) or predictably (e.g. chemotherapy). In roughly two-thirds of patients, no cause is apparent and AA is termed 'idiopathic'. Improved haematopoiesis following immunosuppression suggests that in at least some cases the abnormal stem cell compartment is further compromised by poorly defined immune phenomena.
Table 1 Classification of aplastic anaemia

1. Idiopathic AA
   - Congenital AA
   - Fanconi’s anaemia
   - Dyskeratosis congenita

2. Secondary AA
   - Drugs - idiosyncratic or dose-related
   - Chemicals
   - Ionising radiation
   - Infection

Table 2 Drugs associated with aplastic anaemia

<table>
<thead>
<tr>
<th>Predictable</th>
<th>Cytotoxic agents</th>
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<table>
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<tr>
<th>Idiosyncratic</th>
<th>Chloramphenicol</th>
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<th>Phenylbutazone</th>
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<th>Indomethacin</th>
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<th>Phenytoin</th>
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<th>Acetazolamide</th>
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This is a selective list of more commonly implicated agents

CLINICAL FEATURES

Patients with marrow failure predictably present with anaemia, unusually frequent or severe infections (caused by neutropenia) and a haemorrhagic tendency (caused by thrombocytopenia). The onset may be gradual or fulminant. Symptoms or signs of an underlying systemic disorder (e.g. Fanconi’s) or possible trigger - (e.g. hepatitis) may be present. An exhaustive history, including drug and occupational exposure, and a thorough examination are mandatory.

Fig. 2. Bone marrow trephine in several aplastic anaemia.

DIAGNOSIS
There are really two questions. Is the pancytopenia due to aplastic anaemia? Is this idiopathic AA or aplasia secondary to an identifiable cause?
A reasonable sequence of investigations is as follows:

1. **Blood count and film**
   There is a pancytopenia and reticulocytopenia. Table 3 shows the differential-diagnosis of pancytopenia. In AA there are no abnormal circulating cells, simply a shortage of normal cells.

2. **Bone marrow aspirate and trephine**
   This is the key diagnostic test. The marrow aspirate can be highly suggestive of aplasia with grossly hypocellular particles but a trephine biopsy is necessary to confirm the diagnosis and quantify the degree of hypocellularity. Aplasia may be patchy and if the trephine is surprisingly cellular in the context of the blood count, then further samples should be obtained. In practice the only likely confusion is with hypocellular myelodysplastic syndrome or an atypical presentation of acute leukaemia, the latter particularly in childhood.

3. **Tests for an underlying cause**
   These include liver function tests (?hepatitis), viral titres (?recent infection), and a Ham test (?paroxysmal nocturnal haemoglobinuria). In childhood and adolescence special chronicsome studies are required to exclude Fanconi's anaemia.

4. **Other**
   Ferrokinetic studies will neatly demonstrate the marrow deficit but are rarely performed in practice.

**MEASUREMENT OF SEVERITY**

This is crucial as the severity defined from peripheral blood and bone marrow measurements predicts the response to treatment and survival. The median survival of untreated severe AA is 3-6 months with only 20% of patients surviving longer than one year.

**MANAGEMENT**

**Removal of cause**
Where an agent such as a drug or a chemical is implicated this should be removed.

**Supportive care**
Blood and platelet transfusion may be life-saving but should be used judiciously as patients with AA have intact cellular and humoral immunity and can become sensitised to histocompatibility antigens. Infection in neutropenic patients requires prompt expert management.

**Restoring normal haematopoiesis**
There are two major options, immunosuppression and bone marrow transplantation.

**Immunosuppression**
Although the precise mechanism of action is unknown, immunosuppressive agents provide worthwhile responses and prolonged survival in 50-70% of patients with AA. Responses are
poorer in younger patients and in severe aplastic anaemia (SAA). Agents used include antilymphocyte or antithymocyte globulin (ALG/ATG), cyclosporin and Oxymethalone. The best regimen is probably a combination of ATG (given intravenously over 5-8 days with corticosteroids) and cyclosporin (given orally with monitoring of blood levels). Both drugs are potentially toxic - ATG can produce pyrexia, rashes and hypotension whilst cyclosporin may cause nephrotoxicity and hypertension. Responses to immunosuppressive treatment can take up to 4 months. Oxymethalone also has side-effects including virulisation, salt retention and liver damage. It is less effective than ATG and cyclosporin. As further discussed below there is concern that immunosuppression may often stimulate haematopoiesis but not cure the disease.

Bone marrow transplantation (BMT) The object of allogeneic BMT is to repopulate the patient's marrow with normal stem cells from a healthy compatible donor. Transplantation from an HLA identical sibling donor in younger patients (less than 45 years) produces long-term survival (possibly cure) in about 70% of cases. It is important to transplant early in the course of the disease as multiple transfusions lead to sensitisation and an increased chance of graft rejection.

Immunosuppression or BMT? Younger patients (less than 20 years) with SAA and a matched sibling donor should be transplanted. In VSAA in this age group the lack of a family donor should prompt a search for a matched unrelated donor. In patients 20-45 years with SAA, sibling BMT and immunosuppression produce equivalent survivals over 2-5 years. However, a disturbingly high proportion of patients receiving immunosuppression alone, approximately 50% at 10 years, evolve to clonal marrow diseases such as paroxysmal nocturnal haemoglobinuria, myelodysplastic syndrome and acute myeloid leukaemia. Thus in this group matched sibling BMT gives a better long-term prognosis. In older patients with SAA, and patients with non-severe AA immunosuppression is generally the treatment of choice.

Growth factors Growth factors cannot rectify the stem cell defect and are therefore not used alone in newly diagnosed AA. G-CSF or GM-CSF may, however, be useful in supportive care or in combination with ATG and cyclosporin.

Aplastic anaemia

AA is characterised by a pancytopenia arising from the failure of production of normal cells by the bone marrow. There is a reduction in the number of pluripotential stem cells; there may also be an abnormal marrow microenvironment and ill-defined autoimmunity. AA can be congenital, secondary to well-defined insults (e.g. drugs) or idiopathic. Prognosis relates to severity which is defined from blood and bone marrow indices. Evolution to a clonal marrow disorder such as leukaemia may occur. Good supportive care is vital. Major treatment modalities are immunosuppression and BMT - the choice of treatment is based on patient age, disease severity and availability of a marrow donor.
CHEMOTHERAPY

GENERAL PRINCIPLES

The life cycle of the normal cell is shown schematically in Figure 1. Antileukaemic (and antilymphoma) cytotoxic drugs can be broadly divided into those agents active during only one phase of the cell cycle ('phase-specific') and those acting at all stages ('phase non-specific'). In practice, most antileukaemic drugs act predominantly against proliferating cells and therefore affect only a fraction of the malignant cell population. Thus, if in advanced acute leukaemia the total number of malignant cells is $10^{10}$, a single course of chemotherapy could be expected to kill between 2 and 5 log of cells leaving between $10^5$ and $10^8$ residual leukaemic cells. It can be seen that the chance of eradication of the disease by chemotherapy is favoured by early treatment when the leukaemic mass is small and by repeated courses of cytotoxic drugs. It is also logical to combine different agents to maximise the antileukaemic activity and exploit different toxicities ('combination chemotherapy').

MAJOR CLASSES OF CYTOTOXIC DRUGS

Alkylating agents
Despite their variable structure, all alkylating agents appear to have a common mechanism with cross-linking of DNA the principal cytotoxic action. Alkylating agents commonly used in haematological practice include melphalan, chlorambucil, cyclophosphamide and busulphan. These agents are toxic to rapidly proliferating cells and the dose-limiting toxicity is myelosuppression with a nadir in the blood count 10-28 days after treatment. Other side-effects include infertility, haemorrhagic cystitis (cyclophosphamide) and an increased risk of secondary malignancy.

Antimetabolites
These drugs are compounds which interfere with the utilisation of a natural metabolite by virtue of the similarity of their chemical structure. Most are analogues of nucleic acid precursors. Commonly used examples are the folinic acid analogue methotrexate, the purine analogue 6-mercaptopurine, and the pyrimidine analogue cytosine arabinoside. The main toxic effect of the group is myelosuppression. The cytotoxicity of methotrexate can be partially reversed by

Topoisomerase poisons
This broad class of drugs includes the anthracyclines (doxorubicin, daunorubicin, mitoxantrone, idarubicin) and the epipodophyllotoxins (etoposide). The anthracyclines are cell cycle non-specific drugs. The acute dose-limiting toxicity is bone marrow suppression but the cumulative dosage is limited by cardiotoxicity. Etoposide is a phase specific drug (active in G2) with myelosuppression the major toxicity.
Spindle poisons

Key agents in this group are the vinca alkaloids, vincristine and vinblastine. They are cell-cycle phase-specific, exerting a cytotoxic effect by binding to cellular microtubular protein and inhibiting mitosis. Vincristine's major adverse effect is the causation of mixed motor sensory and autonomic neuropathies (patients usually initially complain of 'pins and needles' in the fingers or toes); vinblastine is less neurotoxic but causes more bone marrow suppression.

Biological agents

The interferons are a family of proteins which have been shown to have anti-tumour activity in addition to an antiviral effect. They are divided on the basis of biochemical properties into three groups; alpha, beta and gamma. Alpha interferon is the most extensively studied in haematological malignancy and is well established in the treatment of hairy cell leukaemia and chronic myeloid leukaemia. Side-effects include influenza like symptoms and a variable degree of myelosuppression. Interleukin 2 has been used experimentally in the treatment of advanced leukaemia; results of clinical trials are awaited.

MAJOR SIDE-EFFECTS OF CYTOTOXIC DRUGS

Some toxic effects are common to many cytotoxic drugs and must be discussed with all patients receiving a relevant single agent or combination chemotherapy. Myelosuppression and alopecia are often unavoidable. However, nausea and vomiting can usually be minimised or even completely avoided by modern antiemetic protocols. The probability of infertility is influenced by the agents used, the total dosage, the duration of administration and the age and sex of the patient. Strategies to minimise infertility include prechemotherapy storage of germ cells (unfortunately, fertility is often abnormal at presentation) or choice of regimens which are relatively non-sterilising. Gonadal failure occurs more commonly in women and may be managed by hormone replacement therapy; androgens are used in men.

NEWER AGENTS AND APPROACHES TO TREATMENT

There are two ways to improve the results of chemotherapy; firstly, by the modulation of the activity of currently available drugs, and secondly, by the introduction of new cytotoxic agents.

BONE MARROW TRANSPLANTATION

The term 'bone marrow transplantation' (BMT) is loosely used to encompass a number of different procedures. It best fits allogeneic BMT where the marrow comes from another donor but is also used to describe autologous BMT where the patient's own marrow is used to reestablish haematopoiesis. The nomenclature has been further strained by the introduction of new cytotoxic agents.

ALLOGENEIC AND SYNGENEIC
(TWIN) BMT
The allogeneic BMT procedure is outlined in Figure 2a. The patient's own haematopoietic stem cells, immune system and, hopefully, any residual tumour cells, are destroyed by high dose chemotherapy and (usually) radiotherapy ('conditioning treatment') prior to intravenous infusion of marrow harvested from the healthy donor. The ideal patient has a disease curable by allogeneic BMT but not by less toxic treatment and is young (less than 40 years). The ideal donor, excepting the presence of a twin, is a sibling genotypically matched with the recipient for HLA-A, B and DR. The genes for HLA are found on chromosome 6 and thus inheritance follows the rules of simple mendelian inheritance; two siblings have a one-in-four chance of sharing the same HLA type. With relatively small family size in the Western world, only around 30% of patients will have an HLA identical sibling. In other patients it is possible to search large panels of unrelated HLA-typed volunteer donors for a phenotypic HLA match. The chance of successfully locating a matched unrelated donor largely depends on the frequency of the patient's HLA type in the population.

Following conditioning treatment there is a period of approximately 3 weeks before 'engraftment' during which the patient is severely pancytopenic and immunosuppressed and requires intensive supportive care with blood products and aggressive treatment of any infection. Major adverse events include graft rejection arising from a failure to immunosuppress the patient adequately, and graft-versus-host disease (GVHD). GVHD is a potentially life-threatening disorder predominantly affecting the skin, gastrointestinal tract and liver which may occur early after transplantation (acute GVHD) or after a few months (chronic GVHD). It is caused by donor immunocompetent cells attacking antigens in the recipient and can be abrogated by removal of T-lymphocytes from the donor marrow. Such 'lymphocyte depletion' also leads to an increased risk of relapse of any underlying malignancy, suggesting that some of the curative potential of the procedure is due to a 'graft-versus-tumour' effect. The profound immunosuppression of allogeneic BMT renders the patient vulnerable to pneumocystis pneumonia, cytomegalo virus pneumonitis and other late viral infections. The indications for allogeneic BMT and results are discussed in the relevant disease sections.

AUTOLOGOUS BMT

The procedure is outlined in Figure 2b. High dose chemotherapy and radiotherapy is followed by reinfusion of previously stored patient marrow. Autologous BMT has less toxicity than allogeneic BMT and therefore can be performed in older patients. It also has the advantage, that the patient is the donor and therefore donor unavailability is not an issue. The main disadvantage of autologous BMT compared with allogeneic BMT is an increased incidence of relapse of malignant disease. It is not clear whether this arises from resistance to the conditioning treatment or reinfusion of tumour cells in the graft. One approach is to 'purge' leukaemic cells from the marrow graft with cytotoxic drugs or monoclonal antibodies but this is controversial. Indeed, the role of autologous BMT in both acute leukaemia and lymphoma remains unclear and further clinical trials are needed.

Chemotherapy and bone marrow transplantation
There are several classes of cytotoxic drugs with different mechanisms of action. In leukaemia and lymphoma it is normal to combine agents in repeated courses to maximise anti-tumour activity and exploit different toxicities.

Bone marrow transplantation (BMT) procedures may be undertaken using an HLA-matched family or unrelated donor (allogeneic BMT), an identical twin donor (syngeneic BMT), or the patient's own stored marrow (autologous BMT).

Allogeneic BMT is a more effective anti-leukaemia treatment than autologous BMT but is associated with greater toxicity including possible graft rejection and GVHD.
HODGKIN'S DISEASE

The lymphomas are malignant disorders of lymphoid tissue and are subdivided into two broad groups - Hodgkin's disease and non-Hodgkin's lymphoma. Hodgkin's disease was first described by Thomas Hodgkin in 1832. In developed countries there is a bimodal age distribution with peak incidences in young adults (20-30 years) and the more elderly (over 50 years). The disease is almost twice as common in men.

AETIOLOGY

Hodgkin's disease is an unusual malignancy in that the malignant cells, termed Reed-Sternberg cells, and mononuclear Hodgkin's cells form only a minority of the tumour. The remainder is composed of very variable numbers of other cells including lymphocytes, granulocytes, fibroblasts and plasma cells. This inflammatory cell infiltrate presumably reflects an immune response by the host against the malignant cells. The nature of Reed-Sternberg (RS) and Hodgkin's cells has been extensively explored with conflicting results. It is likely that they are derived from lymphoid cells at an early stage of differentiation, either before or during the rearrangement of immunoglobulin and T-cell receptor genes. Clustering of cases has been reported and Epstein-Barr virus DNA material is found in a minority of turnouts. One hypothesis is that the bimodal distribution of Hodgkin's disease is due to infection in young adults with other environmental causes in older patients.

Fig. 1 Reed-Sternberg cells in a lymph node biopsy

CLASSIFICATION

Unlike the non-Hodgkin's lymphomas, the traditional classification of Hodgkin's disease is straightforward. The Rye classification divides it into four histological subtypes. In clinical practice the histological subtype is not crucial in the choice of treatment, although there are some correlations with presentation and prognosis.
CLINICAL PRESENTATION

Lymphadenopathy

Asymmetrical and painless lymphadenopathy, most often in the cervical region, is the most common presentation. The nodes usually gradually enlarge but may fluctuate in size. Patterns of disease suggest contiguous spread via the lymphatic chain. Mediastinal involvement is a particular feature of the nodular sclerosing histological subtype. Splenomegaly and hepatomegaly occur but massive enlargement is rare.

Fig. 2 Lymph node biopsy showing bands of collagenous tissue separating malignant cells.

Constitutional symptoms

Significant systemic upset affects a minority of patients (20-30%) at presentation. This includes fever, sweating (often at night), weight loss, pruritis and fatigue. A peculiar symptom is the development of pain at the site of disease after drinking alcohol.

Extranodal disease

Almost any organ can be infiltrated by Hodgkin's disease but at presentation extranodal spread is rarer than in non-Hodgkin's lymphoma.

DIAGNOSIS AND STAGING

Diagnosis

The key investigation is biopsy of a lymph node for histological examination. This is needed to distinguish Hodgkin's disease from other causes of lymphadenopathy.

Staging

Optimal treatment is determined by the stage of disease which is derived from the following investigations:

1. Blood count and bone marrow investigation. A mild normochromic or microcytic anaemia and blood eosinophilia may be present. Bone marrow aspiration and trephine biopsy to detect infiltration by disease is necessary in more advanced cases.
2. **CT scanning.** A whole body CT scan is now the central staging procedure. In difficult cases this may be supplemented by magnetic resonance imaging (MRI).

3. **Lymphangiography.** This is performed less often since the advent of CT scanning but can sometimes demonstrate disease in nodes of normal size on scanning.

4. **Laparotomy.** Laparotomy was previously routinely performed in patients with otherwise early stage disease to exclude abdominal involvement. Its use has dramatically declined with the introduction of better imaging techniques and other non-invasive methods for predicting response to treatment.

At the completion of staging investigations the patient is allotted a stage according to the Cotswold classification.

**MANAGEMENT**

**Early stage disease**
Patients with stage I or II disease who lack adverse features such as systemic symptoms, an elevated erythrocyte sedimentation rate (ESR), multiple sites of involvement and/or bulky disease may be cured by *radiotherapy* alone. This is given over an extended field using a linear accelerator. Nodes above the diaphragm are treated using the 'mantle' field (like the mantle on a suit of armour) whilst the 'inverted Y' field includes all nodes below the diaphragm.

Where adverse features are present *chemotherapy*, either alone or combined with radiotherapy, is required. The classical regimen for treatment of Hodgkin's disease is the MOPP protocol (mustine, vincristine (Oncovin), procarbazine, prednisolone) which is given at four-weekly intervals for a minimum of six cycles. Toxicity is significant and includes nausea, sterility and late secondary malignancy.

**Table 2 Factors predicting a poor prognosis**

<table>
<thead>
<tr>
<th>Advanced stage (most important)</th>
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<tbody>
<tr>
<td>B symptoms</td>
</tr>
<tr>
<td>Increased tumour bulk</td>
</tr>
<tr>
<td>Increased sites of disease</td>
</tr>
<tr>
<td>Advanced age</td>
</tr>
<tr>
<td>Elevated erythrocyte sedimentation rate (ESR)</td>
</tr>
<tr>
<td>Mixed cellularity Lymphocyte depleted histology</td>
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**Advanced stage disease**
All patients with stage III or IV disease require chemotherapy with possible addition of radiotherapy for bulky disease or palliation of symptoms. MOPP or a similar regimen may be used alone but the alternation of MOPP with ABVD (doxorubicin [Adriamycin], bleomycin, vinblastine, dacarbazine) is probably slightly superior. Higher dose chemotherapy may be given in an attempt to rescue patients relapsing after conventional treatment or as first-line treatment in younger patients selected for poor prognostic factors. Studies of autologous bone marrow transplantation and peripheral blood stem cell transplantation are underway.

**PROGNOSIS**
Survival rates are closely linked to stage although within each stage other prognostic factors influence outcome (Table 2). Cure rates for early stage disease are around 85% whilst even more advanced disease is curable in up to 70% of patients with optimal management. As in other haematological malignancies, elderly patients tolerate chemotherapy less well and cure rates are more modest. In long-term survivors there is a risk of secondary malignancy, most commonly leukaemia and non-Hodgkin's lymphoma. The incidence of secondary cancer at 20 years is about 20% with the combination of chemotherapy and radiotherapy for advanced stage disease.

**Hodgkin's disease**

The term 'Hodgkin's disease' describes a group of lymphomas distinct from the 'non-Hodgkin's' lymphomas.
The presumed malignant cells, Reed-Sternberg and mononuclear Hodgkin's cells, compose a minority of tumour cells.
Common clinical presentations are palpable lymphadenopathy and constitutional symptoms.
Prognosis is largely determined by the stage of the disease.
Early disease may be treated by radiotherapy alone. Advanced disease requires combination chemotherapy with the possible addition of radiotherapy.
NON – HODGKIN’S LYMPHOMA

Malignant solid tumours of lymphoid tissue which are not Hodgkin's disease are termed non-Hodgkin's lymphomas (NHL). This group of lymphomas is even more heterogeneous than Hodgkin's disease and this complexity has led to a series of different classification systems, none entirely satisfactory. The disease is the most common haematological malignancy. In terms of years of life lost it is the fourth most important cancer in the Western world and it appears to be increasing in incidence. NHL may occur at any age but the median age of presentation is 50 years.

AETIOLOGY

The cause of the majority of cases of NHL is obscure. However, specific chromosomal translocations are closely associated with particular histological types. Thus, the majority of Burkitt's lymphoma cases demonstrate the t(8, 14) abnormality in which the C-MYC oncogene on chromosome 8 is moved next to the immunoglobulin heavy chain region on chromosome 14. Over 90% of follicular low-grade lymphomas are characterised by t(14, 18) where the bcl-2 gene on chromosome 18 is moved to the immunoglobulin heavy chain region. This leads to excessive expression of bcl-2, an oncogene known to inhibit apoptosis (programmed cell death). It is likely that such chromosome rearrangements require further events - perhaps co-expression of a second proto-oncogene or antigenic stimulus - to produce the clonal malignant cell. An example of multiple events combining to produce a lymphoma occurs in patients with AIDS. The aggressive extranodal lymphomas seen are likely to result from a combination of immunosuppression (due to the HIV virus), deregulation of a proto-oncogene (c-MYC) and secondary viral infection (Epstein-Barr virus). Similar tumours may follow organ transplantation.

Fig. 1 Axillary lymphadenopathy in low-grade non-Hodgkin’s lymphoma

Table 1  **Histological classification (Working Formulation)**
Low grade  
A  Small lymphocytic with/without plasmacytoid differentiation  
B  **Follicular small cleaved**  
C  Follicular mixed small cleaved and large cell  

Intermediate grade  
D  Follicular large cell  
E  Diffuse small cleaved  
F  **Diffuse mixed small and large cell**  
G  Diffuse large cell  

High grade  
H  Large cell immunoblastic  
I  Lymphoblastic (convoluted/non-convoluted)  
J  Small non-cleaved cell (Burkitt/non-Burkitt)  

Other  
Miscellaneous types include composite malignancies and mycosis fungoides  

Table 2  **Classification based on approach to treatment**  

<table>
<thead>
<tr>
<th>Type</th>
<th>Histological examples</th>
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<tbody>
<tr>
<td>Indolent (low grade histology)</td>
<td>Small lymphocytic</td>
</tr>
<tr>
<td></td>
<td>Follicular small cleaved</td>
</tr>
<tr>
<td></td>
<td>Follicular mixed small cleaved and large cell</td>
</tr>
<tr>
<td>Aggressive (intermediate or selected high grade histology)</td>
<td>Follicular large cell</td>
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<tr>
<td></td>
<td>Diffuse small cleaved</td>
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<tr>
<td></td>
<td>Diffuse mixed small and large cell</td>
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<td></td>
<td>Diffuse large cell</td>
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<tr>
<td></td>
<td>Large cell immunoblastic</td>
</tr>
<tr>
<td>Aggressive with high risk of CNS and leukaemic relapse (selected high grade histology)</td>
<td>Lymphoblastic</td>
</tr>
<tr>
<td></td>
<td>Small non-cleaved cell</td>
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**CLASSIFICATION**  

Classification is complicated although some generalisations can be made. NHL is most crudely divided into 'high grade' and 'low grade' types. High grade tumours are composed of large poorly differentiated lymphoid cells and behave aggressively with rapid clinical onset and spread. Low grade tumours are composed of smaller better differentiated cells which are slower growing and accordingly have a more indolent clinical presentation. The histological classification previously most widely used was the Kiel Classification in which NHL was divided into low and high grade types based on cell size with various subdivisions depending on the precise morphological appearance and the cell of origin (B or T-cell). The designation of the lymphoma cell (e.g. centroblast or centrocyte) presumed that it was fixed at an early stage of normal lymphocyte maturation. The most widely used classification system at present is the Working Formulation (Table 1). Here an additional intermediate category is
inserted. Unfortunately even this classification does not accord entirely with current clinical practice. Table 2 is an adaptation of the Working Formulation for clinical use. It acknowledges that low grade tumours are indolent but usefully divides aggressive turnouts on the basis of the likelihood of invasion of the central nervous system (CNS). This is more relevant to management than a histological label of 'high' or 'intermediate' grade.

NHL classification continues to evolve and the recent 'Revised European American Lymphoma' (REAL) system is likely to become the accepted nomenclature.

CLINICAL PRESENTATION

NHL is essentially a disease of lymph nodes but it has a more diverse presentation than Hodgkin's disease with more irregular spread and a higher incidence of extranodal involvement. It may be an indolent disorder, perhaps requiring no immediate treatment, or an aggressive rapidly fatal malignancy.

Fig. 2 Section of cervical lymph node showing extensive infiltration with large poorly differentiated lymphoid cells

Nodal involvement. Painless lymphadenopathy, often in the cervical region, is the most common presentation of NHL.

Extranodal involvement. Symptoms and signs depend on the system involved. Intestinal lymphoma can present with vague abdominal pain, anaemia caused by bleeding or dysphagia. CNS disease frequently leads to headache and cranial nerve palsies and may cause spinal cord compression. Bone marrow involvement is more common in low grade lymphomas and can result in pancytopenia. Appearance of lymphoma cells in the blood is commonplace in low grade NHL but an ominous sign in high grade disease.

Systemic symptoms. Sweating and significant weight loss occurs in less than a quarter of patients and, where present, usually indicates advanced disease. Occasionally, patients present with metabolic complications such as hyperuricaemia, renal failure and hypercalcaemia.

DIAGNOSIS AND STAGING

Diagnosis depends on obtaining a tissue biopsy, usually a lymph node, for histological examination. Additional information is provided by use of monoclonal antibodies directed against specific lymphocyte associated antigens ('immunophenotyping') - these help identify the degree of maturation of the malignant cell and determine whether it is of B- or T-cell origin. B-cell antigenic 'markers' include CD 19, 20 and 22 and T-cell markers CD 2, 3, 5 and 7. Gene rearrangement studies also aid identification. B-cell lymphomas have their immunoglobulin genes clonally rearranged whilst in T-cell lymphomas there is clonal rearrangement of the T-cell
receptor genes. As previously noted, certain chromosomal abnormalities are characteristic of particular NHL subtypes.

The staging system is similar to that used in Hodgkin's disease. Patients are staged with CT scanning or MRI, and a bone marrow aspirate and trephine. However, in NHL the stage plays a more modest role in management than in Hodgkin's disease. The histological type of the tumour is more closely related to the likely clinical course and other factors impinge upon prognosis. This is particularly the case in high grade lymphoma where a number of prognostic factors can be considered together to predict 'high risk' disease with a poor response to conventional treatment. Indicators of a poor prognosis include advanced stage, extranodal disease, poor performance score and high lactate dehydrogenase (LDH) level.

**MANAGEMENT AND PROGNOSIS**

**Indolent (low grade histology)**

Patients may initially require no treatment - the situation is similar to early chronic lymphatic leukaemia. Localised disease may be treated with radiotherapy. Where systemic treatment is needed, the alkylating agent chlorambucil remains the agent of choice. Although initial response rates are high, the disease repeatedly relapses and median survival is between 6 and 10 years. More intensive combination chemotherapy, including autologous in arrow or blood stem cell transplantation, is an option in younger cases. These regimens frequently prolong the time to relapse but cure and even a survival advantage compared with single agent treatment remain elusive. The failure to eradicate this apparently chemosensitive disease may be explained by the low mitotic rate of the turnout cells - cytotoxic treatment is relatively specific for rapidly proliferating cells. Newer agents such as alpha interferon and purine analogues (e.g. fludarabine) and forms of immunotherapy are currently being assessed.

**Aggressive (high/intermediate grade histology)**

Occasional patients with early stage disease lack poor risk factors and may be cured by radiotherapy alone. All others need chemotherapy and the standard regimen remains 'CHOP' (cyclophosphamide, doxorubicin, vincristine and prednisolone) given every 3 weeks for at least six courses. Around 60% of patients attain a remission on CHOP treatment but only 30-40% are cured. Merely increasing the number of cytotoxic drugs has not led to an improved chance of cure. An alternative approach is to identify high risk cases with poor prognostic factors who are unlikely to be cured with CHOP. Such cases may be treated with higher dose chemotherapy combined with autologous bone marrow or peripheral blood stem cell (PBSC) transplantation. This may also be the best treatment for relapse. Early results of PBSC autologous transplants with haematopoietic growth factor support suggest that this is a relatively safe way of giving high doses of chemotherapy and the outcomes of larger studies are awaited. It should be noted that many patients with NHL are elderly and here even conventional chemotherapy doses are likely to be poorly tolerated.

**Aggressive with high risk of CNS and leukaemic relapse (selected high grade histology)**

Treatment of these malignancies is particularly difficult. Most centres rely on drug regimens similar to those used in acute lymphoblastic leukaemia with additional CNS directed prophylactic therapy including intrathecal methotrexate and cranial irradiation.
**Non-Hodgkin's lymphoma**
The term NHL encompasses solid turnouts of lymphoid tissue which are not Hodgkin’s disease. Histological classification is complex. There is great clinical heterogeneity with aggressive types of disease.
Indolent (low grade histology) NHL often initially responds well to chemotherapy (e.g. chlorambucil) but cure is elusive.
Aggressive (high/intermediate grade histology) NHL may be cured with conventional ('CHOP') chemotherapy; PBSC autologous transplants are increasingly used for 'high-risk' and relapsed disease.
MYELOMA

INTRODUCTION

Multiple myeloma is a malignant disorder in which there is an uncontrolled proliferation of clonal plasma cells in the bone marrow. Secretion of a variety of proteins by the malignant cells leads to characteristic symptoms and signs. Myeloma constitutes 10-15% of all haematological malignancies and is essentially a disease of the elderly-only 2% of cases are diagnosed in patients less than 40 years old. For reasons that are unclear the disease is increasing in incidence.

Fig. 1 Blood film in myeloma.  Fig. 2 The bone marrow in myeloma

BASIC BIOLOGY

Long viewed as a neoplasm of mature plasma cells, there is now evidence that the malignant cells are more immature, perhaps preceding B-cell lineage commitment or even at stem cell level. These cells secrete a monoclonal immunoglobulin or immunoglobulin fragments ('M-proteins') composed of a single heavy chain class and a single light chain class, kappa or lambda. Most myelomas produce IgG or IgA but light chains alone are produced in over 10% of cases. Free light chain appearing in the urine is termed Bence-Jones protein. Occasionally myeloma is non-secretory with no detectable M-protein. Although not routinely measured in clinical practice, the malignant cells also produce a variety of cytokines (e.g. interleukin6, tumour necrosis factor, osteoclast activating factor) which contribute to disease characteristics such as osteolysis, hypercalcaemia and renal failure.

CLINICAL FEATURES

More than two-thirds of patients have bone pain at the time of presentation. Pain is most common in the back and chest and may be attributed to 'arthritis'. More advanced bone disease
can lead to pathological fractures or vertebral collapse with loss of height. Infiltration of the bone marrow by plasma cells may lead to symptoms of anaemia or bleeding due to thrombocytopenia. Infections are common due to immune paresis (low level of normal immunoglobulins) and other complications which may lead to symptoms include hypercalcaemia, amyloidosis and renal failure. The major cause of the nephropathy is deposition of obstructive tubular casts composed of immunoglobulin light chains - other possible factors include dehydration, infection and amyloid.

**DIAGNOSIS AND STAGING**

It is worth restating that myeloma is an easy malignancy to miss as the early symptoms such as malaise and backache are common in the population. The combination of backache and a high erythrocyte sedimentation rate (ESR) should be taken seriously as it may indicate myeloma or another metastatic malignancy. Diagnostic criteria for myeloma are shown in Table 1. A pragmatic approach is to regard the minimum criteria as at least 10% immature plasma cells in the marrow or a plasma cytoma (local tumour composed of plasma cells), typical clinical features, and at least one of the following - an M-protein in the serum (usually greater than 3 g/l) or urine and lytic bone lesions on X-ray. Patients who have a paraprotein in the serum but do not meet the criteria for myeloma are said to have a *monoclonal gammopathy of undetermined significance* (MGUS). If followed up for 10 years, 10-20% of these patients than will develop myeloma. Monoclonal gammopathy can be associated with other diseases such as lymphoma, non-haematopoietic malignancies and connective tissue disorders. The prognosis of myeloma can be predicted from presenting clinical and laboratory features. The best known staging system (the 'Durie-Salmon') is based on the levels of haemoglobin, calcium, M-protein and Bence-Jones protein (BJP), and the extent of lytic disease

![Fig. 3 The fundus in hyperviscosity syndrome complicating Waldenström’s macroglobulinaemia](image)

**Table 2 Myeloma: poor prognostic factor**

| Low haemoglobin |
| High calcium' |
| High M-protein or BJP level |
| Multiple lytic lesions on X-ray |
| High creatinine (i.e. renal failure) |
| High beta-2-microglobulin |
| Low albumin |
| Poor response to chemotherapy |

Included in the Durie-Salmon staging system
MANAGEMENT AND OUTCOME

Myeloma may be diagnosed by chance on laboratory screening in patients with limited disease and no symptoms. In this group, about 20% of all patients, the disease may remain stable for several years and there is no advantage in early intervention. Where treatment is required this generally entails chemotherapy, management of specific complications, and palliation.

Chemotherapy

There is virtually no prospect of cure of myeloma with current drugs. The standard first-line treatment is the alkylating agent melphalan, often combined with prednisolone. Both drugs are given orally in intermittent courses. Approximately 40% of patients will enter a remission, defined as a significant fall in M-protein and number of marrow plasma cells. The median duration of remission is around 2 years, the median survival 3 years, and less than 10% of patients live longer than 10 years. More aggressive combination chemotherapy regimens and other approaches such as high-dose steroid and alpha-interferon are being tried but remission rates and survival are not conspicuously better than with melphalan. Alpha-interferon may be more useful in prolonging remissions achieved with chemotherapy. In patients resistant to first-line treatment a combination of vincristine, doxorubicin (Adriamycin and dexamethasone (VAD) is the preferred option. Escalating the dose of melphalan to very high levels or other myeloablative therapy with autologous or allogeneic marrow transplantation may give at least short-term benefits to those younger patients able to tolerate the inevitable side-effects.

Management of complications

The pain of bone disease may require local radiotherapy in addition to analgesia. In spinal compression, radiotherapy and high-dose steroids usually obviate the need for laminectomy. Biphosphates (e.g. pamidronate) inhibit osteoclast activity and are effective both in hypercalcaemia and in reducing bone pain. Renal failure often responds to rehydration and chemotherapy but haemodialysis may be required.

Palliative treatment - a team approach

As there is currently little hope of cure, palliative care and emotional support are key issues. Cooperation is required between doctors, nurses and other healthcare workers in the hospital and community. Particular emphasis is placed on pain relief and the maintenance of independence.

WALDENSTRÖM'S MACROGLOBULINAEMIA

This disease is a form of low-grade lymphoma. It is appropriately considered with myeloma as the malignant cells, which show features of lymphocytes and plasma cells, secrete an IgM paraprotein. The patient may complain only of fatigue, but high IgM levels can lead to the 'hyperviscosity syndrome' with confusion and neurological symptoms. In these cases retinal examination reveals engorged veins, haemorrhages, exudates and rarely papilloedema. Other
possible physical signs include lymphadenopathy and hepatosplenomegaly. First-line therapy for symptomatic cases is chlorambucil or the purine analogue fludarabine which is also a useful new agent for resistant disease. Significant hyperviscosity requires plasmapharesis.

**Myeloma**

Myeloma is a malignant proliferation of plasma cells. Diagnostic features include an 'M-protein' in the serum and/or urine, osteolytic bone lesions and infiltration of the bone marrow by malignant plasma cells. Bone pain is the most common presenting symptom. Complications include renal failure, hypercalcaemia and amyloidosis. Most chemotherapy regimens include melphalan. Cure is elusive. High-dose chemotherapy may prolong survival in younger patients. Good palliative care, especially pain relief, is crucial. Waldenström's macroglobulinaemia is a form of low-grade lymphoma with secretion of an IgM paraprotein and possible hyperviscosity.
POLYCYTHAEMIA

INTRODUCTION
In simple terms, polycythaemia means an increase in red cell count, haemoglobin and packed cell volume (PCV) above the normally accepted levels. These measurements are routinely provided by automated cell counters. Polycythaemia due to an absolute increase in red cell mass may occur as a myeloproliferative disorder (polycythaemia rubra vera (PRV)) or secondary to hypoxia or an abnormal focus of erythropoietin secretion. In 'apparent polycythaemia' the raised haemoglobin and PCV are not accompanied by a significantly raised red cell mass; usually the plasma volume is relatively reduced.

AN APPROACH TO THE PATIENT WITH POLYCYTHAEMIA

This is summarised in Figure 2. The initial decision to investigate further is taken on the basis of a raised haemoglobin and PCV. If true polycythaemia is confirmed by measurement of red cell mass and plasma volume then the next step is to determine whether this is primary (i.e. PRV) or secondary. From the discussion of clinical syndromes which follows it will be seen that the full sequence of investigations is not required in all cases. For example, in a patient with known cardiac or respiratory disease causing chronic hypoxia, a degree of polycythaemia is predictable and does not require investigation.

CLINICAL SYNDROMES
Polycythaemia rubra vera
PRV (or primary proliferative polycythaemia) is a myeloproliferative disorder; other diseases in this category are essential thrombocythaemia and idiopathic myelofibrosis. In PRV, a pluripotential stem cell is mutated. Although neutrophils and platelets may also be produced in excess, polycythaemia is dominant.

Clinical features. The raised red cell mass and total blood volume with associated hyperviscosity causes the symptoms and signs of the disease. Common complaints include headaches, dizziness, lethargy, sweating and pruritis (the latter particularly after a hot bath). Most importantly, there is an increased risk of arterial and venous thrombosis, particularly strokes. Paradoxically, a combination of hyperviscosity and platelet dysfunction can cause a bleeding tendency in some patients. The increased cell turnover may lead to gout. Patients are characteristically plethoric and may have rosacea. Splenomegaly is present in 75% of cases.

Diagnosis. The diagnostic challenge is to differentiate PRV from a secondary polycythaemia. Splenomegaly is highly suggestive of PRV. In PRV the white cell and platelet counts are often also elevated and the increased erythropoiesis can lead to iron deficiency and hypochromic microcytic red cells. Erythropoietin estimation by radioimmunoassay is normal or low. The bone marrow aspirate and trephine in PRV show hypercellularity but there are no pathognomonic features; in about 15% of cases there is an abnormal chromosomal karyotype (e.g. 20q-). Diagnosis of less florid cases of PRV relies on the systematic exclusion of other causes of polycythaemia such as hypoxia (check chest X-ray and blood gases) and renal disease (screen urine and renal ultrasound or intravenous pyelogram).

Management. The dual purpose of treatment is to relieve symptoms and to reduce the risk of complications such as thrombotic disease and bleeding. The aim is to reduce the PCV below 0.45. This is most easily attained by venesections (up to 450 ml of blood removed) which may
initially be required twice weekly. Many patients need no other treatment. In more severe disease the requirement for venesection can become intolerable and cytotoxic drugs are used to suppress erythropoiesis. Hydroxyurea is the usual choice. Busulphan and radioactive phosphorus are effective given intermittently but both are best avoided in younger patients as there is a significant risk of secondary malignancy. Drug treatment is particularly important when there is a need to control coexistent thrombocytosis.

PRV is a relatively benign haematological disorder and if well-controlled is compatible with a median survival of greater than 10 years. However, it is a clonal disease and a few patients eventually transform to myelofibrosis (15%) or even acute leukaemia (5%). The risk of the latter is increased by treatment with alkylating agents (e.g. busulphan).

**Secondary polycythaemia**
This is due to either a physiological response to hypoxia or an inappropriate secretion of erythropoietin. Treatment is essentially that of the underlying cause, although cases with very high PCVs may benefit from venesection.

**Idiopathic erythrocytosis**
These are a heterogeneous group of patients who have an absolute polycythaemia without features of either PRV or secondary polycythaemia. Occasional cases are familial.

**Apparent polycythaemia**
Apparent polycythaemia, where the red cell mass is within normal limits, is more common than PRV. This condition has accumulated several names including spurious, stress or relative polycythaemia, pseudopolycythaemia and Gaisbock's syndrome. Plasma volume is usually in the low normal range with only 20% of cases being below normal. Thus the usual cause of apparent polycythaemia is an increase in red cell mass and a decrease in plasma volume within the normally accepted limits. Patients are most frequently male and middle aged. Other common characteristics are excess weight, hypertension, diuretic use and significant consumption of alcohol and tobacco. The adoption of a healthier lifestyle often leads to resolution of polycythaemia. Most clinicians start venesection at PCVs exceeding 0.54 with a view to maintaining the level below 0.45 and reducing the risk of vascular occlusion.

**Polycythaemia**
Polycythaemia means an increase in haemoglobin and PCV above normally accepted limits, Polycythaemia can be absolute (with an increased red cell mass) or apparent (with a normal red cell mass). The absolute form can be primary or secondary. Primary polycythaemia is myeloproliferative disorder (polycythaemia rubra vera). Secondary polycythaemia arises from a physiological response to hypoxia or inappropriate secretion of erythropoietin. Management of PRV is by venesection alone or with cytotoxic drugs. Treatment of secondary polycythaemia is essentially that of the underlying cause. Apparent polycythaemia may respond to adoption of a healthier lifestyle.
Essential thrombocythaemia (ET) is a myeloproliferative disorder characterized by a persistent increase in platelet count. There is a probable defect in the pluripotential stem cell with a resultant excess platelet production of up to 15 times normal. ET may be associated with either thrombotic or haemorrhagic complications, the latter caused by abnormal platelet function. As there is no specific diagnostic test, diagnosis relies upon exclusion of other causes of a high platelet count. The average age of presentation of ET is around 60 years with an equal incidence in both sexes.

**Fig 1** Bone marrow trephine biopsy in myelofibrosis (a) marked fibrosis and osteosclerosis, (b) increased reticulin fibres

**CLINICAL FEATURES**

ET may be asymptomatic and discovered accidentally on routine blood testing. Symptoms commonly stem from disturbances of the microcirculation. Patients may complain of burning sensations in the soles and palms, cold peripheries and varied neurological symptoms including headache and dizziness. Arteriolar occlusion can cause ischaemia, gangrene or acrocyanosis. Thrombosis of large arteries is of even greater concern. Haemorrhagic problems include ecchymoses, epistaxis, menorrhagia and bleeding into the mouth and gut. Splenomegaly is unusual at least in part because of splenic infarction, which can be painful.

**DIAGNOSIS**

Platelet counts can be as high as 1500 x 10⁹/l and usually exceed 600 x 10⁹/l (the normal range is 150-400 x 10⁹/l). In practice, there is no single test to identify ET - diagnosis is a process of exclusion. As thrombocytosis may accompany a wide range of disorders including infections, inflammatory conditions and malignancy, a thorough history and examination is mandatory. The lack of a measurable 'acute phase response' (i.e. normal erythrocyte sedimentation rate, plasma viscosity and fibrinogen) increases the likelihood of ET as opposed to a 'reactive' thrombocytosis. High platelet counts are also seen in other myeloproliferative disorders, and bone marrow examination is worthwhile to exclude chronic myeloid leukaemia (absence of Philadelphia chromosome). Patients with polycythaemia rubra vera may have thrombocytosis,
while patients with ET can have an increased red cell mass. In clinical practice both such patients are better diagnosed as having myeloproliferative disorders rather than forced into either category. Only about 5% of all raised platelet counts are due to ET, but persistence of the count above 1000, particularly with coexistence of thrombosis or haemorrhage, makes it the likely diagnosis. Abnormal platelet function tests with reduced aggregation to adrenaline suggest ET rather than a reactive thrombocytosis.

**MANAGEMENT**

The treatment of ET is not straightforward. The decision whether to treat at all must follow consideration of the patient's age, the degree of thrombocytosis and the presence or perceived risk of significant thrombotic or haemorrhagic events. Any clinical benefit must be weighed against potential toxicity of cytotoxic drugs. In a patient with a very high platelet count (greater than 1000), characteristic symptoms, or a history of thrombotic/haemorrhagic disease, chemotherapy is indicated to reduce the platelet count. Conventional drugs include busulphan and hydroxyurea while newer agents such as interferon and anagrelide are under investigation. The objective of treatment is to reduce complications by maintaining the platelet count in the normal range. Where thrombotic problems persist the addition of low-dose aspirin may be helpful. There is a case for observation alone in asymptomatic patients with lower counts - particularly in younger patients who seem to have less complications and in whom the risks of long-term cytotoxic drug therapy are considerable. Occasionally, cases of ET transform to acute leukaemia. This is more common where alkylating agents have been used.

**MYELOFIBROSIS**

Idiopathic myelofibrosis is a myeloproliferative disorder characterised by bone marrow fibrosis and splenomegaly. Patients are usually older than 50 years and there is an equal sex incidence. Like ET, myelofibrosis is a neoplastic clonal disorder originating in a single pluripotential stem cell. Abnormal megakaryocytes are produced in increased numbers and it is these cells which release the cytokines, platelet-derived growth factor (PDGF) and platelet factor 4 which stimulate fibroblast proliferation and build-up of collagen in the bone marrow. The scarred marrow is unable to function normally and haematopoietic stem cells move to the spleen and liver (extramedullary haematopoiesis).

**CLINICAL FEATURES**

The disease is often insidious in onset with fatigue and weight loss. Splenomegaly is present in all cases and massive in 10%. Splenic pain is common and a bulky spleen may lead to portal hypertension, bleeding varices and ascites. Hepatomegaly is seen in two thirds of cases.

**DIAGNOSIS**

Sclerosis of bones is common and when X-rays show dense bones in a patient with splenomegaly, myelofibrosis is the likely diagnosis.
Anaemia is almost universal and the blood film shows tear-drop poikilocytes and a 'leucoerythroblastic' picture with nucleated red cells and immature myeloid cells. In the early stages, thrombocytosis and neutrophilia may occur but in more advanced disease low counts are the rule. Bone marrow aspiration characteristically results in a dry tap (i.e. only peripheral blood aspirated), and a marrow trephine showing dense reticulin fibres on silver staining, fibrosis and osteosclerosis is needed for diagnosis. There is usually megakaryocytic hyperplasia. The major differential diagnosis is from other myeloproliferative disorders and myelodysplastic syndromes which may be associated with marrow fibrosis. Systemic causes of marrow fibrosis such as marrow infiltration by carcinoma or lymphoma and disseminated tuberculosis should also be considered. Acute megakaryocytic leukaemia (AML M7) is sometimes termed 'acute myelofibrosis' but it has a presentation more in to acute myeloid leukaemia.

**MANAGEMENT**

Asymptomatic patients may require no treatment. For anaemia a trial of a corticosteroid or androgen is worthwhile but patients usually become dependent on regular transfusion. Cautious use of oral chemotherapeutic agents such as busulphan and hydroxyurea can improve quality of life by reducing systemic upset and shrinking the spleen. Alpha interferon has been found to be helpful in a few cases.

Splenectomy must not be undertaken lightly as it is associated with considerable mortality (around 20%). However, it should be considered where there is painful splenomegaly, unacceptable transfusion requirements, life threatening thrombocytopenia or complications of portal hypertension.

Bone marrow transplantation is the only potentially curative procedure but is unfortunately limited to the rare younger patient with a matched donor.

**Essential thrombocythaemia and myelofibrosis**

ET is a myeloproliferative disorder characterised by a persistent increase in platelet count. ET patients may be asymptomatic or have either thrombotic or haemorrhagic complications. The object of treatment with cytotoxic agents (e.g. hydroxyurea) in ET is to reduce complications by maintaining the platelet count in the normal range.

Myelofibrosis is a myeloproliferative disorder characterised by bone marrow fibrosis and splenomegaly.

Common symptoms in myelofibrosis are fatigue, weight loss and splenic pain.

Treatment of myelofibrosis is problematic. Regular transfusion is often needed for anaemia. Cautious chemotherapy, splenic irradiation and splenectomy can relieve symptoms in some patients.
THROMBOCYTOPENIA

Thrombocytopenia can be simply defined as a blood platelet count of below $150 \times 10^9/l$. With the routine measurement of platelet number by automated cell counters it is a relatively common laboratory finding. Before initiating further investigations it is important to confirm that a low platelet count is genuine by careful inspection of the blood sample and film. Either a small clot in the sample or platelet clumping (Fig. 1) can cause artefactual thrombocytopenia and lead to unnecessary intervention.

CAUSES

Major causes of thrombocytopenia are listed in Table 1. Some of the diseases (e.g. leukaemia) and syndromes (e.g. disseminated intravascular coagulation (DIC)) are discussed elsewhere. In general terms there are four possible processes leading to thrombocytopenia.

**Failure of marrow production.** The bone marrow failure of haematological disease (e.g. aplastic anaemia, leukaemia) usually causes pancytopenia. However, thrombocytopenia may be the only sign of intrinsic marrow disease or marrow suppression associated with infection or chemotherapy.

**Fig. 1** Blood film showing clumping of platelets.

**Shortened life span.** Platelets can be destroyed in the circulation. The most common mechanism is an immunological reaction in clinical syndromes such as idiopathic thrombocytopenic purpura (ITP) and various connective tissue disorders. Infections and drugs may also cause immune platelet destruction. In DIC, platelets are destroyed as part of an abnormal activation of the coagulation system.

**Sequestration.** Splenomegaly can cause low platelet counts because of pooling in the enlarged organ. The spleen is not necessarily massively enlarged.

**Dilution.** Normal platelets are diluted by massive blood transfusion. This happens because platelets are unstable in the conditions of normal blood storage.

CLINICAL PRESENTATION

Patients with thrombocytopenia are particularly prone to bleeding from mucous membranes. It should be emphasized that spontaneous bleeding is usually only seen with platelet counts of less than $10-20 \times 10^9/l$, although patients with associated platelet dysfunction may bleed at higher counts. Conjunctival haemorrhage and nose and gum bleeding are all relatively common, with haematuria, melaena and severe menorrhagia less frequent complications. Intracranial bleeding is of serious import but, thankfully, is rare. Possible examination findings include purpura and more extensive petechial haemorrhages involving the skin and mucous membranes. The retina should be routinely inspected for haemorrhages.

CLINICAL SYNDROMES
IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP)

ITP is a disease characterized by immunological destruction of platelets. It is conventional to divide the disorder into two discrete entities: acute ITP and chronic ITP. This division is convenient for discussion of pathogenesis and apt for most patients, but in 'real life' there is overlap between the two syndromes.

**Acute ITP**
The acute form of the disease is usually seen in childhood. It typically has an abrupt onset a week or so following a trivial viral illness. It is likely that in postviral cases IgG antibody attaches to viral antigen absorbed onto the platelet surface. The resultant sudden fall in platelet count (often to below 20 x 10^9/l) can lead to all the symptoms and signs quoted above. Despite this, serious complications such as intracranial bleeding are very rare and the disease is self-limiting in around 90% of cases. Often only observation is required, but where the bleeding tendency is unusually severe, oral corticosteroids or intravenous immunoglobulin can be given as in chronic ITP. A few children go on to develop chronic thrombocytopenia, but even here the disease is relatively benign and many eventually spontaneously remit.

**Chronic ITP**
Autoantibodies against platelet membrane antigens are detectable in about 80% of patients with chronic ITP. Most are targeted against epitopes on glycoprotein IIb/IIIa which is the most frequent and most immunogenic platelet surface glycoprotein. Platelets sensitised with autoantibody (usually IgG) are destroyed by macrophages in the spleen and liver. Chronic ITP is most common in young women. Patients may be asymptomatic or have insidious onset of bleeding problems. Serious spontaneous bleeding is generally limited to where the platelet count is below 10 x 10^9/l and even then it is unusual. A palpable spleen suggests a diagnosis other than ITP.

The blood film confirms thrombocytopenia; often the platelets are increased in size. A bone marrow aspirate and trephine biopsy show normal or increased numbers of megakaryocytes. There is no routine specific test for ITP although some laboratories are able to detect platelet antibodies using recently developed methods. Other investigations are designed to exclude a co-existent systemic disorder; an autoantibody screen (connective tissue disorder), and anticardiolipin antibodies and a lupus anticoagulant screen (antiphospholipid antibody syndrome) should be checked. HIV infection can cause immune thrombocytopenia and must be considered where patients are at risk. In younger patients congenital thrombocytopenias may be confused with ITP. A thorough drug history is essential.

Patients with asymptomatic mild thrombocytopenia can be merely observed. It is difficult to state a platelet count below which treatment is mandatory 30 x 10^9/l has been suggested but many patients are symptom free even at this level. The normal first line treatment is prednisolone (1 mg/kg body weight). About two-thirds of patients have a significant increase in platelet count within weeks but subsequent dose reduction often leads to relapse. Where there is no response to steroids, an intravenous infusion of immunoglobulin can be efficacious. Platelet transfusions are seldom indicated as the platelets are rapidly destroyed but they may be considered in severe haemorrhage.

If the platelet count cannot be maintained at a satisfactory level on non-toxic doses of corticosteroid then splenectomy is usually performed. It may be delayed a few months to allow
for the small possibility of a spontaneous remission. About two-thirds of patients have a good response. The management of severe/symptomatic thrombocytopenia postsplenectomy is difficult. An accessory spleen should always be excluded. Where treatment is considered necessary a relatively non-toxic dose of prednisolone (e.g. 10 mg alternate days) may be tried. Other options include intermittent immunoglobulin, cyclophosphamide, azathioprine, danazol, interferon, vincristine and high-dose pulsed dexamethasone. All are associated with isolated successes and there is a large element of 'try it and see' in the management of these patients.

**DRUG-INDUCED THROMBOCYTOPENIA**

Many drugs have been linked with isolated thrombocytopenia. The evidence for causation is often circumstantial. Where the pathogenesis has been explored (e.g. quinine), thrombocytopenia has been caused by anti-platelet antibodies. It is likely that in most cases the antigen is a complex formed between the drug (hapten) and some plasma protein or other carrier molecule. The resultant immune complex attaches to the platelets which (as 'innocent bystanders') are removed from the circulation by the cells of the reticuloendothelial system. A few drugs cause idiosyncratic thrombocytopenia by another process: megakaryocyte suppression (e.g. chlorothiazides) or direct platelet damage (e.g. ristocetin). In immune destruction, thrombocytopenia usually develops within a day and the onset of bleeding can be abrupt and severe. Management is withdraw of the offending drug and platelet transfusions (or even exchange transfusion) for dangerous bleeding.

**POST TRANSFUSION PURPURA**

In this very rare syndrome severe thrombocytopenia follows a blood transfusion. In most cases the patient's platelets are negative for the platelet antigen PL$_A^1$ and the transfused platelets are PL$_A^1$ positive. In a way incompletely understood an anti PL$_A^1$ isoantibody destroys the patient's own platelets. Thrombocytopenia develops around one week after transfusion and bleeding may be severe. Intravenous immunoglobulin appears to be an effective treatment.

**Thrombocytopenia**

Thrombocytopenia (a low platelet count) is a relatively common laboratory finding. It is important that it is confirmed by inspection of a blood film. In general thrombocytopenia can be caused by failure of marrow production (e.g. leukaemia), shortened platelet life span (e.g. ITP), sequestration in the spleen and dilution by massive blood transfusion. Idiopathic thrombocytopenic purpura (ITP) is a disease characterised by immunological destruction of platelets. Acute ITP is usually seen in childhood and is typically self-limiting. Chronic ITP classically occurs in young women. There is often an initial response to steroid treatment but splenectomy may ultimately be required.
DISORDERS OF PLATELET FUNCTION AND VASCULAR PURPURAS

Platelet dysfunction should be considered wherever there are the clinical symptoms and signs of thrombocytopenia in the presence of a normal or only moderately reduced platelet count. Disorders of platelet function can be divided into inherited disorders which are rare but well characterised in the laboratory, and acquired disorders which are much more common but often of obscure aetiology. Bleeding problems may also arise in a number of inherited and acquired disorders of the vasculature and its supporting connective tissue the vascular purpuras.

LABORATORY TESTING OF PLATELET FUNCTION

A good starting point is a blood count and blood film. Some disorders of platelet function are associated with a change in platelet number and/or size. The bleeding time is a useful test of platelet function as it specifically assesses the formation of the platelet plug in a skin wound. A small standard incision is made in the forearm skin and the time to cessation of bleeding recorded (normally 2 1/2 to 9 1/2 minutes in the standard template method). A prolonged time is seen in thrombocytopenia and in platelet dysfunction, but the test is a poor predictor of the likelihood of significant haemorrhage.

Platelet aggregation studies assess the ability of platelets to aggregate in response to the addition of a variety of agonists (e.g. ADP, adrenaline, collagen). Disorders of platelet function may cause diminished aggregation responses to one or more of the agonists. Particularly in inherited disorders, the response (or lack of it) to the commonly used agonists has a characteristic pattern (see below).
Other more rarely performed tests include the measurement of platelet surface glycoproteins and platelet granule contents. Ultimately, none of these tests is a substitute for a careful clinical history establishing the nature of any bleeding episodes, drug exposure, and whether there is a family history of bleeding.

INHERITED DISORDERS OF PLATELET FUNCTION

The commonest inherited platelet function and coagulation disorder, von Willebrand's disease.

**Bernard Soulier syndrome**
This is a rare autosomal recessive bleeding disorder. There is a combination of platelet dysfunction, thrombocytopenia and abnormal platelet morphology. The mild thrombocytopenia is probably caused by reduced platelet survival. The functional platelet defect arises from deficiency of the glycoprotein (GP) Ib-IX complex. This complex is crucial for the initial adhesion of platelets to exposed subendothelium at high shear flow and for binding of platelets to fibrin. In platelet aggregation studies there is failure to aggregate with ristocetin. Bleeding can be severe and particularly complicates other predisposing events such as peptic ulcers and pregnancy. Patients require platelet transfusion for severe bleeding and prior to surgery. DDAVP is useful in some cases.

**Glanzman's thrombasthenia**
This rare autosomal recessive disease is also caused by loss of a platelet glycoprotein - GP IIb-IIIa. This normally acts as a receptor for adhesive proteins such as fibrinogen and von Willebrand factor. Platelet numbers and morphology are normal but the platelets fail to aggregate with all agonists. Clinical manifestations are variable but there is typically onset in the neonatal period and subsequent cutaneous and gastrointestinal bleeding, and menorrhagia. The severity of bleeding often decreases with age. Platelet transfusions are indicated where local haemostatic measures fail.

Table 1 *Causes of abnormal platelet function*

<table>
<thead>
<tr>
<th>Inherited</th>
<th>Bernard Soulier syndrome</th>
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<tbody>
<tr>
<td></td>
<td>Glanzman's thrombasthenia</td>
</tr>
<tr>
<td></td>
<td>Storage pool diseases</td>
</tr>
<tr>
<td></td>
<td>Defects of thromboxane synthesis</td>
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<tr>
<td>Acquired</td>
<td>Drugs (e.g. aspirin)</td>
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<td></td>
<td>Foods (e.g. garlic)</td>
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<td></td>
<td>Chronic renal failure</td>
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<tr>
<td></td>
<td>Cardiopulmonary bypass surgery</td>
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<td></td>
<td>Blood diseases</td>
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<tr>
<td></td>
<td>acute myeloid leukaemia</td>
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<td></td>
<td>myelodysplastic syndromes</td>
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<tr>
<td></td>
<td>myeloproliferative disorders</td>
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<tr>
<td></td>
<td>myeloma</td>
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<tr>
<td></td>
<td>Various systemic disorders'</td>
</tr>
</tbody>
</table>
These include disseminated intravascular coagulation (DIC) and thrombotic thrombocytopenic purpura (TTP).

**Other disorders**
Hereditary diseases of platelet function may also result from deficiency of platelet storage organelles (storage pool diseases) or an enzyme defect in thromboxane synthesis. In general these syndromes only cause mild bleeding problems.

**ACQUIRED DISORDERS OF PLATELET FUNCTION**

These disorders are common. Causes include foods, drugs, systemic disorders and diseases of the blood.

**Aspirin**
Many drugs can affect platelet function but aspirin is the best documented and the most frequently prescribed. Aspirin acetylates and irreversibly inactivates the enzyme cyclooxygenase, preventing the production of thromboxane A₂ from arachidonic acid and inhibiting aggregation for the remainder of the platelet's lifespan. Even small doses of aspirin can dramatically prolong the bleeding time and cause haemorrhage in patients with thrombocytopenia or other co-existent bleeding problems.

**Chronic renal failure**
Uraemia can lead to multiple platelet defects. Abnormal platelet aggregation does not correlate well with the severity of renal failure but there may be improvement after dialysis. The nature of bleeding in chronic renal failure is consistent with abnormal platelet function. Epistaxis, gastrointestinal haemorrhage and menorrhagia are common problems. Treatment of the underlying renal disease (e.g. dialysis, transplantation) may need to be supplemented with platelet transfusions and DDAVP.

**Cardiopulmonary bypass**
During and immediately after cardiopulmonary bypass surgery platelet aggregation is decreased, the contents of platelet granules reduced and the bleeding time is longer than would be expected for the degree of thrombocytopenia. Excessive bleeding is uncommon but where this happens platelet transfusion is efficacious.

**Haematological diseases**
Platelet function is impaired in a number of blood diseases, including acute myeloid leukaemia, myelodysplastic syndromes, myeloproliferative disorders and myeloma.

**VASCULAR PURPURAS**
A bleeding tendency caused by a local or general vascular abnormality is referred to as a vascular purpura. Diagnosis of these diseases is made mainly on clinical grounds with laboratory exclusion of other haemostatic defects.
Inherited disorders

Hereditary haemorrhagic telangiectasia (HHT)
The hallmark of this autosomal dominant disease is the development of small, thinwalled venous angiomatous malformations in the skin, mucous membranes and other organs. The characteristic telangiectasia make this relatively rare disease a common occurrence in medical examinations. Clinical problems include recurrent epistaxes (90% of cases), gastrointestinal haemorrhage, haematuria and pulmonary arteriovenous malformations (PAVMs). Chronic bleeding from the gut causes iron deficiency anaemia. Management includes local control of bleeding (e.g. laser treatment of telangiectasia), iron supplements and embolisation of PAVMs. Recent research has identified a putative HHT gene abnormality located at 9q34.

Inherited diseases of connective tissue Several rare inherited disorders of connective tissue predispose to bleeding. The mechanism is either a general failure of support of blood vessels or defective interaction between platelets and abnormal collagen. Specific diseases include Ehlers-Danlos syndrome, pseudoxanthoma elasticum and Marfan's syndrome.

Acquired disorders
This is a very heterogeneous group. Henoch-Schönlein purpura is a syndrome usually seen in childhood where an itchy purpuric rash typically follows an infection. Spontaneous remission is the rule but renal failure may result. Other causes of acquired purpuric rashes include a range of infections, drug reactions, scurvy, trauma, prolonged steroid therapy and simple old age (senile purpura).

Disorders of platelet function and vascular purpuras
Platelet dysfunction should be considered where there are the clinical features of thrombocytopenia in the presence of a normal or only moderately reduced platelet count. Laboratory testing of platelet function normally includes a blood count, a blood film, a bleeding time and platelet aggregation studies.
Inherited disorders of platelet function are generally well characterised but rare (e.g. Bernard Soulier syndrome), whereas acquired disorders are more frequent but often of obscure aetiology. Aspirin is a common cause of acquired platelet dysfunction. A 'vascular purpura' is a disorder with a bleeding tendency caused by a local or general vascular abnormality. Diseases may be inherited (e.g. hereditary haemorrhagic telangiectasia) or acquired (e.g. Henoch-Schönlein purpura).
HAEMOPHILIA

Haemophilia is an inherited disorder of coagulation. The general term haemophilia is usually taken to mean haemophilia A, a deficiency of factor VIII, but a smaller number of cases are caused by a deficiency of factor IX (haemophilia B).

**Fig. 1 a, b** Chronic knee damage in severe haemophilia A

**HAEMOPHILIA A**

Haemophilia A is transmitted as an X-linked recessive disorder. Thus, all males with the defective gene have haemophilia, all sons of haemophiliac men are normal, all daughters are obligatory carriers and daughters of carriers have a 50% chance of also being carriers. The disease affects one in every 8000 males. The gene for factor VIII is situated at the tip of the long arm of the X chromosome. A wide variety of mutations of the gene can lead to underproduction of factor VIII and the clinical syndrome of haemophilia. In 50% of haemophilia families an unusual molecular genetic abnormality involving inversion of the factor VIII gene has been found. A family history is not inevitably present, as up to 30% of all new cases of haemophilia are due to recent sporadic mutations.

**CLINICAL FEATURES**

As factor VIII is a critical component of the blood coagulation pathway, low levels predispose to recurrent bleeding. The likelihood of bleeding can be roughly predicted from the factor VIII level. Clinical features can be divided into those attributable to bleeding and those arising from complications of treatment.

**Bleeding in haemophilia**

The disease usually becomes apparent when the child begins to crawl. Severely affected patients experience 30-50 bleeding episodes each year. The most common problems are
spontaneous bleeds into joints, often elbows or knees, although any joint can be involved. Patients may develop particular target joints which bleed frequently. They often have an innate feeling that a bleed has started prior to any objective signs. Recurrent or inadequately managed joint bleeds lead to chronic deformity of the joint with swelling and pain. Bleeding may also afflict deep-seated muscles, often the flexor muscle groups. If ignored, the enlarging haematoma can compress adjacent nerves and vessels with serious consequences. Haematuria is not unusual and, until recently, intracranial bleeding was the most common cause of death in haemophilia.

Complications of treatment
In affluent countries, factor VIII replacement treatment as described below has been enormously beneficial in allowing early control of bleeding and the avoidance of chronic joint damage. Unfortunately, most haemophiliacs (over 70% of severe cases) treated before 1985 have become infected with pathogenic viruses contaminating factor VIII concentrate, notably HIV and hepatitis C. HIV infection remains asymptomatic for long periods in a significant minority, but most patients eventually develop acquired immunodeficiency syndrome. Kaposi's sarcoma is, however, very rare. The full implications of hepatitis C infection are not fully understood but end-stage liver disease or hepatocellular carcinoma may result.

DIAGNOSIS
Haemophilia is associated with a prolonged activated partial thromboplastin time (APTT) in the routine clotting screen. The diagnosis is confirmed by a factor VIII clotting assay. In the presence of a family history there are usually few problems in identifying the disorder. Tests can be performed on cord blood. In the absence of a family history the disease may present in a young child with bruising and a swollen joint and be mistakenly regarded as non-accidental injury. Mild haemophilia may only cause problems after trauma or surgery. All patients with bleeding or bruising of a severity disproportionate to the trauma sustained should be investigated to exclude a bleeding disorder.

MANAGEMENT
Treatment of haemophilia is complex, and severe disease is best managed in haemophilia centres where an experienced team of doctors, nurses, physiotherapists and social workers can help patients and their families to lead a relatively normal life.

Treatment of bleeding
Most haemophiliacs require replacement therapy with factor VIII concentrate and this is usually self-administered at home when a bleed occurs (on demand treatment). The dose and duration of treatment depends on the patient's size and the locality and magnitude of the bleed. One unit of factor VIII is the amount contained in 1 ml of normal plasma. For spontaneous haemarthroses it is sufficient to raise the factor VIII level to 30% of normal; in a 70 kg man this entails a dose of around 1000 units. More serious bleeding or surgery requires levels of 70-100% maintained until the risk subsides (Fig. 3). The factor VIII products used are increasingly high purity with low levels of protein contamination and recombinant factor VIII is now entering clinical practice. The availability of safe affordable genetically engineered recombinant factor VIII allows prophylactic (thrice weekly) treatment in children with
eradication of bleeding and improved quality of life. An annoying complication of factor VIII treatment is the development of antibodies to factor VIII (inhibitors) in 10-15% of patients. Treatment of such patients is highly specialized. Strategies include the use of porcine factor VIII, factor IX and activated factor IX concentrates (e.g. FEIBA).

In patients with mild disease (factor VIII greater than 10%) 1-amino-8-D-arginine vasopressin (DDAVP), given intravenously or inhaled as snuff, mobilises factor VIII from stores and may avoid the need for concentrate. The antifibrinolytic agent tranexamic acid can also be used to reduce bleeding and thus the requirement for factor VIII - it should, however, be avoided in haematuria where it can induce clot colic.

**The carrier state and genetic counseling**

Female carriers are generally asymptomatic but some will have low enough levels of factor VIII (10-30%) to cause excessive bleeding after trauma. In families with inversion of the X-chromosome (see above), relatively simple molecular biology methods are used in carrier and pre-natal diagnosis (Fig. 4). In other families identification of the mutation requires more advanced techniques and here carrier and pre-natal diagnosis often depends on the detection of DNA polymorphisms which act as indirect markers of the factor VIII gene.

**HAEMOPHILIA B**

Haemophilia B is an X-linked recessive bleeding disorder in which there is a deficiency of factor IX. The incidence is approximately 3-4 per 100000 male births. There are many clinical similarities to haemophilia A severely affected patients suffer recurrent spontaneous joint bleeds. However, inhibitors (antibodies to factor IX) are less common than in haemophilia A, and the incidence of HIV infection may be different depending on the historical source of factor IX. Earlier factor IX concentrates were associated with thrombo-embolic complications but safer high purity preparations are now available for treatment. The half-life of infused factor IX is around 24 hours and thus it can often be given just once daily to maintain levels after spontaneous bleeding or surgery.

**Treatment of viral infection**

Haemophiliacs with HIV infection require state of the art management of the physical and social problems which can arise. Treatment of hepatitis C infection is contentious but alpha interferon does lead to at least short-term improvement of liver disease in a minority of cases.

**Haemophilia**

Haemophilia A is an X-linked recessive disorder characterised by deficiency of factor VIII. Severely affected patients suffer recurrent spontaneous bleeds, most often into joints. Replacement therapy with factor VIII concentrate is needed in all but mild cases; previous contamination of concentrates has led to HIV and hepatitis C infection. DDAVP and tranexamic acid can help control bleeding in mild disease. The management of choice in
severely affected children is prophylactic treatment with genetically engineered recombinant factor VIII. Haemophilia B is characterised by deficiency of factor IX; inheritance and clinical features are similar to haemophilia A.
VON WILLEBRAND'S DISEASE AND OTHER INHERITED COAGULATION DISORDERS

VON WILLEBRAND'S DISEASE

Von Willebrand's disease (VWD) is the most common inherited bleeding disorder. Approximately 125 people per million have symptomatic VWD and asymptomatic deficiencies in von Willebrand factor (VWF) are detectable in nearly 1% of the general population. All VWD is caused by mutations in the gene for VWF. VWF is an adhesive glycoprotein secreted by endothelium and megakaryocytes. It is a multimeric protein with a characteristic normal distribution of multimer sizes in plasma. VWF has two key functions: promotion of platelet adhesion to damaged endothelium and other platelets (Fig. 1) and the transport and stabilisation of factor VIII. Thus, the clinical disorder of VWD is associated with excessive bleeding due to abnormal platelet function and low factor VIII activity. The clinical and laboratory heterogeneity of VWD necessitates the definition of several subtypes. Recently the classification has been simplified by focusing on the phenotype of the VWF protein (i.e. 'multimer pattern') in patient plasma and platelets.

CLASSIFICATION
The current classification of VWD depends on electrophoretic analysis of VWF multimers. In type I VWD, the multimers appear to be normal in structure and function but decreased in concentration. In type 2 VWD there is a qualitative deficiency of VWF divisible into four subtypes. In type 2A there is an absence of high molecular weight VWF multimers and markedly reduced VWF binding to platelets. 2B refers to a variant where defective platelet adhesion results, paradoxically, from increased binding of VWF to platelets. In 2M there is decreased platelet-dependent VWF function despite a relatively normal multimer pattern whilst 2N is characterised by failure of VWF to bind factor VIII. In the rare type 3 form, there is an almost complete deficiency of VWF and the factor VIII level is markedly decreased. Although this may seem complicated, it represents a considerable simplification with only six diagnostic categories compared with around thirty previously. There is correlation between the subtype and the mode of inheritance. Type I VWD is the most common form of the disease (80% of cases) and inheritance is often autosomal dominant. Type 2 VWD (15% of cases) may be dominant or recessive and the type 3 variant is recessive. Because inherited deficiencies of VWF function are common the accidental co-inheritance of otherwise recessive VWD alleles may occur ('compound heterozygosity'). At the molecular level, quantitative deficiencies correlate with promoter, nonsense, and frame shift mutations and with large deletions. Qualitative deficiencies tend to be seen with mis-sense mutations and small in-frame deletions or insertions.

CLINICAL FEATURES
Severe VWD is characterised by spontaneous bleeding, particularly epistaxes, gum bleeding and menorrhagia. Easy bruising is also common but (with the exception of type 3) haemarthroses and muscle haematomas are rare. Milder disease often presents with excessive bleeding following trauma or surgical procedures and the diagnosis can easily be missed. A thorough history is crucial and must include assessment of the severity of recent bleeding, the existence of
previous bleeding pro Diems (particularly after surgery and dental extractions) and the presence of a family history of easy bleeding. Death from bleeding is rare but it may follow massive gastrointestinal haemorrhage.

Table 1 Summary of classification of VWD

Type 1 VWD is a partial quantitative deficiency of VWF
Type 2 VWD is a qualitative deficiency of VWF
Type 3 VWD is a virtually complete deficiency of VWF
Type 2A VWD is a qualitative variant with an absence of high molecular weight VWF multimers
Type 2B VWD is a qualitative variant with *Increased* affinity of VWF for platelet glycoprotein 1b (reduced in other types)
Type 2M VWD is a qualitative variant not caused by absence of high molecular weight multimers
Type 2N VWD is a qualitative variant with reduced affinity of VWF for factor VIII
Note: mixed phenotypes may be caused by compound heterozygosity,

LABORATORY DIAGNOSIS
The diagnosis of classic type I VWD is usually straightforward but recognition of milder forms and rarer variants can be difficult and the following tests may have to be repeated several times before a final conclusion is reached.

![Fig. 1 Prolonged bleeding time in VWD. There is a significant bleeding onto the applied filter paper.](image)

OTHER INHERITED COAGULATION DISORDERS FACTOR DEFICIENCIES
Factor VIII and factor IX deficiencies

Factor XI deficiency
This bleeding disorder is almost entirely confined to Ashkenazi Jews. Inheritance is via an incompletely recessive autosomal gene and homozygous patients have very low factor XI levels (less than 5% of normal). Factor XI concentrate is the treatment of choice in significant bleeding.

**Factor VII deficiency**
This is inherited as an autosomal recessive disorder. Homozygotes can have all the bleeding problems seen in haemophilia. The diagnosis is confirmed by factor VII assay and factor VII concentrate is available for treatment.

**Factor V deficiency**
In this rare autosomal recessive disease the severity of bleeding is more dependent on the patient's platelet factor V level than the plasma concentration of factor V.

**Factor XIII deficiency**
Another rare autosomal recessive disorder, factor XIII deficiency causes a severe haemorrhagic tendency and poor wound healing. Most sufferers present early in life, often with profuse bleeding from the umbilical cord, and death may result from intracranial haemorrhage. Screening coagulation tests are normal. Diagnosis requires the laboratory demonstration of solubility of patient plasma clots in urea 5M (there is defective cross-linking of fibrin). Factor XIII concentrate is available for treatment.

**ABNORMALITIES OF FIBRINOGEN**
Inherited disorders of fibrinogen are broadly divisible into quantitative deficiencies (apofibrinogenaemia and hypofibrinogenaemia) and qualitative abnormalities (dysfibrinogenaemia). Apofibrinogenaemia is an autosomal recessive disease in which blood fails to clot in all coagulation screening tests and plasma fibrinogen is barely detectable by radioimmunoassay. The bleeding tendency can be severe with spontaneous haemorrhage and excessive blood loss after surgery. Hypofibrinogenaemia is a less well-defined entity with milder bleeding problems. The dysfibrinogenaemias are a heterogeneous group of rare autosomal dominant disorders. Patients may have a haemorrhagic disorder or, paradoxically, an increased risk of thrombosis.

**Blood count.** The platelet count is normal except for a moderate reduction in some cases of type 2B disease.

**Activated partial thromboplastin time (APTT).** Usually prolonged due to low Factor VIII: C levels. The prothrombin time (PT) is normal.

**Bleeding time.** Generally prolonged due to platelet dysfunction but may be normal in mild disease.

**Factor VIII: C assay.** Often low. May be borderline or normal in mild type 1 disease.

**VWF antigen.** Reduced in most cases.

**Ristocetin cofactor activity.** Reduced in most cases. Probably the best laboratory indicator of disease severity.

**Platelet aggregation studies.** Ristocetin (an obsolete antibiotic) induces platelet aggregation in normal plasma but not in severe VWD. An exception is the type 2B variant where platelets aggregate at unusually low concentrations of ristocetin.
Multimer analysis. The multimer composition of circulating VWF is assessed by either crossed immunoelectrophoresis or sodium dodecyl sulphate electrophoresis.

MANAGEMENT
Very mild bleeding problems may require little intervention, perhaps just local measures and the prescription of an antifibrinolytic drug such as tranexamic acid. More significant bleeding generally responds to an infusion of DDAVP which stimulates release of VWF from stores. DDAVP is predictably most effective in patients with a partial quantitative impairment of VWF (type 1). It is less effective in most type 2 variants and is possibly contraindicated in type 2B where it may exacerbate bleeding by inducing thrombocytopenia. Patients with type 3 disease do not respond to DDAVP as they lack any capacity to secrete VWF. Where DDAVP is ineffective or contraindicated, then intermediate purity factor VIII concentrates, containing both VWF and factor VIII, are used. An unusually sustained rise in factor VIII levels can be obtained as the VWF in the concentrate prolongs survival of the patient's own factor VIII. Patients with VWD normally require treatment with either DDAVP or factor VIII concentrate prior to surgery. Effective genetic counseling in VWD demands a full understanding of the disease subtype and mode of inheritance. Advice may be completely different classical dominant type I disease and other subtypes or cases of compound heterozygosity.

Von Willebrand's disease and other inherited coagulation disorders
VWD is a relatively common and very heterogeneous inherited bleeding disorder. Deficiency of Von Willebrand factor (VWF) causes abnormal platelet function and low factor VIII activity. Classification of VWD relies on electrophoretic analysis of VWF multimers. Mild bleeding problems in VWD require little intervention. More significant bleeding is treated with either DDAVP or intermediate purity factor VIII concentrates. There are various other inherited coagulation factor deficiencies. In most there are specific concentrates available for treatment. Inherited disorders of fibrinogen include quantitative deficiencies (apofibrinogenaemia and hypofibrinogenaemia) and qualitative abnormalities (dysfibrinogenaemia).
DIC is a complex clinical syndrome which complicates many serious illnesses (Table 1). It is characterised by intravascular deposition of fibrin and accelerated degradation of fibrin and fibrinogen caused by excess activity of proteases, notably thrombin and plasmin, in the blood. DIC is heterogeneous both in its pathophysiology and clinical manifestations. In most cases it probably begins when circulating blood is exposed to tissue factor released from damaged tissues, malignant cells or injured endothelium. This in turn leads to generation of thrombin which causes formation of soluble fibrin, activation of circulating platelets, and secondary fibrinolysis. Figure 1 attempts to summarise the multiple mechanisms at work.

**Fig. 1** Spontaneous bruising in acquired haemophilia.

DIC can cause bleeding, large vessel thrombosis and haemorrhagic tissue necrosis. The coagulation defect arises from consumption of coagulation factors and platelets and increased fibrinolytic activity. In clinical practice acute DIC usually presents as widespread bleeding in an ill patient. Oozing of blood from cannulation sites is characteristic. Microthrombus formation can lead to irreversible organ damage; the kidney, lungs and brain are frequent targets. DIC is not necessarily a fulminant syndrome, more chronic forms may be seen particularly in association with malignancy (e.g. prostatic carcinoma).

Diagnosis depends on the laboratory demonstration of accelerated fibrinolysis accompanied by falling levels of coagulation factors in a patient with a disease known to cause DIC. The following combination of laboratory test abnormalities is typical:
- Reduced platelet count.
- Prothrombin time prolonged and activated partial thromboplastin time (APTT) usually prolonged.
- Thrombin time prolonged.
- Fibrinogen level reduced.
- High levels of fibrinogen degradation products (FDPS) and cross-linked fibrin degradation products (‘D-Dimers’).

The cornerstone of management of DIC is the treatment of the underlying disease. Patients are more likely to die from the underlying disease than from thrombosis or bleeding. However, specific treatment of DIC may be life-saving and if bleeding occurs support with blood products is indicated. Platelets, fresh frozen plasma (FFP - a source of coagulation factors) and cryoprecipitate (a source of fibrinogen) may all be used. Wherever possible the choice of blood products should be guided by the platelet count and coagulation tests. Much more controversial
is the use of pharmacological inhibitors of coagulation and fibrinolysis. Although heparin can reduce clotting factor consumption and secondary fibrinolysis, it can also increase the haemorrhagic risk by its anticoagulant action. Antifibrinolytic drugs (such as tranexamic acid) are generally contraindicated because of their thrombotic risk.

Table 1 Common causes of DIC
Infections - particularly septicaemia
Malignancy - disseminated carcinoma or acute leukaemia
Obstetric emergencies - septic abortion, abruptio placentae
Shock - surgical trauma, burns
Severe haemolytic transfusion reaction
Liver disease

VITAMIN K DEFICIENCY

Vitamin K in the body is derived from dietary vegetables and intestinal flora. Once absorbed it is stored in the liver and following further metabolism it acts as a cofactor for 7-glutamyl carboxylation of coagulation factors 11, VII, IX, and X and proteins C and S. Vitamin K deficiency is probably the most common acquired coagulation disorder encountered in hospital patients. The vitamin K antagonist effect of warfarin is discussed and the vitamin K deficiency of liver disease later in this section.

Dietary deficiency
Normal dietary requirements for vitamin K are low (0.1-0.5 g/kg) and thus patients must be considerably malnourished before overt deficiency occurs. This most commonly occurs in patients receiving intensive medical care, particularly where broad spectrum antibiotics are used. Deficiency is suggested clinically by excessive bleeding and in the laboratory by a prolonged prothrombin time. Supplemental vitamin K should ideally be given before bleeding problems occur.

Malabsorption
Malabsorptive conditions such as coeliac disease and tropical sprue may lead to vitamin K deficiency. Vitamin K can also be lost in chronic biliary obstruction due to failure of bile salts necessary for fat absorption to reach the bowel.

Haemorrhagic disease of the newborn
Vitamin K deficiency may arise in the first weeks of life, most commonly in breast-fed, full-term and otherwise healthy babies. Contributory factors include low placental transfer of vitamin K, low concentrations of vitamin K in breast milk, low intake of milk and a sterile gut. Haemorrhage most commonly occurs on the 2nd to 4th day. A coagulation screen is abnormal with the prothrombin time and APTT both prolonged. In most countries prophylactic vitamin K
(1mg intramuscular injection) is given to newborn babies. Affected babies respond to parenteral vitamin K but fresh frozen plasma may be needed for severe haemorrhage.

**LIVER DISEASE**

The liver is vital to normal haemostasis. It produces all the factors of the intrinsic and extrinsic coagulation pathway and clears potentially damaging products of coagulation such as fibrin degradation products and activated clotting factors. Thus, in advanced liver disease there are often multiple haemostatic abnormalities including reduced synthesis of clotting factors, increased consumption of clotting factors (DIC), qualitative and quantitative platelet abnormalities, qualitative fibrinogen abnormalities and accelerated clot lysis. Where bleeding occurs, the type of therapy is guided by the dominant haemostatic problems. Possible interventions include parenteral vitamin K, fresh frozen plasma, cryoprecipitate and platelet infusions.

**ACQUIRED HAEMOPHILIA**

Antibodies ('inhibitors') that block the action of coagulation factors may appear in patients who have no hereditary disorder of coagulation. Such autoantibodies most commonly target factor VIII and the clinical syndrome is termed 'acquired haemophilia'. Acquired haemophilia may be associated with a number of conditions including rheumatoid arthritis and other autoimmune disorders, skin disorders, drug therapy (particularly penicillin), pregnancy and the puerperium. However, the most common presentation is in an elderly patient (greater than 60 years) with no associated condition. Possible clinical problems include haemorrhage into soft tissues and muscles, haematuria, haematemesis and prolonged bleeding postpartum or postoperatively. Bleeding can be difficult to control and death occurs in 10-20% of cases. In the laboratory, the diagnosis of acquired haemophilia is suggested by a prolonged APTT worsening with incubation and not corrected by the addition of normal plasma, and a low factor VIII level. Laboratory assay of the inhibitor is based on the ability of the patient's plasma to neutralise the activity of a known amount of factor VIII. Management is complex but can be divided into the treatment of the acute bleeding episode and subsequent attempts to eliminate the autoantibody by immunosuppressive treatment. Possible approaches to the acute episode include large doses of human factor VIII concentrate, porcine factor VIII, prothrombin complex concentrate, activated prothrombin complex concentrate and recombinant factor VIIa. Immunosuppressive strategies include intravenous immunoglobulin and plasma-pharesis in the acute episode and longer-term steroids or cyclophosphamide. Many elements of management remain controversial.

**Acquired disorders of coagulation**

Disseminated intravascular coagulation (DIC) is a complex clinical syndrome which complicates serious illness. It causes both haemorrhage and thrombosis. Laboratory tests are needed and monitor to confirm the diagnosis.
Treatment of DIC is essentially that of the underlying cause. Blood products are often indicated where bleeding occurs.
Vitamin K deficiency is a common acquired coagulation disorder.
Advanced liver disease can cause multiple haemostatic abnormalities.
Acquired haemophilia is generally caused by an autoantibody targeted against factor VIII. It may be idiopathic or associated with other autoimmune diseases, pregnancy or drug treatment.

PCC, prothrombin Complex concentrated a, activated, Ig, immunoglobulin.
THROMBOPHILIA

Patients who are predisposed to thrombosis generally either have a disorder of the blood or an abnormality of the vessel wall. Where enhanced coagulation is the major mechanism, the disorder is referred to as 'thrombophilia'. Patients with thrombophilia either tend to have thrombosis at an unusually early age or to develop recurrent thrombotic problems. Venous thrombosis predominates with the chance of thrombosis increased by the coexistence of other risk factors such as obesity, surgery, pregnancy and malignancy. Thrombophilia can be inherited or acquired.

Table 1 Characteristics suggesting possibility of thrombophilia
Venous thrombosis in patient less than 40 years old
Recurrent venous thrombosis or thrombophlebitis
Venous thrombosis in unusual site (e.g. axillary vein)
Arterial thrombosis in patient less than 30 years old
Strong family history of venous thrombosis
Recurrent fetal loss
Skin necrosis in patient receiving warfarin

Table 2 Major risk factors for thrombosis

<table>
<thead>
<tr>
<th>Venous</th>
<th>Arterial</th>
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<tr>
<td>Immobility</td>
<td>Smoking</td>
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<tr>
<td>Obesity</td>
<td>Male sex</td>
</tr>
<tr>
<td>Oral contraceptive pill</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Trauma/surgery</td>
<td>Strong family history</td>
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<td>Thrombophilia (see text)</td>
<td>Hyperlipidaemia</td>
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<tr>
<td>Pregnancy</td>
<td>Diabetes mellitus</td>
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<tr>
<td>Malignancy</td>
<td>Raised fibrinogen</td>
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<td>Increasing age</td>
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WHICH PATIENTS SHOULD BE INVESTIGATED FOR THROMBOPHILIA?

Table 1 summarises factors which should prompt consideration of thrombophilia. Accurate history taking is essential; particular attention should be given to the nature of the recent thrombotic event, the presence of known risk factors (Table 2), a previous history of thrombosis and the family history.

Definition of a 'positive' family history of thrombosis is problematic. If we use the simple definition of a history of deep vein thrombosis (DVT) or pulmonary embolus (PE) in a first or second degree relative, then approximately 25% of all patients will have a positive family history. Even amongst those with a strong family history only a small minority will have a cause of thrombophilia identified.
Basic investigations of thrombophilia should include a blood count (to exclude polycythaemia and other myeloproliferative disorders) and a basic coagulation screen (prothrombin time, activated partial thromboplastin time, and thrombin time or fibrinogen). Further laboratory testing is dictated by the possible causes of familial and acquired thrombophilia detailed below. Testing for thrombophilia should not be undertaken during an acute episode of venous thromboembolism when low levels of coagulation inhibitors are routinely found. It must also be remembered that systemic disorders such as liver disease or disseminated intravascular coagulation (DIC) can depress the levels of coagulation inhibitors and thus simulate the laboratory abnormalities found in familial thrombophilia.

FAMILIAL THROMBOPHILIA

In theory, familial thrombophilia could be caused by any genetically determined defect of the coagulation or fibrinolytic systems that causes accelerated thrombin formation or impaired fibrin dissolution. In practice, the well-defined causes are associated with accelerated thrombin formation either due to a shortage or failure of activation of one of a number of circulating inhibitors of coagulation.

Resistance to activated protein C (APCR)
The anticoagulant property of activated protein C (APC) lies in its capacity to inactivate the activated cofactors Va and VIII, by limited proteolysis. Recent studies have shown that inherited resistance to the anticoagulant action of APC is an important cause of thrombophilia. In most cases resistance is caused by a single point mutation in the factor V gene (factor V Leiden) with replacement of Arg506 with Gln. Arg506 is located at one of the APC cleavage sites in factor V and the mutated V is less sensitive than normal V to APC mediated inactivation. APCR has an autosomal dominant mode of inheritance and is the most common known cause of familial thrombophilia. The increased risk of venous thrombosis in APCR has been estimated as 5-10 fold in heterozygotes and 50-100 fold in homozygotes. The prevalence of the disorder in Western Europe is 3-7% with an incidence of around 20% in unselected cases of venous thrombosis. In practice, the risk of venous thrombosis is highest in patients homozygous for the mutation or in heterozygotes with other risk factors (Table 2). It is not yet clear if APCR is associated with an increased risk of arterial thrombosis.

Protein C deficiency
Hereditary deficiency of protein C is an autosomal dominant disorder found in 2-5% of patients with thromboembolic disease. Heterozygotes have protein C levels approximately 50% of
normal. It is worth noting that an acquired deficiency of protein C can occur in liver disease, DIC and warfarin treatment. Familial protein C deficiency manifests as an increased incidence of venous thromboembolism. Thrombotic events vary from a superficial thrombophlebitis to DVT and PE. They may be spontaneous or triggered by other factors such as surgery or pregnancy. As in other forms of thrombophilia, the first episode of thrombosis may occur at an early age and then be followed by frequent recurrences. In the rare homozygous form of the disease, the infant can be born with undetectable levels of protein C and quickly develop DIC and skin necrosis due to microvascular thrombosis of subcutaneous vessels (purpura fulminans).

**Protein S deficiency**
Protein S is the non-enzymatic cofactor of protein C. Hereditary deficiency is found in 4-8% of patients presenting with venous thrombosis. Deficiency may be acquired in a variety of diseases and in pregnancy. The clinical manifestations of hereditary protein S deficiency are the same as in protein C deficiency.

**Antithrombin III deficiency**
Antithrombin III (AT III) is the major physiological inhibitor of thrombin and clotting factors IXa, Xa, XIa and XIIa. Deficiency can be inherited in an autosomal dominant manner. Its prevalence is unclear but AT III deficiency probably contributes to venous thrombosis in around 2-5% of younger patients. There are several disease subtypes based on the results of functional and immunological assays; the risk of thrombosis varies between subtypes being greater for an abnormality affecting the reactive (thrombin binding) site than for an abnormality affecting the heparin binding site. Overall, it seems that the risk of venous thrombosis is larger in heterozygotes for AT III deficiency than for those with APCR, protein C or protein S deficiency. The risk increases with age, with up to 80% of patients developing venous thrombosis by 55 years. The relationship between AT III deficiency and arterial thrombosis is uncertain.

**Other possible forms of familial thrombophilia**
High factor VIII concentrations have been associated with an increased risk of venous thrombosis. The mechanisms involved and the degree to which they are genetically determined has still to be elucidated. Other candidates for familial thrombophilia status include the dysfibrinogenaemias and factor XII deficiency.

**Management of familial thrombophilia**

**Acute venous thrombosis**
This should be treated with heparin and warfarin. Patients with AT III deficiency may require unusually high doses of heparin. Warfarin should be continued for at least 3 months. Patients with protein C (and occasionally protein S) deficiency can develop warfarin associated skin necrosis; this may be caused by an initial rapid fall in protein C levels after warfarin commencement leading to a hypercoagulable state and thrombosis in the subcutaneous circulation. The risk can be minimised by ensuring full heparinisation and then introducing warfarin gradually. Protein C concentrates have been given to treat purpura fulminans in homozygous disease.

**Other situations**
In patients with recurrent venous thrombosis, long-term oral anticoagulation with warfarin will often be indicated. This approach is, however, not usually appropriate where this is only a family history of thrombosis with no personal thrombotic events. A possible exception is AT III deficiency where the risk of thrombosis in older patients appears considerable. The management of thrombophilia in pregnancy is particularly complex. Warfarin is potentially teratogenic and subcutaneous heparin is normally the mainstay of treatment. AT III concentrate is available and may be helpful in deficient patients at time of delivery.

Counseling
Counseling is frequently not straightforward. Any doubts relating to diagnosis and the probability of thrombosis in asymptomatic family members must be acknowledged. Known risk factors such as immobility, obesity and the oestrogen-containing oral contraceptive should be avoided wherever possible. Recent studies have shown a two to four times increased risk of venous thromboembolism in women receiving hormone replacement therapy (HRT).

ACQUIRED FORMS OF THROMBOPHILIA

Antiphospholipid antibody syndrome
The definition of antiphospholipid antibody syndrome is imprecise, but diagnosis essentially requires a combination of characteristic clinical events and laboratory identification of an antiphospholipid antibody (Table 3). The syndrome can be 'primary' where the patient has no obvious autoimmune disease or 'secondary' if the patient also has systemic lupus erythematos (SLE) or a lupus-like disease. About half of all patients have the primary form of the disorder. Up to 2% of the general population have detectable antiphospholipid antibodies - the probability of clinical problems is greatest where the antibody titre is high. The cause of thrombophilia in antiphospholipid antibody syndrome is not understood. It is possible that the antiphospholipid antibody is merely a marker for an underlying abnormality of coagulation proteins, platelets or endothelial cells. Management is controversial. Where there has been an episode of major thrombosis, warfarin appears to offer the best protection against recurrent thrombosis. It is uncertain if aspirin gives additional benefit. In women with a history of recurrent spontaneous abortion there is no consensus as to best treatment; most studies have focused on aspirin and/or heparin with inconclusive results.

Other acquired forms of thrombophilia
In addition to the well established risk factors (Table 2), there are other acquired disorders predisposing to thrombosis. Myeloproliferative disorders are discussed elsewhere. Recent studies have suggested that increased levels of plasma fibrinogen, von Willebrand factor and tissue plasminogen activator (t-PA) are predictors for coronary artery disease. Whether these abnormalities are constitutional changes predisposing to coronary atherosclerosis and thrombosis or whether they are markers of preexisting inflammation and endothelial dysfunction is currently unclear.

Table 3 Antiphospholipid antibody syndrome

Clinical features
Recurrent venous and arterial thrombosis
Recurrent fetal loss
Immune thrombocytopenia
Livedo reticularis

**Laboratory tests**
Antiphospholipid antibodies: lupus anticoagulant
anticardiolipin antibodies

**Thrombophilia**

The term 'thrombophilia' describes a predisposition to thrombosis caused by abnormally enhanced coagulation. Patients often have venous thrombosis at an early age or develop recurrent thrombotic problems.

Classical familial thrombophilia disorders are deficiencies of the naturally occurring inhibitors of coagulation protein C, protein S, and antithrombin III.

Activated protein C resistance (APCR) is a recently described thrombophilia disorder caused by an inherited mutation in the factor V gene. Heterozygosity is common (3-7% in Western European population).

Rejected patients with familial thrombophilia require lifelong anticoagulation.

Antiphospholipid antibody syndrome is an acquired disorder characterised by laboratory identification of antiphospholipid antibodies and clinical features including thrombophilia, recurrent fetal loss and thrombocytopenia.
ANTICOAGULATION AND THROMBOLYTIC THERAPY

Two major classes of drugs are used in the management of thromboembolic disease. The *anticoagulants* heparin and warfarin are used to prevent thrombosis and limit the extension of an established clot, whilst *thrombolytic agents* such as streptokinase are used to dissolve thrombus.

ANTICOAGULATION

HEPARIN

Unfractionated heparin is a naturally occurring glycosaminoglycan produced by mast cells. Low molecular weight (LMW) heparin is prepared by controlled depolymerisation of the unfractionated form. Both unfractionated and LMW heparin exert their anticoagulant properties by binding to antithrombin III (AT III) and potentiating its activity. AT III is a normal circulating anticoagulant which inhibits the actions of factor Xa and thrombin. LMW heparin differs from unfractionated heparin in having a relatively greater anti-Xa, than antithrombin activity.

Unfractionated heparin

Standard unfractionated heparin may be used therapeutically to treat established thrombosis (usually intravenously at higher dosage) or prophylactically to prevent thrombosis (usually subcutaneously at lower dosage). Most common indications for therapeutic use are deep vein thrombosis (DVT) and pulmonary embolism (PE). A typical regimen is an intravenous loading dose of 5000 units followed by an infusion of 1000-2000 units/hour. Laboratory monitoring should start within 4-6 hours of treatment and continue daily for its duration. The objective is to keep the APTT (see p. 20) at 1.5-2.5. Should the APTT be too high it is generally adequate to stop the infusion for a short time (30-60 minutes) and restart at a reduced dose. In the event of serious bleeding requiring immediate neutralisation of heparin, the antidote prolamine is given. When the APTT is too low the heparin dose should be promptly increased. Heparin is normally continued until oral anticoagulation is therapeutic. Therapeutic doses must be prescribed with caution in patients with a bleeding tendency; examples include recent surgery, thrombocytopenia and liver dysfunction. Prophylactic heparin is most commonly given to prevent DVT and PE in patients undergoing surgery. It is particularly indicated in patients with known risk factors for venous thrombosis and in major procedures. A typical prophylactic regimen is 5000 units subcutaneously preoperatively and 5000 units 8 to 12 hourly after surgery, for 7 days or until the patient is mobile. No laboratory monitoring is necessary in routine cases - where required anti-Xa assays are used. Apart from haemorrhage, patients on heparin may develop thrombocytopenia and prolonged use can cause osteoporosis.

LMW Heparin

These heparins have been developed over the last decade. Compared with standard heparin they have a longer plasma half-life allowing once daily dosage. Less variation in the anticoagulant
response to a fixed dose allows their use without laboratory monitoring. They may also have an improved antithrombotic to haemorrhagic ratio. At present cheaper standard heparin is often still the first choice for established thrombosis and in most prophylactic situations. However, patients at very high risk of thrombosis, for instance following major orthopaedic surgery, seem to benefit from use of LMW preparations. The convenience and probable increased safety of LMW preparations is likely to lead to their increased use in the future.

Fig. 2 The vitamin K cycle and the action of warfarin. The major site of warfarin action is not a direct effect on the carboxylation step needed for coagulation factor activation but on steps needed for resynthesis of active vitamin K from its epoxide form.

WARFARIN

Oral anticoagulant drugs are derived from 4-hydroxycoumarin and the standard agent is warfarin. Warfarin works by antagonising vitamin K, which is needed for the gamma carboxylation of certain glutamic acid residues which facilitate calcium binding of coagulation tors 11, VII, TX and X (Fig. 2). Some indications for warfarin and desired therapeutic ranges are shown in Table 1. A reasonable starting regimen is 10 mg on each of the first 2 days and then

Table 1 Warfarin: indications and therapeutic ranges

<table>
<thead>
<tr>
<th>Indication</th>
<th>Recommended INR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylaxis of DVT including surgery in high risk cases*</td>
<td>2.0-2.5</td>
</tr>
<tr>
<td>Treatment of DVT and PE</td>
<td>2.0-3.0</td>
</tr>
<tr>
<td>Systemic embolism</td>
<td></td>
</tr>
<tr>
<td>Transient ischaemic attacks</td>
<td></td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td></td>
</tr>
<tr>
<td>Mitral stenosis with embolism</td>
<td></td>
</tr>
<tr>
<td>Recurrent DVT and PE</td>
<td>3.0-4.5</td>
</tr>
<tr>
<td>Mechanical prosthetic valves</td>
<td></td>
</tr>
<tr>
<td>Arterial disease including myocardial infarction</td>
<td></td>
</tr>
</tbody>
</table>

*Heparin more commonly used.

adjustment of the dose according to the international normalised ratio (INR). A coagulation screen should always be checked before warfarin is prescribed. The maintenance dose is usually between 3 and 9 mg. Laboratory monitoring depends on the prothrombin time. Thromboplastin reagents used in this test vary and their sensitivity is labelled with an
international sensitivity index (ISI) which permits reporting as an INR such that INR = (prothrombin time)ISI.

As it takes several days for warfarin to become therapeutic, the conventional treatment of established thrombosis is to start heparin and warfarin simultaneously and only to stop the heparin when the desired INR has been achieved. Warfarin should be used with caution in patients with a bleeding tendency. The most common side-effect is haemorrhage, the risk of serious bleeding correlating with the height of the INR. Poor control of anticoagulation and bleeding may arise from poor prescribing or compliance (i.e. over-dosage), intercurrent illness, and interaction with a potentiating drug (Table 2). A prolonged INR in a non-haemorrhagic patient may only require withdrawal of the drug for a few days. Where there is haemorrhage, warfarin can be reversed within hours by intravenous vitamin K (0.5-5 mg) and instantly by infusion of a concentrate of factors II, IX, X and VII or, alternatively, fresh frozen plasma (FFP). Guidelines are complex and significant warfarin over-dosage should be discussed with a haematologist. The duration of warfarin treatment depends on the indication. Anticoagulation may be needed for only six weeks in a patient with a limited DVT and reversible risk factors (e.g. post-surgery). Longer periods are indicated in idiopathic venous thrombosis, and lifelong warfarin treatment may be justified following recurrent episodes of venous thrombosis or where there is a known ongoing thrombotic risk such as a prosthetic heart valve, atrial fibrillation or a thrombophilic state. Community and outpatient warfarin treatment is best monitored in specialist clinics where control is audited and technologies such as computerisation exploited.

Table 2 Drugs interacting with warfarin*

<table>
<thead>
<tr>
<th>Potentiating</th>
<th>Antagonising</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>Oral contraceptives</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>Spironolactone</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>Antihistamines</td>
</tr>
<tr>
<td>Quinine</td>
<td>Barbiturates</td>
</tr>
<tr>
<td></td>
<td>Ritampicin</td>
</tr>
<tr>
<td>Amiodarone</td>
<td></td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td></td>
</tr>
<tr>
<td>Metronidazole</td>
<td></td>
</tr>
<tr>
<td>Tricyclics</td>
<td></td>
</tr>
<tr>
<td>Aspirin and salicylates</td>
<td></td>
</tr>
<tr>
<td>Anabolic steroids</td>
<td></td>
</tr>
<tr>
<td>Thyroxine</td>
<td></td>
</tr>
<tr>
<td>Sulphinpyrazone</td>
<td></td>
</tr>
</tbody>
</table>

These are some commonly implicated agents - this is not a comprehensive list

**THROMBOLYTIC THERAPY**

Until recently the role of thrombolytic agents was limited to systemic use in occasional cases of major pulmonary embolism and iliofemoral venous thrombosis, and local use in acute peripheral artery occlusion. However, it is now known that these drugs benefit patients with acute
myocardial infarction, reducing infarct size, preserving ventricular function and reducing early mortality. All agents are plasminogen activators. Thus they convert plasminogen, the inactive proenzyme of the fibrinolytic system in the blood, to the proteolytic enzyme plasmin. Plasmin dissolves the fibrin of a blood clot (Fig. 3) but may also degrade normal components of the coagulation mechanism. Six agents are either in use or under investigation for patients with myocardial infarction:

- streptokinase
- urokinase
- recombinant tissue-plasminogen activator (rt-PA)
- anisoylated plasminogen streptokinase activator complex (APSAC)
- recombinant single chain urokinase-type plasminogen activator (rscu-PA)
- recombinant staphylokinase.

More recent drugs (e.g. rt-PA) are more specific for fibrin than earlier agents (e.g. streptokinase) but all agents are contraindicated in patients with a significant bleeding tendency.

**Anticoagulation and thrombolytic therapy**

The anticoagulant drugs heparin and warfarin are used to prevent thrombosis and limit the extension of an established clot. Heparin is given intravenously or subcutaneously and acts by potentiating the activity of antithrombin III. Warfarin is given orally and acts by inhibiting vitamin K. Therapeutic treatment with both unfractionated heparin and warfarin requires careful laboratory monitoring. Thrombolytic agents are used to dissolve thrombus. They act by converting plasminogen to the proteolytic enzyme plasmin.

Fig. 3 Action of thrombolytic agents.
THE BLOOD GROUPS

The blood group antigens exist on the surface of the red cell membrane. There are numerous blood group systems encoded by genes on different chromosomes. They are highly variable in their polymorphism and clinical significance. The most important blood group is the ABO system. The genes encoding the ABO antigens are located on chromosome 9 and are inherited in an autosomal dominant fashion. Each antigen is a sugar residue made by a specific glycosyl transferase. The ABO system is crucial in clinical blood transfusion as there are naturally occurring IgM antibodies in the serum targeted against the non-present ABO antigens (Table 1). These antibodies necessitate the use of ABO 'compatible' blood for transfusion. For example, the administration of incompatible group A blood to a group B patient would engender a potentially fatal haemolytic transfusion reaction due to the destruction of the donor's group A red cells by the recipient's anti-A antibody.

In other blood group systems 'naturally occurring' antibodies are rare. However, 'immune antibodies', usually of IgG type, may be induced by transfusion of blood expressing different blood group antigens or maternal exposure to fetal red cell antigens. Where such immune antibodies are present, transfused blood must be matched for the relevant blood group system in addition to ABO. Maternal formation of immune antibodies against antigens of the Rhesus (Rh) blood group system, particularly the strongest antigen D, accounts for most cases of haemolytic disease of the newborn.

Table 1 The occurrence of ABO antigens and antibodies

<table>
<thead>
<tr>
<th>ABO Blood Group</th>
<th>Antigens on red cells</th>
<th>Antibody in serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>Anti-A+B, Anti-B, Anti-A, None</td>
</tr>
<tr>
<td>ABAB</td>
<td></td>
<td>Frequency (%)</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>42-8-3-</td>
</tr>
</tbody>
</table>

* In the United Kingdom Incidences vary greatly in different populations

THE TESTING OF BLOOD

DONOR BLOOD

The safety of blood transfusion is maximised by careful selection of donors. All donors should be in good health and, wherever possible, unpaid volunteers. Particular care is taken to exclude potential donors who may harbour infective diseases which are transmissible by blood transfusion - thus people with recent jaundice (? hepatitis), a history of recent travel to malarial areas or risk factors for HIV infection are not suitable donors. The objective of routine testing of donated blood is to provide blood which can be selected for likely compatibility with a patient and which contains no identifiable infectious agent (See Table 2).
testing before transfusion

Most incompatible transfusions are caused not by errors in the transfusion laboratory but by giving blood to the 'wrong' patient (i.e. not the patient whose serum was tested prior to the transfusion). The source of such mistakes is usually inaccurate documentation on forms and specimens or inadequate procedures for identifying patients prior to transfusion.

If tests on donor and recipient blood confirm matching for ABO and Rhesus groups, the transfusion will be compatible in around 98% of cases. The sequence of tests prior to transfusion thus also includes antibody screening of the patient's serum and crossmatching to ensure compatibility in the remaining 2%.

Most incompatible blood transfusion arise from clerical errors and mistaken patient identity.

blood grouping

The recipient's red cells are tested for ABO and Rhesus antigens and the serum tested for naturally occurring antibodies to confirm the ABO group. Blood grouping tests traditionally rely on the visual identification of agglutination of red cells induced by the presence of antibodies against antigens present on the cell surface (Fig. 1). Newer technologies include the use of gels (Fig. 2).

Table 2 Routine testing of donated blood

<table>
<thead>
<tr>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO group</td>
</tr>
<tr>
<td>Rhesus group (at least D)</td>
</tr>
<tr>
<td>Red cell antibody screen</td>
</tr>
<tr>
<td>Hepatitis B surface antigen</td>
</tr>
<tr>
<td>Antibody to hepatitis C</td>
</tr>
<tr>
<td>Antibody to HIV-1</td>
</tr>
<tr>
<td>Antibody to treponema pallidum (syphilis)</td>
</tr>
<tr>
<td>Antibody to HTLV-1 (in USA)</td>
</tr>
</tbody>
</table>

Fig. 1 ABO blood grouping on a microplate.

Fig. 2 Blood grouping using a gel system. (a) ABO and RhD grouping. (b) Rh and Kell grouping.

Antibody screening

The patient's serum is tested against three standard sets of screening red cells of known antigenic type. This is to detect immune or 'atypical' antibodies (i.e. non-ABO) which might destroy donor red cells. Clinically relevant antibodies are generally reactive at 37°C. If an antibody is found, then blood which is negative for the relevant antigen must be selected. A number of different techniques are used for antibody identification - agglutination may be enhanced by
enzyme treatment of red cells, use of low ionic strength saline (LISS) or the antiglobulin (Coombs') test (Fig. 3).

Crossmatching
The final compatibility check is to mix the patient's serum with red cells from each donor unit. The aim is to highlight any earlier errors in grouping or antibody screening and to identify the presence of antibodies against rare antigens not present on the screening cells. Usually a simple one tube technique with the addition of antihuman globulin reagent is used. The minor crossmatch - mixing of donor serum with patient red cells is not routinely performed.

PRACTICALITIES OF BLOOD ORDERING

Where blood transfusion is definitely required and adequate time is available, tests proceed as above and compatible units are issued. In emergencies, blood is sometimes needed more quickly than this routine testing allows. Normal procedures may be adapted to speed up issue of group specific blood. Only rarely is it necessary to give group 0 Rhesus negative blood. The bulk of blood is crossmatched for use in elective surgical procedures. Where there is only a small chance (less than 10%) that transfusion will be required it is reasonable to limit wastage by adopting a 'group and save' policy. The patient's blood group is determined and the serum screened for atypical antibodies. Provided the screen is negative, blood is not routinely crossmatched. Most hospitals have implemented a formal surgical blood order schedule with guidelines for common operations (Table 3). Such guidelines are generalisations and special provision is made for unusually difficult procedures or patients who are judged to be at a higher than average risk of haemorrhage.

Blood groups and blood testing
The blood group antigens exist on the surface of the red cell membrane. Blood groups are highly variable in their polymorphism and clinical significance.
The ABO system is crucial in blood transfusion as there are naturally occurring IgM antibodies in the serum targeted against non-present ABO antigens - this necessitates the use of ABO 'compatible' blood. Blood donors are carefully selected and donor blood tested to exclude transmissible infections.
Testing of donor and recipient blood for ABO and Rhesus groups, antibody screening of the recipients serum, and crossmatching are routinely performed before transfusion to ensure compatibility.
Most incompatible blood transfusions arise from clerical errors and mistaken patient identity.

Table 3 Possible guidelines for blood ordering in a few common operations. Protocols vary between hospitals and should be based on previous blood usage.

**Procedure Recommendation***

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholecystectomy</td>
<td>Group and save</td>
</tr>
<tr>
<td>Colectomy/hemicolectomy</td>
<td>2</td>
</tr>
<tr>
<td>Procedure</td>
<td>Units of Red Cells Crossmatched</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>Breast biopsy</td>
<td>Group and save</td>
</tr>
<tr>
<td>Heart valve replacement</td>
<td>8</td>
</tr>
<tr>
<td>Resection abdominal aortic aneurysm</td>
<td>8</td>
</tr>
<tr>
<td>Abdominal hysterectomy</td>
<td>Group and save</td>
</tr>
<tr>
<td>Total hip replacement</td>
<td>3</td>
</tr>
<tr>
<td>Transurethral resection of prostate</td>
<td>Group and save</td>
</tr>
</tbody>
</table>

Figures refer to the number of units of red cells crossmatched prior to surgery.
RED CELL TRANSFUSION

Two questions need to be answered before transfusion of red cells is undertaken:

1. Is it indicated?
2. If it is indicated, which red cell preparation should be used?

Some general indications for red cell transfusion are listed in Table 1. Whole blood is now rarely available for the treatment of acute blood loss. Haemorrhage requires transfusion of fluids, including plasma expanders, to maintain blood volume and red cell concentrates to raise haemoglobin. For correction of anaemia, red cell concentrate or concentrate in 'optimal additive solution' are used. It is important to appreciate that many patients with anaemia do not require transfusion, just prescription of the appropriate haematinics (e.g. iron deficiency).

Practicalities of red cell transfusion

All doctors and nurses involved in the prescription and administration of blood should follow local guidelines with respect to patient identification and the checking of the compatibility and viability of the transfused units. Critical information is contained on the blood bag and the attached compatibility label. No discrepancies are permissible. In shocked patients blood is transfused rapidly, the precise rate dependent on the monitoring of vital signs such as pulse, blood pressure and urine output. Transfusion for correction of anaemia is usually a more elective process. Units of red cell concentrate are typically given over 2-4 hours and a rise of around 10g/l of haemoglobin can be expected from each unit. Red cells are infused via specially designed sterile 'giving sets' which contain 170 µm filters. Cannulation for blood transfusion is discussed on page 99. Careful monitoring is particularly important during the first 10 minutes of each unit - subsequently the pulse and blood pressure are checked at least every 30 minutes.

Complications of red cell transfusion

Immediate

Haemolytic transfusion reactions. These potentially fatal reactions arise from the transfusion of incompatible blood (usually for ABO). Symptoms often occur within minutes and may include chest, abdominal and loin pain, vomiting, a 'burning' skin, dyspnoea and headache. Common signs are fever, tachycardia and hypotension. Renal failure and disseminated intravascular coagulation (DIC) can follow. Once a haemolytic reaction is suspected, the transfusion should be stopped and the venous access used to give crystalloid. The transfused unit should be checked (is another patient about to get a 'wrong' unit due to a mix up?) and the blood bank informed. Initial investigations must include blood samples from the patient for a blood count and film, blood group, antibody screen and direct antiglobulin test. The blood bank will also repeat tests on the donated unit. Management of complications will require senior advice and often intensive care. The overall mortality of ABO incompatible transfusion is approximately 10%. Some are fortunate and have trivial or even a complete lack of symptoms.
Table 1 Major indications for red cell transfusion

To replace blood loss
Trauma
Surgery
Other haemorrhage (e.g. gastrointestinal bleed)

To correct anaemia
Marrow failure (e.g. aplastic anaemia, leukaemia) Haemoglobinopathies (e.g. thalassaemia, sickle cell disease)
Chronic disorders (e.g. renal failure, malignancy) Severe haemolysis (e.g. haemolytic disease of the newborn)
The final decision to transfuse requires consideration of the patients age, clinical state, and haemoglobin concentration.

Non-haemolytic transfusion reactions.

The majority of adverse reactions to blood are 'febrile reactions' caused by antileucocyte antibodies in the patient. Uncomplicated febrile reactions are simply managed by slowing the transfusion and giving aspirin. If further transfusions are needed, leucocyte depleted blood can be obtained. Occasionally patients develop allergic reactions with urticaria, wheezing and (rarely) anaphylaxis.

Local thrombophlebitis may occur at the cannulation site.

Circulator overload. Care must be taken not to transfuse too rapidly, especially in elderly patients with heart disease.

Delayed Infection. Bacteria, viruses and parasites may all be transmitted via blood transfusion. As already discussed in the previous section, blood is screened for the relevant agents to minimise this risk. It is now known that the great majority of cases of transfusion associated hepatitis previously described as 'Non-A, Non-B' are attributable to the hepatitis C virus. The significance of transmission of infection from blood can depend on the status of the recipient. Thus, cytomegalovirus (CMV) is of little relevance in healthy adults but potentially life-threatening in a patient receiving a bone marrow transplant or in a low birth-weight premature infant.

Fig. 1 Unit of red cells.

Delayed transfusion reactions. These occur approximately 5-10 days after transfusion and are caused by a previously undetected antibody being boosted by transfusion of incompatible cells. Characteristic features include fever, jaundice and a failing haemoglobin. They are only rarely fatal.
**Iron overload.** A unit of blood contains around 250 mg of iron. Iron is only lost from the body in small amounts and repeated transfusion can lead to accumulation and toxic effects identical to those seen in haemachromatosis. Where repeated transfusion is predictable in a younger person (e.g. in thalassaemia), chelation of iron with parenteral desferrioxamine limits overload and prolongs life.

<table>
<thead>
<tr>
<th>Table 2 Major red cell preparations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation</td>
</tr>
<tr>
<td>Whole blood</td>
</tr>
<tr>
<td>Red cell concentrate (plasma reduced)</td>
</tr>
<tr>
<td>Red cell concentrate in optimal additive solution (SAG-M)</td>
</tr>
</tbody>
</table>

**Massive blood transfusion**

Massive transfusion is defined as replacement of the patient's whole blood volume by stored allogeneic blood in less than 24 hours. Patients receiving such large transfusions are usually already critically ill, but further problems can arise due to the inevitable deficiencies of stored blood. Shortage of clotting factors and platelets in transfused blood may exacerbate haemorrhage. It is important to monitor haemostasis by checking the basic coagulation screen (page 20) and replacing components accordingly. Metabolic disturbances are less common but include hyperkalaemia, hypocalcaemia, acidosis and citrate toxicity. Rapid transfusion can cause hypothermia; this can be minimised by carefully controlled blood warming.

**Autologous blood transfusion**

Use of the patient's own blood for transfusion rather than allogeneic blood minimises the risk of infection. The most common method is to arrange for patients to 'predeposit' up to four units of blood in the weeks prior to elective surgery. It has been calculated that up to 10% of all transfusions could be provided in this way. An alternative approach is the use of specially designeci equipment to salvage blood lost during surgery and reinfuse it back into the patient.

**TRANSFUSION OF PLATELETS AND GRANULOCYTES**

**Platelet transfusion**

This is used to treat or prevent haemorrhage in patients with significant thrombocytopenia. It is more useful where platelets are low due to underproduction (i.e. marrow failure) or dilution than where thrombocytopenia is due to immune destruction as in ITR Platelets are collected either from routine blood n donations or from a single donor by plasmapharesis. They should ideally be matched with the patient for ABO and Rhesus. The standard dose for an adult is either a single plasmapharesis donation or 4-6 pooled standard donations. Where repeated platelet transfusions are given, patients can become sensitised against class I HLA antigens (HLA-A, B and C) absorbed onto the platelet surface with the result that they derive a lower increment in platelet count than would be predicted ('platelet refractoriness'). In these cases, platelet donors
matched with the recipient's HLA class I type can be selected. Platelet transfusion can cause non-haemolytic reactions and can transmit infection as for red cells.

Table 3  **Possible indications for use of fresh frozen plasma**

<table>
<thead>
<tr>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disseminated intravascular coagulation (DIC)</td>
</tr>
<tr>
<td>Severe liver disease (e.g. prior to liver biopsy)</td>
</tr>
<tr>
<td>Coagulopathy of massive blood transfusion</td>
</tr>
<tr>
<td>Reversal of oral anticoagulation where significant bleeding</td>
</tr>
<tr>
<td>Replacement therapy of some rare congenital factor deficiencies</td>
</tr>
<tr>
<td>Bleeding in haemorrhagic disease of newborn/malabsorption vitamin K</td>
</tr>
<tr>
<td>Thrombotic thrombocytopenic purpura (with plasma exchange)</td>
</tr>
<tr>
<td>Depletion of coagulation factors following thrombolysis</td>
</tr>
</tbody>
</table>

Prothrombin complex and factor VII concentrate probably better where available.

**Granulocyte (neutrophil) transfusion**

Neutrophils for transfusion are collected by plasmapharesis of healthy donors who preferably should be cytomegalovirus (CMV) seronegative. Granulocytes transfusion is infrequently used and the indications uncertain, particularly now that the neutrophil count may be increased with growth factors such as G-CSF. However, granulocytes may be helpful in a patient with severe neutropenia and a focus of infection not responsive to treatment with antibiotics and growth factors.

**TRANSFUSION OF PLASMA AND PLASMA PRODUCTS**

A wide range of plasma products is available for therapeutic use:

**Fresh frozen plasma (FFP).** Plasma is collected from whole blood or derived from plasmapharesis prior to rapid freezing. FFP contains the full range of coagulation factors and indications for use are shown in Table 3. The normal dose in an adult is one hire. FFP can transmit infection and cause immunological reactions - it is not suitable for volume expansion alone.

**Cryoprecipitate.** This is prepared from FFP by slow thawing and separation of the resultant precipitate. It is rich in fibrinogen and may be useful in the treatment of DIC and management of massive blood transfusion. In haemophilia and von Willebrand's disease it has been superseded by factor concentrates.

**Factor VIII and IX concentrate.** Use of these is discussed.

**Albumin.** This is produced by fractionation of pooled plasma. Solutions for clinical use include human albumin 4.5/5%, human albumin 20% and plasma protein fraction (PPF). Major clinical indications are in resuscitation (often combined with crystalloid), replacement of plasma protein in severe burns and symptoms arising from hypoproteinaemia.

**Immunoglobulins.** These can be specific' and used in passive prophylaxis against a range of infections (e.g. varicella-zoster, tetanus) or to prevent haemolytic disease of the newborn (anti-Rhesus D). 'Non-specific' immunoglobulins are used for passive prophylaxis against hepatitis A, treatment of congenital or acquired hypogaminaglobulinaemia and in selected autoimmune disorders (e.g. ITP).
Blood transfusion - clinical practice

Before red cell transfusion is undertaken the indication should be confirmed and the optimal red cell preparation selected. Red cell transfusion can cause both immediate complications (e.g. haemolytic transfusion reaction) and delayed complications (e.g. infection, iron overload). Platelet transfusion may be helpful in the management of thrombocytopenia. Granulocyte transfusion is occasionally indicated in neutropenia. A wide range of plasma products is available for transfusion. Selection of the appropriate product requires an understanding of the therapeutic benefit and possible side effects.
THE IMMUNOSUPPRESSED PATIENT

Many patients with blood disorders are immunosuppressed. Patients with aggressive haematological malignancies such as leukaemia and high grade non-Hodgkin's lymphoma have their immune function initially compromised by the disease and then further depressed by chemotherapy. Others have more subtle deficiencies; patients with low-grade non-Hodgkin's lymphoma and with 'benign' diseases such as idiopathic thrombocytopenic purpura (ITP) and hereditary spherocytosis who have had splenectomy performed are also at increased risk of infection.

Fig. 1 Aspergillosis complicating prolonged neutropenia

An increased susceptibility to infection can arise from multiple factors (Table 1). Neutropenia and neutrophil dysfunction are probably the most important causes of infectious complications in patients with leukaemia. Unlike many other forms of immunosuppression, neutropenia is easy to quantify - the risk of infection rises appreciably at counts below 0.5 x 10^9/l and is greatest where the count is below 0.1. Lymphopenia and lymphocyte dysfunction are seen in lymphoid malignancy and after chemo- and radiotherapy. Defects in humoral immunity are particularly seen in patients with chronic lymphoid malignancies and in myeloma. The likelihood of infection is related to the severity of hypogammaglobulinaemia. Other common immunosuppressive factors are the loss of mucosal or skin integrity due to damage from disease or treatment, and the presence of indwelling venous catheters.

TYPES OF INFECTION

Bacteria
Bacterial infections in neutropenic patients are usually caused by the spread of commensal flora to previously sterile sites. Fatal septicaemia can result from bowel-associated gram-negative bacilli such as Pseudomonas aeruginosa, E. Coli, Klebsiella spp. and Proteus spp. Gram-positive cocci are becoming increasingly common causes of nosocomially acquired
infections. The skin pathogen *Staphylococcus epidermidis* often colonises indwelling venous catheters. The use of broad-spectrum antibiotics can lead to the emergence of toxin-producing *Clostridium difficile* in the stools. Bacterial infection in neutropenic patients may be overt - for instance a chest infection with a productive cough or the presence of infected skin lesions (Fig. 1). However, bacterial sepsis can equally present with non-specific malaise and a pyrexia. In the latter case extensive cultures including blood, nose, throat, stool and urine are indicated.

**Fig. 2** Herpes zoster following an allogeneic bone marrow transplantation

**Table 1** Possible factors predisposing to infection in haematology patients

**Cellular defects**
- Neutropenia and neutrophil dysfunction
- Lymphopenia and lymphocyte dysfunction

**Humoral defects**
- Reduced antibody production

**Anatomic defects**
- Reduced mucosal barriers (e.g., mucositis)
- Indwelling venous catheters
- Splenectomy

**Fungi**
The incidence of invasive fungal infections is increasing and they are a major cause of morbidity and mortality in patients with haematological malignancy. The most widespread fungal pathogen is *Candida*. Oral and colonic carriage of the organism is common in healthy people. Oropharyngeal candidiasis frequently complicates neutropenia and becomes invasive in 25% of cases. Disseminated candidiasis usually presents with a persistent fever and no diagnostic clinical features. Possible organ involvement includes the kidney, lung, heart and liver. Cutaneous emboli may lead to a nodular skin eruption whilst exudative retinal lesions can be seen through the ophthalmoscope. Unfortunately, *Candida* spp. are grown from blood cultures in only 20% of patients with definite candidiasis. A number of tests are available to detect circulating antigen but all have limited sensitivity.

The second most common fungal species in immunosuppressed patients is *Aspergillus*. Infection is usually via the inhalation of airborne spores and is mainly pulmonary. A chest X-ray may show pneumonia and cavitation (Fig. 2). Other infected sites can include the
paranasal sinuses, skin, central nervous system and eye. Even in disseminated disease, blood and sputum cultures are rarely positive. Open lung biopsy gives the best diagnostic yield from cultures with bronchoscopy (biopsy or lavage) a better tolerated alternative in ill patients. *Aspergillus* is present in up to 20% of neutropenic patients at autopsy.

**Viruses**

Most viral infection in immunosuppressed patients is caused by reactivation of latent organisms. Patients with deficient cell mediated immunity (e.g. acute lymphoblastic leukaemia (ALL), bone marrow transplantation, chronic lymphatic leukaemia) are particularly susceptible. Important pathogens include herpes simplex, varicella-zoster and cytomegalovirus (CMV). Clinical manifestations range from relatively trivial mouth ulcers attributable to herpes simplex through herpes zoster (shingles) (Fig. 3) with the risk of dissemination to the potentially fatal CMV pneumonitis which complicates allogeneic bone marrow transplantation. Measles can be a fatal illness in children with ALL. There may be no specific diagnostic features of viral infection and it must be considered as a possible cause of a febrile illness in the immunosuppressed patient. Newer methods for detecting viruses include molecular probes for viral DNA and monoclonal antibodies specific for viral antigens. These technologies are already proving useful in diagnosing CMV infection after marrow transplantation.

**Pneumocystis carinii**

*Pneumocystis* causes a potentially fatal bilateral pneumonia in patients with depressed cell mediated immunity. In haematological practice it mostly affects patients receiving intensive chemotherapy regimens or bone marrow transplantation, and haemophiliacs infected with the HIV virus.

**PREVENTION OF INFECTION IN THE IMMUNOSUPPRESSED PATIENT**

**Neutropenia**

General measures include the isolation of the patient, laminar airflow rooms, strict hygiene and avoidance of possible contaminants (e.g. uncooked food). Simple measures such as hand washing by staff are crucial in reducing infection rates.

As many bacterial infections are attributable to endogenous bowel associated organisms, antibiotics have been used to 'decontaminate' the gut during periods of neutropenia. A more recent approach is that of selective decontamination of the aerobic bowel flora. The combination of cotrimoxazole and colistin reduces the incidence of infections. An increasingly popular alternative is the quinolone antibiotic ciprofloxacin. Antifungal prophylaxis is available in the form of ketoconazole, itraconazole and fluconazole. Ketoconazole can cause serious liver damage and the choice is between the other agents. Fluconazole has superior bioavailability and offers effective protection against *Candida* but is less effective against a *Aspergillus* than itraconazole. Viral infections are frequently seen in the context of neutropenia and the antiviral drug acyclovir is accordingly often added to prophylactic regimens.

**Depressed cell mediated immunity and hypogammaglobulinaemia**
Impaired cell mediated immunity leads to an increased risk of *Pneumocystis carinii* pneumonia and viral infections. Standard prophylaxis against *Pneumocystis* is oral cotrimoxazole three times weekly. Nebulised pentamidine may be substituted where cotrimoxazole is not tolerated. Acyclovir is effective in reducing the incidence of viral infections. The more toxic drug gancyclovir can be used after marrow transplantation to give additional protection against CMV.

Patients with low-grade lymphoproliferative disorders and myeloma can have significant hypogammaglobulinaemia and suffer recurrent infection. Regular infusions of immunoglobulin are often helpful in these cases.

**Post-splenectomy**

**TREATMENT OF INFECTION**

**The pyrexial neutropenic patient**

A common clinical problem in haematology is the management of the patient with neutropenia (less than $1 \times 10^9$ neutrophils) who develops a pyrexia (a single temperature above 39°C or two successive measurements above 38°C). Such patients can rapidly succumb to bacterial infection and need prompt **empirical** treatment with broad spectrum intravenous antibiotics even before the infectious pathogen is identified. Blood and other cultures are taken prior to starting antibiotics and a chest X-ray is helpful; investigations, however, should not substantially delay treatment. A microbiological diagnosis is made in only half of these cases.

The empirical antibiotic regimens are designed to provide protection against commonly implicated organisms, particularly those causing life-threatening infection (e.g. *Pseudomonas*). Regimens are constantly changing - the major groups of drugs are summarised in Table 2. Most clinicians rely on a combination of antibiotics. Monotherapy with ceftazidime or ciprofloxacin may be adequate to prevent death from gram-negative infection, but the high incidence of grampositive infections make the addition of a glycopeptide (e.g. teicoplanin) advisable.

Persistent pyrexia or clinical deterioration on first line antibiotics is a difficult management problem. Often the infectious agent is unknown. The usual approach is to continue investigations whilst making a change in the antibiotic regimen. A lack of response necessitates the introduction of amphotericin B as empirical antifungal treatment. Growth factors (e.g. G-CSF) may be given to shorten the period of neutropenia and granulocyte transfusions are worth consideration in well documented bacterial infections unresponsive to antibiotics.

**Treatment of specific infections** Intravenous amphotericin B is indicated for proven systemic aspergillosis as well as in the empirical role outlined above. Oropharyngeal candidiasis can often be treated with local agents (e.g. nystatin) with the addition of oral fluconazole in resistant cases. Amphotericin B is effective against systemic candidiasis with intravenous fluconazole a possible alternative. Herpes simplex and varicella zoster infections are best treated with acyclovir. Gancyclovir is used for CMV infection after marrow transplantation. *Pneumocystis carinii* pneumonia is equally effectively treated by either high dose cotrimoxazole or pentamidine.

**Table 2 Groups of antibiotics used in the empirical treatment of infection in neutropenia**

<table>
<thead>
<tr>
<th>Group</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antipseudomonal panic</td>
<td>Azlocillin, piperacillin</td>
</tr>
</tbody>
</table>
Aminoglycosides  Gentamycin, amikacin
Cephalosporins  Ceftazidime
Quinolones  Ciprofloxacin
Carbapenems  Imipenem
Glycopeptides  Teicoplanin, vancomycin

Note: some agents have been used alone (e.g. ceftazidime) but combinations are more commonly used (e.g. penicillin + aminoglycoside, cephalosporin + glycopeptide).

The immunosuppressed patient
Many patients with blood disorders are immunosuppressed. Possible factors predisposing to infection include neutropenia, lymphopenia, reduced antibody levels and anatomical defects. Bacteria, fungi and viruses can all cause severe systemic infection in an immunosuppressed patient. Measures to prevent infection in the immunosuppressed patient include isolation of the patient, strict hygiene and prophylactic use of antimicrobial agents. Infection in a neutropenic patient generally requires empirical treatment with broad-spectrum antibiotics. Persisting fever or clinical deterioration necessitates a change in antibiotics and/or empirical antifungal treatment.
HAEMATOLOGICAL CHANGES

Several haematological changes occur in normal pregnancy (Fig. 1). Beginning in the sixth week there is an increase in plasma volume accompanied by an increase in red cell mass. The plasma volume expansion peaks at around 24 weeks when it is approximately 40% greater than in a non-pregnant woman. As the increase in red cell mass is more is modest (15-25%) a dilutional anaemia is inevitable. In practice the haematocrit and haemoglobin level start to fall at 6-8 weeks and reach a trough at around 20 weeks. It is unusual for the haemoglobin level to fall below 100g/l and if this happens another cause for anaemia should be sought. Negative iron balance can be regarded as routine in pregnancy and as discussed below frank iron deficiency commonly occurs.

The other major changes which may be regarded as a physiological consequence of pregnancy affect the coagulation system. There are increases in the levels of the coagulation factors VII, VIII and X and a marked increase in plasma fibrinogen. The resulting hypercoagulability is helpful in limiting the likelihood of life-threatening bleeding at delivery but it does lead to an increased risk of thromboembolism. Surprisingly, there is conflicting evidence regarding platelet count, size and function in pregnancy. There may be a small fall in platelet number and an increase in mean platelet volume (MPV) in the last few weeks.

ANAEMIA IN PREGNANCY

There are several causes of anaemia in pregnancy. The most common scenario is an exacerbation of the usual dilutional anaemia by deficiency of iron and/or folate.

The identification of iron deficiency relies upon normal laboratory tests. However, even in women with no overt clinical deficiency there is a progressive fall in serum iron and increase in total iron binding capacity (TIBC) through pregnancy. Routine dietary supplementation with modest amounts of iron (e.g. ferrous sulphate 200 mg daily) leads to a significant increase in haemoglobin level at term compared with women receiving no supplements.

The other major type of anaemia in pregnancy is megaloblastic anaemia. This usually results from deficiency of folate. As for iron, folate requirements are increased during pregnancy and the diet is frequently inadequate to meet this demand. Megaloblastic anaemia most often presents as a macrocytic anaemia in the third trimester or postpartum. It is normal practice to give folate supplements in pregnancy. The amount of folate administered orally should be large enough to routinely avoid megaloblastic anaemia but not so large as to risk masking pernicious anaemia with vitamin B12 deficiency which does occasionally occur in pregnancy. The usual dose is 200-500 mcg daily. Folate deficiency in pregnancy has been linked with an increased incidence of neural tube defects in the fetus and recent recommendations for planned pregnancies are the use of folate supplements (400 mcg daily) prior to conception and then particularly in the first twelve weeks. Higher doses of folate are recommended to prevent recurrence of neural tube defects. There is no justification for the prescription of multi-ingredient vitamin preparations in pregnancy but a combined iron and folate tablet of adequate dosage may be prescribed.
It should be remembered that not all anaemia in pregnancy is caused by deficiency states. Other blood disorders may present in pregnancy and chronic blood diseases such as sickle cell anaemia can be especially difficult to manage at this time.

Fig. 1 Common haematological changes in normal pregnancy.

THROMBOCYTOPENIA IN PREGNANCY

With the introduction of automated cell counters which routinely provide a platelet count, the incidence of thrombocytopenia in pregnancy has increased and it is now a common clinical problem.

Some women have an obvious systemic disorder such as pre-eclampsia; disseminated intravascular coagulation (DIC) in pregnancy is further discussed below. However, the majority of women are systemically well with an apparently normal pregnancy. In these cases thrombocytopenia can be divided into two categories, with differing clinical implications for the mother and fetus.

Incidental thrombocytopenia

Incidental thrombocytopenia of pregnancy accounts for about three-quarters of all cases. Thrombocytopenia is mild to moderate (70-150 x 10^9/l) and the woman is otherwise well. There is no past history suggesting a cause for the low platelet count and particularly no history of idiopathic thrombocytopenic purpura (ITP). The overwhelming majority of these mothers can have normal antenatal observations and a normal delivery. The incidence of thrombocytopenia in their babies is not greater than in the general population. Diagnosis of incidental thrombocytopenia is made by exclusion as there is no specific diagnostic test.

ITP in pregnancy

The management of pregnancy in a woman with known chronic ITP is problematic as severe thrombocytopenia may be a threat to the mother and there is also a risk of the child becoming thrombocytopenic. The latter complication arises as the causative IgG anti-platelet autoantibody in the mother freely crosses the placenta and can target fetal platelets. Fortunately, the majority of babies escape - thrombocytopenia is seen in approximately 5-10% of neonates and is severe (less than 20 x 10^9/l) in only half of these cases. All management decisions must acknowledge that fetal thrombocytopenia is uncommon and fetal morbidity rare. In this context, aggressive treatment of all mothers with ITP with corticosteroids and/or intravenous immunoglobulin and routine delivery by caesarian section are probably not justified. Attempts have been made to try and predict the likelihood of thrombocytopenia in the neonate by measuring fetal scalp blood platelet counts and platelet antibodies but success has been limited. A conservative approach with normal delivery and an immediate neonatal platelet count is gaining support. If the baby's count is low or failing, intravenous immunoglobulin can be given. Because of its low incidence of side effects, intravenous immunoglobulin is probably the treatment of choice for severe maternal thrombocytopenia.

COAGULATION ABNORMALITIES IN PREGNANCY
Thromboembolism and anticoagulant therapy
Pulmonary embolism (PE) remains a major cause of maternal death. Approximately half of fatal PEs occur antepartum and half postpartum, the majority of the latter in the first two weeks of the puerperium. The precise incidence of deep vein thrombosis (DVT) and non-fatal PE in pregnancy is not known as there is a lack of reliable data. Once a DVT has occurred the risk of recurrence is around 15%. Other factors increasing the risk of thrombosis in pregnancy include caesarian section, obesity, incidental surgical procedures, a history of thrombotic problems or familial thrombophilia, and systemic disorders where there is significant blood loss or dehydration.
Both the anticoagulants commonly used in clinical practice, heparin and warfarin, require special consideration in pregnancy.

Heparin. Neither unfractionated standard heparin or low molecular weight heparin cross the placenta and there is no evidence of a risk of fetal haemorrhage or any teratogenic effect. However, prolonged heparin therapy may lead to maternal osteopenia and the drug can also be associated with thrombocytopenia and allergic reactions.

Warfarin. Warfarin is not significantly secreted in breast milk and treatment is safe during lactation. However, it readily crosses the placenta and is a known teratogen producing a specific warfarin embryopathy at around 6-9 weeks (approximately 5% incidence). Thus, heparin should be substituted for warfarin in the first trimester. There may be a risk of fetal haemorrhage secondary to warfarin throughout pregnancy, particularly if anticoagulant control is poor, and the risk to mother and fetus becomes unacceptable in the antepartum period. It should therefore be discontinued at 36 weeks and heparin substituted until after delivery wherever possible.

DIC in pregnancy
DIC is associated with a wide variety of situations in pregnancy (Fig. 2). The chief characteristics and pathogenesis of DIC are discussed. In pregnancy, DIC may manifest as a chronic compensated state or as life-threatening haemorrhage. The latter is a frightening medical emergency and there should be a planned regime of management with input from an obstetrician, haematologist, physician, anaesthetist and nurse (Table 1). It is imperative that the source of bleeding is identified and addressed as soon as possible. It is often shock which triggers DIC with a resultant increase in bleeding.

HELLP syndrome
HELLP is an acronym for microangiopathic haemolysis (H), elevated liver enzymes (EL) and low platelets (LP). The syndrome complicates severe preeclampsia, and there are the laboratory abnormalities of DIC. The mainstay of treatment is delivery of the fetus.

Table 1 General guidelines for the management of acute obstetric haemorrhage

Secure venous access and consider insertion of central line to measure central venous pressure (CVP)
Seek additional (preferably senior) medical help
Collect samples for urgent blood count, crossmatching and coagulation screen; liaise with haematology laboratory
Restore blood volume - may have to use unmatched blood of patient's ABO and Rh group
(preferred to group 0 Rh negative)
Address source of bleeding
Blood product replacement as necessary

PREGNANCY

Normal pregnancy is accompanied by a modest dilutional anaemia.
Deficiency of iron and/or folate frequently exacerbates the normal dilutional anaemia.
Thrombocytopenia is most often 'incidental' and of little significance. Idiopathic
thrombocytopenic purpura (ITP) may require treatment but a normal delivery is usual and severe
neonatal thrombocytopenia is rare.
There is a hypercoagulable state in pregnancy and pulmonary embolism remains a major cause
of maternal death.
Disseminated intravascular coagulation (DIC) can complicate pregnancy and cause
life-threatening haemorrhage.
PEDIATRIC HAEMATOLOGY

Many of the blood disorders encountered in children have been discussed in the preceding pages. For instance, acute lymphoblastic leukaemia is the most common leukaemia of childhood, haemophilia is usually diagnosed in infancy and the haemoglobinopathies are a significant cause of ill health in children worldwide. Chronic and severe diseases of the blood pose particular problems in childhood and usually are best managed by a paediatrician with a special interest in haematology or in a combined paediatric/haematology clinic. The child's growth and development, and educational needs often require special attention. In this section we discuss some haematological disorders encountered in paediatric practice which are not addressed elsewhere.

NORMAL VALUES

It is important to appreciate that the normal ranges for many haematological tests vary with age. Table I illustrates reference values for the total white cell count (WCC) and the differential count in children. More detailed listings of normal ranges of laboratory tests in childhood can be found in specialized paediatric haematology texts.

Table 1 Normal white cell counts in children (x10^9/l)

<table>
<thead>
<tr>
<th>Age</th>
<th>White Cell Count</th>
<th>Neutrophils</th>
<th>Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth (full term)</td>
<td>18 ± 8</td>
<td>5-13</td>
<td>3-10</td>
</tr>
<tr>
<td>Day 3</td>
<td>15 ± 8</td>
<td>3-5</td>
<td>2-8</td>
</tr>
<tr>
<td>1 month</td>
<td>12 ± 7</td>
<td>3-9</td>
<td>3-16</td>
</tr>
<tr>
<td>2-6 months</td>
<td>12 ± 6</td>
<td>1.5-9</td>
<td>4-10</td>
</tr>
<tr>
<td>2-6 years</td>
<td>10 ± 5</td>
<td>1.5-8</td>
<td>6-9</td>
</tr>
<tr>
<td>6-12 years</td>
<td>9 ± 4</td>
<td>2-8</td>
<td>1-5</td>
</tr>
</tbody>
</table>

NEONATAL DISORDERS

Haemolytic disease of the newborn

Haemolytic disease of the newborn (HDN) is a disease of the fetus and newborn child. The haemolysis is caused by maternal IgG antibodies traversing the placenta and attaching to fetal red cells which are destroyed in the child's reticuloendothelial system. The antibodies are directed against a fetal red cell antigen not shared by the mother. Incompatability for one of a large number of different red cell blood group systems can cause HDN but most cases of clinically significant disease affect a Rhesus (Rh)D-positive child where the mother is RhD negative. Sensitisation of the mother (i.e. the formation of anti-D) occurs following the
haemorrhage of fetal red cells into the maternal circulation. This usually occurs at parturition following a normal pregnancy but may also arise earlier in pregnancy or following abortion. ABO incompatibility between mother and fetus gives some protection against sensitisation to RhD as fetal red cells are quickly destroyed by the mother's naturally occurring anti-A or anti-B antibodies. Unfortunately, in most cases baby and mother are ABO compatible. With the considerable success of prophylaxis against HDN due to RhD incompatibility (see below) the most common cause of the disorder is the formation of immune antibodies against ABO; most cases are associated with only mild haemolysis.

**Diagnosis**

Severe HDN can result in intrauterine death. In the newborn child the presentation is entirely dependent on the degree of haemolysis but common features include anaemia, jaundice, oedema and hepatosplenomegaly. High levels of circulating unconjugated bilirubin may lead to high frequency deafness or deposition in the basal ganglia with spasticity and other neurological symptoms and signs ('kernicterus'). Further investigation of the anaemia reveals features typical of haemolysis (Fig. 1) with a positive direct antiglobulin test (DAT). In HDN due to RhD incompatibility the baby is RhD positive and the mother RhD negative with a high level of anti-D.

**Management**

Management of HDN is complex, requiring close liaison between the haematology laboratory and obstetrician. The severity of disease in the fetus may initially be gauged by serial measurements of maternal anti-D; where there is a possibility of severe disease amniocentesis with spectroscopic measurement of bile pigment in amniotic fluid allows assessment of the degree of haemolysis. Intrauterine transfusion or premature delivery are indicated for life-threatening disease. At birth exchange transfusion is considered for neonates with significant anaemia and/or high bilirubin levels. Phototherapy may be used in the management of kernicterus.

Fig. 1 *Peripheral blood film in a newborn child with severe HDN.* Note the numerous nucleated red cells and polychromasia.

**RhD prophylaxis in RhD-negative mothers**

The breakthrough in the prevention of HDN has been the introduction of Rh prophylaxis (Fig. 2). A dose of Rh anti-D immunoglobulin (Ig) is given to all Rh-negative mothers who deliver an
Rh-positive infant. A larger than average feto-maternal haemorrhage necessitates a greater dose of anti-D Ig. It is possible that anti-D administration prevents HDN by simply removing the fetal D-positive cells, or another immunological mechanism may be operating. General recommendations for Rh prophylaxis are shown in Table 2. As some women undoubtedly become sensitised earlier in a normal pregnancy routine antenatal prophylaxis has been recommended; however, this is not currently universally performed.

Table 2 Recommendations for Rh prophylaxis

Rh prophylaxis after delivery
Anti-D (usually 500 iu) is given within 72 hours in Rh-negative mothers where the infant is Rh-D positive (or group undetermined). If there is a large fetomaternal haemorrhage (assessed in a Kleihauer test) additional anti-D is given

Rh prophylaxis and abortions
In Rh-D negative mothers anti-D is given after all therapeutic abortions and after spontaneous threatened abortions later than 12-13 weeks gestation (usual dose 250 iu before 20 weeks and 500 iu after 20 weeks)

Rh prophylaxis during pregnancy
Anti-D is given after possible sensitising events in Rh-negative women. These include: amniocentesis chorionic villous sampling, abdominal trauma, external cephalic version, antepaum haemorrhage, ectopic pregnancy (usual dose of anti-D is 250 iu before 20 weeks and 500 iu after 20 weeks)

Anaemia of prematurity
The haemoglobin concentration falls after birth in all babies but in premature infants it falls faster and to a lower level. At 1-3 months of age haemoglobin concentrations of less than 70 g/l are common and in babies born at less than 32 weeks gestation this anaemia is often associated with inadequate adaptive responses including tachycardia, tachypnoea and apnoeic attacks. The anaemia is due in part to shortened red cell life-span and the effects of rapid growth but the fundamental problem appears to be a poor erythropoietin response. Erythropoietin levels are highest in premature infants with the most severe anaemia and hypoxia but even in these cases levels are inadequate compared to those achieved in anaemic adults. Recombinant erythropoietin is of benefit in some infants.

Polycythaemia in the neonate
Polycythaemia in the neonate is most simply defined as a packed cell volume (PCV) exceeding 0.7. Causes include placental transfusion (e.g. delayed clamping of the cord), intrauterine hypoxia, endocrine disorders (e.g. maternal diabetes) and genetic disorders (e.g. Down's syndrome). Significant polycythaemia may cause hyper viscosity with congestive heart failure, respiratory distress, neurological disturbances and even gangrene. Venesection with plasma
replacement is indicated where a high PCV is associated with symptoms and signs of hyperviscosity.

**Thrombocytopenia in the neonate**
Some causes of thrombocytopenia in neonates are listed in Table 3. In practice the major divide is between seriously ill infants where the low platelet count is caused by disseminated intravascular coagulation (DIC), and relatively well infants where thrombocytopenia is most often of immune actiology or occurs secondary to a specific inherited syndrome. Idiopathic thrombocytopenic purpura (ITP) may be seen in infants born to mothers with ITP where there is passive transfer of IgG across the placenta. Alloimmune thrombocytopenia arises where the healthy mother becomes sensitised against a fetal platelet antigen in a manner analogous to HDN; the platelet antigen PI" is most commonly implicated.

**IRON DEFICIENCY IN INFANCY**
Iron deficiency has already been discussed but some aetiological factors in infancy are unique to this period of life. Blood loss may still be the major cause of deficiency but other factors worthy of consideration are decreased total body iron at birth (e.g. prematurity, feto-maternal haemorrhage, twins), the impact of growth with increased demands for iron, and dietary inadequacy (e.g. excessive dependence on unsupplemented cow's milk).

**RED CELL APLASIA IN CHILDHOOD AND ADOLESCENCE**
Pure red cell aplasia (PRCA) is characterised by anaemia, reticulocytopenia and reduced or absent erythroid precursor cells in the bone marrow. There are many causes of PCRA including infection (e.g. parvovirus B19), connective tissue disorders and malignancies (e.g. thymoma). However, two types of PCRA are unique to childhood: Diamond-Blackfan anaemia and transient erythroblastopenia.

**Diamond-Blackfan anaemia**
This is a heterogeneous disorder. The majority of cases are sporadic but various patterns of inheritance have been documented. An anaemia with the features of red cell aplasia usually presents within the first twelve months of life. This runs a chronic course and can be combined with other anomalies and an increased risk of leukaemia and myelodysplasia. Beyond blood transfusion, therapeutic options include conicosteroids, androgens, immunosuppression, growth factors (erythropoietin, interleukin-3) and allogeneic bone marrow transplantation in severe cases.

**Transient erythroblastopenia of childhood**
This is a transient form of red cell aplasia of probable immune origin which must be distinguished from Diamond-Blackfan anaemia. It generally affects older children (1-4 years) and may be diagnosed simultaneously in siblings or in seasonal clusters. In over half of cases there is a previous viral illness. The anaemia may be complicated by worrying neurological symptoms. Full recovery within 4-8 weeks is the rule.

**CONGENITAL DYSERYTHROPOIETIC ANAEMIAS (CDAs)**
This is a group of rare inherited anaemias. There are various subtypes but common features include ineffective erythropoiesis and multi-nucleated erythroblasts. The white cell and platelet counts are normal. Anaemia is usually first diagnosed in infancy or childhood. It may be of normocytic or macrocytic type. Transfusion is required in more severe cases.

Table 3 **Some causes of thrombocytopenia in the neonate**
DIC in various severe systemic disorders
Intrauterine infection (e.g. rubella, cytomegalovirus)
Platelet antibodies:
- autoimmune (maternal ITP)
- alloimmune
- drugs
Hereditary / congenital disorders:
- Wiskott-Aldrich syndrome
- Thrombocytopenia with absent radii (TAR) syndrome
Post exchange transfusion
Neonatal leukaemia
Giant haemangloma

**Paediatric haematology**

Chronic and severe blood disorders in children are usually best managed by a paediatrician with a special interest in haematology or in a combined paediatric/haematology clinic.
In haemolytic disease of the newborn (HDN), haemolysis is caused by maternal IgG antibodies crossing the placenta and attaching to fetal red cells. Most clinically significant cases affect a RhD positive fetus or newborn child where the mother is RhD negative.
RhD prophylaxis has much reduced the incidence of severe HDN.
Prematurity is associated with a particular type of anaemia.
In pure red cell aplasia in children it is important to distinguish between Diamond-Blackfan anaemia and the more benign transient erythroblastic anaemia of childhood.
SYSTEMIC DISEASE

Clinical haematologists spend a considerable part of their time investigating blood abnormalities in patients with diseases of other organ systems. Some of the more common diagnostic challenges are discussed here.

**RENAL DISEASE**

Diseases of the kidney are associated with a remarkably wide range of possible haematological abnormalities (Table 1).

Anaemia is almost inevitable in chronic renal failure. The pathogenesis is complex but impaired erythropoietin production is the principal cause. Other possible contributory factors include the release of inhibitors of erythropoiesis, mild haemolysis and iron deficiency. The anaemia of renal failure is typically normocytic and normochromic. A characteristic finding in the blood film is the presence of *burr cells* (Fig. 1). The best treatment of anaemia is resolution of the underlying renal problem (e.g. by transplantation), but where this is not feasible, recombinant erythropoietin is the treatment of choice. Intermittent bolus administration generally leads to a marked improvement in anaemia and transfusion independence.

A failure of the anaemia to respond to erythropoietin should prompt a search for other aetiologies such as iron deficiency.

Paradoxically, some forms of renal disease can lead to increased red cell production and clinical polycythaemia (Table 1). This arises either from inappropriate secretion of erythropoietin by a kidney tumour or from local renal hypoxia promoting erythropoietin release from normal cells. Polycythaemia can be the presenting feature of renal carcinoma and rapid identification of the malignancy may allow curative surgical treatment. Benign diseases such as polycystic disease and hydronephrosis probably cause polycythaemia by inducing renal ischaemia. The polycythaemia of renal disease is not an appropriate physiological response and patients with high-packed cell volumes (e.g. greater than 0.5) can derive benefit from regular venesection.

Chronic renal failure is also associated with a large number of possible platelet and coagulation abnormalities. The increased risk of bleeding in these patients is generally caused by the complex interaction of abnormalities shown in Table 1. Anaemia tends to worsen bleeding by interfering with the normal interaction between platelets and vascular endothelium.

**LIVER DISEASE**

Advanced liver disease is often associated with abnormal haemostasis.

**Table 1  Haematological changes in renal disease**

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Clinical association</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Red cells</strong></td>
<td></td>
</tr>
<tr>
<td>Anaemia</td>
<td>Chronic renal failure</td>
</tr>
<tr>
<td>Polycythaemia</td>
<td>Renal carcinoma, cystic disease, hydronephrosis, parenchymal disease, Bartter's syndrome, renal transplantation</td>
</tr>
<tr>
<td>Burr cells</td>
<td>Renal failure</td>
</tr>
<tr>
<td><strong>Haemostasis</strong></td>
<td></td>
</tr>
<tr>
<td>Abnormal platelet function</td>
<td></td>
</tr>
</tbody>
</table>
MALIGNANCY

Anaemia is seen in around half of patients with non-haematological malignant tumours. The anaemia of chronic disease is the most common aetiology but other causes include chemotherapy, blood loss, haemolysis and marrow infiltration. Invasion of the bone marrow by solid tumours can result in a pancytopenia and a characteristic leucoerythroblastic blood picture with circulating nucleated red cells and myelocytes (Fig. 2a). Clumps of malignant cells may be seen in a bone marrow aspirate but a bone marrow trephine is a more reliable way of demonstrating solid malignancy (Fig. 2b).

Malignancy can be associated both with a hypercoagulable state and a bleeding tendency. The presence of hypercoagulability was first suggested by the increased incidence of deep vein thrombosis and pulmonary embolism seen in cancer patients. The mechanism is thought to be activation of the normal clotting system with low-grade intravascular coagulation and secondary fibrinolysis. In the laboratory, common findings are elevated levels of clotting factors and a shortened prothrombin time and activated partial thromboplastin time. It is presumed that cancer cells secrete thromboplastin which initiates clot formation. Treatment of thrombosis in malignancy is difficult, as anticoagulant control is often poor. Antiplatelet agents such as aspirin and dipyrimadole are a possible alternative approach both in treatment of established thrombosis and in prophylaxis.

Disseminated intravascular coagulation (DIC) can complicate malignancy. It may be an acute haemorrhagic state but is more often a chronic low-grade disorder with no bleeding. It is particularly likely to accompany carcinomas of the prostate, stomach, colon, breast, ovary, lung, gallbladder and melanoma.

Fig. 1 Burr cells in the blood in renal failure.

CONNECTIVE TISSUE DISORDERS

Systemic disorders such as rheumatoid arthritis, systemic Jupus erythematosis (SLE) and mixed connective tissue disease often lead to abnormal blood counts. In practice the most common finding, particularly in rheumatoid arthritis, is the anaemia of chronic disease. Immune thrombocytopenia is more often seen in SLE and this heterogeneous disorder may also be complicated by the presence of the lupus anticoagulant. Neutropenia can arise in several
connective tissue disorders; the triad of long-standing rheumatoid arthritis, splenomegaly and neutropenia is termed Felty's syndrome. There may be a small increased risk of haematological malignancy in patients with rheumatoid arthritis. In Sjögren's syndrome there is a substantially increased risk of non-Hodgkin's lymphoma.

Fig. 2 Patient with prostatic carcinoma and invasion of the bone marrow. (a) Leucoerythroblastic blood picture: note the nucleated red cell. (b) Bone marrow trephine specimen showing replacement of normal haematopoiesis by carcinoma.

INFECTIONS

Infections are ubiquitous clinical problems in the community and hospital, and they are probably the most common cause of abnormal blood counts in a typical haematology laboratory. Different infections are associated with different abnormalities but it is possible to make some generalisations.

Bacterial infections commonly cause a neutrophil leucocytosis. The neutrophils are classically 'left-shifted' (i.e. reduced nuclear segmentation) with increased cytoplasmic granulation (toxic granulation). Very severe bacterial infections such as disseminated tuberculosis can induce a leukaemoid reaction with immature myeloid cells appearing in the blood.

Viral infections most commonly cause a transient lymphocytosis with reactive changes in the cells. Two types of viral infection merit more detailed description: infectious mononucleosis and HIV infection.

Infectious mononucleosis
Infectious mononucleosis (or glandular fever) is a disorder caused by the Epstein-Barr virus (EBV). It predominantly affects adolescents and young adults. Clinical features often include malaise, fever, pharyngitis, lymphadenopathy, splenomegaly and hepatitis. The haematological hallmark of the disease is the presence of numerous atypical lymphocytes in the blood (Fig. 3). These lymphocytes are mainly activated T-cells produced as an immunological response to EBV-infected B lymphocytes. Other possible blood changes are neutropenia, thrombocytopenia and a cold-type autoimmune haemolytic anaemia. The differential diagnosis is essentially other
viral diseases, but where the blood abnormalities are severe the disease may be confused with acute lymphoblastic leukaemia. The diagnosis is confirmed by the Paul-Bunnel or Monospot tests which rely on the detection of heterophile antibodies which appear in the serum. Treatment of infectious mononucleosis is essentially symptomatic, although corticosteroids can be helpful in unusually difficult cases.

**HIV infection**
Progressive HIV infection has many possible haematological consequences (Table 2). These result from a combination of a direct effect of the virus, opportunistic infection and side-effects from the drugs used in treatment. The blood changes are often similar to those seen in other viral infections but a chronic decline in the lymphocyte count is a particular feature. Examination of the bone marrow often reveals non-specific changes such as fibrosis, gelatinous transformation, trilineage myelodysplasia, increased lymphocytes and plasma cells, and prominent haemophagocytosis. The presence of granulomas can signify infection by atypical mycobacteria or other opportunistic pathogens. In clinical practice the major haematological problems associated with HIV infection are immune thrombocytopenia (ITP) and lymphomas. The latter are typically aggressive B-cell malignancies with extra-nodal involvement.

**Fig. 3** Atypical lymphocyte in infectious mononucleosis.

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### Systemic disease

**Table 2** Possible haematological changes in HIV infection

<table>
<thead>
<tr>
<th>Blood</th>
<th>Lymphopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaemia</td>
<td>Neutropenia</td>
</tr>
<tr>
<td></td>
<td>Thrombocytopenia</td>
</tr>
<tr>
<td></td>
<td>Atypical lymphocyte morphology</td>
</tr>
<tr>
<td></td>
<td>Anisopoikilocytosis</td>
</tr>
<tr>
<td></td>
<td>Macrocytosis’</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bone marrow</th>
<th>Variable changes in cellularity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gelatinous transformation</td>
</tr>
<tr>
<td></td>
<td>Myelodysplasia</td>
</tr>
<tr>
<td></td>
<td>Increased lymphocytes and plasma cells</td>
</tr>
<tr>
<td></td>
<td>Increased fibrosis</td>
</tr>
<tr>
<td></td>
<td>Opportunistic infection (e.g. granulomas)</td>
</tr>
</tbody>
</table>
Lymphoma

**Other**
- Positive direct antiglobulin test (DAT)
- Lupus anticoagulant

particularly in patients receiving the drug zidovudine.

**SYSTEMIC DISEASE**

Renal disease can cause anaemia, polycythaemia and abnormalities in platelets and coagulation. Malignancy often causes anaemia. Invasion of the bone marrow by solid tumour is a cause of a leucoerythroblastic blood picture. Bacterial and viral infections are common causes of abnormal blood counts. Infectious mononucleosis is a disease caused by the Epstein-Barr virus. Numerous atypical lymphocytes are seen in the blood. The many possible blood changes of HIV infection result from a combination of a direct viral effect, opportunistic infection and drugs used in treatment.
THE DEVELOPING WORLD

The term 'developing world' is used to describe the majority of tropical countries which are 'hot, humid and poor'. An alternative term is the 'less economically sound' nations, as these countries are often advanced in human and cultural resources. Haematological practice is different to that in most developed countries. Genetic diseases such as the haemoglobinopathies and red cell enzymopathies are frequent in many tropical regions. Deficiency anaemia and haemolytic anaemia are often secondary to infections such as ancylostomiasis (hookworm) and malaria. Medical treatment regarded as routine in the developed countries is commonly unavailable. For instance, only about 20% of the world's haemophiliac population has access to factor VIII replacement therapy. With the ever-increasing availability of 'exotic' holidays and regular foreign travel within immigrant populations, doctors in the developed world are seeing more tropical diseases. For the patient with unexplained symptoms such as malaise and fever, or signs such as splenomegaly, a history of travel should not be overlooked.

MALARIA

Malaria is a protozoal disease, the infectious agent being Plasmodium falciparum, P vivax, P ovale or P malariae. It is a growing health risk throughout the tropics and subtropics where insecticide resistance of anopheline mosquitos and multiple drug resistance of malarial parasites have made control and treatment increasingly difficult. It has been estimated that worldwide one hundred million people are attacked annually with a mortality of around 1%, largely in children.

Pathogenesis
The life cycle of the malaria parasite is illustrated in Figure 1. When taking a meal of blood an infected mosquito initiates human infection by the inoculation of malarial sporozoites. These rapidly pass to the liver where they enter hepatocytes and divide. After several days, enormously increased numbers of parasites (merozoites) depart the liver and invade red cells. Here the merozoites develop via ring forms and trophozoites into schizonts. Rupture of the schizont releases 12-20 merozoites back into the blood thus perpetuating the cycle. The duration of the blood cycle varies between malarial species, explaining the different periodicity of fever in each type. A further mosquito becomes infected when it feeds on blood containing gametocytes, the sexual form of the parasite.

Diagnosis
Although malarial parasites may be detected in normal blood films, their identification is generally easier in Leishmann or Giemsa stain at a higher pH. A thick film is best for detection and a thin film for determination of the species. Prolonged inspection of the film is sometimes necessary to spot malarial parasites as there can be a low level of parasitaemia. Where malaria is suspected on clinical grounds repeated samples may be needed to make or exclude the diagnosis. P. falciparum is often associated with higher parasite counts. Paradoxically, some very ill patients with malaria initially have no detectable parasites in the blood as there is sequestration of parasite-laden red cells in the tissues. An experienced microscopist will be able to identify the malarial species; it is beyond the scope of this book to make a detailed comparison.
of the four species but some typical appearances are shown in Figure 2. Other methods of parasite detection under evaluation include the quantitative huffy coat technique, antigen detection by monoclonal antibody and nucleic acid methods.

Clinical features
Malaria has a different clinical presentation in non-immune and immune patients.

Non-immune patient
The interval between the mosquito bite and the onset of symptoms is typically one to two weeks. Common symptoms are rigors, sweats, headache, vomiting, diarrhoea and muscle pains. *P. vivax* and *P. ovale* are classically associated with bouts of fever on alternate days and *P. malariae* on every third day. Possible clinical signs include a rising temperature, tachycardia, herpes labialis, jaundice, dehydration and splenomegaly. *P. falciparum* infection is the most dangerous form of malaria. The onset can be insidious and the fever has no particular pattern. Life-threatening complications such as cerebral malaria (with development of coma), acute renal failure and blackwater fever (rapid intravascular haemolysis), can suddenly develop in a patient previously not particularly ill. Children are particularly at risk of a sudden demise.

Endemic malaria

In indigenous populations, malaria presents variably depending on the degree of endemicity the age of the patient and the development of immunity. Thus in hyperendemic areas where there are seasonal variations, adults develop considerable immunity, malaria causing only short episodes of fever and a palpable spleen. In holoendemic areas there is infection through the year and

Fig. 2 Malarial parasites in the blood. (a) Ring-forms in *P. falciparum* malaria. (b) Schizont in *P. ovale* malaria. (c) Gametocytes in *P. vivax* malaria.
usually the disease manifests as a transient low parasitaemia with no symptoms. In hypoendemic areas, epidemics occur and the disease resembles that in the non-immune. Tropical splenomegaly syndrome is the development of massive splenomegaly in adults in hyperendemic areas. The patient has a low parasitaemia with an exaggerated immune response and very high levels of IgM.

**Treatment and prophylaxis**

Ill patients should be rested and rehydrated. A rational choice of drug treatment requires a knowledge of both the clinical syndrome and the likelihood of drug resistance. Chloroquine remains the choice for sensitive P falciparum malaria. Quinine can be useful in areas of chloroquine resistance. Artemether, the active ingredient of a traditional Chinese remedy for fever, may be effective in quinine-resistant severe malaria. Patients with life-threatening complications of falciparum malaria require intensive medical support and prompt intravenous drug treatment.

Chemoprophylaxis is advised for nonimmune travelers entering malarial areas. Chloroquine, proguanil and pyrimethamine have been widely used. Mefloquine may give the best protection in areas with chloroquine resistance. Where there is doubt, expert advice should be sought as all recommendations are constantly reviewed. Simple preventative measures protective clothes mosquito nets and insect repellent creams also help reduce the risk of infection.

**VISCERAL LEISHMANIASIS (KALA-AZAR)**

This protozoal disease is caused by the organism Leishmania donovani. It is a cause of massive splenomegaly. The organism may be detected in a blood film within monocytes or neutrophils but bone marrow aspiration is more sensitive.

**OTHER PARASITIC DISEASES DETECTABLE IN THE BLOOD**

These include the following:

- **Filaria.** Microfilariae are released into the blood during an acute attack of disease. As the organisms are motile, examination of a wet preparation is useful.
- **Babesiosis.** This tick-born disease only occasionally affects man. Trophozoites which resemble small ring-forms of Pfalciparum can be found in red cells.
- **Trypanosomiasis.** The parasites are extracellular and motile.

**IRON DEFICIENCY IN ANCYLOSTOMIASIS (HOOKWORM)**

Ancylostomiasis affects approximately 20% of the world's population. It is a major cause of gastrointestinal blood loss and iron deficiency anaemia in tropical regions. Worms attach to the upper small intestine and remove blood from the host; the daily loss can be as great as 250 mls. Management of anaemic patients should include both treatment of worms with an effective anti-helminthic agent and oral iron supplements to replenish stores.

**ENDEMIC BURKITT'S LYMPHOMA**
Endemic Burkitt's lymphoma is an aggressive B-lymphoblastic lymphoma which is found particularly in African children. In areas where malaria is holoendemic it is the most common childhood cancer. The disease is associated (EBV) infection with Epstein-Barr virus and the chromosomal rearrangement t(8, 14). The classical clinical presentation is with a massive tumour of the jaw or other extranodal disease. There is often a rapid response to chemotherapy but relapses are frequent and cure rates low.

The developing world

The incidence of many haematological disorders and the availability of treatment is different in the developing world and developed countries. Malaria is a protozoal disease transmitted to man by anopheline mosquitoes. It is a major health problem in tropical and sub-tropical regions. Laboratory diagnosis of malaria depends on the identification of parasites in thick and thin blood films. Optimum drug treatment of established malaria and the best choice of prophylaxis require expert knowledge of clinical syndromes and possible drug resistance. Ancylostomiasis (hookworm) is a major cause of iron deficiency in tropical areas.
RECENT ADVANCES IN INVESTIGATION

An account of recent advances in the investigation of blood disorders must inevitably be dominated by molecular techniques and particularly the polymerase chain reaction. Flow cytometry has also found new applications and is briefly discussed.

MOLECULAR BIOLOGY

OVERVIEW OF TECHNIQUES USED IN THE ANALYSIS OF DNA

Southern blotting
In this methodology, DNA is extracted from cells and then cut at specific sites by incubation with restriction endonucleases. The fragments of DNA are then separated out by electrophoresis prior to transfer to a nitrocellulose or nylon membrane by a blotting technique. DNA is then fixed to the membrane and specific sequences detected by the use of a radiolabelled probe. The method is relatively straightforward but time-consuming, taking at least seven days for normal analysis.

Polymerase chain reaction (PCR)
The advent of PCR has revolutionised molecular work in many medical specialities. Its rapidly increasing use is in part due to its unusual simplicity. The object of PCR is to amplify a preselected sequence of DNA many times over. This amplification greatly facilitates subsequent analysis of the DNA sequence for point mutations and polymorphisms, and often allows direct analysis of the product by gel electrophoresis without the use of probes. The method is shown schematically in Figure 1. Essentially two specific oligonucleotide primers are added to the DNA. These have sequences matching the regions flanking the region of interest. A DNA polymerase is added and the mixture heated causing the DNA to dissociate into two single strands. Following cooling the single strands bind to the oligonucleotides which are in excess. The oligonucleotide then acts as a primer for DNA polymerase and is extended to form a new double stranded molecule. With each repeat of the cycle the amount of DNA is doubled. Generally about 30 cycles are used and amplification of approximately 10^7 can be achieved.

Fluorescence-in-situ-hybridisation (FISH)
FISH describes the hybridisation of specific DNA or RNA sequences in situ to cellular targets attached to microscope slides. The most popular probes are chromosome specific DNA sequences which generate a brilliant signal in both metaphase and interphase nuclei. The technique is particularly useful in the demonstration of chromosomal monosomies or trisomies but chromosome translocations (Fig. 2), deletions and amplification of specific genes can also be detected.

APPLICATION OF MOLECULAR BIOLOGY IN HEMATOLOGY
Carrier detection and antenatal detection in genetic disorders  Molecular techniques now play a central role in genetic counseling and antenatal diagnosis in genetic disorders of the blood. Restriction fragment length polymorphism (RFLP) analysis is used as a method of tracking an abnormal gene in affected families. A major disadvantage of this approach is the necessity to study other family members. A PCR-based technique (mismatched amplification refractory mutation system or ARMS) can detect an abnormal thalassaemia gene; no gene product is obtained unless the primer corresponds to the mutated gene. In haemophilia A most gene mutations were thought to be private (i.e. only found in isolated families). However, a common inversion of the factor VIII gene has recently been identified in many haemophilia families. By southern blot analysis one or other of the two types of the inversion can be detected in around 40% of patients with severe disease. In these cases the inversion simplifies both carrier detection and antenatal diagnosis.

Haematological malignancy

Diagnosis
Leukaemias and lymphomas have traditionally been diagnosed on the basis of their morphological appearance and immunophenotype. In most cases a precise diagnosis is possible but on occasion there is doubt as to the lineage of the malignant cell or even as to whether the cell proliferation is malignant (i.e. clonal). In this context the development of probes for the immunoglobulin genes and T-cell receptor gene has been an advance. Firstly, a distinction can be made between B and T-lymphocyte malignancies. In almost all tumours derived from B-cells (e.g. B-ALL, B-CLL, B-cell non-Hodgkin's lymphoma) a monoclonal rearrangement of immunoglobulin (1g) genes is found, whereas in T-cell malignancies there is a corresponding rearrangement of the T-cell receptor (TCR) gene. Secondly, the presence of a clonal rearrangement of the Ig or TCR genes differentiates (with rare exceptions) a malignant proliferation of cells from a 'reactive' proliferation where the presence of many different rearrangements does not lead to the formation of specific bands on Southern blotting.

Where there are specific molecular markers of leukaemia (e.g. bcr-abl in CML, PML-RARα in acute promyelocytic leukaemia) then molecular techniques are likely to play some part in diagnosis. They can be used to complement conventional cytogenetics and may provide additional information. The molecular abnormality can have prognostic significance: detection of the bcr- abl rearrangement in ALL indicates a low likelihood of cure by chemotherapy. It is less clear whether precise determination of the breakpoint within the breakpoint cluster region affects the prognosis in CML.

Minimal residual disease
Traditional definitions of remission in leukaemia rely on crude morphological criteria. Many patients in remission subsequently relapse implying the existence of occult neoplastic cells undetectable by normal morphological or cytogenetic methods - so-called minimal residual disease (MRD) (Fig. 3). Reliable detection of MRD would allow improved management with escalation of therapy for patients with persistent disease and the avoidance of excessive treatment in patients showing a good response to previous intervention. Now two techniques can detect MRD in selected disorders - immunological marker analysis (see below) and PCR. Successful use of PCR relies on the presence of a disease marker which can be targeted.
Examples are junctional rearrangements of Ig and TCR genes, and chromosomal rearrangements defined at the molecular level, such as bcr-abl and PML-RARα. Currently in the order of 10-45% of leukaemias carry a definable marker, Preliminary clinical studies suggest that early detection of MRD by PCR can predict later frank relapse, but larger trials are needed to establish its routine role in management.

**Bone marrow transplantation**
Molecular techniques can be used both to monitor MRD posttransplant and to improve the level of HLA matching between unrelated donors and recipients.

**FLOW CYTOMETRY**

**HAEMATOLOGICAL MALIGNANCY**

Immunophenotyping using a flow cytometer is a standard part of leukaemia diagnosis and classification. New ideas are under investigation. Detection of particular antigens on leukaemic cells may give useful prognostic information at presentation (e.g. presence of myeloid antigens on ALL cells). Multiparameter immunophenotyping enables the identification of leukaemia-associated phenotypes in many cases of acute leukaemia. This differentiation between leukaemic cells and normal progenitors allows monitoring of MRD. It is likely that the best strategy for detecting and monitoring MRD will be a combination of molecular and immunological methods.

**OTHER APPLICATIONS**

Flow cytometry is now a routine automated method for reticulocyte counting. In the blood transfusion laboratory the technology has a number of potential serological applications (e.g. detecting red cell bound Ig), and could also be used in the evaluation of haemolytic transfusion reactions, in predicting the survival of transfused red cells and in quantifying feto-maternal haemorrhage.

**Recent advances in investigation**

Molecular biology techniques used in haematology in Southern blotting, the polymerase chain reaction (PCR) fluorescence in situ hybridisation (FISH). Molecular techniques now play a key role in carrier detection and antenatal detection in genetic disorders such as thalassaemia and haemophilia.

In haematological malignancy, molecular techniques can aid diagnosis, distinguish between malignant and reactive cell proliferations, and improve detection of minimal residual disease (MRD).
Newer uses of flow cytometry include the detection of cells with leukaemia-associated phenotypes in acute leukaemia, automated reticulocyte counting and various serological methods in the blood transfusion laboratory.
RECENT ADVANCES IN TREATMENT

This section addresses in more detail some recent developments in treatment previously alluded to in the coverage of specific diseases. Inevitably any listing of recent advances is subjective. Most clinicians would probably choose to emphasise developments in chemotherapy, the expanding role of growth factors and the imminent introduction of gene therapy for single gene disorders such as haemophilia and thalassaemia.

CHEMOTHERAPY FOR HAEMATOLOGICAL MALIGNANCY: NEW APPROACHES

New agents
There are basically two approaches to the development of new drugs for the treatment of haematological (or other) malignancy. Large scale screening programmes evaluate considerable numbers of drugs with potential anti-cancer activity. Many promising agents have been identified with subsequent transfer into routine clinical practice. However, it is likely that this random approach will gradually give way to a second strategy where increased understanding of the cellular biology and biochemistry of cancer allows the rational design of drugs. Such agents will probably target specific stages of the malignant process within the cell.

DNA, the target of traditional drugs such as alkylating agents, is an attractive target for the next generation of chemotherapeutic agents. Antisense oligonucleotides are small synthetic nucleotide sequences complimentary to specific DNA or RNA sequences which selectively inhibit the transcription or translation of a gene. They may be directed against particular oncogenes. Anti-bcr/abl antisense oligonucleotides have already been investigated as a means of in vitro purging of malignant cells from the bone marrow of patients with chronic myeloid leukaemia. Other oncogenes implicated in haematological malignancies (e.g. C-MYC) may also be inhibited in this way. The delivery of these agents to malignant cells in vivo is problematic and may require the use of gene therapy strategies.

Targets for anti-cancer drugs are not limited to the cell nucleus. Drugs may be designed to block malignant proliferation by selective inhibition of growth factors, cell surface receptors and intracellular molecules mediating transmission of signals from the membrane to the nucleus. Agents could target components of the second messenger cascade including tyrosine kinase and protein C. Other drugs could interact with membrane molecules involved in cell-cell or cell-matrix combinations crucial in local malignant invasion and metastases.

Peripheral blood stem cell transplantation (PBSCT)
Although novel drugs are under development, there remains dependence on more traditional cytotoxic drugs. One way to try and increase cure rates in haematological malignancy is to escalate the dose of conventional agents whilst designing strategies to minimise the inevitable toxicity. In this context, autologous PBSCT is a rapidly growing treatment modality. PBSCT has now largely superseded the use of autologous bone marrow in the support of patients receiving high-dose chemotherapy. The procedure relies upon the harvest of haematopoietic stem cells from the patient's blood by leucapheresis during the recovery phase from moderate doses of chemotherapy (Fig. 1). The growth factor G-CSF is often used to facilitate this mobilisation of stem cells. The stem cell harvest is cryopreserved and is ultimately reinfused following the administration of high-dose chemotherapy.
The principle is the same as for autologous bone marrow transplantation but PBSCT has the advantage of giving more rapid recovery of a normal blood count than the traditional procedure. This translates into a shorter hospital stay, less dependence on antibiotics and blood products, and probably a reduction in the morbidity and mortality associated with high doses of cytotoxic drugs. In haematological practice PBSCT has been mainly used for younger patients with myeloma and lymphoma. Its use may be gradually extended to patients with chemotherapy responsive non-haematopoietic solid tumours.

Multi-drug resistance: possible solutions
The major problem in the treatment of leukaemia and other haematological malignancies is the emergence of cells resistant to chemotherapy. Genes capable of conferring resistance to cytotoxic drugs have been characterised. Of particular interest is the P-glycoprotein or multi-drug resistance gene (MDR 1), as its overexpression can lead to resistance to many of the agents used in the treatment of leukaemia. The MDR 1 gene encodes a membrane protein which acts as an ATP-dependent efflux pump transporting organic compounds out of the cell (Fig. 2). Elevated MDR 1 levels appear to be a poor prognostic factor in acute myeloid leukaemia. In an effort to overcome MDR a number of MDR-reversing agents have been given in conjunction with normal chemotherapy regimens. Cyclosporin A is currently generating most interest with encouraging early results in myeloma and acute myeloid leukaemia refractory to chemotherapy alone.

OTHER APPROACHES TO THE TREATMENT OF HAEMATOLOGICAL MALIGNANCY

Differentiating agents: an isolated success
In most cases attempts to induce maturation of malignant cells have been disappointing. One remarkable exception is the drug all-trans-retinoic-acid (ATRA) in acute promyelocytic leukaemia (APL, the FAB M3 variant of acute myeloid leukaemia). Initial treatment with ATRA gives a high proportion of complete remissions without marrow hypoplasia. There is also a reduction in the incidence of the coagulopathy non-nally associated with this type of leukaemia. The clinical complete remission is usually accompanied by disappearance of the molecular marker of APL the PML/RARa gene rearrangement. There is now evidence that induction of remission by ATRA followed by consolidation chemotherapy gives a significant survival advantage over the use of chemotherapy alone.

Alpha interferon: an expanding role
Alpha interferon is an anti-viral protein with immunomodulatory and anti-cancer activities. Its use in haematology generally exploits the latter attributes, although it has a role in chronic hepatitis C infection which affects many adults with severe haemophilia. For a lengthy period, interferon's main use was in hairy cell leukaemia. More recently the drug has become the treatment of choice for the majority of patients with chronic phase chronic myeloid leukaemia. The use of interferon as an adjunct to chemotherapy in myeloma and lymphoma also holds promise.

HAEMATOPOIETIC GROWTH FACTORS
Much in the early days of chemotherapy the precise indications for their use and the best dosages and combinations are gradually being worked out. G-CSF and GM-CSF shorten the period of neutropenia following intensive chemotherapy with a resultant reduction in the number of infections and duration of stay in hospital. These agents can also be used to mobilise haematopoietic stem cells in the blood for subsequent harvesting (see above). More contentious is the inclusion of growth factors as an integral part of chemotherapy regimens to amplify the anti-leukaemic effect of cytotoxic drugs (e.g. combination of G-CSF, fludarabine and cytosine arabinoside in acute myeloid leukaemia). G-CSF is given long-term in various forms of congenital or acquired chronic neutropenia to minimise the risk of infection. There is currently no effective platelet growth factor in routine clinical use but thrombopoietin (c-mpl ligand) is undergoing testing. 

Immunological factors are important in preventing relapse of leukaemia after allogeneic bone marrow transplantation (see p. 55). Immunotherapy is therefore an attractive option for the treatment of leukaemia outside the transplant setting, particularly for the eradication of minimal residual disease. Several agents are being investigated (e.g. interleukin-2) but results to date are inconclusive.

**GENETHERAPY**

Gene therapy will be a major part of the medicine of the future. The principle is straightforward and illustrated in Figure 3. A new functional gene is inserted into a cell. Physical methods may be used (e.g. electroporation or liposomes), but most gene therapy to date has depended on viruses (usually retroviruses) as vectors of the gene. Current problems with this technology include the attainment of adequate expression of the new gene and the maintenance of this response for a prolonged period of time. There are also concerns regarding potential toxicity from the transfected DNA and viral vectors. As single gene disorders, both thalassaemia and haemophilia are good candidates for cure by gene therapy. However, there are still sizable problems to overcome and it is likely to be several years before procedures are routinely performed for either of these diseases. Gene therapy's role in haematological malignancy is more speculative. The technique may eventually be used to insert informational drugs and cytokines into cancer cells or even to restore the function of defective tumour suppressor genes.

**Recent advances in treatment**

Recent developments in chemotherapy for haematological malignancy include the design of new drugs, peripheral blood stem cell transplantation (PBSCT) to escalate the dose of conventional drugs and the use of multi-drug resistance (MDR)-reversing agents. Haematopoietic growth factors are increasingly used following chemotherapy to reduce toxicity and to mobilise stem cells for PBSCT. Gene therapy is likely to become the treatment of choice for single gene disorders such as haemophilia and thalassaemia. Its role in haematological malignancy is more speculative.
VENEPUNCTURE AND VENOUS ACCESS

Obtaining a sample of venous blood from a patient is the most commonly performed practical procedure in haematology. The technique is apparently straightforward but poorly performed venepuncture can both upset the patient and compromise the quality of the sample. Gaining venous access for the delivery of fluids, blood or drugs is also fundamental to good haematological practice. This section is an overview of venepuncture and venous cannulation. These skills are best learnt by practice with expert supervision.

TAKING A VENOUS BLOOD SPECIMEN (VENEPUNCTURE)

The patient should be the correct patient - check their identity! Most serious haemolytic transfusion reactions arise from careless identification of patients and incorrect form labelling. Patients should sit or lie comfortably in such a way that no serious injury could result from a faint. The operator washes his hands and wears plastic gloves - insist on gloves that fit properly. The procedure is briefly explained to the patient. The presence of a little transient pain when the needle is inserted should be acknowledged but not exaggerated.

Under normal circumstances blood is most easily taken from a vein in the antecubital fossa; the median cubital vein is preferred (Figs 1 & 2). It is considerate to ask whether the patient is left- or right-handed and then to choose the non-dominant arm. A tourniquet is applied well proximal to the site. This should cause distension of the veins but not discomfort. Gentle palpation is the best method of identifying a vein and checking its patency. If a suitable vein proves elusive it may help to gently tap the area or to warm the arm in water. The skin over the chosen vein is thoroughly cleaned with antiseptic solution. Usually a 19 or 20 gauge needle is used but a smaller size (e.g. 21 or 23) can be used where the veins are fragile and in children. The syringe should be adequate for the sample - where larger blood samples necessitate more than one syringe a 'butterfly needle' may be preferred to a conventional venepuncture needle. The needle is inserted bevel uppermost along the line of the vein at an angle of around 20. There is a distinctive 'give' as the vein is entered. Blood is aspirated into the syringe slowly to avoid haemolysis. The tourniquet is released and the needle withdrawn after a dry swab has been held to the site. Pressure should be applied by the patient or an assistant with the arm held straight or slightly elevated. The needle is removed from the syringe - not resheathed - and placed directly into a sharps container. The specimen is expelled gently from the syringe into the relevant bottles. Mixing with anticoagulant is best achieved by gently inverting the bottle several times - violent shaking will damage the sample. An adhesive plaster can be applied to the venepuncture site (check for allergy) when bleeding has stopped.

The above describes the procedure for a conventional needle and syringe. Increasingly, venepuncture is performed using closed evacuated container systems where a double-ended venepuncture needle is screwed into a holder and the evacuated tube inserted into the holder following entry of the vein. Blood is automatically aspirated into the tube as the vacuum is released. It is important to understand how the system works before undertaking venepuncture.

Precautions
Blood should not be taken from a vein proximal to an intravenous infusion as the sample can be diluted. Neither should eczematous or infected areas be used for venepuncture. If patients are known to have a blood transmissible infection (e.g. Hepatitis B or C, HIV) or are at increased risk of such an infection this must be indicated on the specimen bottle and request form. Due care must be taken as evidently this is sensitive information - special labels stating an infective risk are available. In view of the possibility of needle-stick injuries, those performing venepuncture should be vaccinated against hepatitis B.

**Common problems**
Venepuncture is not always easy. If blood is not aspirable following perceived entry of the vein it is worth withdrawing the needle slowly with suction applied as the vein may have been transfixed. If a vein cannot be located in the antecubital fossa it is permissible to use veins at the wrist or on the dorsum of the hand. If two attempts fail a more experienced colleague should be sought. As a last resort a sample can be taken from the femoral vein. The operator must be familiar with the anatomy of the femoral region as the vein lies close to the femoral artery and nerve.

**Children**
In babies and infants a blood sample is often more easily obtained from a stab wound made with a lancet (capillary blood). The usual site is the heel, although fingers and earlobes can be used. Venepuncture may also be from scalp veins.

**VENOUS ACCESS**

**Peripheral venous cannulation**
Almost all haematology patients admitted to hospital require at some stage a drip to infuse fluids, blood products or drugs. Before inserting a cannula into a vein, an appropriate giving set should be prepared in accordance with instructions and the bag or bottle containing the infusion fluid inverted and hung on the drip stand. The set should be properly primed and all bubbles excluded. The operator must wash hands and wear gloves. It is vital to ensure that the patient is comfortable and fully understands the procedure. The choice of cannula depends both on the quality of the veins and the duration and type of infusion. For short-term infusions or small veins a winged metal cannula (butterfly needle) is often suitable. In other circumstances a larger gauge plastic cannula is used (Fig. 3). An 18-gauge cannula is appropriate for crystalloid solutions but a 14- or 16-gauge is needed for blood.

The best site is the non-dominant forearm or the dorsum of the hand. The antecubital fossa is best avoided as it is uncomfortable to have the elbow immobilised. A tourniquet is applied and the skin cleaned as for venepuncture. The skin at the site may be stretched slightly to immobilise the vein. The cannula assembly (metal needle and surrounding plastic cannula) is introduced through the skin and into the vein. Once blood enters the cannula chamber or is easily drawn into a syringe, the tourniquet is released and the metal needle withdrawn from the plastic cannula which may be advanced further into the vein.

The pre-prepared giving set is attached to the cannula and fluid allowed to enter the vein whilst the insertion site is carefully inspected for possible extravasation. The needle is promptly disposed of in a sharps receptacle. To minimise the chance of the drip being infected or dislodged the site is protected with a sterile dressing and the cannula secured with a bandage or adhesive tape. The most common problem is failure to locate a vein in the favoured sites. A more
experienced operator (e.g. an anaesthetist) may be successful. Where problems persist in experienced hands, other veins such as those in the region of the ankle or the subclavian, jugular or saphenous veins may be cannulated. Regular inspection of the drip site and careful hygiene will minimise the chance of infection. Where there is local inflammation or an otherwise unexplained bacteraemia, the cannula should be removed and another site used.

**Central venous cannulation**
Insertion of wide lumen silicon rubber catheters (generally referred to as Hickman catheters) is routinely undertaken in clinical haematology where recurrent intravenous access is required. Examples include:
- patients with haematological malignancy receiving intensive chemotherapy
- patients with thalassaemia having regular blood transfusions

The catheter is normally inserted into the subclavian vein and the location of the distal tip checked on X-ray (Fig. 4). The proximal end of the catheter can be tunneled under the skin with an exit site on the anterior chest wall. A catheter cuff within the tunnel promotes the formation of fibrous tissue which helps secure the device. The procedure is usually performed in the operating theatre by a surgeon or anaesthetist. Once in place the catheter may be used for several months. Strict aseptic technique is necessary as infection with coagulase negative staphylococci is the most common complication.

**Venepuncture and venous access**
Obtaining a venous blood sample (venepuncture) is a commonly performed practical procedure in haematology; poor technique can upset the patient and ruin the sample. In babies and infants, capillary blood sampling is often easier than venepuncture. Peripheral venous cannulation is commonly performed to infuse fluids, blood products and drugs. Where there is serious difficulty in locating a vein for venepuncture or cannulation, more experienced help should be sought. For recurrent venous access, insertion of an indwelling central venous catheter can be helpful.
BONE MARROW ASPIRATION AND TREPHINE BIOPSY

The indications for performing bone marrow aspiration and trephine biopsy procedures have previously been discussed. In this section the practical aspects of obtaining these samples are outlined. More detailed accounts can be found in books of practical procedures, but ultimately the only way to perfect technique is to practise under expert supervision.

Although the anterior iliac crest is occasionally preferred, most operators get the best specimens from the posterior iliac crest. The sternum is now less frequently used. This is, in part, due to the small risk of causing catastrophic damage to the mediastinum, but mainly because it is not possible to obtain a trephine biopsy. Only the posterior iliac crest approach is described here.

BONE MARROW ASPIRATION

As for all procedures the sequence of events should be explained to the patient and reassurance given. A degree of discomfort should be acknowledged but it should be emphasised that this is transitory. In most adults, local analgesia is adequate but sedation is considered where patients are unusually anxious. A general anaesthetic is the norm in children. A clean, no touch technique is mandatory and most operators now wear gloves. Stringent asepsis is needed in immunosuppressed cases.

The patient lies in the left or right lateral position and the skin over the posterior iliac crest is cleaned with antiseptic prior to screening with sterile drapes. The crucial next stage is to properly identify the bony landmarks (Fig. 1). This is straightforward in most patients but can be problematic in obese subjects. If there are real difficulties in locating the posterior iliac crest then the anterior crest or the sternum may be considered. A local anaesthetic (normally 1 or 2% plain lignocaine) is infiltrated into the skin and then down to the periosteum. The Salah aspirate needle is shown in Figure 2. Before use it should be checked that the stylet is easily withdrawn and the guard is removed (this is only required for sternal aspirates). The needle is inserted through the skin and subcutaneous tissues at the site of lignocaine infiltration until the periosteum is encountered. It is pushed through the periosteum with a deliberate screwing motion (alternating clockwise and anti-clockwise) - a 'give' is felt as the marrow cavity is entered. The stylet is withdrawn and a syringe attached to the needle (Fig. 3). Approximately 1 ml of marrow is aspirated into the syringe. The patient should be warned that this stage often causes pain but that it is momentary.

Marrow aspirate smears must be made promptly at the bedside before the marrow clots. If a larger volume is needed for tests such as cytogenetics and immunophenotyping, it is best to use a second syringe as large samples dilute the marrow with peripheral blood and reduce the quality of the morphological preparations. If it proves difficult or impossible to aspirate marrow it is worth replacing the stylet and carefully advancing or retracting the needle a short distance before repeating aspiration. It is important to remember that a 'dry tap' can result from marrow pathology (particularly fibrosis or solid malignancy) and is not always caused by poor technique. Once the aspirate needle is withdrawn, firm pressure is applied to the site for a few minutes and then a sterile dressing or plaster used as protection. The patient lies on his/her back for 15 minutes to ensure a period of recuperation and further light pressure is applied to the puncture.
Outpatients should probably be observed for at least an hour before being allowed home (evidently more if sedated). Troublesome haemorrhage from the site is rare but it is sensible to correct a severe coagulation defect before undertaking the procedure. Thrombocytopenia alone is generally not a problem.

Patients often ask how quickly the 'results' will be available. Aspirate slides can be processed for microscopy within a few hours but most ancillary tests (see Table 1) take longer.

Table 1 Ancillary tests which may be performed on bone marrow aspirate samples
- Cytochemistry
- Cytogenetics
- Immunophenotyping
- Molecular studies
- Microbiological culture
- Cell culture studies
- Drug resistance studies

**BONE MARROW TREPINE BIOPSY**

In practice the trephine procedure is usually performed immediately following the aspirate at the same site. It is helpful to enlarge the aspiration puncture site slightly with a scalpel blade. There is more prolonged discomfort than in the aspirate procedure and sedation is indicated in anxious adults, and a general anaesthetic is necessary in children. A number of different needles are available - the Jamshidi type is illustrated in Figure 4. Smaller needles are available for paediatric use.

It is important to ensure that the device is complete and that the stylet can be easily withdrawn. The trephine needle is inserted in a similar fashion to the aspirate needle through the periosteum and approximately 1/2 cm into the cortex - when properly inserted the needle should easily support its own weight (Fig. 5). The stylet is removed prior to advancing the needle 2-3 cm using the same oscillatory movement. The needle is aimed towards the anterior iliac crest. The method for breaking off the biopsy varies with the needle used. For a Jamshidi needle the usual technique is to cut the core off by carefully withdrawing the needle a few millimetres and then readvancing the same distance in a different direction. The needle is then withdrawn taking care not to catch the skin and lose the biopsy in subcutaneous tissue. A special blunt probe is provided to push the biopsy out of the needle. The probe is inserted (with great care to avoid injury to the operator) at the sharp end of the needle so as not to traumatise the sample.

If the aspirate is a 'dry tap' it is worthwhile gently dabbing the trephine biopsy onto a glass slide before putting it into histological fixative. This 'touch preparation' is not useful for subtle morphological diagnosis but can permit rapid identification of malignant infiltration. It usually takes several days to process the trephine biopsy. Aftercare is the same as for the aspirate, although as it is a slightly more invasive procedure the patient also having a trephine may require a longer period of recuperation. Nevertheless, trephine biopsies are routinely performed in the outpatient clinic.
BONE MARROW HARVESTING

Bone marrow can be harvested from a patient (for autologous bone marrow transplantation) or from a donor (for allogeneic bone marrow transplantation). The procedure is performed under a general anaesthetic, the marrow being collected from the iliac crests using multiple punctures with specialised harvest needles. Normally, approximately one litre is harvested from an adult in under an hour. Donors are hospitalised for around 48 hours. Serious side-effects are rare but some short-lived discomfort over the aspiration sites is common.

Bone marrow aspiration and trephine biopsy

The optimal site for both bone marrow aspiration and trephine biopsy procedures is the posterior iliac crest. Local analgesia is often adequate but nervous adults require sedation and children normally require a general anaesthetic. Marrow aspiration smears may be stained for microscopy immediately after the procedure whereas trephine biopsies are processed over several days. Serious side-effects from posterior iliac crest aspiration and trephine biopsy are very rare. Occasionally there can be excessive haemorrhage or local infection at the site. Bone marrow can be harvested from the iliac crests in patients (for autologous bone marrow transplantation) or healthy donors (for allogeneic bone marrow transplantation).