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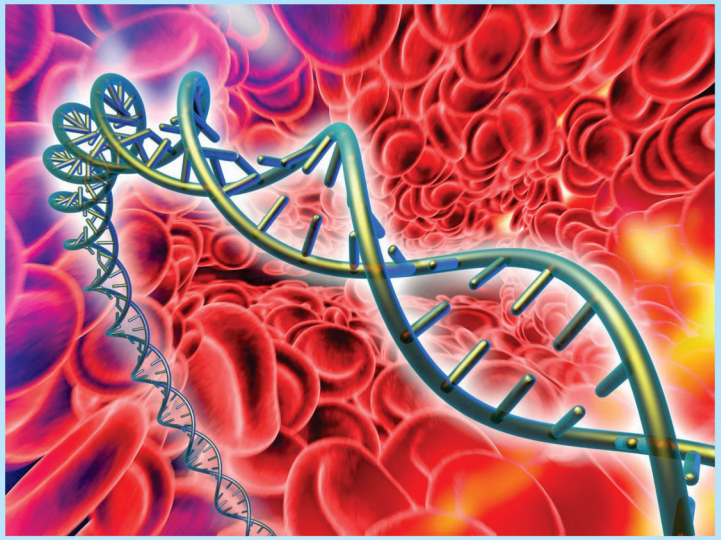

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ډاکټر زکریا امیرزاده

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هیماتولوژي (انګلیسي)
Hematology (in English)
ډاکټر زکریا امیرزاده
۱۳۹۶

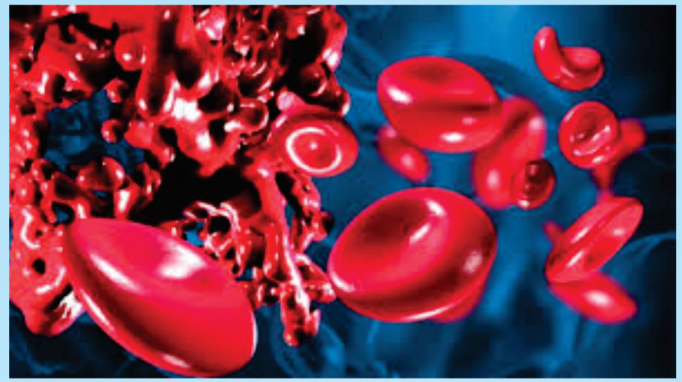
Kandahar Medical Faculty

Afghanic

Dr Zekria Amirzada

Hematology

(in English)



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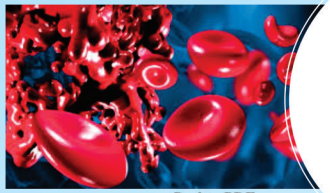

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Hematology

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Dr Zekria Amirzada

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هیماتولوژي

(انګلیسي)

ډاکترمحمد زکریا امیرزاده

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In the name of Allah, Most Gracious, Most Merciful.

Dedication

This try of mine is dedicated to momin, sincere, sympathetic and kind lecturers of Kandahar university, who educat Islamic morality and transfer knowledge to our poor nation with ultimate love and sincerity.

تقریظ

د کندهار پوهنتون علمی معاونیت ته!

محترما!

د کندهار پوهنتون د طب پوهنځی د داخله ځانګی د استاد پوهندوی ډاکتر محمد زکریا امیرزاده لیکلی کتاب می ولوست.

نوموړی کتاب چی په ۳۸۹ پاڼو او ۸ فصلونو کی په روانه ، اسانه او عام فهمه انګلیسی ژبه د پنځم ټولګی د دوهم سمستر د کریکولم په پام کی نیولو سره لیکل سویدی، یو جامع او د محصلینو د اړتیا سره سمون لری.

په نوموړی کتاب کی د محصلینو د ښی او اسانه زده کړی لپاره د تصویرونو او جد ولونو څخه کار اخستل سویدی، چی بر سیره پر دی چی د محصلینو سره د اړینو ټکوپه یا دولو کی زیاته مرسته کوی، کتاب ته یی هم زیاته ښکلا ور ببښلی ده.

زه پخپل وار سره د محترم پوهندوی ډاکتر محمد زکریا امیرزاده دا هڅی ستایم او نوموړی کتاب نه یوازی د پنځم ټولګی د محصلینو بلکه د ستاژیر ډاکترانو او نوی فارغ سوی ډاکترانو د معلوماتو د زیاتوالی لپاره د مطالعی ښه منبع ګنم.

محترم پوهندوی ډاکتر محمد زکریا امیر زاده ته په دی برخه کی د لابر یاوو هیله لرم.

په درناوی
پوهنوال ډاکتر عبدالواحد وثیق
د داخله ځانګی مشر

Preface

Haematology has advanced more rapidly in the last ten years more than any branch of medicine. The increased understanding of blood diseases particularly their genetic basis and changes in their treatment is such substantial changes. The classification of the neoplasms of the haemopoietic and lymphoid diseases has been revised by WHO and the names and definitions of many of these diseases have changed. We have made changes in all the relevant chapters but, in a book intended primarily for undergraduates, we have simplified some of the classification tables and omitted detailed descriptions of rare diseases. As previously, we have used a colour line in the margin to indicate text that we consider more advanced than is needed for under graduate medical students and more appropriate for postgraduates.

The *Amirzada's short text of Hematology,* First edition has updated topics reflecting the most current definition, etiology, epidemiology, pathology/pathogenesis, clinical features, investigations, treatment recommendations, prevention and prognosis of haematologic diseases. The book has been organized into eight chapters namely Introduction, Erythrocyte disorders, Leukocytes disorders, Thrombocytes & Coagulation disorders, Blood Transfusion, Disorders of Immune System, Vitamines Deficiency and Hemoglobinopathies. This book is the result of my teaching try in Medical faculty of Kandahar University.

Dr M. Zakarya Amirzada

Contents

Chapter one

Intruduction to hematologic diseases

- *Introduction*
- *Haematopoiesis*
- *Investigation of blood diseases*
- *Clinical examination in blood disease*

Introduction

Blood flows throughout the body in the vascular system, and consists of plasma and three cellular components:

1. Red cells, which transport oxygen from the lungs to the tissues
2. White cells, which protect against infection
3. Platelets, which interact with blood vessels and clotting factors to maintain vascular integrity and prevent bleeding. (3)

Haematopoiesis

Haematopoiesis describes the formation of blood cells, an active process that must maintain normal numbers of circulating cells and be able to respond rapidly to increased demands such as bleeding or infection.

1. ***During development***, haematopoiesis occurs in:
   - The liver
   - Spleen
   - Red bone marrow in the medullary cavity of all bones
2. ***In childhood***, red marrow is progressively replaced by fat (yellow marrow)
3. ***Adults***' normal haematopoiesis is restricted to:
   - Vertebrae
   - Pelvis
   - Sternum
   - Ribs
   - Clavicles
   - Skull
   - Upper humeri
   - Proximal femora (3)

However, red marrow can expand in response to increased demands for blood cells. (3)

Bone marrow contains a range of immature haematopoietic precursor cells and a storage pool of mature cells for release at times of increased demand.

Haematopoietic cells interact closely with surrounding connective tissue stroma, made of:

1. Reticular cells
2. Macrophages
3. Fat cells
4. Blood vessels
5. Nerve fibres (Fig. 1.1) (3)

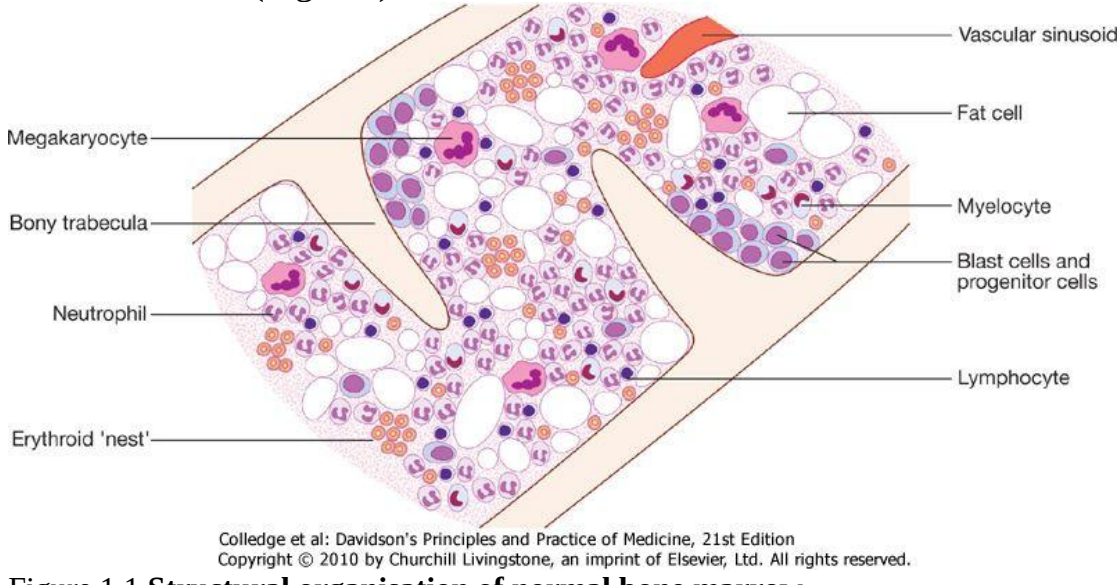


Figure 1.1 **Structural organisation of normal bone marrow.**

In normal marrow, nests of red cell precursors cluster around a central macrophage, which provides iron and phagocytoses extruded nuclei. Megakaryocytes are large cells which produce and release platelets into vascular sinuses. White cell precursors are clustered next to the bone trabeculae; maturing cells migrate into the marrow spaces towards the vascular sinuses. Plasma cells

are antibody-secreting mature B cells which normally represent < 5% of the marrow population and are scattered throughout the intertrabecular spaces. (3)

Stem cells

All blood cells are derived from pluripotent stem cells. These comprise only 0.01% of the total marrow cells, but they can self-renew (i.e. make more stem cells) or differentiate to produce a hierarchy of lineage-committed stem cells. The resulting primitive progenitor cells cannot be identified morphologically, so they are named according to the types of cell (or colony) they form during cell culture experiments. (3)

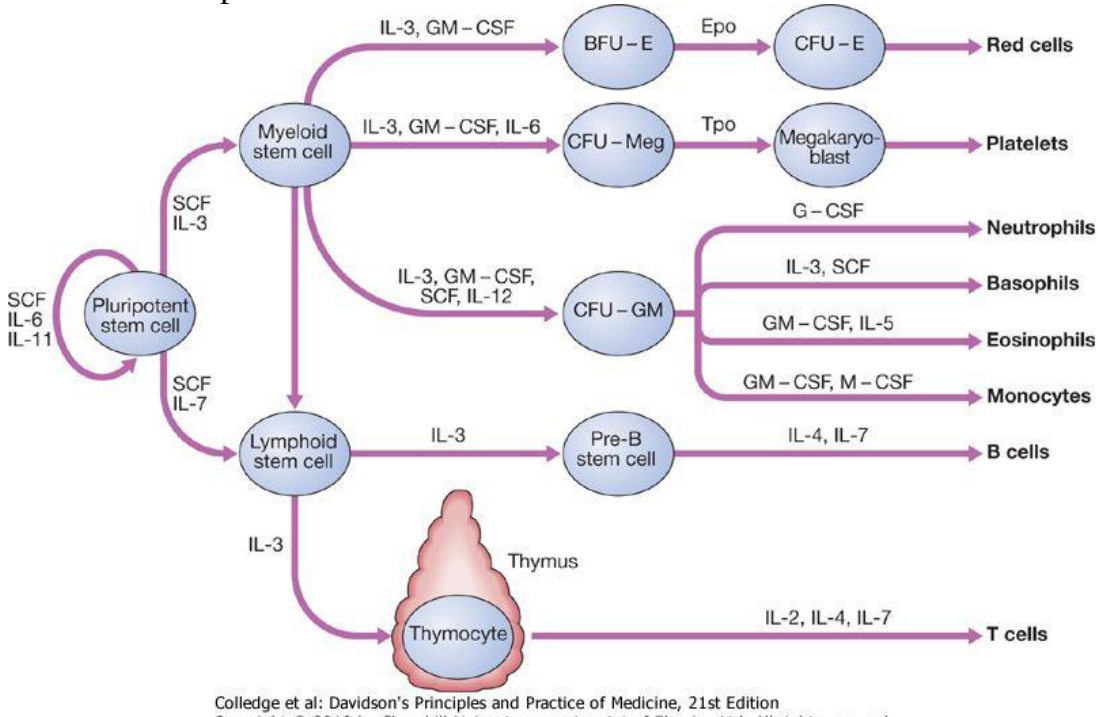


Figure 1.2 **Stem cells and growth factors in haematopoietic cell development.** (BFU-E = burst-forming unit-erythroid; CFU-E = colony-forming unit-erythroid; CFU-GM = colony-forming unit-granulocyte, monocyte; CFU-Meg = colony-forming unit-megakaryocyte; Epo = erythropoietin; G-CSF = granulocyte-colony-stimulating factor; GM-CSF = granulocyte macrophage-colony-stimulating factor; IL = interleukin; M-CSF = macrophage-colony-stimulating factor; SCF = stem cell factor; Tpo = thrombopoietin)

CFU-GM (colony-forming unit-granulocyte, monocyte) is a stem cell that produces granulocytic and monocytic lines, CFU-E produces erythroid cells, and CFU-Meg produces megakaryocytes and ultimately platelets (Fig. 1.2). [3]

Growth factors

A range of growth factors, produced in bone marrow stromal cells and elsewhere, controls:

1. The survival
2. Proliferation
3. Differentiation
4. Function of stem cells and their progeny

Types

Act on a wide number of cell types

1. Granulocyte macrophage-colony stimulating factor (GM-CSF)
2. Interleukin-3 (IL-3)
3. Stem cell factor (SCF)

Lineage-specific

1. Erythropoietin (Epo)
2. Granulocyte-colony stimulating factor (G-CSF)
3. Thrombopoietin (Tpo)

Many of these growth factors are now synthesised by recombinant DNA technology and used as treatments. [3]

Blood cells and their functions

Red cells

Red cell precursors formed in the bone marrow from the erythroid (CFU-E) progenitor cells are called erythroblasts or normoblasts (Fig. 1.3). These divide and acquire haemoglobin which turns the cytoplasm pink; the nucleus condenses and is extruded from the cell. The first non-nucleated red cell is a reticulocyte which still contains ribosomal material in the cytoplasm, giving these large cells a faint blue tinge ('polychromasia'). Reticulocytes lose their

ribosomal material and mature over 3 days, during which time they are released into the circulation. Increased numbers of circulating reticulocytes (reticulocytosis) reflect increased erythropoiesis. Proliferation and differentiation of red cell precursors is stimulated by erythropoietin, a polypeptide hormone produced by renal tubular cells in response to hypoxia. Failure of erythropoietin production in patients with renal failure causes anaemia, which can be treated with exogenous recombinant erythropoietin. [3]

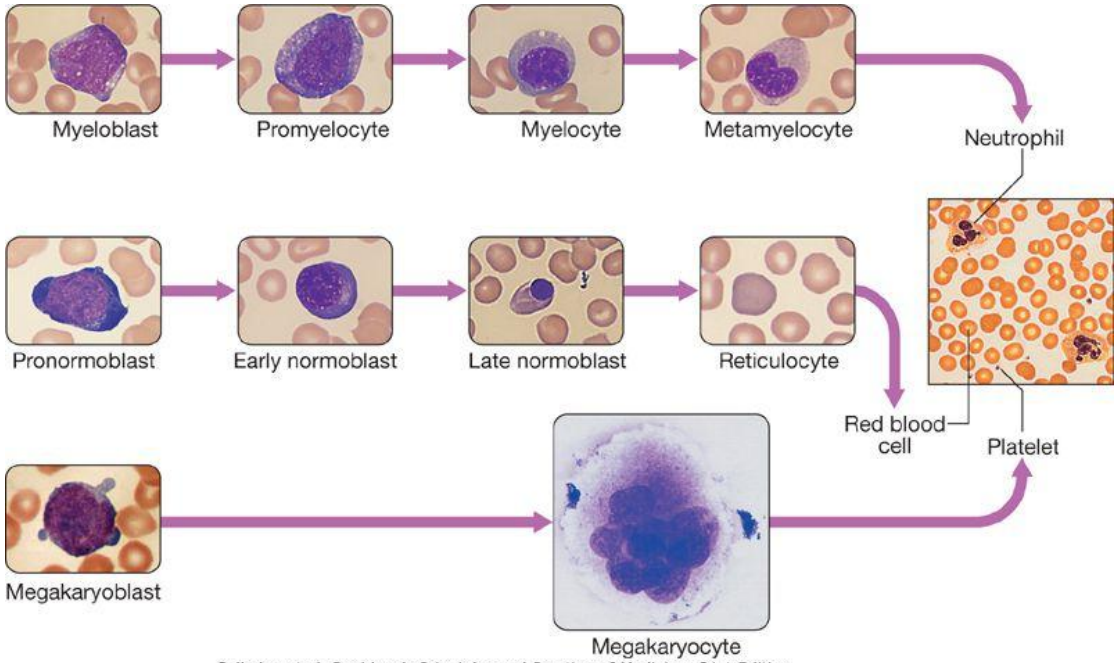


Figure 1.3 **Maturation pathway of red cells, granulocytes and platelets.**

Mature red cells circulate for about 120 days. They are 8 μm biconcave discs lacking a nucleus but filled with haemoglobin, which delivers oxygen to the tissues from the lungs. In order to pass through the smallest capillaries the red cell membrane is adapted to be deformable, with a lipid bilayer to which a 'skeleton'

of filamentous proteins is attached via special linkage proteins (Fig. 1.4). [3]

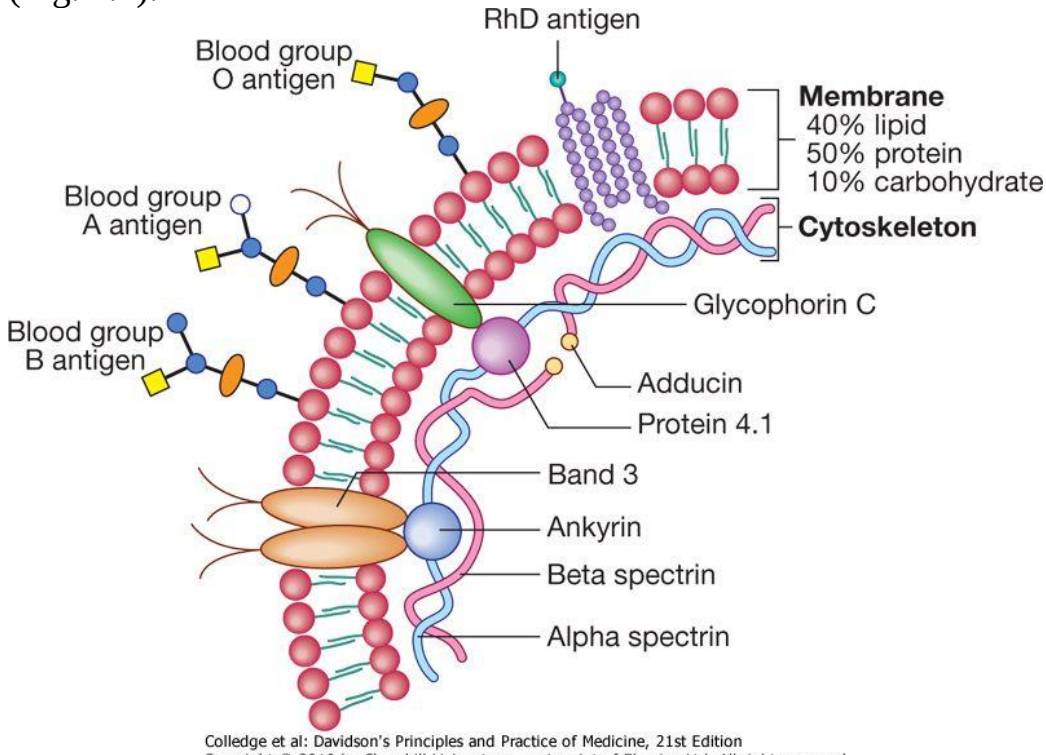


Figure 1.4 **Normal structure of red cell membrane.** Red cell membrane flexibility is conferred by attachment of cytoskeletal proteins. Important transmembrane proteins include band 3 (an ion transport channel) and glycophorin (involved in cytoskeletal attachment and gas exchange, and a receptor for *Plasmodium falciparum* in malaria). Antigens on the red blood cell determine an individual's blood group. There are about 22 blood group systems (groups of carbohydrate or protein antigens controlled by a single gene or by multiple closely linked loci); the most important clinically are the ABO and Rhesus (Rh) systems. The ABO genetic locus has three main allelic forms: A, B and O. The A and B alleles encode glycosyltransferases that introduce N-acetylgalactosamine (open circle) and D-galactose (blue circle), respectively, on to antigenic carbohydrate molecules on the membrane surface. People with the O allele produce an O antigen which lacks either of these added sugar groups. Rh antigens are transmembrane proteins.

Inherited abnormalities of any of these proteins result in loss of membrane as cells pass through the spleen, and the formation of abnormally shaped red cells called spherocytes or elliptocytes (Fig. 1.10). Red cells are exposed to osmotic stress in the pulmonary and renal circulation; to maintain homeostasis, the membrane contains ion pumps which control intracellular levels of sodium, potassium, chloride and bicarbonate. In the absence of mitochondria, the energy for these functions is provided by anaerobic glycolysis and the pentose phosphate pathway in the cytosol. Membrane glycoproteins inserted into the lipid bilayer also form the antigens recognised by blood grouping (Fig. 1.4). The ABO and Rhesus systems are the most commonly recognised, but over 400 blood group antigens have been described. (3)

Haemoglobin

Haemoglobin is a protein specially adapted for oxygen transport. It is composed of four globin chains, each surrounding an iron-containing porphyrin pigment molecule termed haem. Globin chains are a combination of two alpha and two non-alpha chains; haemoglobin A (αα/ββ) represents over 90% of adult haemoglobin, whereas haemoglobin F (αα/γγ) is the predominant type in the fetus. Each haem molecule contains a ferrous ion (Fe^{2+}) to which oxygen reversibly binds; the affinity for oxygen increases as successive oxygen molecules bind. When oxygen is bound, the beta chains 'swing' closer together; they move apart as oxygen is lost. In the 'open' deoxygenated state, 2,3 diphosphoglycerate (DPG), a product of red cell metabolism, binds to the haemoglobin molecule and lowers its oxygen affinity. These complex interactions produce the sigmoid shape of the oxygen dissociation curve (Fig. 1.5). The position of this curve depends upon the concentrations of 2,3 DPG, H^+ ions and CO_2; increased levels shift the curve to the right and cause oxygen to be released more readily, e.g. when red cells reach hypoxic tissues.

Haemoglobin F is unable to bind 2,3 DPG and has a left-shifted oxygen dissociation curve which, together with the low pH of fetal blood, ensures fetal oxygenation. [3]

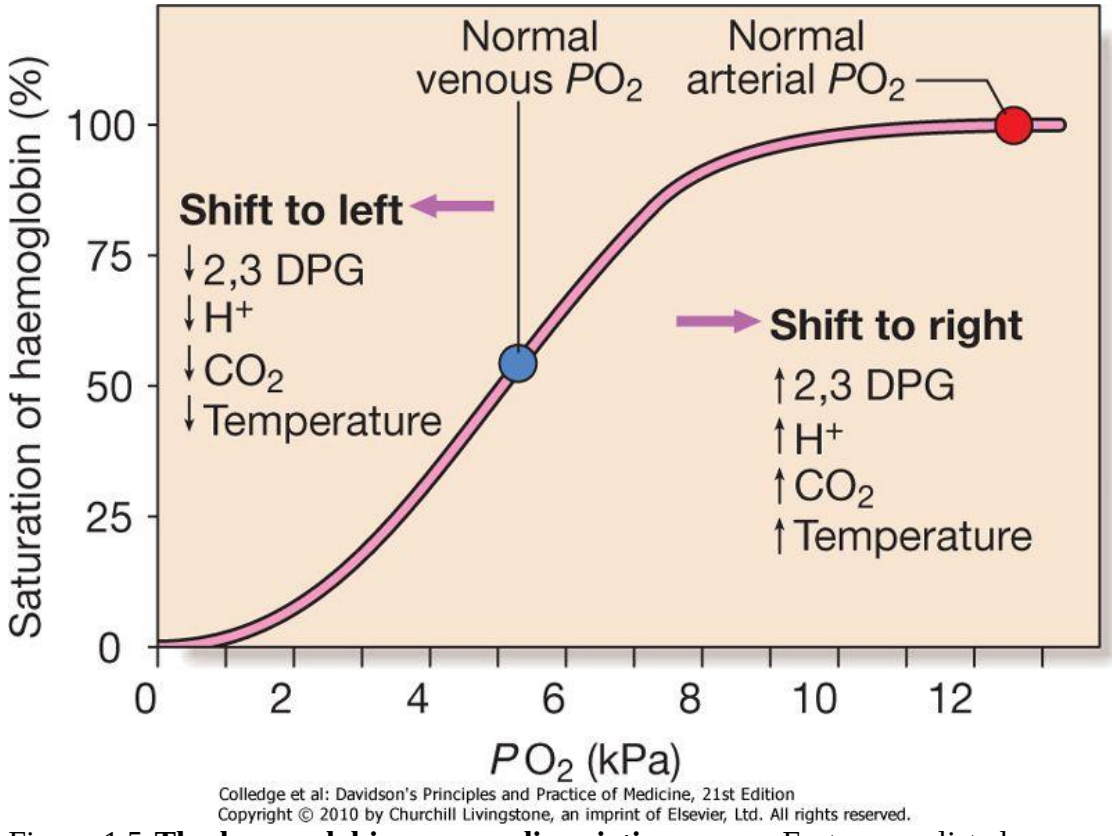


Figure 1.5 **The haemoglobin oxygen dissociation curve.** Factors are listed which shift the curve to the right (more oxygen released from blood) and to the left (less oxygen released) at given PO_2. (To convert kPa to mmHg, multiply by 7.5.)

Genetic mutations affecting the haem-binding pockets of globin chains or the 'hinge' interactions between globin chains result in haemoglobinopathies or unstable haemoglobins. Alpha globin chains are produced by two genes on chromosome 16 and beta globin chains by a single gene on chromosome 11; imbalance in

the production of globin chains produces the thalassaemias. Defects in haem synthesis cause the porphyrias. (3)

Destruction

Red cells at the end of their lifespan of approximately 120 days are phagocytosed by the reticulo-endothelial system. Amino acids from globin chains are recycled and iron is removed from haem for reuse in haemoglobin synthesis. The remnant haem structure is degraded to bilirubin and conjugated with glucuronic acid before being excreted in bile. In the small bowel, bilirubin is converted to stercobilin; most of this is excreted, but a small amount is reabsorbed and excreted by the kidney as urobilinogen. Increased red cell destruction due to haemolysis or ineffective haematopoiesis results in jaundice and increased urinary urobilinogen. Free intravascular haemoglobin is toxic and is normally bound by haptoglobins, which are plasma proteins produced by the liver. (3)

White cells

White cells or leucocytes in the blood consist of granulocytes (neutrophils, eosinophils and basophils), monocytes and lymphocytes. Granulocytes and monocytes are formed from bone marrow CFU-GM progenitor cells. The first recognisable granulocyte in the marrow is the myeloblast, a large cell with a small amount of basophilic cytoplasm and a primitive nucleus with open chromatin and nucleoli. As the cells divide and mature, the nucleus segments and the cytoplasm acquires specific neutrophilic, eosinophilic or basophilic granules (Fig. 1.3). This takes about 14 days. The cytokines G-CSF, GM-CSF and M-CSF are involved in the production of myeloid cells and G-CSF can be used clinically to hasten recovery of blood neutrophil counts after chemotherapy. (3)

Myelocytes or metamyelocytes are normally only found in the marrow but may appear in the circulation in infection or toxic

states. The appearance of more primitive myeloid precursors in the blood is often associated with the presence of nucleated red cells and is termed a 'leucoerythroblastic' picture; this indicates a serious disturbance of marrow function. (3)

Neutrophils

Neutrophils, the most common white blood cells in the blood of adults, are 10-14 μm in diameter with a multilobular nucleus containing 2-5 segments and granules in their cytoplasm. Their main function is to recognise, ingest and destroy foreign particles and microorganisms. A large storage pool of mature neutrophils exists in the bone marrow. Every day some 10^{11} neutrophils enter the circulation, where cells may be freely circulating or attached to endothelium in the marginating pool. These two pools are equal in size; factors such as exercise or catecholamines increase the number of cells flowing in the blood. Neutrophils spend 6-10 hours in the circulation before being removed, principally by the spleen. Alternatively, they pass into the tissues and either are consumed in the inflammatory process or undergo apoptotic cell death and phagocytosis by macrophages. (3)

Eosinophils

Eosinophils represent 1-6% of the circulating white cells. They are a similar size to neutrophils but have a bilobed nucleus and prominent orange granules on Romanowsky staining. Eosinophils are phagocytic and their granules contain a peroxidase capable of generating reactive oxygen species and proteins involved in the intracellular killing of protozoa and helminths. They are also involved in allergic reactions. (3)

Basophils

These cells are less common than eosinophils, representing less than 1% of circulating white cells. They contain dense black granules which obscure the nucleus. Mast cells resemble basophils

but are only found in the tissues. These cells are involved in hypersensitivity reactions. [3]

Monocytes

Monocytes are the largest of the white cells, with a diameter of 12-20 μm and an irregular nucleus in abundant pale blue cytoplasm containing occasional cytoplasmic vacuoles. These cells circulate for a few hours and then migrate into the tissue where they become macrophages, Kupffer cells or antigen-presenting dendritic cells. The former phagocytose debris, apoptotic cells and microorganisms. [3]

Lymphocytes

Lymphocytes are derived from pluripotent haematopoietic stem cells in the bone marrow. There are two main types: T cells (which mediate cellular immunity) and B cells (which mediate humoral immunity). Lymphoid cells which migrate to the thymus develop into T cells, whereas B cells develop in the bone marrow. [3]

The majority of lymphocytes (approximately 80%) in the circulation are T cells. Lymphocytes are heterogeneous, the smallest cells being the size of red cells and the largest being the size of neutrophils. Small lymphocytes are circular with scanty cytoplasm but the larger cells are more irregular with abundant blue cytoplasm. Lymphocyte subpopulations can be defined with specific functions and their lifespan can vary from a few days to many years. [3]

Haemostasis

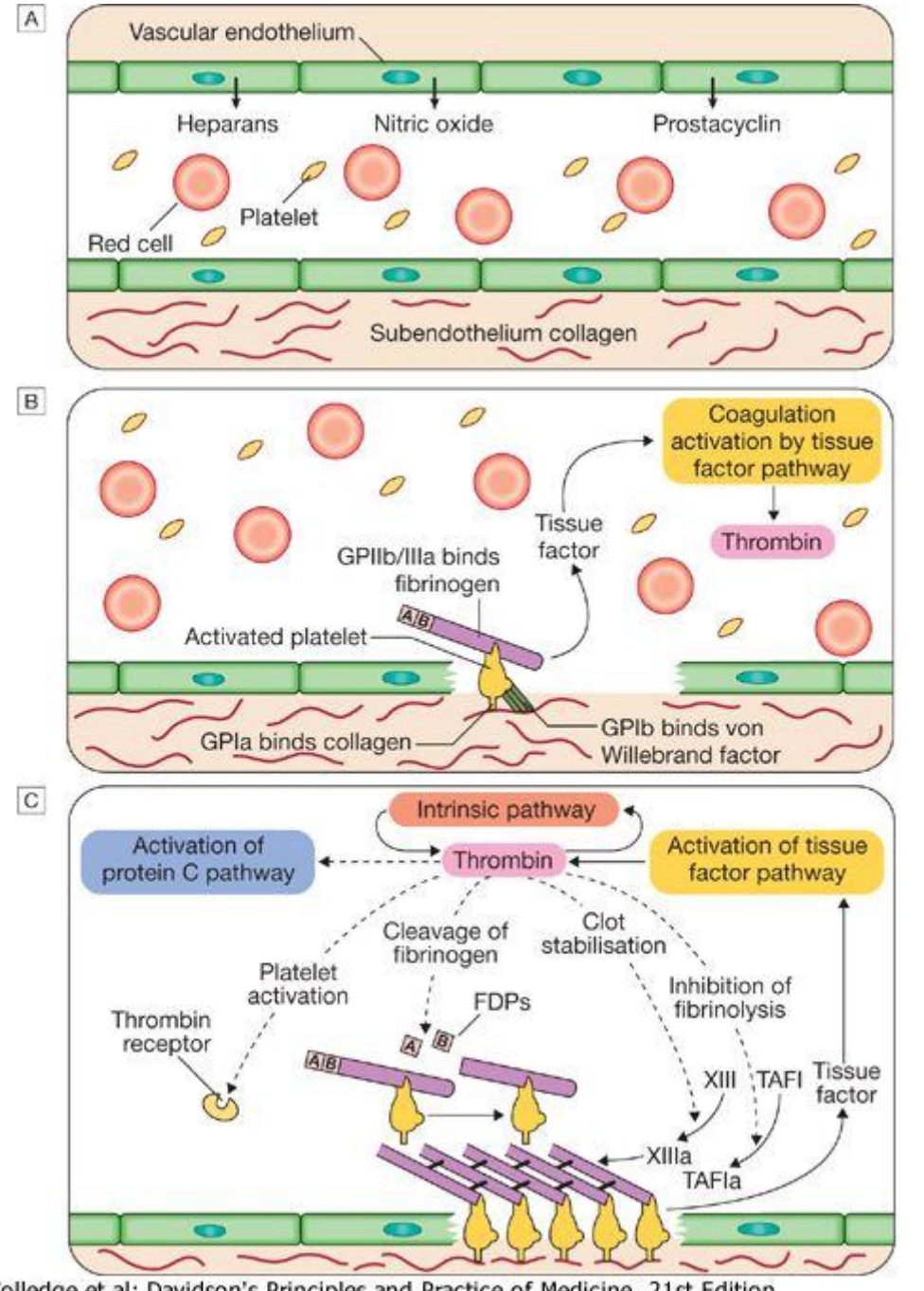
Blood must be maintained in a fluid state in order to function as a transport system, but must be able to solidify to form a clot following vascular injury to prevent excessive bleeding, a process known as haemostasis. Successful haemostasis is localised to the area of tissue damage and is followed by removal of the clot and tissue repair. This is achieved by complex interactions between

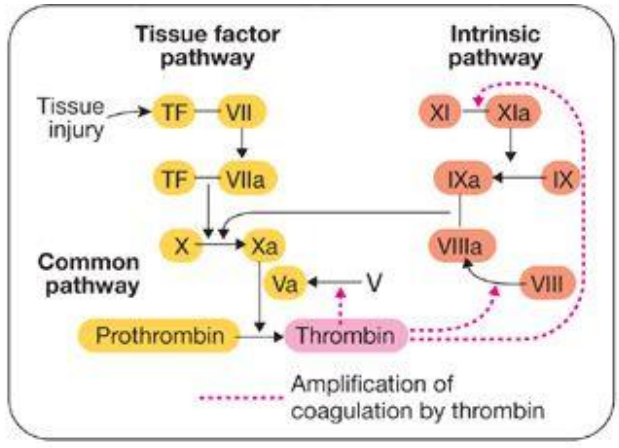
the vascular endothelium, platelets, coagulation factors, natural anticoagulants and fibrinolytic enzymes, as detailed in Figure 24.6. Dysfunction of any of these components may result in haemorrhage or thrombosis. (3)

Platelets

Platelets are formed in the bone marrow from megakaryocytes. Megakaryocytic stem cells (CFU-Meg) divide to form megakaryoblasts, which undergo a process called 'endomitotic reduplication', in which there is division of the nucleus but not the cell. This creates mature megakaryocytes, large cells with several nuclei and cytoplasm containing platelet granules. Up to 3000 platelets then fragment off from each megakaryocyte into the circulation in the marrow sinusoids. The formation and maturation of megakaryocytes are stimulated by thrombopoietin produced in the liver. Platelets circulate for 8-10 days before they are destroyed in the reticulo-endothelial system. Some 30% of peripheral platelets are normally pooled in the spleen and do not circulate. (3)

Under normal conditions platelets are discoid, with a diameter of 2-4 μm (Fig. 1.7). The surface membrane invaginates to form a tubular network, the canalicular system, which provides a conduit for the discharge of the granule content following platelet activation. Drugs which inhibit platelet function and thrombosis include aspirin (cyclo-oxygenase inhibitor), clopidogrel (inhibits adenosine diphosphate (ADP)-mediated activation), dipyridamole (inhibits phosphodiesterase), and the IIb/IIIa inhibitors abciximab, tirofiban and eptifibatide (prevent fibrinogen binding). (3)





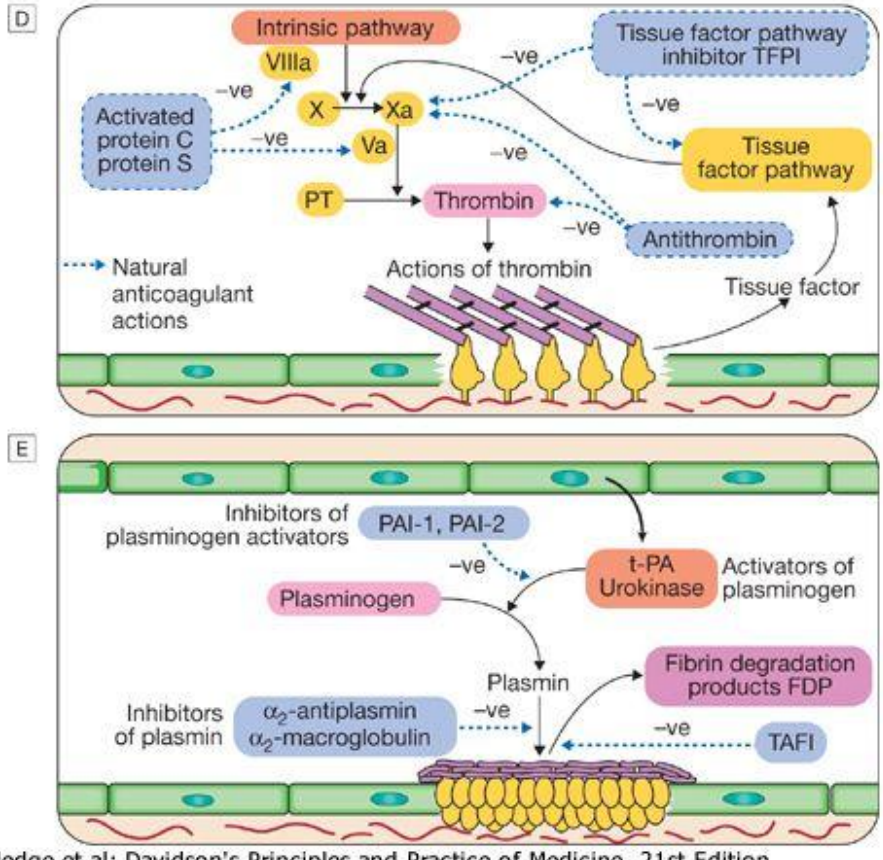


Figure 1.6 **The stages of normal haemostasis**

The stages of normal haemostasis.[1]

[1] **A** *Stage 1 Pre-injury conditions encourage flow* The vascular endothelium produces substances (including nitric oxide, prostacyclin and heparans) to prevent adhesion of platelets and white cells to the vessel wall. Platelets and coagulation factors circulate in a non-activated state.
B *Stage 2 Early haemostatic response: platelets adhere; coagulation is activated*. At the site of injury the endothelium is breached, exposing subendothelial collagen. Small amounts of tissue factor (TF) are released. Platelets bind to collagen via a specific receptor, glycoprotein Ia (GPIa), causing a change in platelet shape and its adhesion to the area of damage by the binding of other receptors (GPIb and GPIIb/IIIa) to von Willebrand factor and fibrinogen respectively. Coagulation is activated by the tissue factor (extrinsic) pathway, generating small amounts of thrombin.
C *Stage 3 Fibrin clot formation: platelets become activated and aggregate; fibrin formation is supported by the platelet membrane; stable fibrin clot forms*. The adherent platelets are activated by many pathways, including binding of ADP, collagen, thrombin and adrenaline (epinephrine) to surface receptors. The cyclo-oxygenase pathway converts arachidonic acid from the platelet membrane into thromboxane A_2, which causes aggregation of platelets. Activation of the platelets results in release of the platelet granule contents, enhancing coagulation further (see Fig. 1.7). Thrombin plays a key role in the control of coagulation: the small amount generated via the TF pathway massively amplifies its own production; the 'intrinsic' pathway becomes activated and large amounts of thrombin are generated. Thrombin enhances clot formation by cleaving fibrinogen to produce fibrin. Fibrin monomers are cross-linked by factor XIII, which is also activated by thrombin. Having had a key role in clot formation and stabilisation, thrombin then starts to regulate clot formation in two main ways: (a) activation of the protein C (PC) pathway (a natural anticoagulant), which reduces further coagulation; (b) activation of thrombin-activatable fibrinolysis inhibitor (TAFI), which inhibits fibrinolysis (see D and E).
D *Stage 4 Limiting clot formation: natural anticoagulants reverse activation of coagulation factors*. Once haemostasis has been secured, the propagation of clot is curtailed by anticoagulants. Antithrombin is a serine protease inhibitor synthesised by the liver, which destroys activated factors such as XIa, Xa and thrombin (IIa). Its major activity against thrombin and Xa is enhanced by heparin and fondaparinux, explaining their anticoagulant effect. Tissue factor pathway inhibitor (TFPI) binds to and inactivates VIIa and Xa. Activation of PC occurs following binding of thrombin to membrane-bound thrombomodulin; activated protein C (aPC) binds to its co-factor protein S (PS), and cleaves Va and VIIIa. PC and PS are vitamin K-dependent and are depleted by coumarin anticoagulants such as warfarin.
E *Stage 5 Fibrinolysis: plasmin degrades fibrin to allow vessel recanalisation and*

Clotting factors

The coagulation system consists of a cascade of soluble inactive zymogen proteins designated by Roman numerals. When proteolytically cleaved and activated, each is capable of activating one or more components of the cascade. Activated factors are designated by the suffix 'a'. Some of these reactions require phospholipid and calcium. Coagulation occurs by two pathways; it is initiated by the extrinsic (or tissue factor) pathway and amplified by the intrinsic pathway (Fig. 1.6). [3]

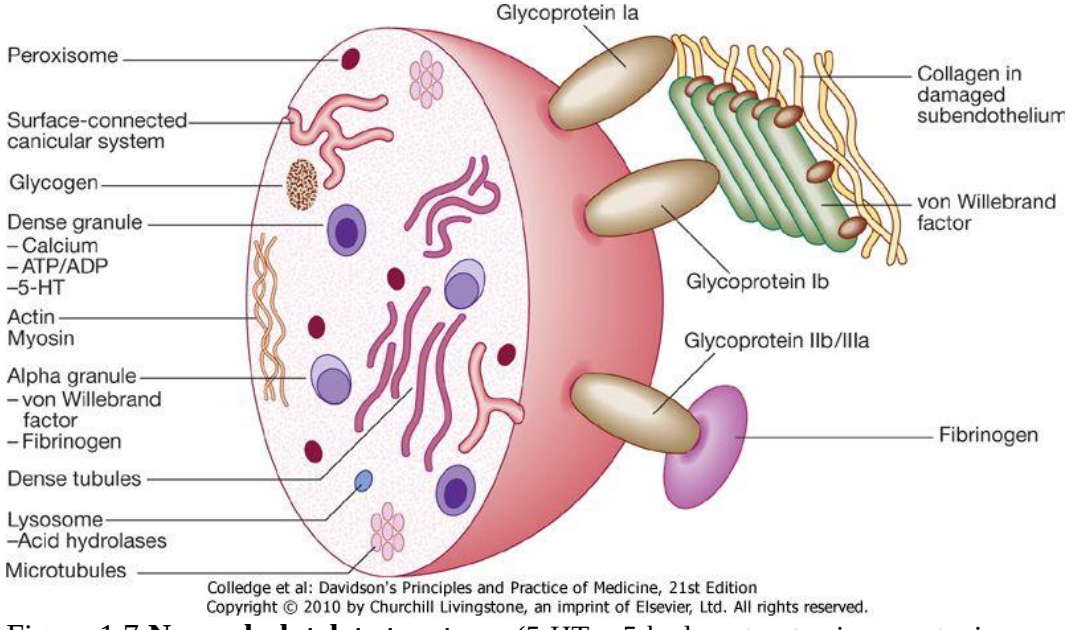


Figure 1.7 **Normal platelet structure.** (5-HT = 5-hydroxytryptamine, serotonin; ADP = adenosine diphosphate; ATP = adenosine triphosphate)

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tissue repair. The insoluble clot needs to be broken down for vessel recanalisation. Plasmin, the main fibrinolytic enzyme, is produced when plasminogen is activated, e.g. by tissue plasminogen activator (t-PA) or urokinase in the clot. Plasmin hydrolyses the fibrin clot, producing fibrin degradation products including the D-dimer. This process is highly regulated; the plasminogen activators are controlled by an inhibitor called plasminogen activator inhibitor (PAI), the activity of plasmin is inhibited by α_2-antiplasmin and α_2-macroglobulin, and fibrinolysis is further inhibited by the thrombin-activated TAFI. [3]

Clotting factors are synthesised by the liver, although factor V is also produced by platelets and endothelial cells. Factors II, VII, IX and X require post-translational carboxylation to allow them to participate in coagulation. The carboxylase enzyme responsible for this in the liver is vitamin K-dependent. Vitamin K is converted to an epoxide in this reaction and must be reduced to its active form by a reductase enzyme. This reductase is inhibited by warfarin, and this is the basis of the anticoagulant effect of coumarins. Congenital (e.g. haemophilia) and acquired (e.g. liver failure) causes of coagulation factor deficiency are associated with bleeding. [3]

Investigation of blood diseases

The full blood count (FBC)

To obtain an FBC, anticoagulated blood is processed through automatic blood analysers which use a variety of technologies (particle-sizing, radiofrequency and laser instrumentation) to measure the haematological parameters. These include numbers of circulating cells and platelets, the proportion of whole blood volume occupied by red cells (the haematocrit, Hct), and the red cell indices which give information about the size of red cells (mean cell volume, MCV) and the amount of haemoglobin present in the red cells (mean cell haemoglobin, MCH). Modern blood analysers can differentiate types of white blood cell and give automated counts of neutrophils, lymphocytes, monocytes, eosinophils and basophils. It is important to appreciate, however, that a number of conditions can lead to spurious results (Table 1.1). [3]

1.1 **Spurious FBC results from autoanalysers** [3]	
Result	**Explanation**
Increased haemoglobin	Lipaemia, jaundice, very high white cell count
Reduced haemoglobin	Improper sample mixing, blood taken from vein into which an infusion is flowing
Increased red cell volume (MCV)	Cold agglutinins, non-ketotic hyperosmolarity
Increased white cell count	Nucleated red cells present
Reduced platelet count	Clot in sample, platelet clumping

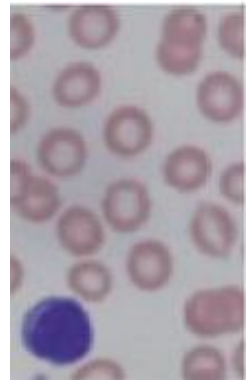
Blood film examination

Although the technical advances of modern full blood count analysers have resulted in fewer blood samples requiring manual examination, scrutiny of blood components prepared on a microscope slide (the 'blood film') can often yield invaluable information. Analysers cannot identify abnormalities of red cell shape and content (e.g. Howell-Jolly bodies, basophilic stippling, and malaria parasites) or fully define abnormal white cells such as blasts (Table 1.2). [3]

How to interpret red cell appearances

Microcytosis (reduced average cell size, MCV < 76 fL)

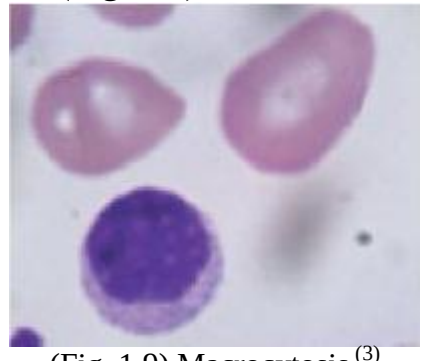
- Iron deficiency
- Thalassaemia
- Sideroblastic anaemia
- Anemia of inflammation
- Rarely, lead poisoning
- Vitamin B_6 deficiency (Fig. 1.8) [5 & 3]



(Fig. 1.8) Microcytosis [3]

Macrocytosis (increased average cell size, MCV > 100 fL)

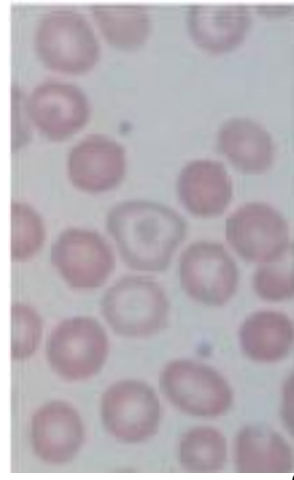
- Vitamin B_{12} or folate deficiency
- Liver disease, alcohol
- Hypothyroidism
- Drugs (e.g. zidovudine)
- Polychromatophilia (reticulocytes)
- Myelodysplasia (Fig. 1.9) [5 & 3]



(Fig. 1.9) Macrocytosis [3]

Target cells (central area of haemoglobinisation)

- Liver disease
- Thalassaemia
- Post-splenectomy
- Haemoglobins C, D, E, and S disease
- Abetalipoproteinemia (Fig. 1.10) [5 & 3]



(Fig. 1.10) Target cells [3]

Spherocytes (dense cells, no area of central pallor)

- Autoimmune haemolysis
- Post-splenectomy
- Hereditary spherocytosis (Fig. 1.11) [3]

(Fig. 1.11) Spherocytes [3]

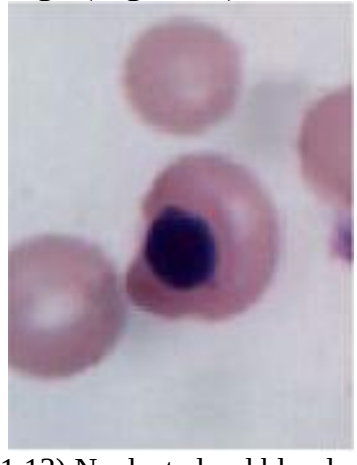
Red cell fragments (intravascular haemolysis)

- Disseminated intravascular coagulation (DIC)
- Haemolytic uraemic syndrome (HUS)
- Thrombotic thrombocytopenic purpura (TTP) (Fig. 1.12) [3]

(Fig. 1.12) Red cell fragments [3]

Nucleated red blood cells (normoblasts)

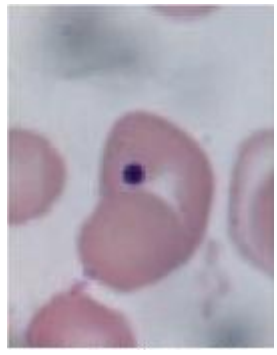
- Marrow infiltration
- Severe haemolysis
- Myelofibrosis
- Acute haemorrhage (Fig. 1.13) [3]



(Fig. 1.13) Nucleated red blood cells [3]

Howell-Jolly bodies (small round nuclear remnants)

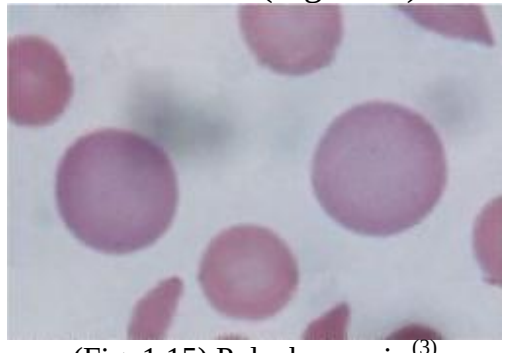
- Hyposplenism
- Post-splenectomy
- Dyshaematopoiesis (Fig. 1.14) [3]



(Fig. 1.14) Howell-Jolly bodies [3]

Polychromasia (young red cells-reticulocytes present)

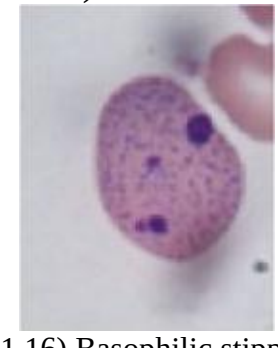
- Haemolysis, acute haemorrhage
- Increased red cell turnover (Fig. 1.15) [3]



(Fig. 1.15) Polychromasia [3]

Basophilic stippling (abnormal ribosomes appear as blue dots)

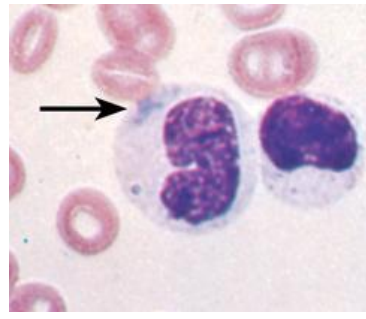
- Dyshaematopoiesis
- Lead poisoning (Fig. 1.16) [3]



(Fig. 1.16) Basophilic stippling [3]

Neutrophil band with Döhle body They represent aggregates of rough endoplasmic reticulum. They found in the periphery of the cytoplasm of the neutrophil in:

- Infections
- Other toxic states (Fig. 1.17) [10]



(Fig. 1.17) **Neutrophil band with Döhle body**. The neutrophil with a sausage-shaped nucleus in the center of the field is a band form. Döhle bodies are discrete, blue-staining nongranular areas found in the periphery of the cytoplasm. [10]

Bone marrow examination

In adults bone marrow for examination is usually obtained from the posterior iliac crest. After a local anaesthetic, marrow may be sucked out from the medullary space, stained and examined under the microscope (bone marrow aspirate). In addition, a core of bone may be removed (trephine biopsy), fixed and decalcified before sections are cut for staining (Fig. 1.18). A bone marrow aspirate is used to assess the composition and morphology of haematopoietic cells or abnormal infiltrates. Further investigations may be performed, such as cell surface marker analysis (immunophenotyping), chromosome and molecular studies to assess malignant disease, or marrow culture for suspected tuberculosis. A trephine biopsy is superior for assessing marrow cellularity, marrow fibrosis, and infiltration by abnormal cells

such as metastatic carcinoma. Bone marrow aspiration can usually be performed safely in a thrombocytopenic patient. [3]

Table 1.2 **Red blood cell morphologic abnormalities as clues to diagnosis of anemias**

Red Blood Cell Morphology	**Representative Causes of Anemia**
Microcytosis	Iron deficiency, anemia of inflammation, thalassemia, and rarely, lead poisoning, vitamin B_6 deficiency, or hereditary sideroblastic anemias
Macrocytosis	Polychromatophilia (reticulocytes), vitamin B_{12} (cobalamin) or folate deficiency, myelodysplasia, use of drugs that inhibit DNA synthesis
Basophilic stippling	Hemolysis, lead poisoning, thalassemia
Target cells	Thalassemia; hemoglobins C, D, E, and S; liver disease; abetalipoproteinemia
Microspherocytes	Autoimmune hemolytic anemia, alloimmune hemolysis, hereditary spherocytosis, some cases of Heinz body hemolytic anemias
Schistocytes and fragmented RBCs	Thrombotic thrombocytopenic purpura, disseminated intravascular coagulation, vasculitis, malignant hypertension, eclampsia, traumatic hemolysis secondary to a prosthetic heart valve or damaged vascular graft, thermal injury (burns), post-splenectomy status
Teardrop cells	Myelofibrosis, myelophthisis (bone marrow infiltration by neoplastic cells)
Sickle cells	Hemoglobin SS, SC, or S–β-thalassemia
Acanthocytes (spur cells)	Severe liver disease, malnutrition, McLeod blood group phenotype
Echinocytes (burr cells)	Renal failure, hemolysis from malnutrition with hypomagnesemia and hypophosphatemia, pyruvate kinase deficiency, common in vitro artifact

Red Blood Cell Morphology	Representative Causes of Anemia
Stomatocytes	Alcoholism, hereditary stomatocytosis
"Bite" cells or "blister" cells	Glucose-6-phosphate dehydrogenase deficiency, other oxidant-induced hemolysis, unstable hemoglobins
Howell-Jolly bodies	Post-splenectomy status, hyposplenism
Intraerythrocytic parasitic or bacterial inclusions	Malaria (parasites), babesiosis (parasites), bartonellosis (gram-negative coccobacilli)
Agglutinated RBCs	Cold agglutinin disease, in vitro artifact
Rouleaux formation	Multiple myeloma, monoclonal gammopathy of undetermined significance

Microscopic examination of the morphology of RBCs in a peripheral blood smear is an essential part of the evaluation of defective production and excessive destruction of RBCs [5]

Investigation of coagulation

Bleeding disorders

In patients with clinical evidence of a bleeding disorder, there are recommended screening tests.

Coagulation tests measure the time to clot formation in vitro in a plasma sample after the clotting process is initiated by activators and calcium. The result of the test sample is compared with normal controls. The extrinsic pathway is assessed by the prothrombin time (PT) and the intrinsic pathway by the activated partial thromboplastin time (APTT), sometimes known as the partial thromboplastin time with kaolin, (PTTK). Clotting is delayed by deficiencies of coagulation factors and the presence of inhibitors of coagulation, e.g. heparin. The approximate normal ranges and causes of abnormalities are shown in Box 24.3. If both the PT and APTT are prolonged, there is deficiency or inhibition

of the final common pathway which includes factors X, V, prothrombin and fibrinogen, or global coagulation factor deficiency involving more than one factor. Further specific tests may be performed based on interpretation of the clinical scenario and results of these screening tests. (3)

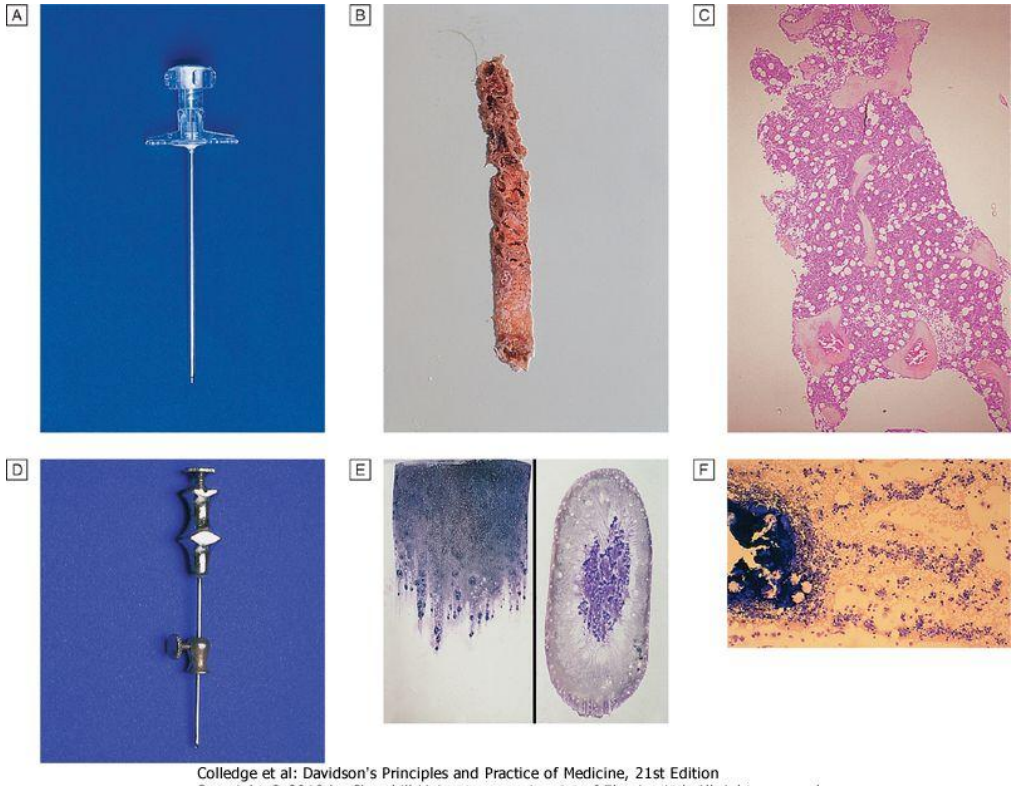


Figure 1.18 **Bone marrow aspirate and trephine**.
A Trephine biopsy needle.
B Macroscopic appearance of a trephine biopsy.
C Microscopic appearance of stained section of trephine.
D Bone marrow aspirate needle.
E Stained macroscopic appearance of marrow aspirate: smear (left) and quash (right).
F Microscopic appearance of stained marrow particles and trails of haematopoietic cells.

A mixing test with normal plasma allows differentiation between a coagulation factor deficiency (the prolonged time corrects) and the presence of an inhibitor of coagulation (the prolonged time does not correct); the latter may be chemical (heparins) or an antibody (most often a lupus anticoagulant but occasionally a specific inhibitor of one of the coagulation factors, typically factor VIII). Von Willebrand disease may present with a normal APTT. (3)

1.3 **Coagulation screening tests**

Investigation	**Normal range**	**Situations in which tests may be abnormal**
Platelet count	150-400 × 10^9/L	Thrombocytopenia
Bleeding time	< 8 mins	Thrombocytopenia
		Abnormal platelet function
		von Willebrand disease
		Vascular and connective tissue abnormalities
Prothrombin time (PT)	9-12 secs	Deficiencies of factors II, V, VII or X Severe fibrinogen deficiency
Activated partial thromboplastin time (APTT)	26-36 secs	Deficiencies of factors II, V, VIII, IX, X, XI, XII Severe fibrinogen deficiency Unfractionated heparin therapy Antibodies against clotting factors Lupus anticoagulant
Fibrinogen concentration	1.5-4.0 g/L	Hypofibrinogenaemia, e.g. liver failure, disseminated intravascular coagulation

N.B. International normalised ratio (INR) is used only to monitor coumarin therapy and is not a coagulation screening test. (3)

Platelet function has historically been assessed by the bleeding time, using a standardised incision. However, many centres have

abandoned this test as it is non-specific, being affected by the coagulation factor disorders shown in Table 1.3. Platelet function can be assessed in vitro by measuring aggregation in response to various agonists such as adrenaline (epinephrine), collagen, thrombin or ADP, or by measuring the constituents of the intracellular granules, e.g. ATP/ADP. (3)

Coagulation screening tests are also performed in patients with suspected disseminated intravascular coagulation (DIC) when clotting factors and platelets are consumed, resulting in thrombocytopenia and prolonged PT and APTT. In addition, there is evidence of active coagulation with consumption of fibrinogen and generation of fibrin degradation products (D-dimers). Note, however, that fibrinogen is an acute phase protein which may also be elevated in inflammatory disease. (3)

Monitoring anticoagulant therapy

The international normalised ratio (INR) is validated only to assess the therapeutic effect of coumarin anticoagulants, including warfarin. INR is the ratio of the patient's prothrombin time to that of a normal control, raised to the power of the international sensitivity index of the thromboplastin used in the test (ISI, derived by comparison with an international reference standard material).

Monitoring of heparin therapy is on the whole only required with unfractionated heparins. Therapeutic anticoagulation prolongs the APTT relative to a control sample by a ratio of ~1.5-2.5. Low molecular weight heparins have such a predictable dose response that monitoring of the anticoagulant effect is not required, except in patients with renal impairment (GFR < 30 mL/min). (3)

Thrombotic disorders

Measurement of plasma levels of D-dimers derived from fibrin degradation is useful in excluding the diagnosis of active thrombosis in some patients.

A variety of tests exist which may help to explain an underlying propensity to thrombosis, especially venous thromboembolism. Examples of indications for testing are given in. In most patients, the results do not affect clinical management but they may influence the duration and intensity of anticoagulation (e.g. antiphospholipid antibodies, justify family screening in inherited thrombophilias, or suggest additional management strategies to reduce thrombosis risk. Anticoagulants can interfere with some of these assays; for example, warfarin reduces protein C and S levels and affects measurement of lupus anticoagulant, while heparin interferes with antithrombin and lupus anticoagulant assays. [3]

Investigation of possible thrombophilia

Full blood count

Plasma levels

- Antithrombin
- Protein C
- Protein S (free)
- Antiphospholipid antibodies/lupus anticoagulant and anticardiolipin antibody

Thrombin/reptilase time (for dysfibrinogenaemia) Genetic testing

- Factor V Leiden
- Prothrombin G20210A
- *JAK-2* mutation

Flow cytometry

- Screen for GPI-linked cell surface proteins (CD 14, 16, 55, 59), deficient in paroxysmal nocturnal haemoglobinuria

Indications for thrombophilia testing*

- Venous thrombosis < 45 years
- Recurrent venous thrombosis
- Family history of unprovoked or recurrent thrombosis

- Combined arterial and venous thrombosis
- Venous thrombosis at an unusual site
    - Cerebral venous thrombosis
    - Hepatic vein (Budd-Chiari syndrome)
    - Portal vein, mesenteric vein [3]

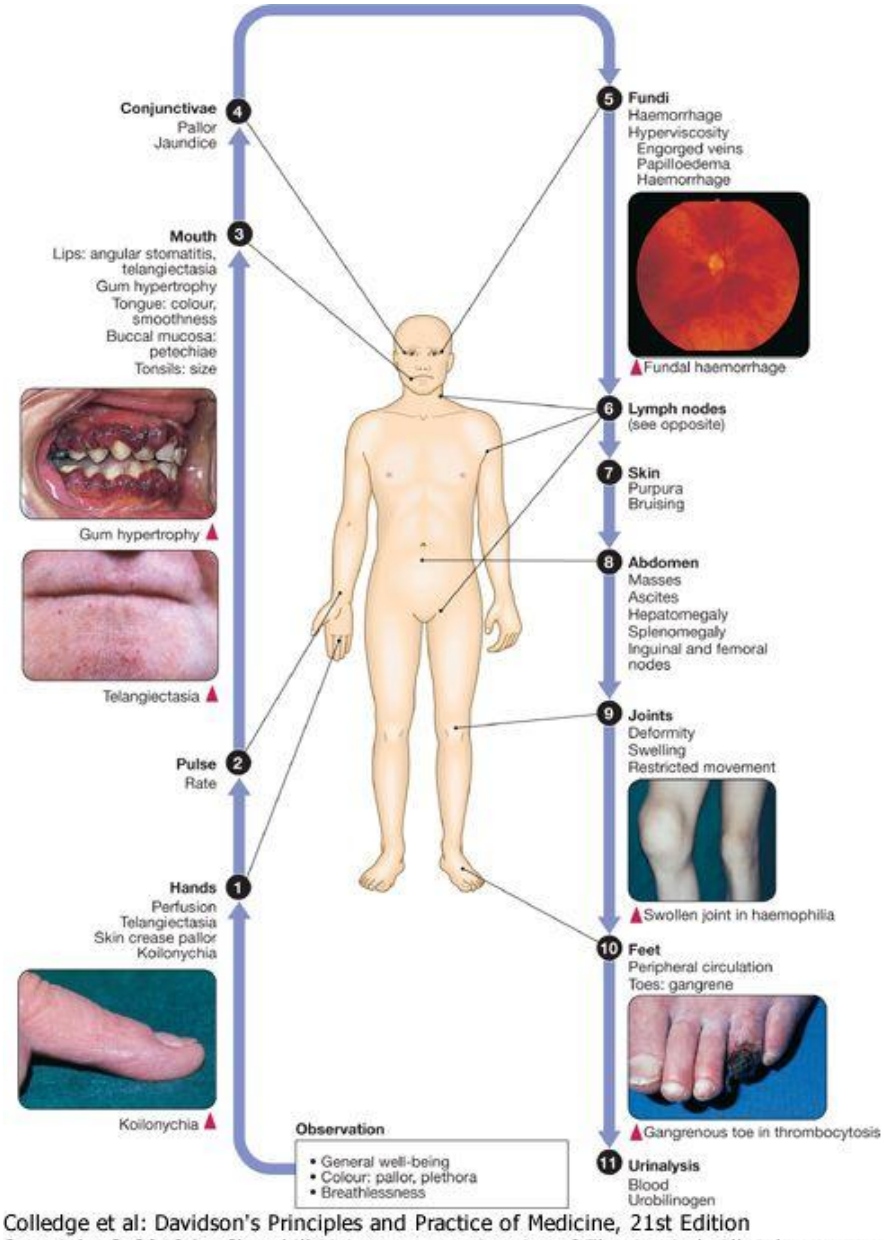
Haematological investigations in old age

Blood cell counts and film components: not altered by ageing alone.

- Ratio of bone marrow cells to marrow fat: falls.
- Neutrophils: maintained throughout life, although leucocytes may be less readily mobilised by bacterial invasion in old age.
- Lymphocytes: functionally compromised by age due to a T cell-related defect in cell-mediated immunity.
- Clotting factors: no major changes, although mild congenital deficiencies may be first noticed in old age.
- Erythrocyte sedimentation rate (ESR): raised above the normal range, but usually in association with chronic or subacute disease. In truly healthy older people the ESR range is very similar to that in younger people. [3]

Clinical examination in blood disease

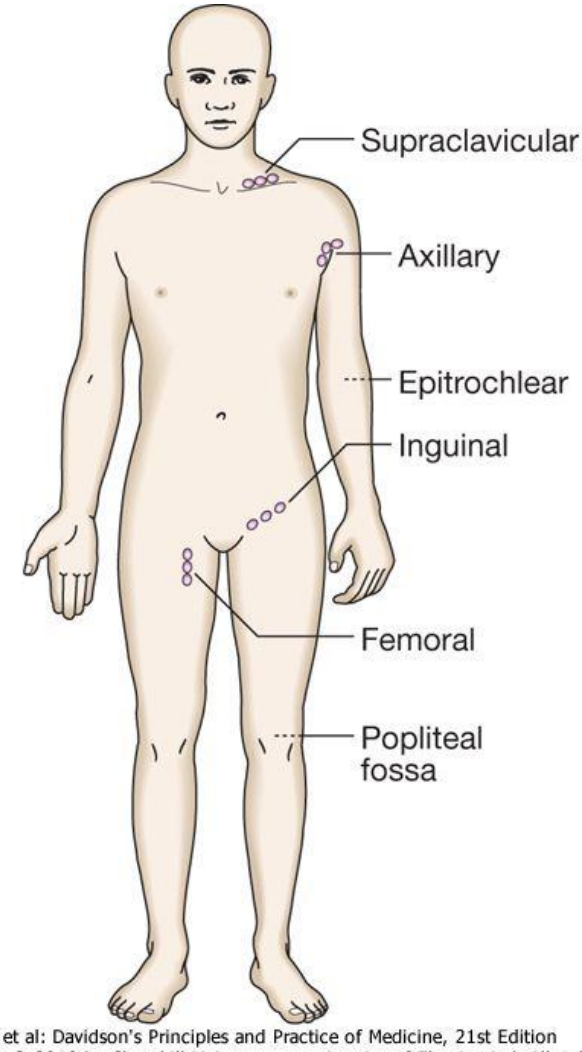
Abnormalities detected in the blood are caused not only by primary diseases of the blood and lymphoreticular systems, but also by diseases affecting other systems of the body. The clinical assessment of patients with haematological abnormalities must include a general history and examination, as well as a search for symptoms and signs of abnormalities of red cells, white cells, platelets, bleeding and clotting systems, lymph nodes and lymphoreticular tissues. [3]



(Fig. 1.19) **Clinical examination in blood disease**

Lymphadenopathy

Lymphadenopathy can be caused by benign or malignant disease.



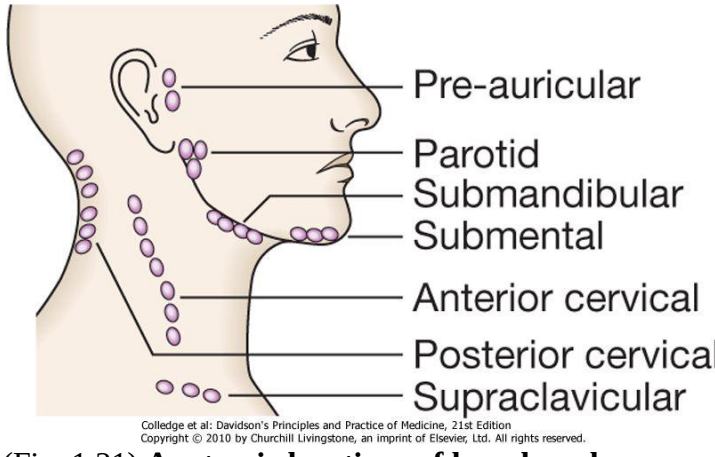
(Fig. 1.20) **Lymphadenopathy**

History

- Speed of onset, rate of enlargement
- Painful or painless
- Associated symptoms: weight loss, night sweats, itch

Examination

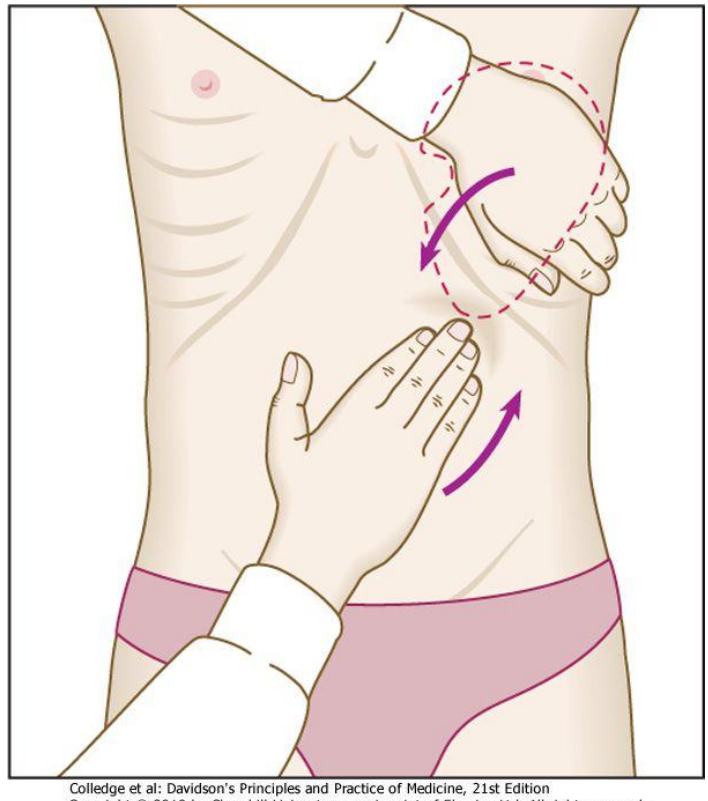
- Sites: localised, generalised
- Size (cm)
- Character: hard, soft, rubbery
- Fixed, mobile
- Search area that node drains for abnormalities (e.g. tooth abscess)
- Other general examination (e.g. joints, rashes, finger clubbing) (3)



(Fig. 1.21) **Anatomic locations of lymph nodes**

Examination of the spleen

- Move hand up from right iliac fossa, towards left upper quadrant on expiration.
- Keep hand still and ask patient to take a deep breath through the mouth to feel spleen edge being displaced downwards.
- Place your left hand around patient's lower ribs and approach costal margin to pull spleen forward.
- To help palpate small spleens, roll patient on to the right side and examine as before (Fig. 1.22). (3)



(Fig. 1.22) **Examination of the spleen**

Characteristics of the spleen

- Notch
- Superficial
- Dull to percussion
- Cannot get between ribs and spleen
- Moves well with respiration [3]

Anaemia

The box shows the symptoms and signs that will help to indicate the clinical severity of anaemia. A full history and examination is needed to identify clues to the underlying cause.

Non-specific symptoms Anaemia

- Tiredness
- Lightheadedness
- Breathlessness
- Ankle-swelling
- Development/worsening of ischaemic symptoms e.g. angina or claudication

Non-specific signs

- Mucous membrane pallor
- Tachypnoea
- Raised jugular venous pressure
- Flow murmurs
- Ankle oedema
- Postural hypotension
- Tachycardia [3]

Bleeding

Bleeding can be due to congenital or acquired abnormalities in components of the clotting system. The history and examination will help to clarify the severity and underlying cause of the bleeding problem. [3]

History of bleeding

- Site of bleed
- Duration of bleed
- Precipitating causes, including previous surgery or trauma
- Family history
- Drug history
- Other medical conditions, e.g. liver disease [3]

Examination

There are two major patterns of bleeding:

1. Mucosal bleeding

Reduced number or function of platelets (e.g. bone marrow failure or aspirin) or von Willebrand factor (e.g. von Willebrand disease)

- Skin: petechiae, bruises, post-surgical bleeding
- Gum and mucous membrane bleeding
- Fundal haemorrhage

2. Coagulation factor deficiency (e.g. haemophilia or warfarin)

- Bleeding into joints (haemarthrosis) or muscles
- Bleeding into soft tissues
- Intracranial haemorrhage
- Post-surgical bleeding [3]

Disorders of the blood cover a wide spectrum of illnesses, ranging from some of the most common disorders affecting mankind (anaemias), to relatively rare conditions such as leukaemias and congenital coagulation disorders. Although the latter are uncommon, advances in cellular and molecular biology have had major impacts on their diagnosis, treatment and prognosis. Haematological changes occur as a consequence of diseases affecting any system and give important information in the diagnosis and monitoring of many conditions. [3]

Chapter two

Disorders of erethrocytes

- *Anemia*
- *Iron deficiency anaemia*
- *Megaloblastic anaemia*
- *Haemolytic anaemia*
- *Bone marrow failure*
- *Aplastic anaemia*
- *Polycythemia*
- *Polycythemia Vera*

Anaemias

Definition

Anaemia is usually defined clinically as a reduction of the haemoglobin concentration to less than 12 g/dl (females) or less than 13 g/dl (males).[1] (12)

It has been very difficult to produce an adequate definition of anaemia. 'Normal' haematological values vary with:

- Age
- Between sexes
- At different altitudes and
- Possibly, between races

On the other hand, it is helpful to have a standard set of haemoglobin levels at different ages below which 'anaemia' is defined. The World Health Organization (WHO) have attempted to set out criteria of these kind, summarized in Table 2.1. (12)

Table 2.1 **Definition of haemoglobin levels below which anaemia is said to exist in populations at sea level (WHO 1968)**(12)

	Haemoglobin (g/dl) below
Children, 6 months–6 years	11.0
Children, 6–14 years	12.0
Adult males	13.0
Adult females (nonpregnant)	12.0
Adult females (pregnant)	11.0

[1] **Anemia** is defined as a decrease in circulating red blood cell (RBC) mass; the usual criteria being hemoglobin (Hb) < 12 g/dL or hematocrit (Hct) < 36% for women and Hb < 14 g/dL or Hct < 41% in men. (W) A functional definition of anaemia is 'a state in which the circulating red-cell mass is insufficient to meet the oxygen requirements of the tissues'.(12)

Anemia is defined as a reduction in the number of circulating erythrocytes (Ce)

Anemia is present in adults if the hematocrit is < 41% (hemoglobin < 13.5 g/dL) in males or < 36% (hemoglobin < 12 g/dL) in females. (5)

Classification

On the base of etiology

Anemia can be broadly classified into three etiologic groups:

1. Blood loss (hemorrhage)
   - Acute or
   - Chronic
2. Decreased RBC production
   - Iron deficiency
   - Vitamin B12 deficiency
   - Folate deficiency
3. Increased RBC destruction (hemolysis) Table 2.2.[4]

Table 2.2 **Classification of anemias by pathophysiology.**[11]
Decreased production
1. Hemoglobin synthesis lesion: iron deficiency, thalassemia, anemia of chronic disease
2. DNA synthesis lesion: megaloblastic anemia
3. Stem cell lesion: aplastic anemia, myeloproliferative leukemia
4. Bone marrow infiltration: carcinoma, lymphoma
5. Immune-mediated inhibition: aplastic anemia, pure red cell aplasia
Increased destruction
1. Acute blood loss
2. Hemolysis (intrinsic)
• Membrane lesion: hereditary spherocytosis, elliptocytosis
• Hemoglobin lesion: sickle cell, unstable hemoglobin
• Glycolysis: pyruvate kinase deficiency, etc
• Oxidation lesion: glucose-6-phosphate dehydrogenase deficiency
3. Hemolysis (extrinsic)
• Immune: warm antibody, cold antibody
• Microangiopathic: thrombotic thrombocytopenic purpura, hemolytic-uremic syndrome, mechanical cardiac valve, paravalvular leak
• Infection: Clostridium perfringens, malaria
• Hypersplenism

By mean cell volume

Classification of anemias by mean cell volume (Table2.3)

1. Microcytic (Figure 2.1)
   - Iron deficiency
   - Thalassemia
   - Anemia of chronic disease
2. Macrocytic (Megaloblastic)
   - Vitamin B12 deficiency
   - Folate deficiency
3. Normocytic
   - Kidney disease
   - Acute heamorrhage
   - Heamolysis [11]

Table 2.3 **Classification of anemias by mean cell volume**.[11]

1. Microcytic
   - Iron deficiency
   - Thalassemia
   - Anemia of chronic disease
   - Lead toxicity
2. Macrocytic
   - Megaloblastic
     a. Vitamin B12 deficiency
     b. Folate deficiency
     c. DNA synthesis inhibitors
   - Nonmegaloblastic
     a. Myelodysplasia, chemotherapy
     b. Liver disease
     c. Increased reticulocytosis
     d. Myxedema
     e. Bone marrow failure state
     (eg, aplastic anemia, marrow infiltrative disorder, etc.)
3. Normocytic
   - Kidney disease
   - Mild form of most acquired etiologies of anemia

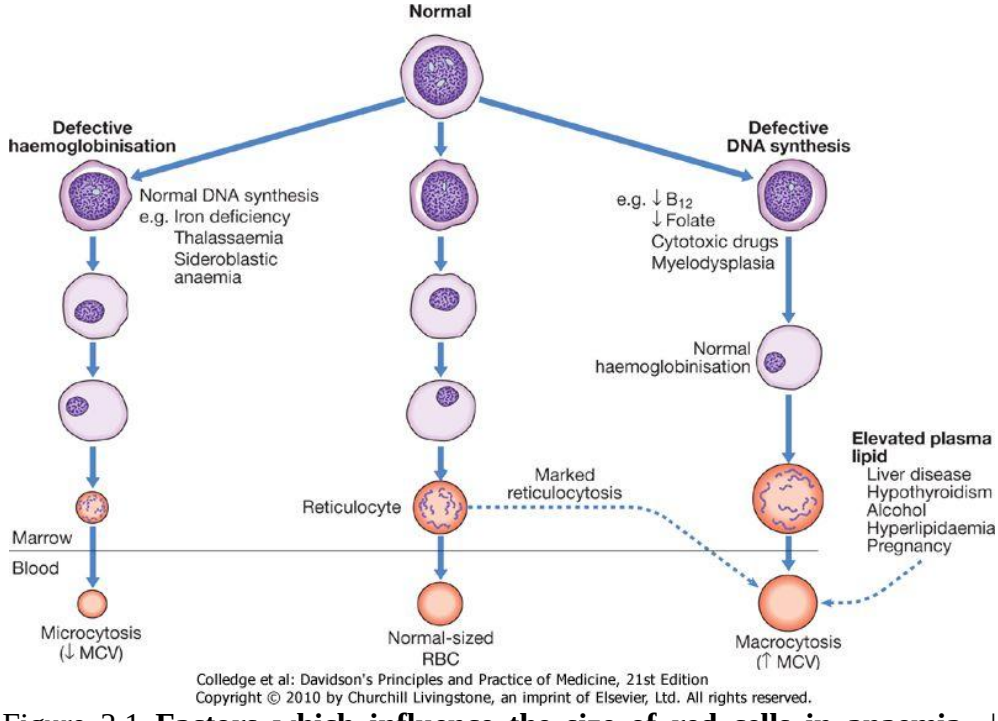


Figure 2.1 **Factors which influence the size of red cells in anaemia.** ↓ MCV is < 76 fL; ↑ MCV is > 100 fL. [3]

Aetiology

Important causes of anaemia in the developing countries are:

Acquired

1. Nutritional
   - Iron
   - Folate
   - Vitamin B_{12}
2. Chronic infection
   - Malaria
   - Leishmaniasis
   - Schistosomiasis
   - Tuberculosis

- AIDS

3. Blood loss
   - Hookworm
   - Schistosomiasis
4. Protein–energy malnutrition
5. Malabsorption
   - Tropical sprue and related disorders

Hereditary

1. Thalassaemias
2. Haemoglobin variants
3. Glucose-6-phosphate dehydrogenase deficiency
4. Ovalocytosis [12]

Epidemiology

1. Prevalence: Around 30% of the total world population is anaemic and half of these, some 600 million people, have iron deficiency (Table 2.4). [3]
2. Geographical variation: Common in developing world

Table 2.4 **Estimated prevalence of anaemia by region and sex**

Region	Percentage anaemic				
	Children		Women 15–49 years		Men
	0–4 years	5–12 years	Pregnant	All	15–59 years
Developing	51	46	59	47	26
Developed	12	7	14	11	3
World	43	37	51	35	18

Data from DeMaeyer EM, Adiels-Tegman M (1985). The prevalence of anemia in the world. *World Health Statist Quart*, **38**, 302–16. [12]

5. Age group and Sex:

- About 47 % of women aged 15 to 49 years have Hb less than 12 g/dl
- About 59 % of pregnant women have Hb less than 11 g/dl

- About 26 % of men aged 15 to 49 years have Hb less than 13 g/dl [12]

Pathogenesis/Pathology

The basic mechanisms of anemia can be divided into two conditions:

1. Accelerated destruction or loss of RBCs
2. Impaired Production of Erythrocytes by Bone Marrow [5]

Clinical Features (C/F)

Non-specific symptoms:

1. Fatigue
2. Headaches
3. Faintness

(Are very common)

4. Malaise
5. Dizziness
6. Tiredness
7. Lightheadedness
8. Tinnitus
9. Breathlessness
10. Syncope
11. Angina
12. Intermittent claudication
13. Palpitations
14. Ankle-swelling [3, 8 & 4]

Non-specific signs:

1. Mucous membrane and skin pallor (Figure 2.2, 2.3)
2. Tachypnoea
3. Raised jugular venous pressure
4. Systolic flow murmurs
5. Ankle oedema
6. Postural hypotension

7. Tachycardia [3]
8. Cardiac failure [8]

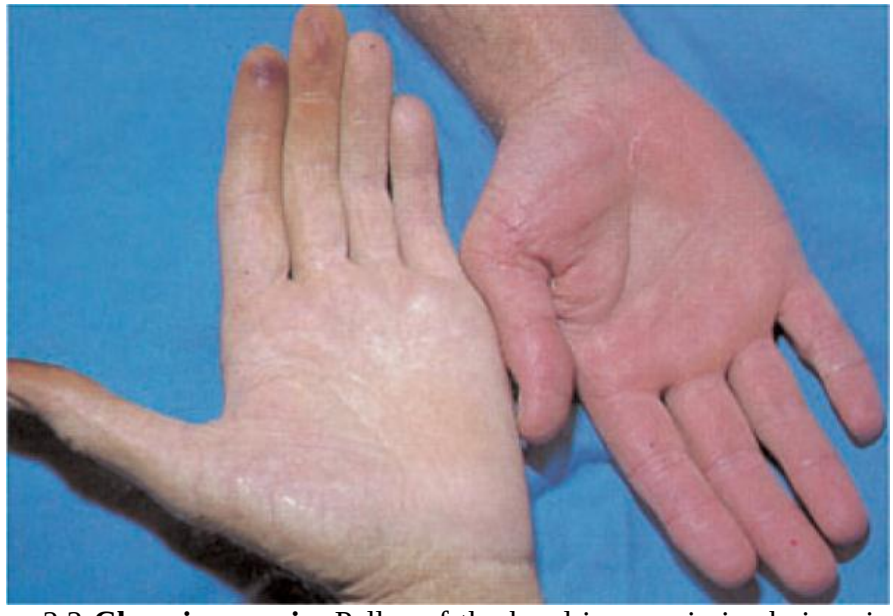


Figure 2.2 **Chronic anemia.** Pallor of the hand in anemia is obvious in this patient, especially when compared with the physician's hand on the right. The patient's hemoglobin concentration was 7 g/dL. The hand also shows that the patient was a heavy smoker. His anemia resulted from chronic blood loss from a carcinoma in the esophagus, a site where the risk for carcinoma is increased in smokers. *(From Forbes CD, Jackson WF: Color Atlas and Text of Clinical Medicine, 3rd ed. London, Mosby, 2003.)* [5]

Figure 2.3 Pallor of palmar skin creases. [7]

Iron deficiency anaemia

Definition

Iron deficiency anaemia is the anaemia in which there is inadequate iron for haemoglobin synthesis.

A normal level of Hb is maintained for as long as possible after the iron stores are depleted; ***latent iron deficiency*** is said to be present during this period:

1. Blood loss
2. Increased demands such as growth and pregnancy
3. Decreased absorption (e.g. postgastrectomy)
4. Poor intake. (8)

Iron Metabolism

Role of iron

1. To carry O_2 as part of hemoglobin
2. O_2 is also bound by myoglobin in muscle
3. Iron-containing enzymes, including the cytochrome system in mitochondria Table 2.5. (10)

Table 2.5 **Body Iron Distribution** (10)

	Iron Content, mg	
	Adult Male, 80 kg	**Adult Female, 60 kg**
Hemoglobin	2500	1700
Myoglobin/enzymes	500	300
Transferrin iron	3	3
Iron stores	600–1000	0–300

The Iron Cycle in Humans

1. Iron absorbed from:
   - The diet or

- Released from stores circulates in the plasma

2. Iron bound to *transferrin*
3. Transferrin is a glycoprotein with two iron binding sites
4. Iron transported by transferrin is delivered to the erythroid marrow
5. Erythropoiesis is markedly stimulated (Fig. 2.4, 2.5) [10]

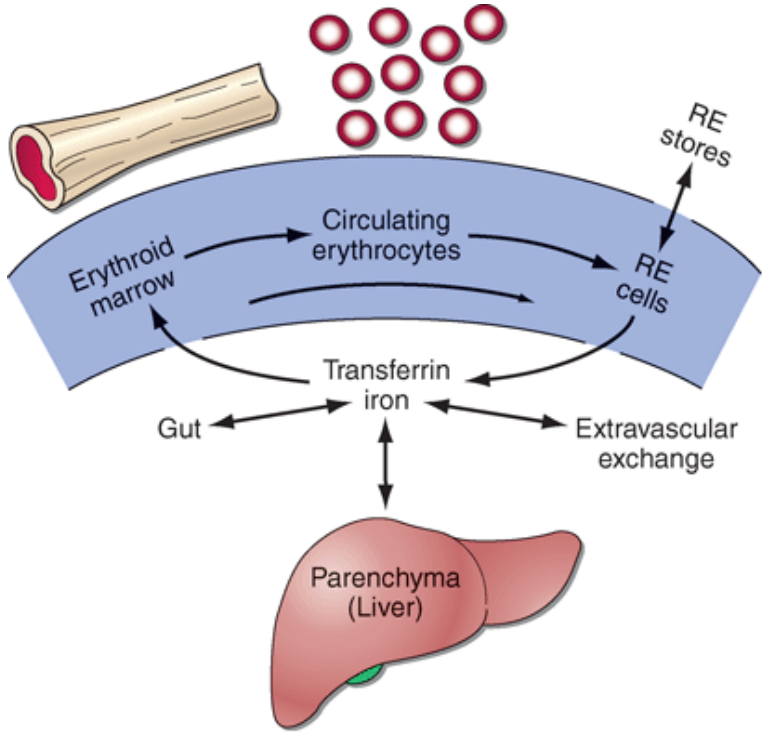


Figure 2.4 **Internal iron exchange.** Normally approximately 80% of iron passing through the plasma transferrin pool is recycled from broken-down red cells. Absorption of approximately 1 mg/d is required from the diet in men, and 1.4 mg/d in women to maintain homeostasis. As long as transferrin saturation is maintained between 20–60% and erythropoiesis is not increased, use of iron stores is not required. However, in the event of blood loss, dietary iron deficiency, or inadequate iron absorption, up to 40 mg/d of iron can be mobilized from stores. RE, reticuloendothelial. [10]

Aetiology

Conditions that increase demand for iron, increase iron loss, or decrease iron intake or absorption can produce iron deficiency.

1. Increased Demand for Iron
   - Rapid growth in infancy or adolescence
   - Pregnancy
   - Erythropoietin therapy
2. Increased Iron Loss [1]
   - Chronic blood loss
   - Menses
   - Acute blood loss
   - Blood donation
   - Phlebotomy as treatment for polycythemia vera
3. Decreased Iron Intake or Absorption
   - Inadequate diet
   - Malabsorption from disease (sprue, Crohn's disease)
   - Malabsorption from surgery (postgastrectomy)
   - Acute or chronic inflammation (10)

---

[1] **Blood loss:** The most common explanation in men and post-menopausal women is gastrointestinal blood loss.This may result from:

1. Occult gastric or colorectal malignancy
2. Gastritis & Peptic ulceration
3. Inflammatory bowel disease
4. Diverticulitis
5. Polyps
6. Angiodysplastic lesions
7. World-wide, hookworm and schistosomiasis are the most common causes of gut blood loss
8. Gastrointestinal blood loss may be exacerbated by the chronic use of aspirin or NSAIDs
9. In women of child-bearing age, menstrual blood loss, pregnancy and breastfeeding
10. Very rarely, chronic haemoptysis or haematuria may cause iron deficiency. (3)

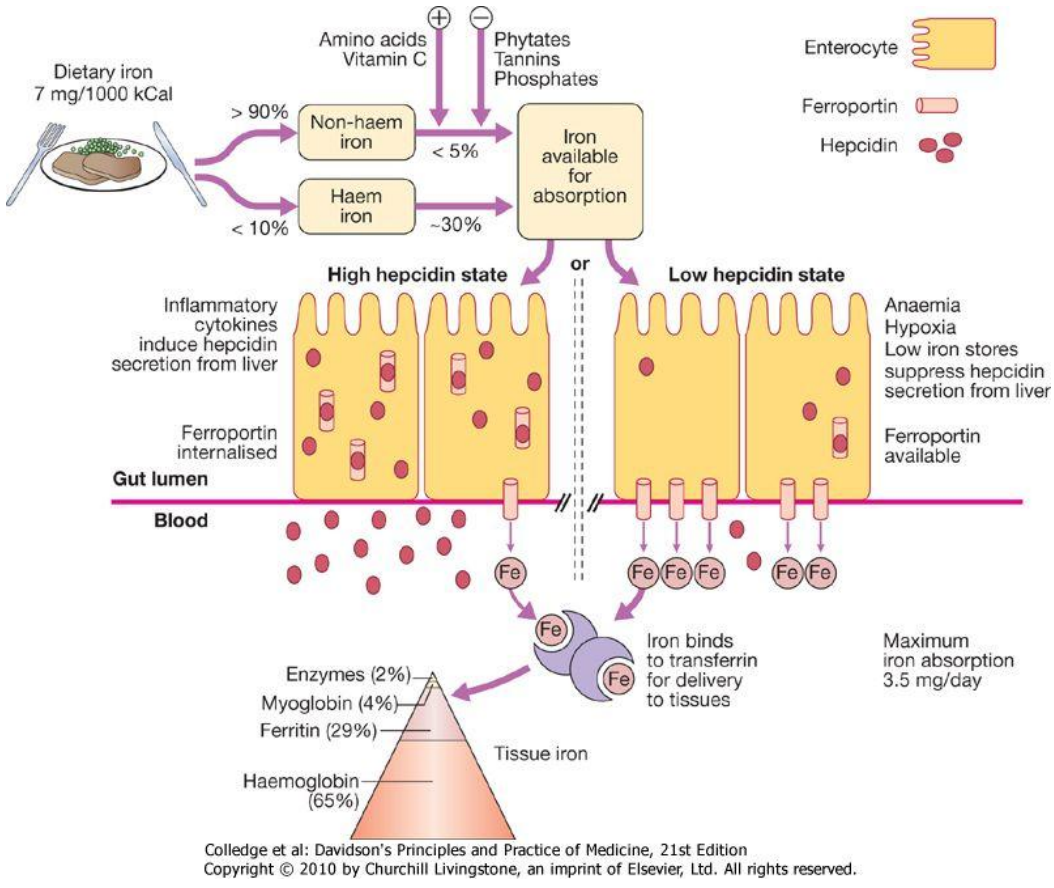


Figure 2.5 **The regulation of iron absorption, uptake and distribution in the body.** The transport of iron is regulated in a similar fashion to enterocytes in other iron-transporting cells such as macrophages. [3]

Epidemiology

1. Prevalence:
   - Most common cause of anaemia in the world
   - Affecting 30% of the world's population (500 million people)

- Globally, 50% of anemia is attributable to iron deficiency and
- Causes 841,000 deaths annually worldwide

2. Incidence:
3. Geographical variation:
   - Africa and parts of Asia bear 71% of the global mortality
   - North America represents only 1.4% of the total morbidity [10]
4. Age group:
   - Adolescence
   - Periods of rapid growth
   - Childbearing women
5. Sex: Common in female [5]

Clinical Features

History

1. Pregnancy
2. Adolescence
3. Periods of rapid growth
4. Gastrointestinal blood loss
5. History of any blood loss

Symptoms

1. Asymptomatic (in mild anaemia)
2. Nonspecific symptoms
   - Weakness
   - Pallor (Fig. 2.6)
   - Dizziness
   - Decreased exercise tolerance
   - Irritability
3. Manifestations of skin and mucus membrane
   - Dysphagia

- Esophageal stricture or web (Plummer-Vinson syndrome)
- Glossitis
- Angular stomatitis

4. Pica or unusual craving for certain non-nutritional substances
   - Ice (pagophagia)
   - Clay (geophagia)
   - Starch (amylophagia)[5]

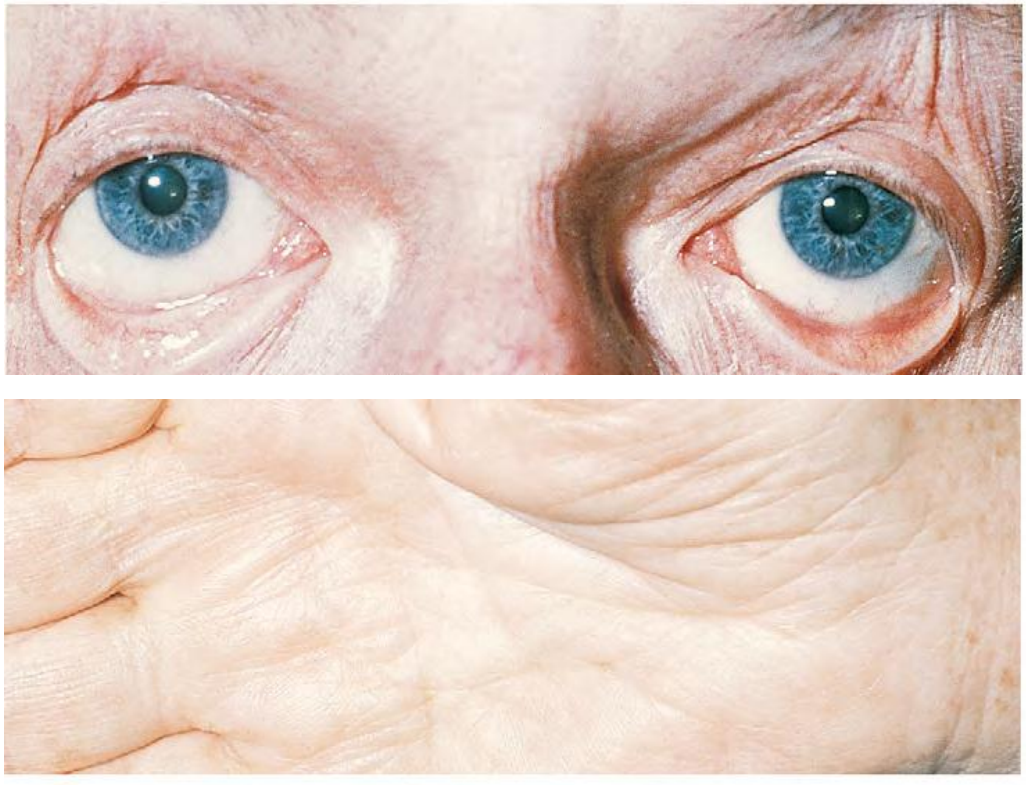


Figure 2.6 **Iron-deficiency anemia. A**, Pallor of conjunctival mucosa; mucous membrane pallor becomes clinically apparent when the hemoglobin concentration is below 9 g/dl. **B**, Pallor of palmar skin creases. [7]

Signes

1. Glossitis(Figure 2.7)

2. *Cheilosis* (fissures at the corners of the mouth)
3. Angular stomatitis (Figure 2.8)
4. *Koilonychia* (spooning of the finger nails) (Figure 2.11)
5. Blue-tinged sclerae [10 & 5]

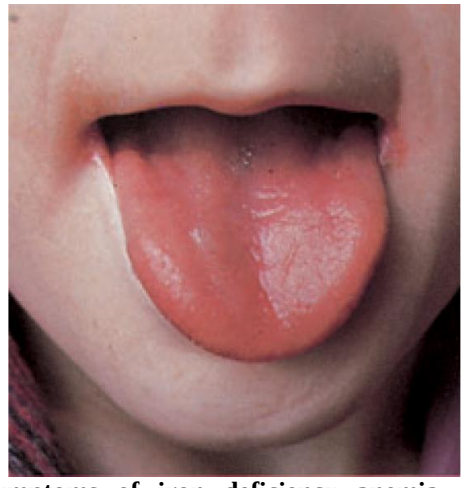


Figure 2.7 **Clinical symptoms of iron deficiency anemia.** Iron deficiency anemia commonly leads to pallor of the face, lips, and tongue and, if chronic, to atrophic glossitis and angular stomatitis. All of these symptoms are seen in this young woman whose iron deficiency anemia resulted from excessive menstrual bleeding. The anemia responded to oral iron supplementation. *(From Forbes CD, Jackson WF: Color Atlas and Text of Clinical Medicine, 3rd ed., London, Mosby, 2003, with permission.)*

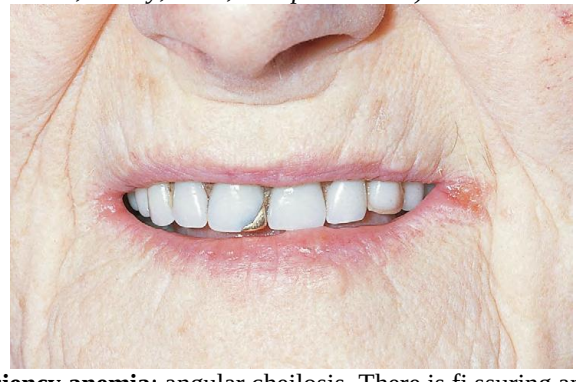


Fig. 2.8 **Iron-defi ciency anemia**: angular cheilosis. There is fi ssuring and ulceration at the corners of the mouth. The biochemical mechanism is uncertain but may be similar to that for nail, mucosal, and pharyngeal changes. [7]

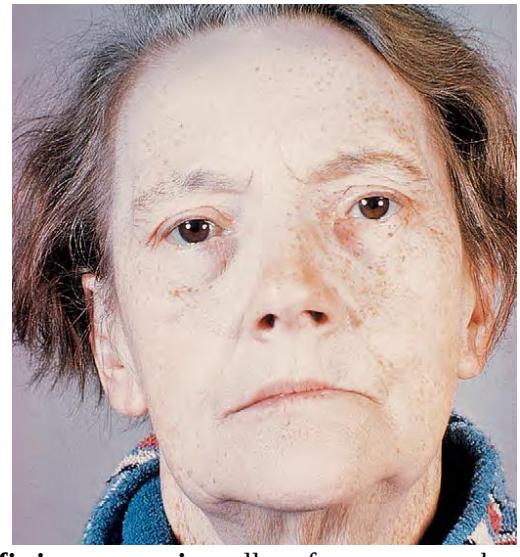


Figure 2.9 **Iron-defi ciency anemia**: pallor of mucous membranes (lips) and skin in a 69-year-old woman. (Hb, 8.1 g/dl; RBC, 4.13 1012/L; PCV, 26.8%; MCV, 65 fl ; MCH, 19.6 pg.) [7]

Figure 2.10 **Iron-defi ciency anemia**: marked pallor of the nail beds in a dark-skinned patient. The nails are fl attened. 78 Hypochromic [7]

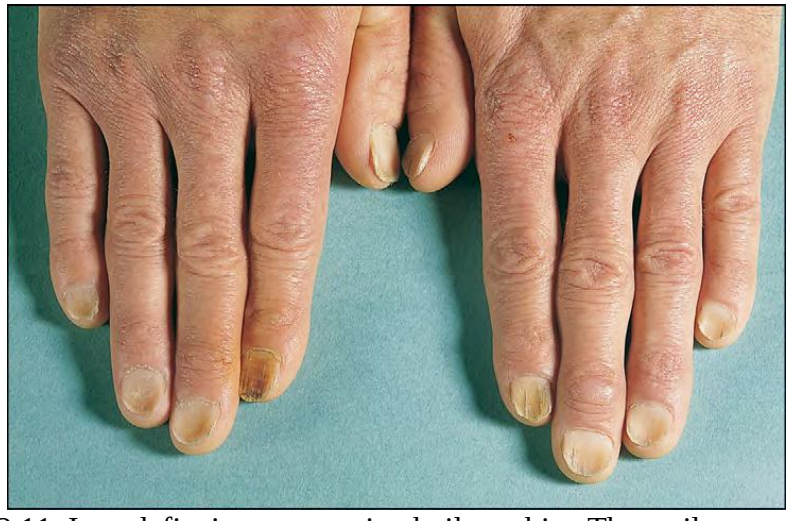


Figure 2.11 Iron-defi ciency anemia: koilonychia. The nails are concave, ridged, and brittle. This patient's anemia had been rapidly corrected by blood transfusion before an operation for cecal carcinoma. The cause of the nail changes in iron deficiency is uncertain but may be related to the iron requirement of many enzymes present in epithelial and other cells. (Courtesy of Dr. S. M. Knowles.) [7]

Investigations

Confirmation of iron deficiency

1. Plasma ferritin is a measure of iron stores in tissues

Subnormal level is due to:

- Iron deficiency
- Hypothyroidism
- Vitamin C deficiency

Raised levels:

- Liver disease
- Acute phase response [3]

2. Plasma iron and total iron binding capacity (TIBC)

Very low during an acute phase response

Raised in:

- Liver disease

- Haemolysis

3. Levels of transferrin, the binding protein for iron

Lowered by:

- Malnutrition
- Liver disease
- Acute phase response
- Nephrotic syndrome

Raised by:

- Pregnancy (Table 2.6)
- Oral contraceptive pill

4. A transferrin saturation (i.e. iron/TIBC × 100) of less than 16% is consistent with iron deficiency but is less specific than a ferritin (Table 2.7).[3]

2.6 **Haematological physiology in pregnancy** [3]
• **Full blood count:** increased plasma volume (40%) lowers normal Hb (reference range reduced to > 105 g/L at 28 weeks). The MCV may increase by 5 fL. A progressive neutrophilia occurs. Gestational thrombocytopenia (rarely < 60×10^9/L) is a benign phenomenon. • **Depletion of iron stores:** iron deficiency is a common cause of anaemia in pregnancy and, if present, should be treated with oral iron supplement. • **Vitamin B_{12}:** serum levels are physiologically low in pregnancy but deficiency is uncommon. • **Folate:** tissue stores may become depleted, and folate supplementation is recommended in all pregnancies. • **Coagulation factors:** from the second trimester, procoagulant factors increase approximately three-fold, particularly fibrinogen, von Willebrand factor and factor VIII. This causes activated protein C resistance and a shortened APTT, and contributes to a prothrombotic state. • **Anticoagulants:** levels of protein C increase from the second trimester while levels of free protein S fall as C4b binding protein increases.

Table 2.7 **Investigations to differentiate anaemia of chronic disease from iron deficiency anaemia**					
	Ferritin	**Iron**	**TIBC**	**Transferrin saturation**	**Soluble transferrin receptor**
Iron deficiency anaemia	↓	↓	↑	↓	↑
Anaemia of chronic disease	↑/Normal	↓	↓	↓	↓/Normal

(TIBC = total iron binding capacity)

Blood film: A blood film shows the presence of:

1. Hypochromic microcytic red cells (Figure 2.12)
2. With abnormally shaped cells:
   - "Pencil" or cigarshaped
   - Poikilocytes
   - Occasional target cells
3. Platelet count is often raised, particularly in hemorrhage (Ho)

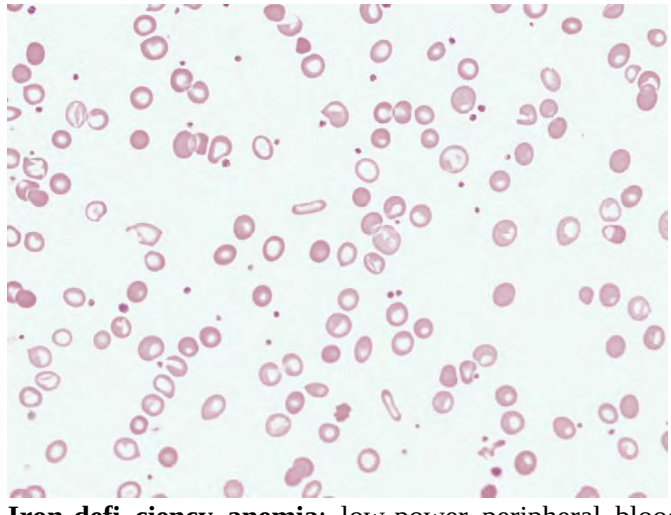


Figure 2.12 **Iron-defi ciency anemia**: low-power peripheral blood fi lm taken during therapy with oral iron. There is a dimorphic population of hypochromic microcytic cells and target cells and well-hemoglobinized cells of normal size, but there are some large polychromatic cells. (7)

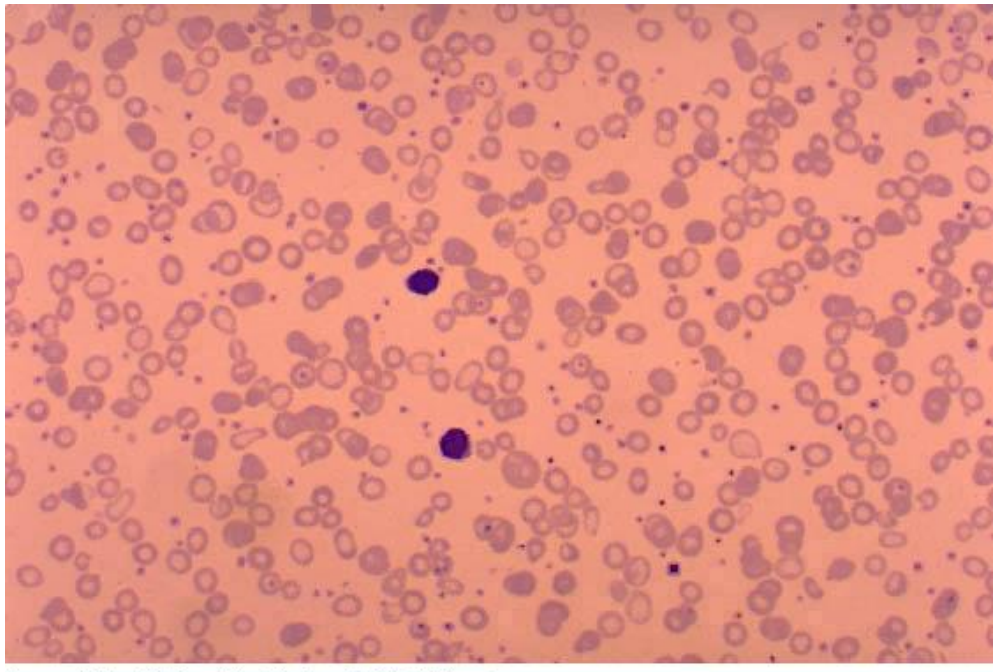


Figure 2.13 **Iron deficiency anemia.** (Peripheral blood, 50 x.) Hypochromic and microcytic cells due to iron deficiency. The diameter of the normal red blood cell should be approximately the same as that of the nucleus of a small lymphocyte. This smear shows that most of the red cells are much smaller than the lymphocytes. This patient also has an increased platelet count—a common finding in patients with iron deficiency. (Courtesy of L Damon.)

Bone marrow appearances

1. Bone marrow is of normal cellularity
2. Sometimes with normoblastic hyperplasia
3. The ragged vacuolated cytoplasm (Figure 2.14)
4. Complete absence of iron stores (Figure 2.15)
5. Siderotic granules absence (Figure 2.16) [7]

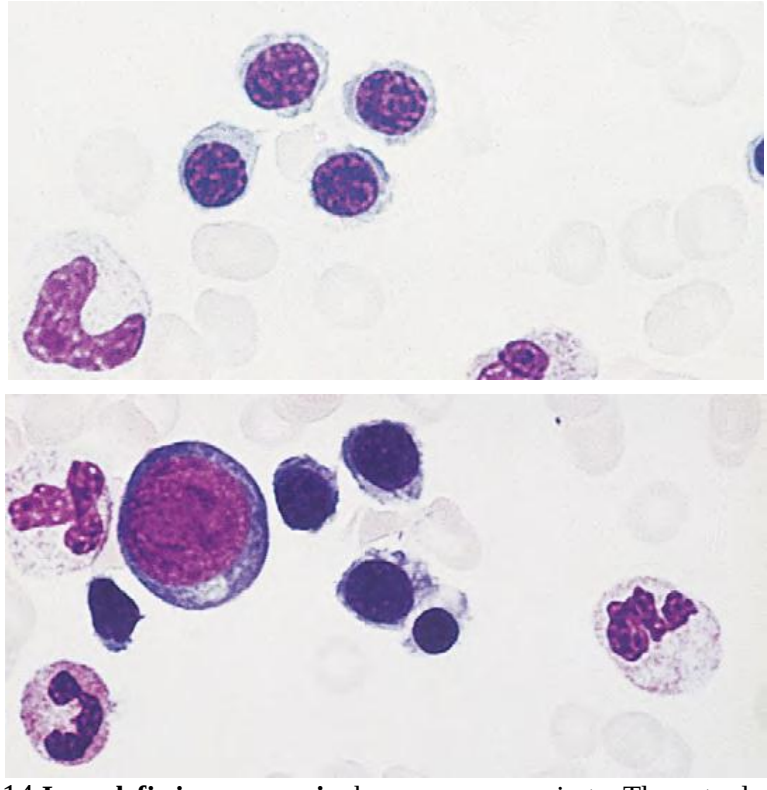


Figure 2.14 **Iron-defi ciency anemia**: bone marrow aspirate. The cytoplasm of polychromatic and pyknotic erythroblasts is scanty, vacuolated, and irregular in outline. This type of erythropoiesis has been described as micronormoblastic. (7)

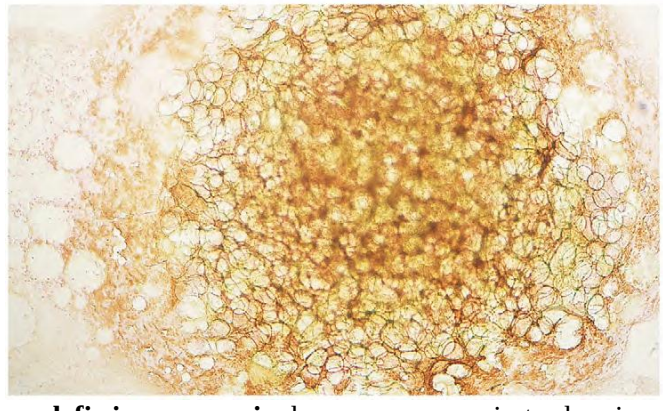


Figure 2.15 **Iron-defi ciency anemia**: bone marrow aspirate showing absence of stainable iron in a bone marrow fragment. The appearances are similar in irondefi ciency anemia and latent iron defi ciency (absent iron stores without anemia). (7)

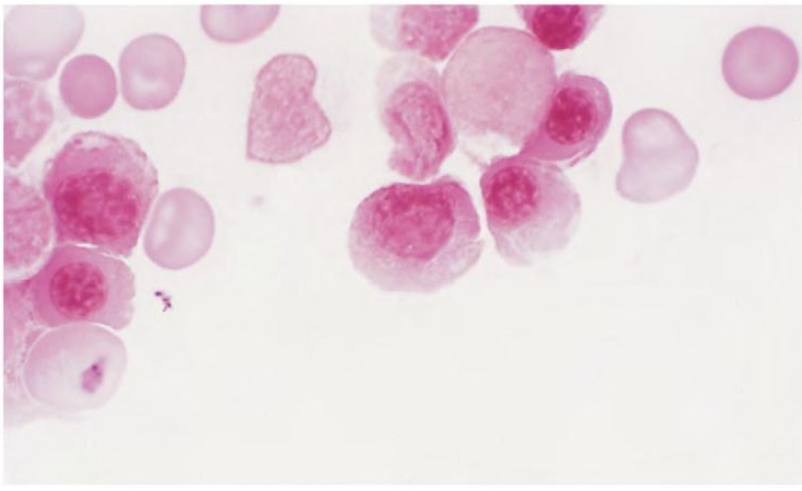


Figure 2.16 **Iron-defi ciency anemia**: bone marrow aspirate showing lack of siderotic granules in developing erythroblasts. (7)

Investigation of the cause[2]

1. Endoscopy or (Fig. 2.17)
2. Barium studies (Fig. 2.18)
3. Serum anti-endomysial antibodies &
4. Duodenal biopsy to detect coeliac disease
5. In the tropics, stool and urine exam for parasites (3)

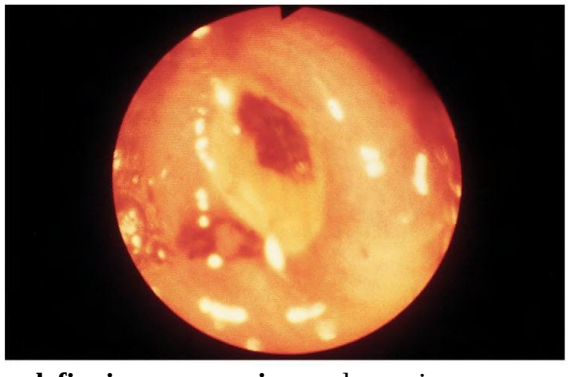


Figure 2.17 **Iron-defi ciency anemia**: endoscopic appearance of a bleeding duodenal ulcer in a 45-year-old man with symptoms of anemia. (Courtesy of Professor R. E. Pounder.) (7)

[2] This will depend upon the age and sex of the patient, as well as the history and clinical findings.

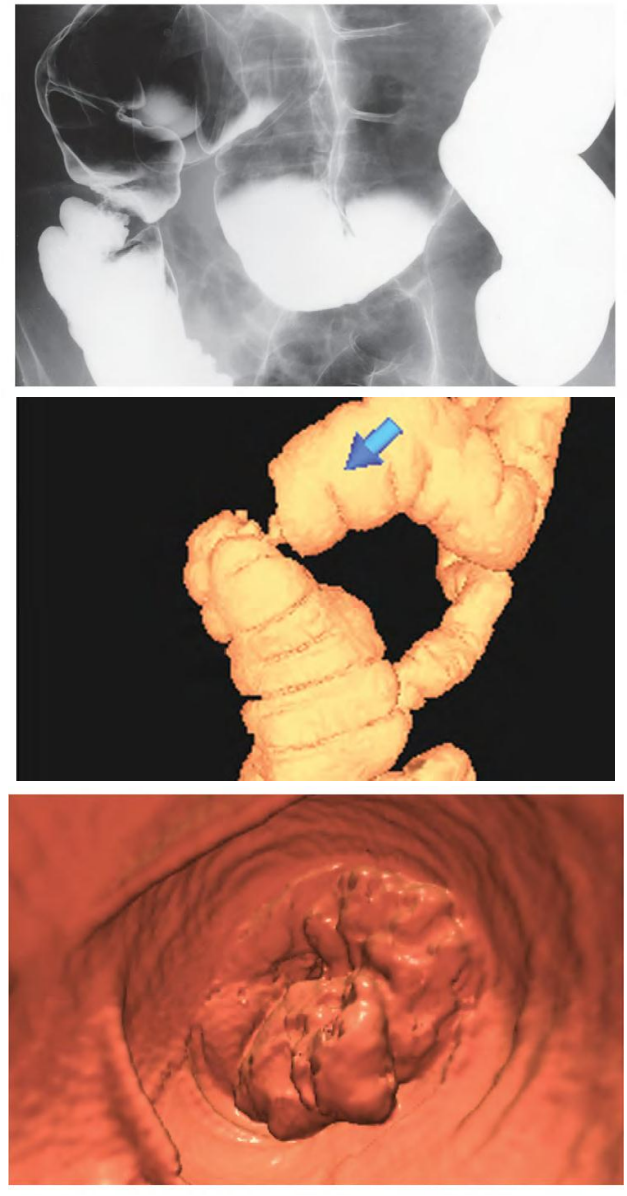


Figure 2.18 **Iron-defi ciency anemia**. **A**, Barium enema radiograph showing an annular filling defect (arrow) of the ascending colon due to adenocarcinoma. **B**, Virtual colonoscopy: annular ("apple-core") narrowing of the colon 126 cm from the anal verge. **C**, Luminal views reveal fungating adenocarcinoma (same case as B). (B and C, Courtesy of Dr. J. Bell.) [(7)]

Management

1. **Oral iron therapy**. In stable patients with mild symptoms, this consists of ferrous sulfate, 325 mg PO OD to TID
   - Iron is best absorbed on an empty stomach
   - Oral iron ingestion may induce GI side effects:
     a. Epigastric distress
     b. Bloating
     c. Constipation
   - Ferrous gluconate and fumarate at a similar dose may be better-tolerated alternative therapies.
   - Iron polysaccharide complex (Niferex) contains 150 mg of elemental iron, given twice daily.
   - Vitamin C along with the iron improves absorption
2. **Parenteral iron therapy**. may be useful in patients with:
   - Poor absorption (e.g., inflammatory bowel disease, malabsorption).
   - Very high iron requirements that cannot be met with oral supplementation (e.g., ongoing bleeding).
   - Intolerance to oral preparations.
   - The total amount of iron necessary to replete the deficiency can be estimated by a formula using the starting Hb level; however, in practice, parenteral iron is often infused to a dose of 1 to 1.2 g.
   - Iron dextran. IV iron dextran therapy (INFeD, Dexferrum) may have serious side effects including anaphylaxis
   - Delayed reactions to IV iron, such as arthralgia, myalgia, fever, pruritus, and lymphadenopathy may be seen within 3
3. **Alternatives to iron dextran** include sodium ferric gluconate (Ferrlecit) and iron sucrose (Venofer)..

- The dosage of sodium ferric gluconate is 125 mg diluted in 100 mL of NS infused IV over 1 hour or as a slow IV push over 10 minutes (12.5 mg/min). This can be repeated weekly until circulating iron (to a normal Hct) and storage iron (1 to 3 g) are replenished.
- Iron sucrose is administered as a 100 to 200 mg IV push or up to 400 mg over a 2.5-hour IV infusion.(4)

4. **Transfusion** is necessary if the patient has:
   - Angina
   - Heart failure or
   - Evidence of cerebral hypoxia[3] (3)

---

[3]

1. Oral iron therapy. In stable patients with mild symptoms, this consists of ferrous sulfate, 325 mg (65 mg elemental iron) PO one to three times per day.
   - Iron is best absorbed on an empty stomach, and between 3 and 10 mg of elemental iron can be absorbed daily.
   - Oral iron ingestion may induce a number of GI side effects, including epigastric distress, bloating, and constipation, as a result noncompliance is a common problem. These side effects can be decreased by initially administering the drug with meals or once per day and increasing the dose as tolerated. Concomitant treatment with a stool softener can also alleviate these symptoms.
   - Ferrous gluconate and fumarate at a similar dose may be better-tolerated alternative therapies.
   - Iron polysaccharide complex (Niferex) contains 150 mg of elemental iron, given twice daily, is as effective as other preparations at a similar cost and seems to have fewer GI side effects.
   - Administration of vitamin C along with the iron improves absorption by maintaining the iron in the reduced state.
2. Parenteral iron therapy. Parenteral iron therapy may be useful in patients with:
   - Poor absorption (e.g., inflammatory bowel disease, malabsorption).
   - Very high iron requirements that cannot be met with oral supplementation (e.g., ongoing bleeding).
   - Intolerance to oral preparations.
   - The total amount of iron necessary to replete the deficiency can be estimated by a formula using the starting Hb level; however, in practice, parenteral iron is often infused to a dose of 1 to 1.2 g.

Prognosis

1. About 20% of perinatal mortality and 10% of maternal mortality in developing countries [12]
2. Approximately 841,000 deaths annually worldwide
3. Africa and parts of Asia bear 71% of the global mortality
4. North America represents only 1.4% of the total mortality [10]

---

3. Iron dextran. IV iron dextran therapy (INFeD, Dexferrum) can be complicated by serious side effects including anaphylaxis; therefore, an IV test dose of 25 mg in 50 mL of normal saline (NS) should be administered over 5 to 10 minutes. Methylprednisolone, diphenhydramine, and 1:1,000 epinephrine 1-mg ampule (for subcutaneous administration) should be immediately available at all times during the infusion. For an online dose calculator, go to www.globalrph.com/irondextran.htm.
    - Delayed reactions to IV iron, such as arthralgia, myalgia, fever, pruritus, and lymphadenopathy may be seen within 3 days of therapy and usually resolve spontaneously or with NSAIDs.
4. Alternatives to iron dextran include sodium ferric gluconate (Ferrlecit) and iron sucrose (Venofer).
    - The side effect profile for these preparations appears to be better than that of iron dextran, with less hypersensitivity infusion reactions.
    - However, they cannot be used to replenish the entire iron deficit with a single infusion.
    - The recommended dosage of sodium ferric gluconate is 125 mg diluted in 100 mL of NS infused IV over 1 hour or as a slow IV push over 10 minutes (12.5 mg/min). This can be repeated weekly until circulating iron (to a normal Hct) and storage iron (1 to 3 g) are replenished.
    - Iron sucrose is administered as a 100 to 200 mg IV push or up to 400 mg over a 2.5-hour IV infusion. [4]

Anaemia of chronic disease (ACD)

1. This is a common type of anaemia, particularly in hospital populations.
2. It occurs in the setting of:
   - Chronic infection
   - Chronic inflammation
   - Neoplasia
3. The anaemia is not related to:
   - Bleeding
   - Haemolysis or
   - Marrow infiltration
   - Hb in the range of 85-115 g/L
   - Normal MCV (normocytic, normochromic)
   - May be reduced in long-standing inflammation
4. The serum iron is low but iron stores are normal or increased, as indicated by the ferritin or stainable marrow iron. (3)

Pathogenesis

1. Production of hepcidin[4] by the liver
2. High levels of production are encouraged by pro-inflammatory cytokines, especially IL-6.
3. Hepcidin binds to ferroportin on the membrane of iron exporting cells, such as small intestinal enterocytes and macrophages
4. Internalizing the ferroportin
5. Inhibiting the export of iron from these cells into the blood (and hence to the main target cells and proteins of iron).

[4] Key regulatory protein

6. The iron remains trapped inside the cells in the form of ferritin, levels of which are therefore normal or high in the face of significant anaemia.
7. Inhibition or blockade of hepcidin is a likely target for potential treatment of this form of anaemia. [3]

Diagnosis and management

1. It is often difficult to distinguish ACD associated with a low MCV from iron deficiency.
2. Examination of the marrow may ultimately be required to assess iron stores directly. A trial of oral iron can be given in difficult situations.
3. A positive response occurs in true iron deficiency but not in ACD.
4. Measures which reduce the severity of the underlying disorder generally help to improve the ACD. [3]

Megaloblastic anaemia

Definition

Megaloblastic anemias, is a group of disorders characterized by a morphologic pattern in hematopoietic cells, are commonly due to deficiency of vitamin B_{12} or folates.[1] (5)

[1] This results from a deficiency of vitamin B_{12} or folic acid, or from disturbances in folic acid metabolism.

Folate is an important substrate of, and vitamin B_{12} a co-factor for, the generation of the essential amino acid methionine from homocysteine. This reaction produces tetrahydrofolate, which is converted to thymidine monophosphate for incorporation into DNA. Deficiency of either vitamin B_{12} or folate will therefore produce high plasma levels of homocysteine and impaired DNA synthesis. (3)

The end result is cells with arrested nuclear maturation but normal cytoplasmic development: so-called nucleocytoplasmic asynchrony. All proliferating cells will exhibit megaloblastosis; hence changes are evident in the:

- Buccal mucosa
- Tongue
- Small intestine
- Cervix
- Vagina
- Uterus

The high proliferation rate of bone marrow results in striking changes in the haematopoietic system in megaloblastic anaemia. Cells become arrested in development and die within the marrow; this ineffective erythropoiesis results in an expanded hypercellular marrow. The megaloblastic changes are most evident in the early nucleated red cell precursors, and haemolysis within the marrow results in a raised bilirubin and lactate dehydrogenase (LDH), but without the reticulocytosis characteristic of other forms of haemolysis. (3)

Table 2.8 **Vitamin B_{12} and folate** [12]		
	Vitamin B_{12}	Folate
Parent form	Cyanocobalamin (cyano-B_{12}), mol. wt. 1355	Folic acid (pteroyglutamic acid), mol. wt. 441.4
Crystals	Dark-red needles	Yellow, spear-shaped
Natural forms	Deoxyadenosylcobalamin	Reduced (di- or tetrahydro-), methylated, formylated, other single carbon additions; mono- and polyglutamates
	Methylcobalamin	
	Hydroxocobalamin	
Foods	Animal produce (especially liver) only	All, especially liver, kidney, yeast, greens, nuts
Adult daily requirements	2 μg	100 μg
Adult body stores	2–5 mg	6–20 mg
Length of time to deficiency	2–4 years	4 months
Daily diet content	5–30 μg	About 200–250 μg
Cooking	Little effect	Easily destroyed
Absorption	Intrinsic factor (+ neutral pH + Ca^{2+}) via ileum	Deconjugated, reduction, and methylation via duodenum and jejunum
Plasma transport	Tightly and specifically bound to transcobalamins	One-third loosely bound albumin, other proteins; ?specific protein
Enterohepatic circulation	3–9 μg/day	60–90 μg/day

Vitamin B_{12} (Cobalamins)

Requirement & source

1. Daily diet contains 5-30 μg of vitamin B_{12} (Table 2.8)
2. Mainly in:

- Meat
- Fish
- Eggs
- Milk

3. The daily requirement is 1 μg [3]

Vitamin B_{12} absorption

1. In the stomach, gastric enzymes release vitamin B_{12} from food

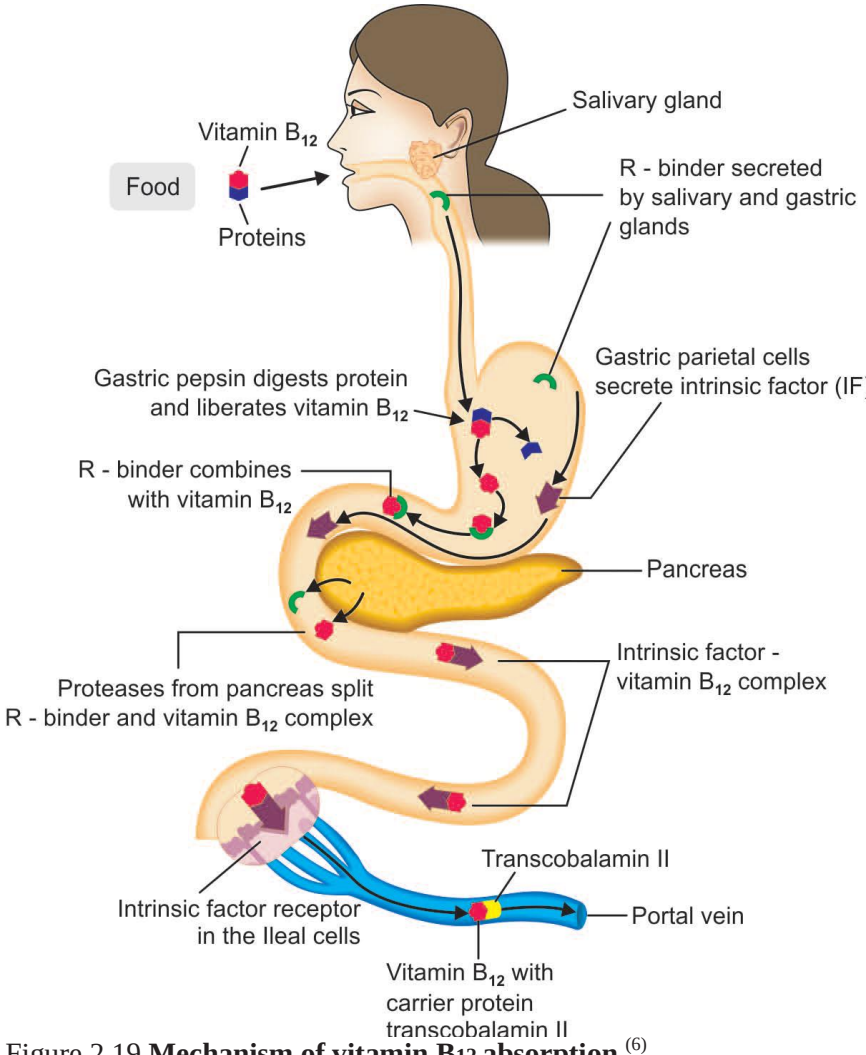


Figure 2.19 **Mechanism of vitamin B12 absorption** [6]

2. Binds to a carrier protein termed R protein [2]
3. Parietal cells produce intrinsic factor, a vitamin B_{12}-binding protein
4. Bile also contains vitamin B_{12} which is available for reabsorption (Fig. 2.19)
5. Vitamin B_{12} intrinsic factor complex binds to specific receptors in the terminal ileum
6. Vitamin B_{12} is actively transported
7. In plasma it binds to transcobalamin II [3]
8. The liver stores enough vitamin B_{12} for 3 years (3)

Role of cobalamin

Vitamin B12 serves as a cofactor for two reactions in humans

1. As methylcobalamin, it is a cofactor for methionine synthetase in the conversion of homocysteine to methionine
2. As adenosylcobalamin for the conversion of methylmalonyl-coenzyme A (CoA) to succinyl-CoA (Fig. 2.20) (11)

1. Parietal cells produce intrinsic factor, a vitamin B_{12}-binding protein
2. which optimally binds vitamin B_{12} at pH 8. As gastric emptying occurs, pancreatic secretion raises the pH and vitamin B_{12} released from the diet switches from the R protein to intrinsic factor.

[3] A transport protein produced by the liver, which carries it to the tissues for utilisation.

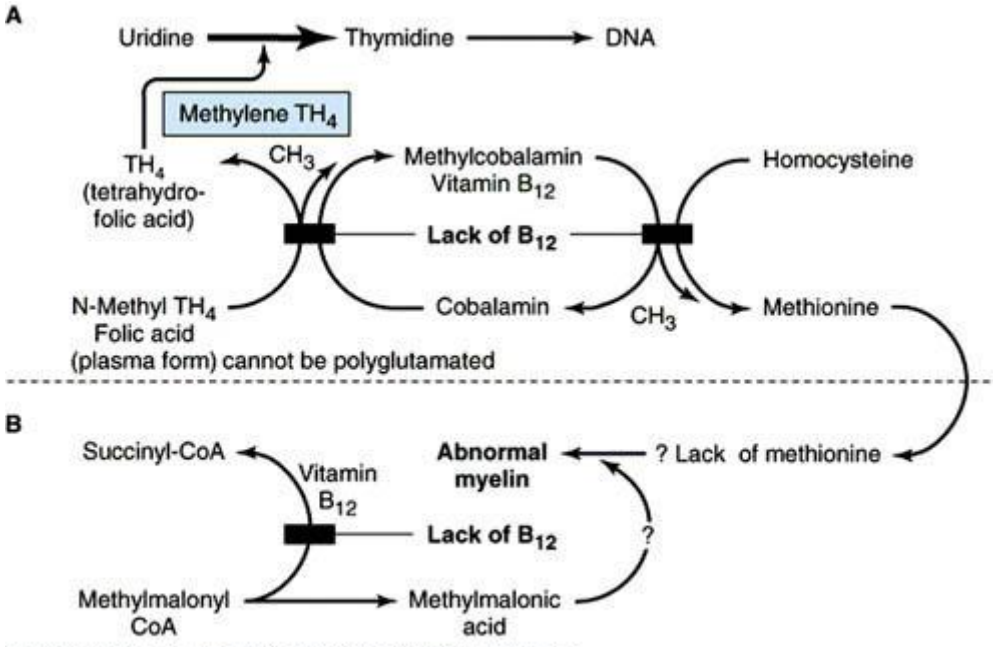


Figure 2.20 **Role of cobalamin (vitamin B12) and folic acid in nucleic acid and myelin metabolism.** Lack of either cobalamin or folic acid retards DNA synthesis **(A)**, and lack of cobalamin leads to loss of folic acid, which cannot be held intracellularly unless polyglutamated. Lack of cobalamin also leads to abnormal myelin synthesis, probably via a deficiency in methionine production **(B)**. (Modified and reproduced, with permission, from Chandrasoma P, Taylor CR: *Concise Pathology*, 3rd ed. Originally published by Appleton & Lange. Copyright © 1998 by The McGraw-Hill Companies, Inc.)

Aetiology

1. Dietary deficiency in strict vegans (rare)
2. Decreased production of intrinsic factor
3. Pernicious anemia
4. Total gastrectomy
5. *Helicobacter pylori* infection
6. Competition for vitamin B12 in gut
7. Blind loop syndrome

8. Fish tapeworm (rare)
9. Pancreatic insufficiency
10. Decreased ileal absorption of vitamin B12
11. Surgical resection
12. Crohn's disease
13. Transcobalamin II deficiency (rare)
14. Hypochlorhydria in elderly patients (Table 2.9) [11& 3]

Table 2.9 **Causes of B_{12} deficiency and malabsorption of B_{12}** [12]
1. Causes of severe B_{12} deficiency
(a) Nutritional:
Vegans
long-continued extremely poor diet (rarely)
(b) Malabsorption:
gastric causes
acquired (addisonian) pernicious anaemia
congenital intrinsic-factor deficiency or abnormality
total and partial gastrectomy
destructive lesions of stomach
intestinal causes
gut flora associated with (jejunal diverticulosis, ileocolic, fistula, anatomical blind loop, stricture, Whipple's disease, scleroderma, HIV disease)
ileal resection and Crohn's disease
chronic tropical sprue
selective malabsorption with proteinuria
irradiation to cervix
HIV disease
fish tapeworm
transcobalamin II deficiency
2. Causes of malabsorption of B_{12} usually without severe B_{12} deficiency
Simple atrophic gastritis, gastric bypass, severe chronic pancreatitis
Zollinger–Ellison syndrome, adult gluten-induced enteropathy, giardiasis
Drugs:
PAS, colchicine, neomycin, slow K, ethanol, metformin, phenformin, anticonvulsants
Deficiencies of folate, B_{12}, protein

Epidemiology

Pernicious anaemia[4] has an incidence of 25/100 000 population over the age of 40 years in developed countries, but an average age of onset of 60 years (3)

Clinical features

Symptoms

1. Malaise (90%)
2. Breathlessness (50%)
3. Paraesthesiae (80%)
4. Sore mouth (20%)
5. Anorexia
6. Diarrhea
7. Weight loss
8. Altered skin pigmentation
9. Grey hair
10. Impotence
11. Poor memory
12. Depression
13. Personality change
14. Hallucinations
15. Visual disturbance (3&11)

Signs

1. pallor
2. Smooth tongue (Fig. 2.22)

[4] It is more common in individuals with other autoimmune disease

- Hashimoto's thyroiditis
- Graves' disease
- Vitiligo
- Hypoparathyroidism
- Addison's disease (3)

3. Angular cheilosis (Fig. 2.24)
4. Vitiligo
5. Skin pigmentation
6. Heart failure
7. Pyrexia
8. May be mildly icteric (Fig. 2.21) [11& 3]

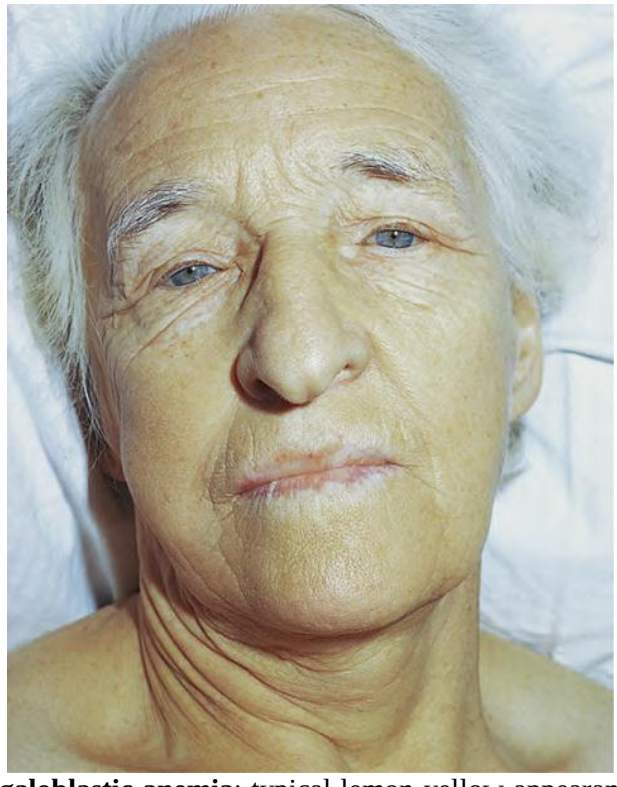


Figure 2.21 **Megaloblastic anemia**: typical lemon-yellow appearance of a 69-year-o1d woman with pernicious anemia and severe megaloblastic anemia (Hb, 7.0 g/dl; MCV, 132 fl). The color is from the combination of pallor (from anemia) and jaundice (from ineffective erythropoiesis). [7]

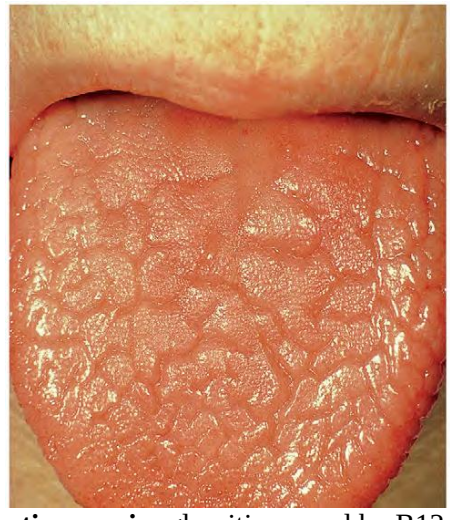


Figure 2.22 **Megaloblastic anemia**: glossitis caused by B12 deficiency in a 55-year-old woman with untreated pernicious anemia. The tongue is beefy red and painful, particularly with hot and acidic foods. An identical appearance occurs in folate defi ciency because of impaired DNA synthesis in the mucosal epithelium. (7)

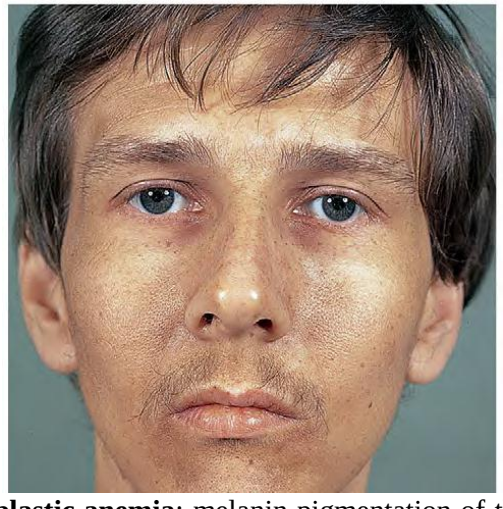


Figure 2.23 **Megaloblastic anemia**: melanin pigmentation of the skin in a 24-year-old man with B12 defi ciency caused by pernicious anemia. Similar pigmentation affected the nail beds, skin creases, and periorbital areas. Such pigmentation also occurs in patients with folate defi ciency. In both, the pigmentation rapidly disappears with appropriate vitamin therapy. The biochemical basis for the melanin excess is unknown. (7)

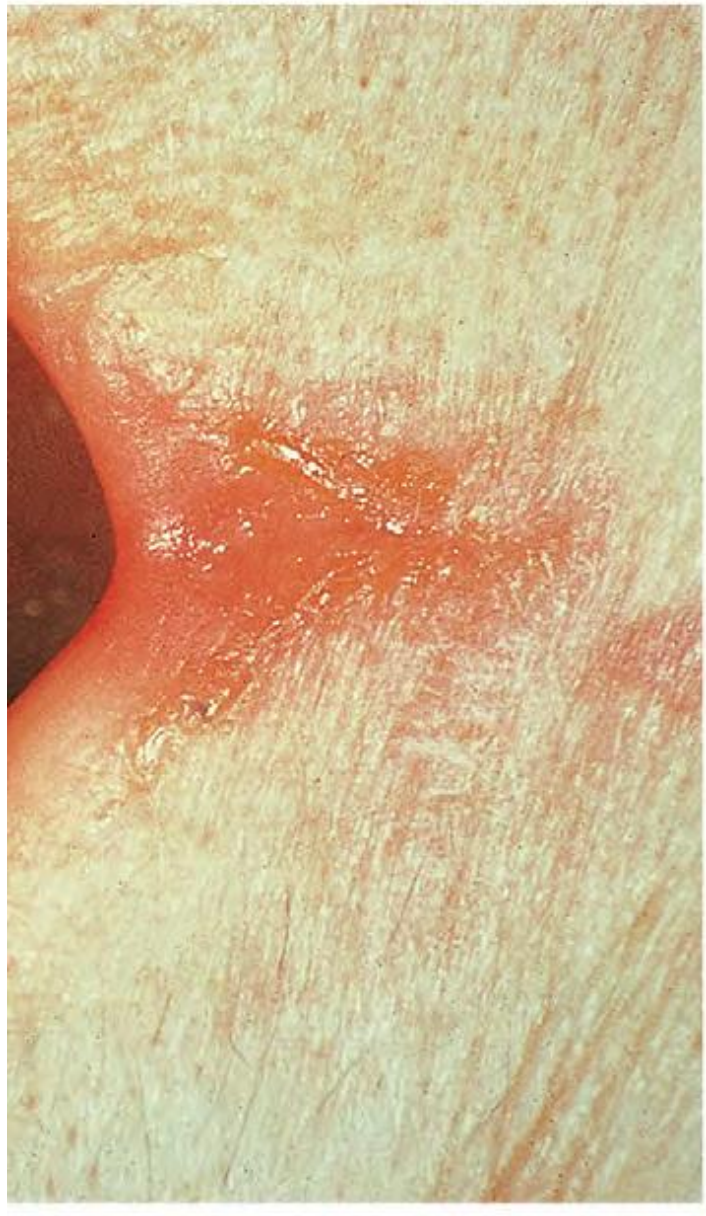


Figure 2.24 **Megaloblastic anemia**: angular cheilosis. This is also thought to result from impaired proliferation of epithelial cells. It is unusual for this abnormality to be so marked. [7]

Neurological findings

1. Peripheral nerves
   - Glove and stocking paraesthesiae
   - Loss of ankle reflexes
2. Spinal cord
   - Subacute combined degeneration of the cord
   a. Posterior columns-diminished vibration sensation and proprioception
   b. Corticospinal tracts-upper motor neuron signs
3. Cerebrum
   - Dementia
   - Optic atrophy
4. Autonomic neuropathy [3]

Investigation

1. Haemoglobin is low
2. MCV is raised
3. Erythrocyte count is low
4. The peripheral blood smear:
   - Megaloblastes
   - Anisocytosis (different in size)
   - Poikilocytosis (different in shape)
   - Macro-ovalocyte (Fig. 2.25, 2.26)
   - Hypersegmented neutrophils
   - Four or six-lobed neutrophils
   - Reticulocyte count is reduced
   - Pancytopenia

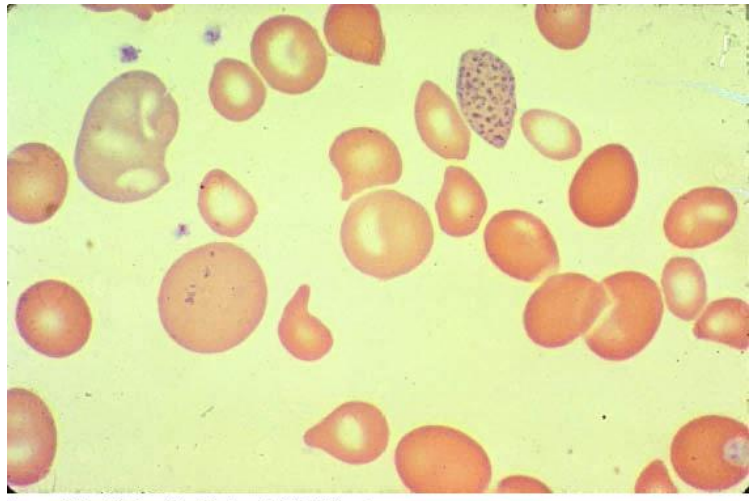


Figure 2.25 Vitamin B12 deficiency. (Peripheral blood, 100 x.) Shown are several hallmark features of vitamin B_{12} deficiency, including macro-ovalocytes, basophilic stippling, and bizarre-shaped red cell forms. (Courtesy of L Damon.)

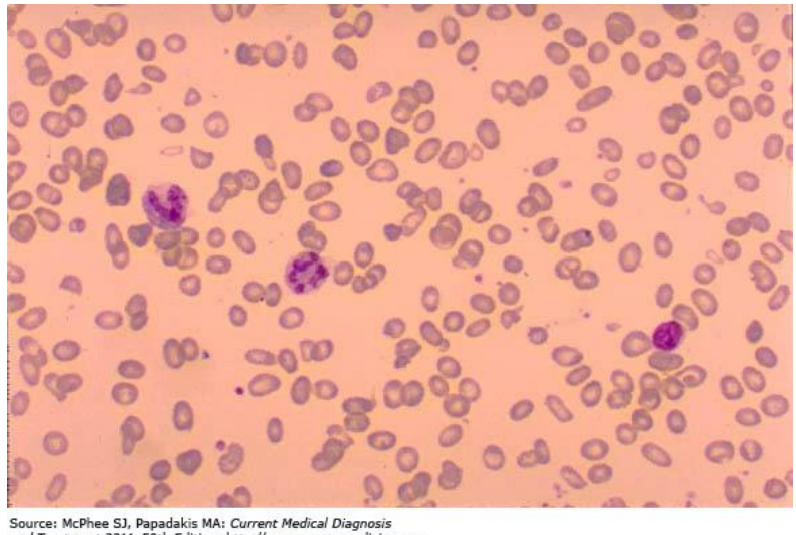


Figure 2.26 Vitamin B12 deficiency. (Peripheral blood, 50 x.) Hypersegmentation of a neutrophil associated with vitamin B_{12} deficiency. The combination of polymorphonuclear neutrophil nuclear hypersegmentation plus macro-ovalocytes renders the smear megaloblastic, consistent with vitamin B_{12} or folate deficiency. (Courtesy of L Damon.)

5. Bone marrow morphology:
   - Marked erythroid hyperplasia (Fig. 2.27, 2.28)
   - Defective red blood cell production
   - Megaloblastic changes
   - Giant metamyelocytes

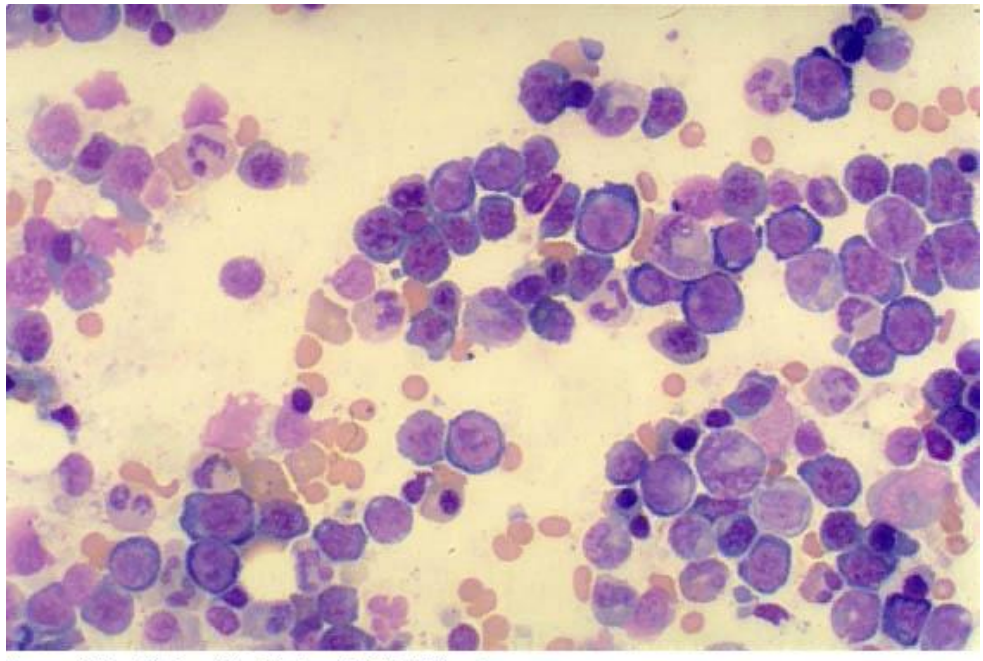


Figure 2.27 **Vitamin B12 deficiency**. (Bone marrow aspirate, 50 x.) Intense erythroid activity is shown. There is inversion of the usual myeloid-to-erythroid ratio (normally 2–4:1), with predominance of early erythroid cells (pronormoblasts) with deep basophilic cytoplasm. Giant band forms are also present. There is dyssynchrony between nuclear and cytoplasmic maturation—so-called megaloblastic changes. (Courtesy of L Damon.)

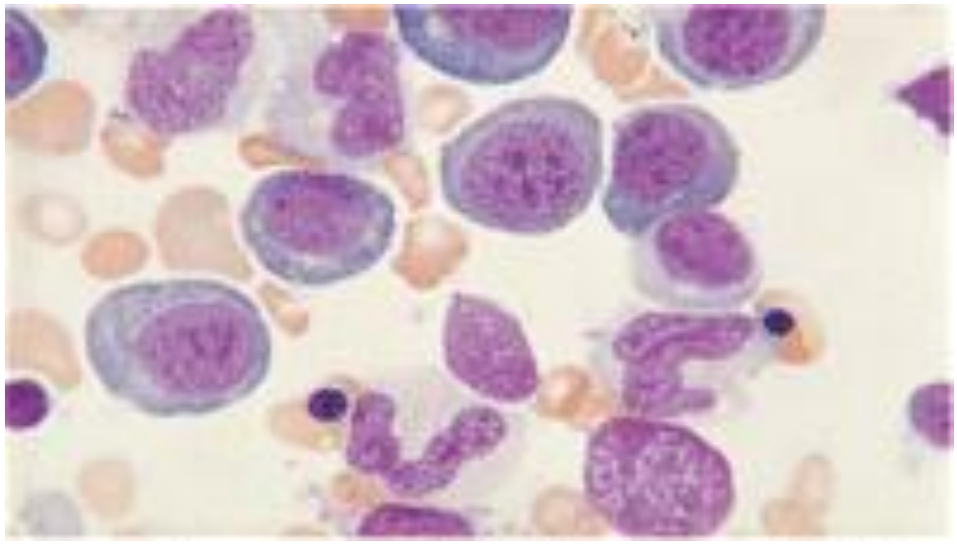


Figure 2.28 **A Megaloblastic anaemia**. Bone marrow aspirate showing megaloblasts at different stages and giant metamyelocytes.

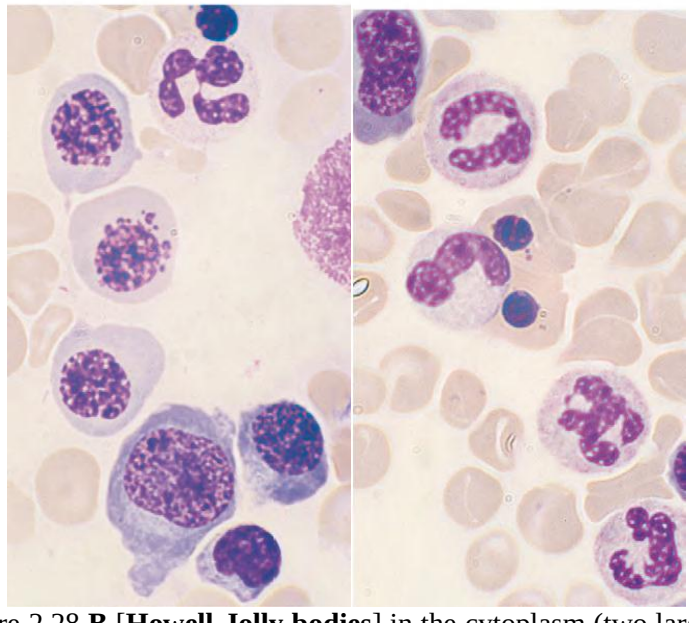


Figure 2.28 **B** [**Howell-Jolly bodies**] in the cytoplasm (two large band-form neutrophils are also present); and the central orthochromatic cells have karyorrhectic pyknotic nuclei linked by a thin chromatin bridge. [7]

6. Other laboratory abnormalities:
   - Elevated serum lactate dehydrogenase (LDH)
   - Modest increase in indirect bilirubin
7. The diagnosis is made by low vitamin B_{12} serum level
   - Normal vitamin B_{12} level is > 240 pg/mL
   - Overt vitamin B_{12} deficiency will have serum levels < 170 pg/mL (Table 2.10)
   - Symptomatic patients usually having levels < 100 pg/mL
8. Elevated level of serum methylmalonic acid (> 1000 nmol/L)[5] (11& 3)
9. A two-part Schilling test (Fig. 2.29)
10. Anti-intrinsic factor antibodies detection[6]

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[5] However, elevated levels of serum methylmalonic acid can be due to kidney insufficiency. (11)

[6] The finding of anti-intrinsic factor antibodies in the context of B_{12} deficiency is diagnostic of pernicious anaemia without further investigation. Antiparietal cell antibodies are present in over 90% of cases but are also present in 20% of normal females over the age of 60 years; a negative result makes pernicious anaemia less likely but a positive result is not diagnostic. Patients with low B_{12} levels and negative anti-intrinsic factor antibodies should have a Schilling test performed to determine whether there is B_{12} malabsorption, and if so, where it is occurring. (3)

Small bowel factors

One-third of patients with pancreatic exocrine insufficiency fail to transfer dietary vitamin B_{12} from R protein to intrinsic factor. This usually results in slightly low vitamin B_{12} values but no tissue evidence of vitamin B_{12} deficiency. (3)

Motility disorders or hypogammaglobulinaemia can result in bacterial overgrowth and the ensuing competition for free vitamin B_{12} can lead to deficiency. This is corrected to some extent by appropriate antibiotics.

A small number of people heavily infected with the fish tapeworm develop vitamin B_{12} deficiency. (3)

Inflammatory disease of the terminal ileum, such as Crohn's disease, may impair the interaction of the vitamin B_{12}-intrinsic factor complex with its

Table 2.10 **Investigations in megaloblastic anaemia** [3]

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

receptor, as will surgery on part of the bowel. Both may result in vitamin B_{12} malabsorption. [3]

It is possible to distinguish pernicious anaemia from intestinal problems with a two-part Schilling test. The patient must be vitamin B_{12}-replete, have normal renal function and be able to comply with a 24-hour urine collection. This latter criterion is important, as up to 25% of tests are invalidated by an incomplete urine collection. It is important to note that this is not a test of gut function, but simply distinguishes pernicious anaemia from the other causes of vitamin B_{12} deficiency. [3]

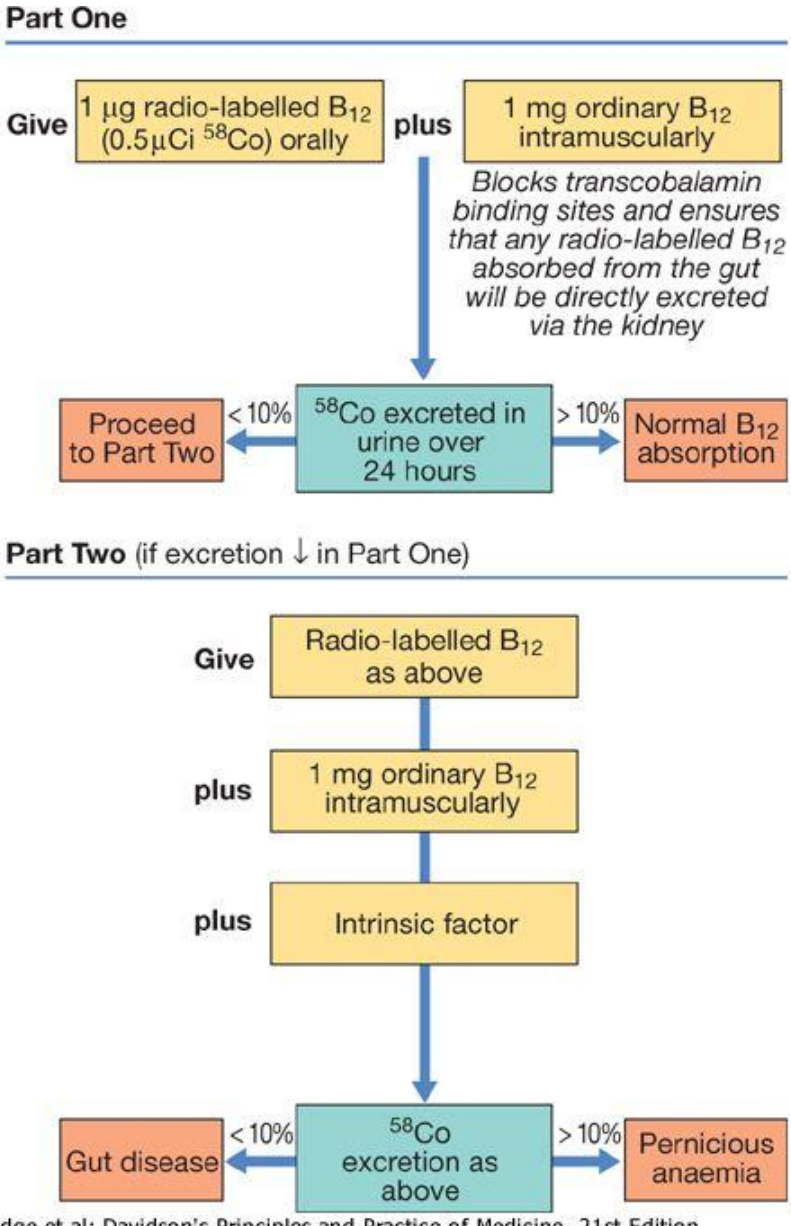


Figure 2.29 **The two-part Schilling test in the diagnosis of the cause of vitamin B_{12} deficiency.**

Folate

Folates are produced by plants and bacteria;

Rich source

1. Dietary leafy vegetables
   - Spinach
   - Broccoli
   - Lettuce
2. Fruits
   - Bananas
   - Melons
3. Animal protein
   - Liver
   - Kidney

Absorption

1. Western diet contains daily intake of 50 μg
2. Excess cooking for longer than 15 minutes destroys folates
3. Most dietary folate is present as polyglutamates
4. These are converted to monoglutamate in the upper small bowel
5. Actively transported into plasma
6. Plasma folate is loosely bound to plasma proteins such as albumin
7. There is an enterohepatic circulation.
8. Total body stores of folate are small and deficiency can occur in a matter of weeks. [(3)]

Folate deficiency

1. The elderly or psychiatric patient is particularly susceptible to dietary deficiency and
2. This is exacerbated in the presence of gut disease or malignancy.

3. Pregnancy-induced folate deficiency is the most common cause of megaloblastosis world-wide.[7]

Aetiology

1. Diet
   - Poor intake of vegetables
2. Malabsorption
   - e.g. Coeliac disease
3. Loss: dialysis
4. Increased demand
   - Cell proliferation, e.g. haemolysis
   - Pregnancy
   - Exfoliative skin disease

4. Drugs*[8]
   - Certain anticonvulsants (e.g. phenytoin)
   - Contraceptive pill
   - Certain cytotoxic drugs (e.g. methotrexate) (Table 2.11) (3)

[7] The edentulous elderly or psychiatric patient is particularly susceptible to dietary deficiency and this is exacerbated in the presence of gut disease or malignancy. Pregnancy-induced folate deficiency is the most common cause of megaloblastosis world-wide and is more likely in the context of twin pregnancies, multiparity and hyperemesis gravidarum. Serum folate is very sensitive to dietary intake; a single meal can normalise it in a patient with true folate deficiency, whereas anorexia, alcohol and anticonvulsant therapy can reduce it in the absence of megaloblastosis. For this reason red cell folate levels are a more accurate indicator of folate stores and tissue folate deficiency.

[8] *Usually only a problem in patients deficient in folate from another cause

Table 2.11 **Causes of folate deficiency** [12]
1. Poor diet
Especially poverty, psychiatric disturbance, alcoholism, dietary fads, scurvy, kwashiorkor, goat's milk anaemia, partial gastrectomy, other gastrointestinal disease
2. Malabsorption
Gluten-induced enteropathy (child or adult or associated with dermatitis herpetiformis)
Tropical sprue
Congenital specific malabsorption
Minor factor: partial gastrectomy, jejunal resection, inflammatory bowel disease, lymphoma, systemic infections
Drugs: cholestyramine, sulphasalazine, methotrexate, ? others (see (5) below).
3. Excessive requirements
Physiological
Pregnancy
Prematurity and infancy
Pathological:
(a) Malignancies—leukaemia, carcinoma, lymphoma, myeloma, sarcoma, etc.
(b) Blood disorders—haemolytic anaemia (especially sickle-cell anaemia, thalassaemia major), primary myelofibrosis
(c) Inflammatory—tuberculosis, malaria, Crohn's diseases, psoriasis, exfoliative dermatitis, rheumatoid arthritis, etc.
(d) Metabolic—homocystinuria (some cases)
4. Excess urinary excretion
Congestive heart failure, acute liver damage, chronic dialysis
5. Drugs
Mechanism uncertain
Anticonvulsants (diphenylhydantoin, primidone, barbiturates)
? nitrofurantoin
? alcohol
Also drugs causing malabsorption of folate (see (2) above)
6. Liver disease
Mixed causes above, and poor storage

Clinical Findings

Symptoms and Signs

The features are similar to those of vitamin B12 deficiency, with megaloblastic anemia and megaloblastic changes in mucosa. However, there are none of the neurologic abnormalities associated with vitamin B_{12} deficiency. (11)

Investigation

1. Diagnostic findings
   - Low serum folate levels (fasting blood sample)
   - Red cell folate levels low (but may be normal if folate deficiency is of very recent onset) (3)
2. proprioception findings Broccoli
   - Macrocytic dysplastic blood picture
   - Megaloblastic marrow (3)

Management of megaloblastic anaemia

1. If a patient is very ill treatment with folic acid and vitamin B_{12} must be started before vitamin B_{12} and red cell folate results
2. The use of folic acid alone in the presence of vitamin B_{12} deficiency may result in worsening of neurological deficits. (D)
3. Rarely in severe angina or heart failure transfusion can be used
4. In chronic anaemia exchange transfusion or slow administration of 1 unit each day with diuretic cover may be given cautiously. (3)

Vitamin B_{12} deficiency

1. Hydroxycobalamin 1000 μg i.m. in five doses 2 or 3 days apart
2. Maintenance therapy of 1000 μg every 3 months for life[9]

[9] The reticulocyte count will peak by the 5th-10th day after therapy and may be as high as 50%
The haemoglobin will rise by 10 g/L every week

3. Additional iron therapy
4. A sensory neuropathy may take 6-12 months to correct
5. Long-standing neurological damage may not improve [3]

Folate deficiency

1. Oral folic acid 5 mg daily for 3 weeks will treat acute deficiency
2. Maintenance therapy 5 mg once weekly
3. Prophylactic folic acid in pregnancy prevents megaloblastosis and reduces the risk of fetal neural tube defects
4. Prophylactic supplementation is also given in:
   - Autoimmune haemolytic anaemia
   - Haemoglobinopathies
5. Supraphysiological supplementation (400 μg/day) can reduce the risk of coronary and cerebrovascular disease by reducing plasma homocysteine levels [3]

The response of the marrow is associated with a fall in plasma potassium levels and rapid depletion of iron stores.

If an initial response is not maintained and the blood film is dimorphic (i.e. shows a mixture of microcytic and macrocytic cells), the patient may need additional iron therapy.

A sensory neuropathy may take 6-12 months to correct; long-standing neurological damage may not improve. [3]

Haemolytic anaemia

Definition

Anemias due to increased destruction of red cells, which we know as hemolytic anemias (HAs)[1]

Types

- May be *inherited* or *acquired* (Table 2.12)
- Clinically may be *acute* or *chronic*
- They may vary from mild to very severe
- The site of hemolysis may be *intravascular* or *extravascular*
- With respect to mechanisms, HAs may be due to *intracorpuscular* or to *extracorpuscular* causes (10)

Table 2.12 **Classification of Hemolytic Anemias***(10)

	Intracorpuscular Defects	**Extracorpuscular Factors**
Hereditary	Hemoglobinopathies Enzymopathies Membrane-cytoskeletal defects	Familial (atypical) hemolytic uremic syndrome
Acquired	Paroxysmal nocturnal hemoglobinuria (PNH)	Mechanical destruction (microangiopathic) Toxic agents Drugs Infectious Autoimmune

Hereditary causes correlate with ***intracorpuscular defects*** because these defects are due to inherited mutations. The one exception is PNH because the defect is due to an acquired somatic mutation.
Acquired causes correlate with ***extracorpuscular factors*** because mostly these factors are exogenous. The one exception is familial hemolytic uremic syndrome (HUS) (10)

[1] The normal red cell lifespan of 120 days may be shortened by a variety of abnormalities (3)

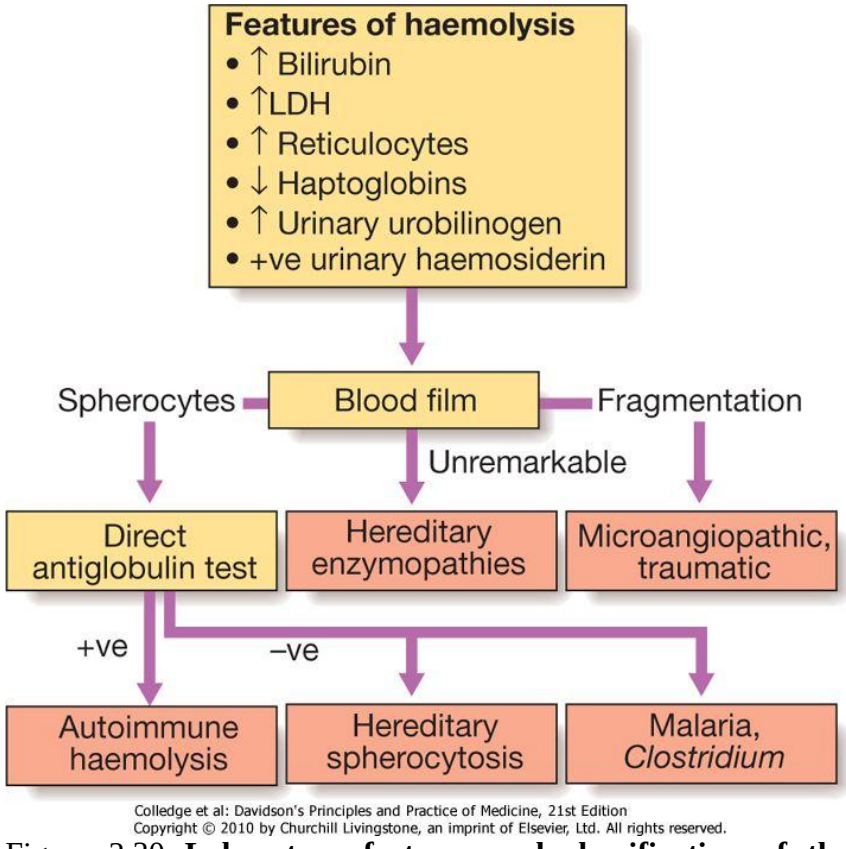


Figure 2.30 **Laboratory features and classification of the causes of haemolysis.** (LDH = lactate dehydrogenase; DAT = direct antiglobulin test)

Investigation and differential diagnosis of haemolysis are outlined in Figure 2.30 Red cell destruction overloads pathways for haemoglobin breakdown, causing a modest rise in unconjugated bilirubin in the blood and mild jaundice. Increased reabsorption of urobilinogen from the gut results in an increase in urinary urobilinogen. Red cell destruction releases LDH into the serum. The bone marrow compensation results in a reticulocytosis, and nucleated red cell precursors may also appear in the blood. Activation of the bone marrow can result in a neutrophilia and immature granulocytes appearing in the blood to cause a leuco-erythroblastic blood film. The appearances of the red cells may give an indication of the likely cause of the haemolysis [(3)]

Aetiology

The causes of haemolytic anaemia can be classified as:

Inherited[2]

1. Red cell membrane defect (Fig. 2.30, 2.31)
   - Hereditary spherocytosis
   - Hereditary elliptocytosis
2. Haemoglobin abnormalities
   - Thalassaemia
   - Sicklecell disease
3. Metabolic defects
   - Glucose-6-phosphate dehydrogenase deficiency
   - Pyruvate kinase deficiency
   - Pyrimidine kinase deficiency
4. Miscellaneous
   - Infections
     a. Malaria
     b. Mycoplasma
     c. Clostridium welchii
     d. Generalized sepsis
   - Drugs and chemicals causing
     a. Damage to the red cell membrane or
     b. Oxidative haemolysis
   - Hypersplenism

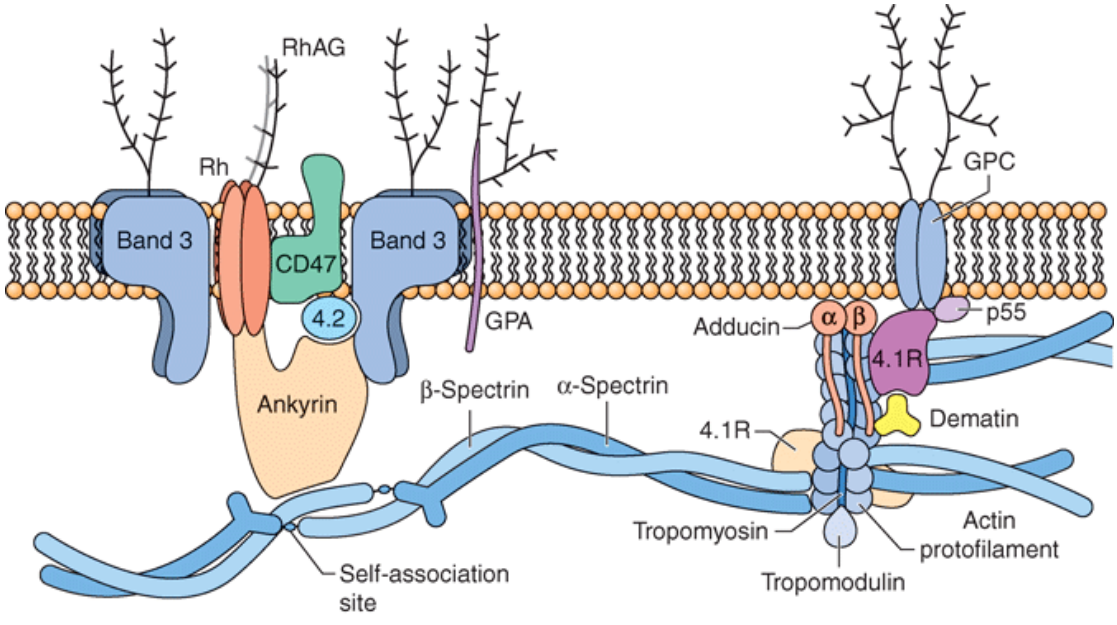
Acquired

1. Immune
   - Autoimmune
     a. Warm
     b. Cold
   - Alloimmune

[2] *Inherited* red cell abnormalities resulting in chronic haemolytic anaemia

   a. Haemolytic transfusion reactions
   b. Haemolytic disease of the newborn
   c. After allogeneic bone marrow or organ transplantation
- Drug-induced
   a. Dapsone
   b. Sulfasalazine

2. Non-immune
   - Acquired membrane defects (Table 2.13)
      a. Paroxysmal nocturnal haemoglobinuria
   - Mechanical
      a. Microangiopathic haemolytic anaemia
      b. Valve prosthesis
      c. March haemoglobinuria
   - Secondary to systemic disease
   - Renal and liver failure [8]
   - Toxins
      a. Arsenic
      b. Products of *Clostridium welchii* [3]
   - Infective
      a. *Plasmodium*
      b. *Toxoplasma gondii* [12]



Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com

Figure 2.31 **Diagram of red cell membrane-cytoskeleton.** (For explanation see text.) *(From N Young et al: Clinical Hematology. Copyright Elsevier, 2006; with permission.)*

Due to Abnormalities of the Membrane-Cytoskeleton

The lipid bilayer incorporates phospholipids and cholesterol, and it is spanned by a number of proteins that have their hydrophobic transmembrane domains embedded in the membrane. Most of these proteins have hydrophilic domains extending toward both the outside and the inside of the cell. Other proteins are tethered to the membrane through a glycosylphosphatidylinositol (GPI) anchor, and they have only an extracellular domain. These proteins are arranged roughly perpendicular to or lying across the membrane: they include ion channels, receptors for complement components, receptors for other ligands, and some of unknown function. The most abundant of these proteins are glycophorins and the so-called band 3, an anion transporter. The extracellular domains of many of these proteins are heavily glycosylated, and they carry antigenic determinants that correspond to blood groups. Underneath the membrane, and tangential to it, is a network of other proteins that make up the cytoskeleton: the main cytoskeletal protein is spectrin, the basic unit of which is a dimer of -spectrin and -spectrin. [(10)]

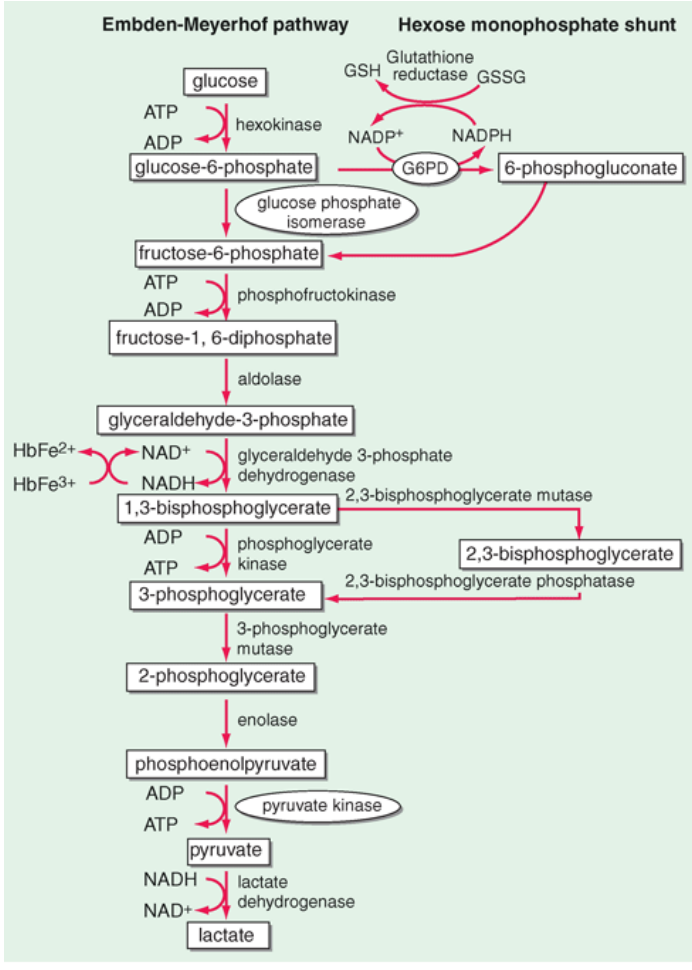
Table 2.13 Inherited Diseases of the Red Cell Membrane-Cytoskeleton [10]

Gene	**Chromosomal Location**	**Protein Produced**	**Disease(s) with Certain Mutations (Inheritance)**	**Comments**
SPTA1	1q22-q23	-Spectrin	HS (recessive)	Rare
			HE (dominant)	Mutations of this gene account for about 65% of HE. More severe forms may be due to coexistence of an otherwise silent mutant allele
SPTB	14q23-q24.1	-Spectrin	HS (dominant)	Rare
			HE (dominant)	Mutations of this gene account for about 30% of HE, including some severe forms
ANK1	8p11.2	Ankyrin	HS (dominant)	May account for majority of HS
SLC4A1	17q21	Band 3 (anion channel)	HS (dominant)	Mutations of this gene may account for about 25% of HS
			Southeast Asia ovalocytosis (dominant)	Polymorphic mutation (deletion of 9 amino acids);

				clinically asymptomatic; protective against *Plasmodium falciparum*
			Stomatocytosis	Certain specific missense mutations shift protein function from anion exchanger to cation conductance
EPB41	1p33-p34.2	Band 4.1	HE (dominant)	Mutations of this gene account for about 5% of HE: mostly with prominent morphology but no hemolysis in heterozygotes; severe hemolysis in homozygotes
EPB42	15q15-q21	Band 4.2	HS (recessive)	Mutations of this gene account for about 3% of HS.
RHAG	6p21.1-p11	Rhesus antigen	Chronic nonspherocytic hemolytic anemia	Very rare; associated with total loss of all Rh antigens

Abbreviations: HE, hereditary elliptocytosis; HS, hereditary spherocytosis.

In either case, the life span of the red cell is reduced, which is the definition of a *hemolytic disorder*.



Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com

Figure 2.32 **RBC metabolism.** The Embden-Meyerhof pathway (glycolysis) generates ATP for energy and membrane maintenance. The generation of NADPH maintains hemoglobin in a reduced state. The hexose monophosphate shunt generates NADPH that is used to reduce glutathione, which protects the red cell against oxidant stress. Regulation of 2,3-bisphosphoglycerate levels is a critical determinant of oxygen affinity of hemoglobin. Enzyme deficiency states in order of prevalence: glucose-6-phosphate -dehydrogenase (G6PD) > pyruvate kinase > glucose-6-phosphate isomerase > rare deficiencies of other enzymes in the pathway. The more common enzyme deficiencies are encircled. [(10)]

General Clinical Features

Clinical presentation

The clinical presentation depends to:

1. The onset (abrupt or gradual)
2. Autoimmune HA or with favism may be a medical emergency
3. Mild hereditary spherocytosis or with cold agglutinin disease may be diagnosed after years[3]

Signs

1. Jaundice (Table 2.14)
2. Discoloration of the urine
3. Spleenomegaly
4. Liver may be enlarged
5. Skeletal changes due to overactivity of the bone marrow

(In all severe congenital forms of HA) (10)

Table 2.14 **Some Common Features of Hemolytic Disorders** (10)

General examination	Jaundice, pallor
Other physical findings	Spleen may be enlarged; bossing of skull in severe congenital cases
Hemoglobin level	From normal to severely reduced
MCV, MCH	Usually increased
Reticulocytes	Increased
Bilirubin	Increased (mostly unconjugated)
LDH	Increased (up to 10x normal with intravascular hemolysis)
Haptoglobin	Reduced to absent (if hemolysis is part intravascular)

Abbreviations: LDH, lactate dehydrogenase; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume.

[3] This is due in large measure to the remarkable ability of the body to adapt to anemia when it is slowly progressing

Laboratory features

1. Unconjugated bilirubin
2. Aspartate transaminase (AST)
3. Urobilinogen will be increased in both urine and stool
4. Hemoglobinuria
5. Hemosiderinuria
6. Increased hemoglobin (in serum)
7. Lactate dehydrogenase (LDH) is increased
8. Haptoglobin is reduced[4]
9. Bilirubin level may be normal or only mildly elevated
10. Increased in reticulocytes
11. Increased mean corpuscular volume (MCV)
12. Macrocytes
13. Polychromasia
14. Nucleated red cells
15. Erythroid hyperplasia
16. Specific tests (10)
    - Antiglobulin (Coombs') test
    - Schumm's test

[4] Haptoglobin is an α_2-globulin produced by the liver which binds free haemoglobin, resulting in a fall in levels of haptoglobin. Once haptoglobins are saturated, free haemoglobin is oxidised to form methaemoglobin which binds to albumin, in turn forming methaemalbumin which can be detected spectrophotometrically in the Schumm's test. (3)

Autoimmune haemolytic anaemia

Definition

Autoimmune haemolytic anaemias (AIHA) are acquired disorders resulting from increased red cell destruction due to red cell autoantibodies and characterized by the presence of a positive direct antiglobulin (Coombs') test, which detects the autoantibody on the surface of the patient's red cells. [8]

Immune destruction of red cells

1. IgM or IgG red cell antibodies
   - Which fully activate the complement cascade?
   - Cause lysis of red cells in the circulation
2. IgG antibodies
   - Frequently do not activate complement
   - The coated red cells undergo extravascular haemolysis
   - In the spleen through an interaction with Fc receptors [8]

Classification

Autoimmune haemolytic anaemia is best classified according to the temperature at which the antibody optimally binds to the erythrocyte.

The four major types of autoimmune haemolytic anaemia are:

1. Warm autoimmune haemolytic anaemia
2. Cold agglutinin syndrome (Table 2.15)
3. Paroxysmal cold haemoglobinuria
4. Mixed-type autoimmune haemolytic anaemia [12]

Table 2.15 Causes and major features of autoimmune haemolytic anaemias [8]

	Warm	Cold
Temperature at which antibody attaches best to red cells	37°C	Lower than 37°C
Type of antibody	IgG	IgM
Direct Coombs' test	Strongly positive	Positive
Causes of primary conditions	Idiopathic	Idiopathic
Causes of secondary condition	Autoimmune disorders e.g. Systemic lupus erythematosus Chronic lymphocytic leukaemia Lymphomas Hodgkin's lymphoma Carcinomas Drugs, many including methyldopa, penicillins, cephalosporins, NSAIDs, quinine, interferon	Infections, e.g. infectious mononucleosis, Mycoplasma pneumoniae, other viral infections (rare) Lymphomas Paroxysmal cold haemoglobinuria (IgG)

Warm autoimmune haemolysis

Definition

The anaemia in which:

1. *Warm antibodies* bind best at 37 °C
2. Account for 80% of cases
3. The majority are IgG
4. Often react against Rhesus antigens
5. Direct antiglobulin test is positive for IgG and/or complement (3& 12)

Aetiology

1. No underlying cause in up to 50% of cases

2. Secondary to a wide variety of other conditions:
   - Lymphoid neoplasms
     a. Lymphoma
     b. Chronic lymphocytic leukaemia
     c. Myeloma
   - Solid tumours:
     a. Lung
     b. Colon
     c. Kidney
     d. Ovary
     e. Thymoma
   - Connective tissue disease:
     a. SLE
     b. Rheumatoid arthritis
   - Drugs:
     a. Methyldopa
     b. Mefenamic acid
     c. Penicillin
     d. Quinidine
   - Miscellaneous:
     a. Ulcerative colitis
     b. HIV [3]

Epidemiology

1. The incidence is 1/100 000 population per annum
2. It occurs at all ages but is more common in middle age
3. Women are affected slightly more often than men [3& 12]

Clinical features

Symptomatic patients present with:

1. Anaemia
2. Jaundice (Fig. 2.33, 2.34)

3. Splenomegaly
4. Most patients have a chronic, stable anaemia
5. In severest form, patients present with:
    - Fulminant intravascular haemolysis
    - Progressive anaemia
    - Congestive heart failure
    - Respiratory distress
    - Neurological abnormalities [12]

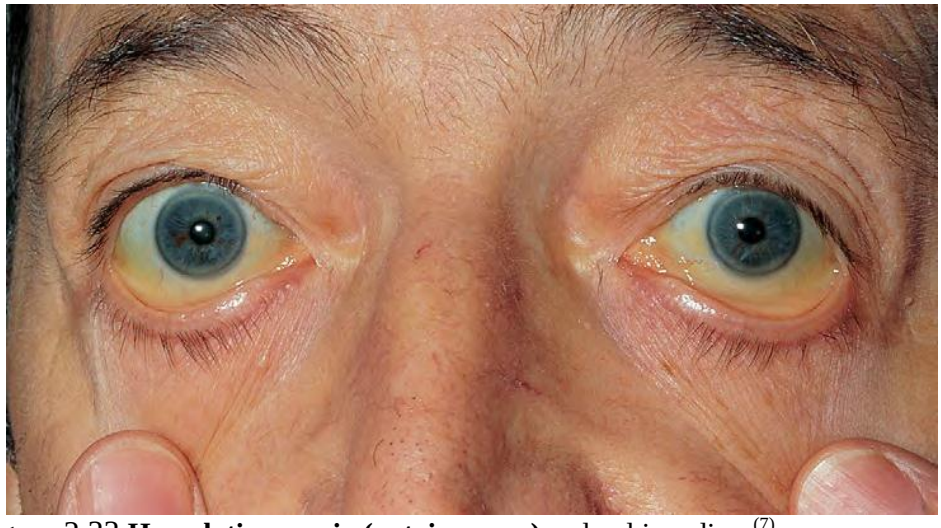


Figure 2.33 **Hemolytic anemia (autoimmune):** scleral jaundice. [7]

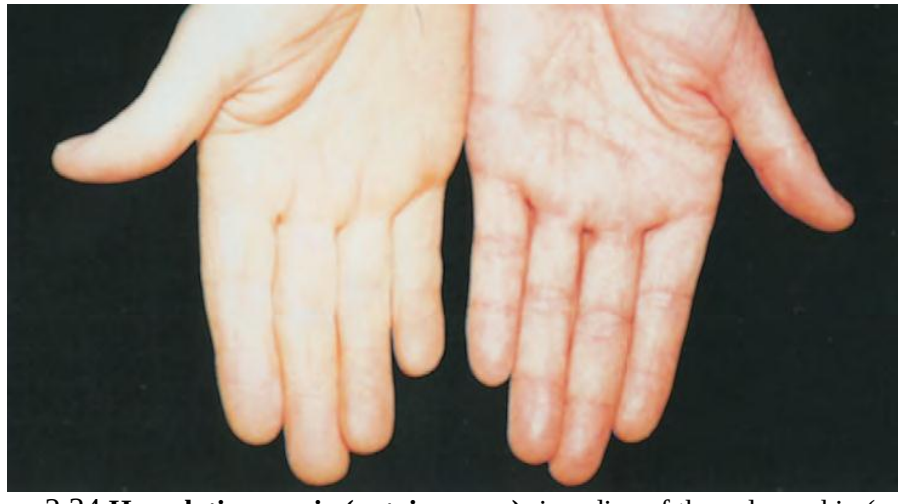


Figure 2.34 **Hemolytic anemia (autoimmune):** jaundice of the palmar skin (on the left) contrasted with normal skin color. (7)

Investigations

1. Peripheral smear
    - Anisocytosis
    - Reticulocytosis (Fig. 2.35)
    - Spherocytes
    - Macrocytes

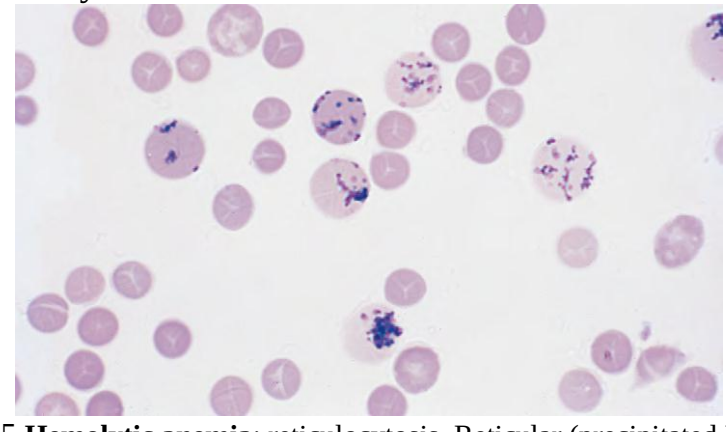


Figure 2.35 **Hemolytic anemia**: reticulocytosis. Reticular (precipitated RNA) material is seen in the larger cells. New methylene blue stain (Giemsa counterstain). (7)

2. The platelet count is normal[5]
3. Bone marrow erythroid hyperplasia (Fig. 2.36)
4. Rarely, patients with clinical and laboratory findings consistent with a warm autoimmune haemolytic anaemia may have a negative DAT
5. These cases have been attributed to IgA, IgM, or low affinity IgG antibodies.
6. Alternatively, bound IgG has been reported below the level of routine DAT detection
7. The diagnosis is confirmed by the direct Coombs or antiglobulin test[6] (Fig. 2.37). (12& 3)

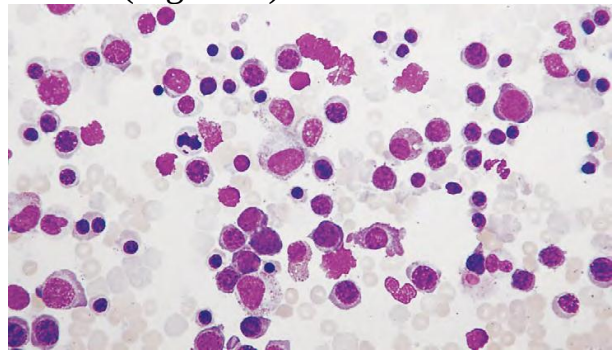


Figure 2.36 **Hemolytic anemia**: This bone marrow cell trail with erythroid hyperplasia shows a dominance of erythroblasts. (7)

[5] Except in patients with Evans' syndrome where the autoantibody destroys both red cells and platelets (12)

[6] The diagnosis is confirmed by the direct Coombs or antiglobulin test (Fig. 24.23). The patient's red cells are mixed with Coombs reagent, which contains antibodies against human IgG/M/complement. If the red cells have been coated by antibody in vivo, the Coombs reagent will induce their agglutination and this can be detected visually. The relevant antibody can be eluted from the red cell surface and tested against a panel of typed red cells to determine against which red cell antigen it is directed. The most common specificity is Rhesus and most often anti-e; this is helpful when choosing blood to cross-match. The direct Coombs test can be negative in the presence of brisk haemolysis. A positive test requires about 200 antibody molecules to attach to each red cell; with a very avid complement-fixing antibody, haemolysis may occur at lower levels of antibody-binding. The standard Coombs reagent will miss IgA or IgE antibodies. (3)

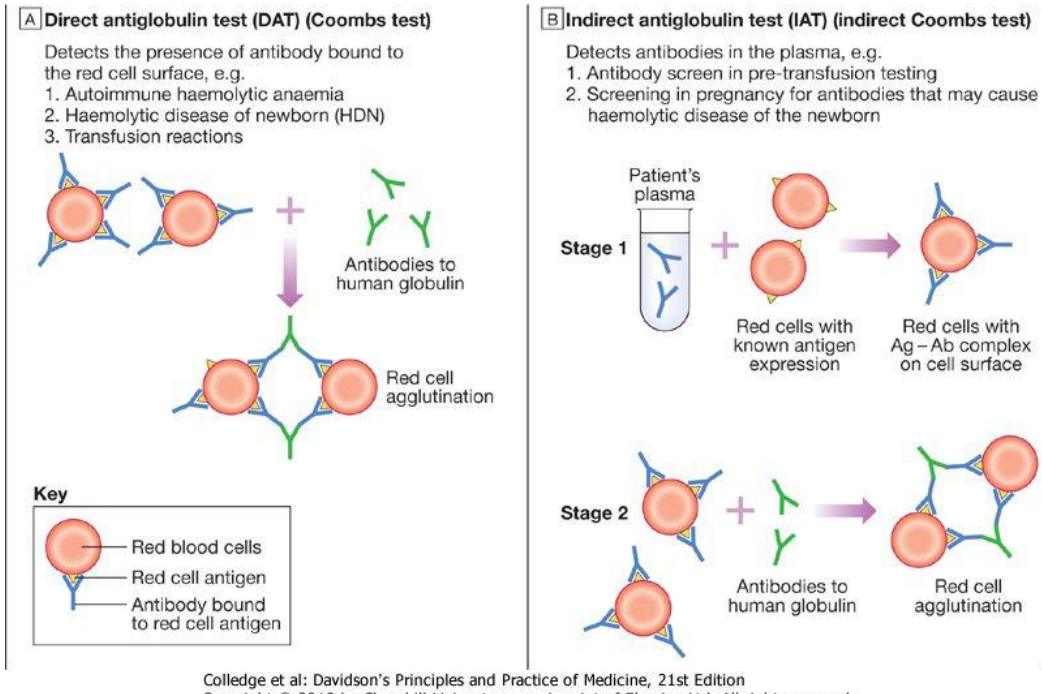


Figure 2.37 **Direct and indirect antiglobulin tests.**

Treatment

1. Corticosteroids, are the primary therapy which:
   - Block macrophage Fc receptor activity
   - Inhibit antibody production
2. Prednisone at a dose of 1 to 2 mg/kg body weight in divided doses is effective
3. Higher doses rarely provide additional benefit[7]

[7] Higher doses rarely provide additional benefit, but do increase the number and severity of side-effects. Treatment continues until the haemoglobin levels stabilize. The initial dose of prednisone can then be tapered at a rate of 5 to 10 mg/week. Once a dose of 10 mg/day is reached, the steroid taper should progress more slowly in order to determine the minimum controlling dose. Side effects may be reduced by using an alternate-day schedule.

4. Splenectomy should be performed only in steroid-refractory patients
5. Alternative therapies include:
   - Azathioprine
   - Cyclophosphamide
   - Intravenous immunoglobulin (IVIG)
   - Danazol
   - Plasma exchange[8]
6. Recently, rituximab (chimeric anti-CD20 monoclonal antibody)
7. The decision to transfuse requires careful consideration (12)

Cold agglutinin syndrome

Definition

The anemia in which:

1. *Cold antibodies* bind best at 4 °C but can bind up to 37 °C
2. They are usually IgM
3. Bind complement
4. They account for the other 20% of cases
5. Occurs as an acute or chronic condition (3& 12)

Aetiology

1. Idiopathic
2. Infections(following infection)
   - Infectious mononucleosis
   - Mycoplasma pneumonia
   - Cytomegalovirus

[8] These therapeutic options should be reserved for patients unfit for splenectomy or who have failed to respond to steroids and surgery.

- Epstein–Barr virus (EBV)

3. Lymphomas
4. Chronic lymphocytic leukaemia
5. Waldenström's macroglobulinaemia [8&12]

Clinical features

Acute form

1. Commonly seen in adolescents and young adults (Fig. 2.38)
2. Following infection with
3. *Mycoplasma pneumoniae* or
4. Infectious mononucleosis
5. Haemolysis occurs 1 to 2 weeks after infection
6. Associated with a rise in polyclonal anti-I IgM ab. with:
   - *Mycoplasma pneumonia*
   - Infectious mononucleosis [12]

Chronic form

1. Occurs commonly in older people
2. Either idiopathically or
3. Associated with lymphoma
4. Chronic lymphocytic leukaemia, or
5. Waldenström's macroglobulinaemia
6. Chronic intravascular haemolysis and anaemia
7. That are exacerbated by cold temperature
8. Episodes of Raynaud's phenomenon [12]

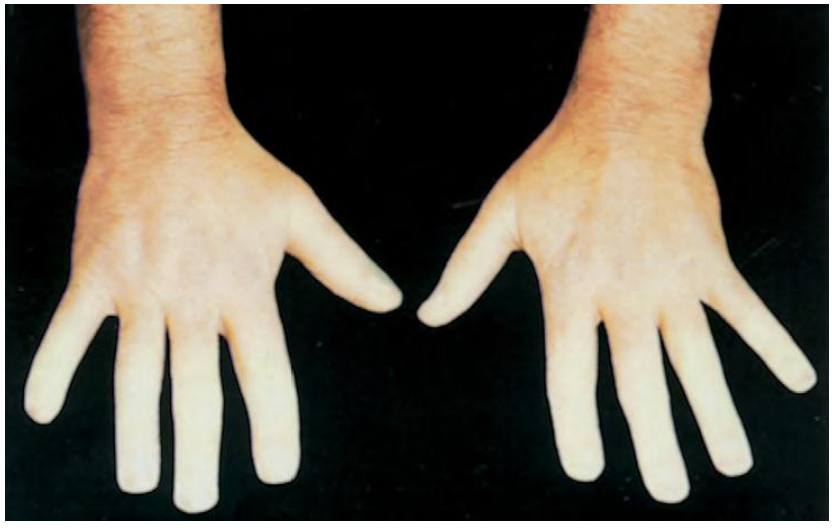


Figure 2.38 Autoimmune hemolytic anemia (cold type): peripheral blood film showing autoagglutination of red cells. (7)

Investigations

1. Peripheral smear (Fig. 2.39)
2. Red-cell agglutination
3. Direct antiglobulin test is positive for complement (12)

Treatment

Acute cold agglutinin disease is a rare form

1. Always self-limited
2. Transfusions
3. Avoidance of cold
4. Treatment of the mycoplasma infection
5. Corticosteroids are usually not helpful
6. Splenectomy is almost never indicated (12)

Severe cold autoimmune haemolytic anaemia secondary to a B-cell neoplasm can be treated with:

1. Chlorambucil
2. Cyclophosphamide
3. Or α-interferon

4. Blood transfusion should be avoided
5. In situations of life-threatening anaemia, the blood should be given slowly through a blood warmer
6. Hypothermia must be avoided during surgery
7. Plasma exchange may be helpful
8. Corticosteroids and splenectomy are rarely effective
9. Rituximab, anti-CD-20 monoclonal antibody [12]

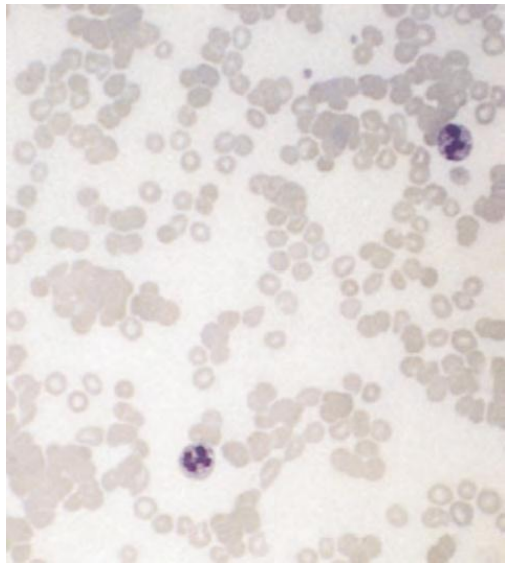


Figure 2.39 Autoimmune hemolytic anemia (cold type): peripheral blood film showing autoagglutination of red cells. [7]

Paroxysmal cold haemoglobinuria

Aetiology

Rarest form of autoimmune haemolytic anaemia

The disorder is caused by the complement-fixing Donath–Landsteiner IgG antibody

In the cold, this antibody binds to, and irreversibly fixes, complement to the red-cell membrane

Upon return to warmer temperatures, the antibody dissociates from the red cell leaving activated complement to lyse the cell. The Donath–Landsteiner antibody appears to have an anti-P specificity allowing it to bind to practically all red cells. [12]

Clinical features

1. Acute intravascular haemolysis
2. Abdominal pain
3. Peripheral cyanosis
4. Raynaud's phenomenon
5. Haemoglobinaemia
6. Haemoglobinuria after exposure to cold
7. In the past, paroxysmal cold haemoglobinuria was commonly associated with congenital syphilis but most cases are now associated with viral infections in children or are idiopathic in adults
8. During or shortly after a haemolytic episode, the direct antiglobulin test may be positive for complement but will be negative for IgG. [12]

Treatment

1. No specific therapy
2. Steroids are not useful
3. Most postinfectious cases are self-limited
4. Avoidance of cold
5. Transfusion is indicated only for severe haemolysis and life-threatening anaemia [12]

Mixed-type autoimmune haemolytic anaemia

Aetiology

1. Approximately 8% of all autoimmune haemolytic anaemias
2. Both IgG and complement are present on the red cells

3. Both warm IgG and cold IgM autoantibodies are present in the serum
4. The warm-reactive IgG autoantibodies are indistinguishable from antibodies encountered in warm autoimmune haemolytic anaemia.
5. The IgM autoantibodies are unlike those in cold-agglutinin syndrome in that they generally have low titres at 4°C and have high thermal amplitudes, reacting at 30°C or above.
6. These IgM autoantibodies usually have no distinguishable specificity, but on occasion have I or i specificities. [12]

Clinical features

1. May be idiopathic or
2. Secondary with systemic lupus erythematosus
3. Often severe and chronic
4. With intermittent exacerbations
5. Exposure to cold does not increase the haemolysis [12]

Treatment

1. Steroids
2. Splenectomy
3. Cytotoxic agents
4. If blood transfusions are necessary, selection of blood should adhere to transfusion guidelines outlined earlier for warm autoimmune haemolytic anaemia [12]

Bone marrow failure

Nonspecific anaemia is a common feature of bone marrow failure. It may occur in the pure red cell aplasias or as part of aplastic anaemia. (12)

Definition

Bone marrow failure is the failure of production of circulating blood cells by the bone marrow, resulting in single cytopenias or pancytopenia. Most often they are acquired, but there are also rare congenital disorders. (12)

1. Hypoproliferative anemia is also a prominent feature of hematologic diseases that are described as bone marrow failure (10)
2. The hypoproliferative anemias are normochromic, normocytic, or macrocytic and are characterized by a low reticulocyte count.
3. Deficient production of red blood cells (RBCs) occurs with marrow damage and dysfunction, which may be secondary to infection, inflammation, and cancer. (10)
4. Anemia in these disorders is often not a solitary or even the major hematologic finding. (10)
5. More frequent in bone marrow failure is pancytopenia: anemia, leukopenia, and thrombocytopenia. (10)
6. Low blood counts in the marrow failure diseases result from deficient hematopoiesis, as distinguished from blood count depression due to peripheral destruction of red cells (hemolytic anemias), platelets [idiopathic thrombocytopenic purpura (ITP) or due to splenomegaly], and granulocytes (as in the immune leukopenias). (10)
7. Hematopoietic failure syndromes are classified by dominant morphologic features of the bone marrow (Table 2.16). (10)

8. Although practical distinction among these syndromes usually is clear, they can occur secondary to other diseases, and some processes are so closely related that the diagnosis may be complex. (10)
9. Patients may seem to suffer from two or three related diseases simultaneously, or one diagnosis may appear to evolve into another.
10. Many of these syndromes share an immune-mediated mechanism of marrow destruction and some element of genomic instability resulting in a higher rate of malignant transformation. (10)

Table 2.16 **Differential Diagnosis of Pancytopenia** (10)

Pancytopenia with Hypocellular Bone Marrow	
Acquired aplastic anemia	
Constitutional aplastic anemia (Fanconi's anemia, dyskeratosis congenita)	
Some myelodysplasia	
Rare aleukemic leukemia	
Some acute lymphoid leukemia	
Some lymphomas of bone marrow	
Pancytopenia with Cellular Bone Marrow	
Primary bone marrow diseases	Secondary to systemic diseases
Myelodysplasia	Systemic lupus erythematosus
Paroxysmal nocturnal hemoglobinuria	Hypersplenism
	B_{12}, folate deficiency
Myelofibrosis	Overwhelming infection
Some aleukemic leukemia	Alcohol
Myelophthisis	Brucellosis
Bone marrow lymphoma	Sarcoidosis
Hairy cell leukemia	Tuberculosis
	Leishmaniasis
Hypocellular Bone Marrow ± Cytopenia	
Q fever	
Legionnaires' disease	
Anorexia nervosa, starvation	
Mycobacterium	

It is important that the internist recognize the marrow failure syndromes, as:

1. Their prognosis may be poor if the patient is untreated
2. Effective therapies are available but complex in choice [10]

Classification

1. Pure red-cell aplasia (PRCA)
2. Aplastic anaemia
3. Infiltration
4. Myelodysplastic syndrome (MDS)
5. Myelophthisis [12& 10]

Aplastic anaemia

Definition

Aplastic anemia is a disorder of hematopoiesis characterized by pancytopenia and a marked reduction or depletion of erythroid, granulocytic, and megakaryocytic cells in bone marrow.
In aplastic anemia, hematopoietic stem cells are unable to:

- Proliferate
- Differentiate, or
- Give rise to mature blood cells and
- Their precursors (5)

Epidemiology

1. Incidence
   - In Western countries about two new cases per 1 million persons per year.
   - In Asia, four new cases per 1 million persons per year in Bangkok and rural Thailand.[1]
   - The incidence is similar in males and females.
2. Age group
   - The disease occurs at all ages
   - More common in young adults aged 15 to 30 years
   - In persons older than 60 (5)

Etiology

Aplastic anemia may occur as the result of inherited abnormalities, such as Fanconi's anemia, but most cases are acquired. (Table 2.17) (5)
Causative factors include:
1. Drugs (Table 2.18) [2]

[1] In Thailand and China, rates of five to seven per million have been established. (10)

- Cytotoxic drugs
- Antibiotics
  a. Chloramphenicol
  b. Sulphonamides
- Antirheumatic agents
  a. Penicillamine
  b. Gold
  c. Phenylbutazone
  d. Indomethacin
- Antithyroid drugs
- Anticonvulsants
- Immunosuppressives-azathioprine

2. Chemicals
   - Benzene toluene solvent misuse-glue-sniffing
   - Insecticides
     a. Chlorinated hydrocarbons (DDT)
     b. Organophosphates
     c. Carbamates
3. Radiation
4. Viral hepatitis
5. Pregnancy
6. Paroxysmal nocturnal haemoglobinuria [3]

[2] For more than 50% of patients, however, no cause can be determined. Even when a well-defined association exists between an exposure and the subsequent development of aplastic anemia (e.g., chloramphenicol), it remains unclear why the disease develops in only a small proportion of exposed individuals. Furthermore, the mechanisms by which certain agents or classes of agents (e.g., viruses, drugs) contribute to the pathogenesis of aplastic anemia are still poorly understood. [5]

Table 2.17 **Classification of Aplastic Anemia and Single Cytopenias** [10]

Acquired	**Inherited**
Aplastic Anemia	
Secondary	Fanconi's anemia
Radiation	Dyskeratosis congenital
Drugs and chemicals	Shwachman-Diamond syndrome
Regular effects	Reticular dysgenesis
Idiosyncratic reactions	Amegakaryocytic thrombocytopenia
Viruses	Familial aplastic anemias
Epstein-Barr virus (infectious mononucleosis)	Preleukemia (monosomy 7, etc.)
Hepatitis (non-A, non-B, non-C hepatitis)	Nonhematologic syndrome (Down, Dubowitz, Seckel)
Parvovirus B19 (transient aplastic crisis, PRCA)	
HIV-1 (AIDS)	
Immune diseases	
Eosinophilic fasciitis	
Hyperimmunoglobulinemia	
Thymoma/thymic carcinoma	
Graft-versus-host disease in immunodeficiency	
Paroxysmal nocturnal hemoglobinuria	
Pregnancy	
Idiopathic (50 %)	
Cytopenias	
PRCA	Congenital PRCA (Diamond-Blackfan anemia)
Neutropenia/agranulocytosis	
Idiopathic	Kostmann's syndrome
Drugs, toxins	Shwachman-Diamond syndrome
Pure white cell aplasia	Reticular dysgenesis
Thrombocytopenia	
Drugs, toxins	Amegakaryocytic thrombocytopenia
Idiopathic amegakaryocytic	Thrombocytopenia with absent radii

Abbreviation: PRCA, pure red cell aplasia.

Infections: Hepatitis is the most common preceding infection, and posthepatitis marrow failure accounts for approximately 5% of etiologies in most series. Patients are usually young men who have recovered from a bout of liver inflammation 1 to 2 months earlier; the subsequent pancytopenia is very severe. The hepatitis is seronegative (non-A, non-B, non-C) and possibly due to an as yet undiscovered infectious agent. Fulminant liver failure in childhood also follows seronegative hepatitis, and marrow failure occurs at a high rate in these patients. Aplastic anemia can rarely follow infectious mononucleosis. Parvovirus B19, the cause of transient aplastic crisis in hemolytic anemias and of some PRCAs (see below), does not usually cause generalized bone marrow failure. Mild blood count depression is frequent in the course of many viral and bacterial infections but resolves with the infection.

Immunologic Diseases: Aplasia is a major consequence and the inevitable cause of death in *transfusion-associated graft-versus-host disease* (GVHD) that can occur after infusion of nonirradiated blood products to an immunodeficient recipient. Aplastic anemia is strongly associated with the rare collagen vascular syndrome *eosinophilic fasciitis* that is characterized by painful induration of subcutaneous tissues. Pancytopenia with marrow hypoplasia can also occur in systemic lupus erythematosus (SLE).

Pregnancy: Aplastic anemia very rarely may occur and recur during pregnancy and resolve with delivery or with spontaneous or induced abortion.

Paroxysmal Nocturnal Hemoglobinuria: An acquired mutation in the *PIG-A* gene in a hematopoietic stem cell is required for the development of PNH, but *PIG-A* mutations probably occur commonly in normal individuals. If the PIG-A mutant stem cell proliferates, the result is a clone of progeny deficient in glycosylphosphatidylinositol-linked cell surface membrane proteins. Small clones of deficient cells can be detected by sensitive flow cytometry tests in approximately one-half of patients with aplastic anemia at the time of presentation [and PNH cells are also seen in MDS (see below)]. Functional studies of bone marrow from PNH patients, even those with mainly hemolytic manifestations, show evidence of defective hematopoiesis. Patients with an initial clinical diagnosis of PNH, especially younger individuals, may later develop frank marrow aplasia and pancytopenia; patients with an initial diagnosis of aplastic anemia may suffer from hemolytic PNH years after recovery of blood counts.

Constitutional Disorders: Fanconi's anemia, an autosomal recessive disorder, manifests as congenital developmental anomalies, progressive pancytopenia, and an increased risk of malignancy. Chromosomes in Fanconi's anemia are peculiarly susceptible to DNA cross-linking agents, the basis for a diagnostic assay. Patients with Fanconi's anemia typically have short stature, café au lait spots, and anomalies involving the thumb, radius, and genitourinary tract. At least 12 different genetic defects (all but one with an identified gene) have been defined; the most common, type A Fanconi's anemia, is due to a mutation in *FANCA*. Most of the Fanconi's anemia gene products form a protein complex that activates FANCD2 by monoubiquitination to play a role in the cellular response to DNA damage and especially interstrand cross-linking.

Dyskeratosis congenita is characterized by mucous membrane leukoplasia, dystrophic nails, reticular hyperpigmentation, and the development of aplastic anemia in childhood. Dyskeratosis is due to mutations in genes of the telomere repair complex, which acts to maintain telomere length in replicating cells: The X-linked variety is due to mutations in the *DKC1* (*dyskerin*) gene; the more unusual autosomal dominant type is due to mutation in *TERC*, which encodes an RNA template, and *TERT*, which encodes the catalytic reverse transcriptase, telomerase. Mutations in TNF2, a component of the shelterin, proteins that bind the telomere DNA, also occur in dyskeratosis. (10)

In Shwachman-Diamond syndrome, marrow failure is seen with pancreatic insufficiency and malabsorption; most patients have compound heterozygous mutations in *SBDS* that may affect marrow stroma function.

Mutations in *TERT*, *TERC*, *TNF2*, and *SBDS* also can occur in patients with apparently acquired aplastic anemia (TERT and TERC mutations also are etiologic in familial pulmonary fibrosis and in some hepatic cirrhosis). (10)

Table 2.18 **Some Drugs and Chemicals Associated with Aplastic Anemia**

Agents that regularly produce marrow depression as major toxicity in commonly employed doses or normal exposures:
Cytotoxic drugs used in cancer chemotherapy: *alkylating agents*, *antimetabolites*, *antimitotics*, some antibiotics
Agents that frequently but not inevitably produce marrow aplasia:
Benzene
Agents associated with aplastic anemia but with a relatively low probability:
Chloramphenicol
Insecticides
Antiprotozoals: *quinacrine* and chloroquine, mepacrine
Nonsteroidal anti-inflammatory drugs (including *phenylbutazone*,
indomethacin, ibuprofen, sulindac, aspirin)
Anticonvulsants (*hydantoins*, *carbamazepine*, phenacemide, felbamate)
Heavy metals (*gold*, arsenic, bismuth, mercury)
Sulfonamides: some antibiotics, antithyroid drugs (methimazole, methylthiouracil, propylthiouracil), antidiabetes drugs (tolbutamide, chlorpropamide), carbonic anhydrase inhibitors (acetazolamide and methazolamide)
Antihistamines (*cimetidine*, chlorpheniramine)
d-Penicillamine
Estrogens (in pregnancy and in high doses in animals)
Agents whose association with aplastic anemia is more tenuous:
Other antibiotics (streptomycin, tetracycline, methicillin,
mebendazole, trimethoprim/sulfamethoxazole, flucytosine)
Sedatives and tranquilizers (chlorpromazine, prochlorperazine,
piperacetazine, chlordiazepoxide, meprobamate, methyprylon)
Allopurinol
Methyldopa
Quinidine
Lithium
Guanidine
Potassium perchlorate
Thiocyanate
Carbimazole

Note: Terms set in italic show the most consistent association with aplastic anemia.

Pathogenesis

1. Aplastic anaemia is characterized by both a quantitative and qualitative defect in the haemopoietic stem cell compartment, while the bone marrow microenvironment functions normally in most patients, as assessed by long-term bone marrow cultures.
2. The primitive long term culture-initiating cells and more mature haemopoietic progenitors in the bone marrow (colony-forming cells) of all cell lineages are reduced or absent.
3. There is a reduction in the percentage of CD34+ bone marrow cells, and they are more apoptotic than normal CD34+ cells.
4. Aplastic anaemia bone marrow cells also have shortened telomere length compared with normal bone marrow cells.

Table 2.19 **Currently licensed drugs and occupational exposures reported as a probable cause of aplastic anaemia** (12)	
(a) Currently licensed drugs	
Antibiotics	Chloramphenicol[a], sulphonamides, co-trimoxazole, linezolid
Anti-inflammatories	Phenylbutazone, indomethacin, diclofenac, naproxen, piroxicam, gold, penicillamine
Anticonvulsants	Phenytoin, carbamazepine
Antithyroid	Carbimazole[b], thiouracil
Antidepressants	Dothiepin, phenothiazides
Antidiabetic	Chlorpropamide, tolbutamide
Antimalarial	Chloroquine
Others	Mebendazole, thiazides[c], allopurinol
(b) Occupational and environmental exposures	
Evidence base	**Agent**
Benzene	Large industrial studies, case–

	control study from Thailand
• Pesticides: • organochlorines e.g. lindane; organophosphates; pentachlorophenol	Literature review of case reports and UK case–control study
Cutting oils and lubricating agents	UK case control study
Recreational drugs e.g. methylenedioxymethamphetamine (MDMA, ecstasy)	Case reports
a No association with chloramphenicol tablets was observed in case control study from Thailand. There is no evidence for an association between chloramphenicol eye drops and aplastic anaemia. b More likely to cause neutropenia; c From case–control study in Thailand.	

Observations which support an autoimmune basis for the disease are:

1. There is evidence of HLA restriction with over-representation of HLA DR15
2. Stem cell transplants from identical twins fail to correct the defect unless prior immunosuppression is given
3. 60 to 80% of patients respond to immunosuppressive therapy with antithymocyte globulin ± ciclosporin
4. Increased levels of interferon-γ and tumour necrosis factor-α, cytokines that inhibit haemopoiesis, are produced by mononuclear cells in aplastic anaemia
5. There is increased Fas-antigen expression on bone marrow CD34+ cells, indicating increased apoptosis
6. Activated autoreactive cytotoxic T-cells are present in blood and bone marrow
7. T-cell repertoire analysis shows oligoclonal expansion of CD8 T-cells
8. There is upregulation of apoptosis and immune response genes (12)

Clinical Features

History

1. Abruptness or a more insidious onset
2. A family history of:
   - Hematologic diseases
   - Blood abnormalities
   - Pulmonary or liver fibrosis
3. Bleeding is the most common early symptom; a complaint of days to weeks of:
   - Easy bruising
   - Oozing from the gums
   - Nose bleeds
   - Heavy menstrual flow
   - Sometimes petechiae
4. With thrombocytopenia, retinal hemorrhage (10)
5. Symptoms of anemia are including:
   - Lassitude
   - Weakness
   - Shortness of breath
   - A pounding sensation in the ears
6. Infection is unusual unlike in agranulocytosis
7. Feature of the hematologic system
8. Systemic complaints
9. Weight loss (10)

Physical Examination

1. Petechiae
2. Ecchymoses
3. Retinal hemorrhages may be present
4. Pelvic and rectal
   - Bleeding from the cervical os
   - Blood in the stool
5. Pallor
   - Skin

- Mucous membranes

6. Infection on presentation may occur
7. Lymphadenopathy
8. Splenomegaly[3]
9. Café au lait spots and short stature suggest Fanconi's anemia
10. Peculiar nails and leukoplakia suggest dyskeratosis congenita (10)

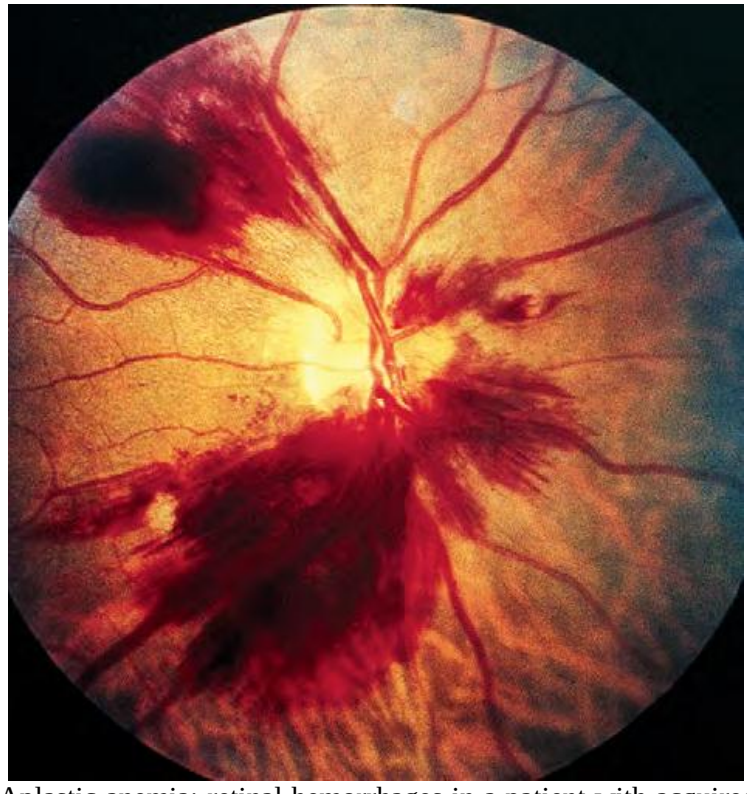


Figure 2.40 Aplastic anemia: retinal hemorrhages in a patient with acquired disease and profound thrombocytopenia. (7)

[3] Lymphadenopathy and splenomegaly are highly atypical of aplastic anemia. (10)

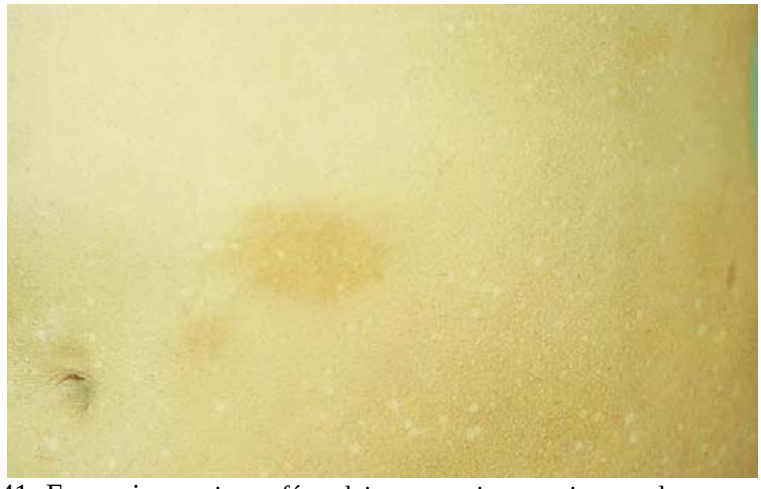


Figure 2.41 Fanconi anemia: café-au-lait spot, pigmentation, and punctate areas of depigmentation over the abdominal wall. (Courtesy of Professor E. C. Gordon-Smith.) [7]

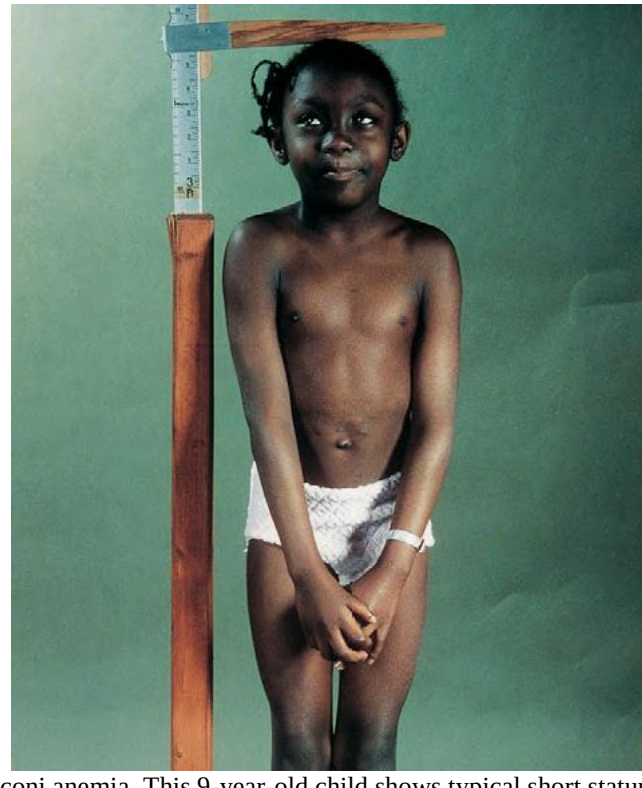


Figure 2.42 Fanconi anemia. This 9-year-old child shows typical short stature of 1.06 m (42 inches). (Courtesy of Dr. B. Wonke.) [7]

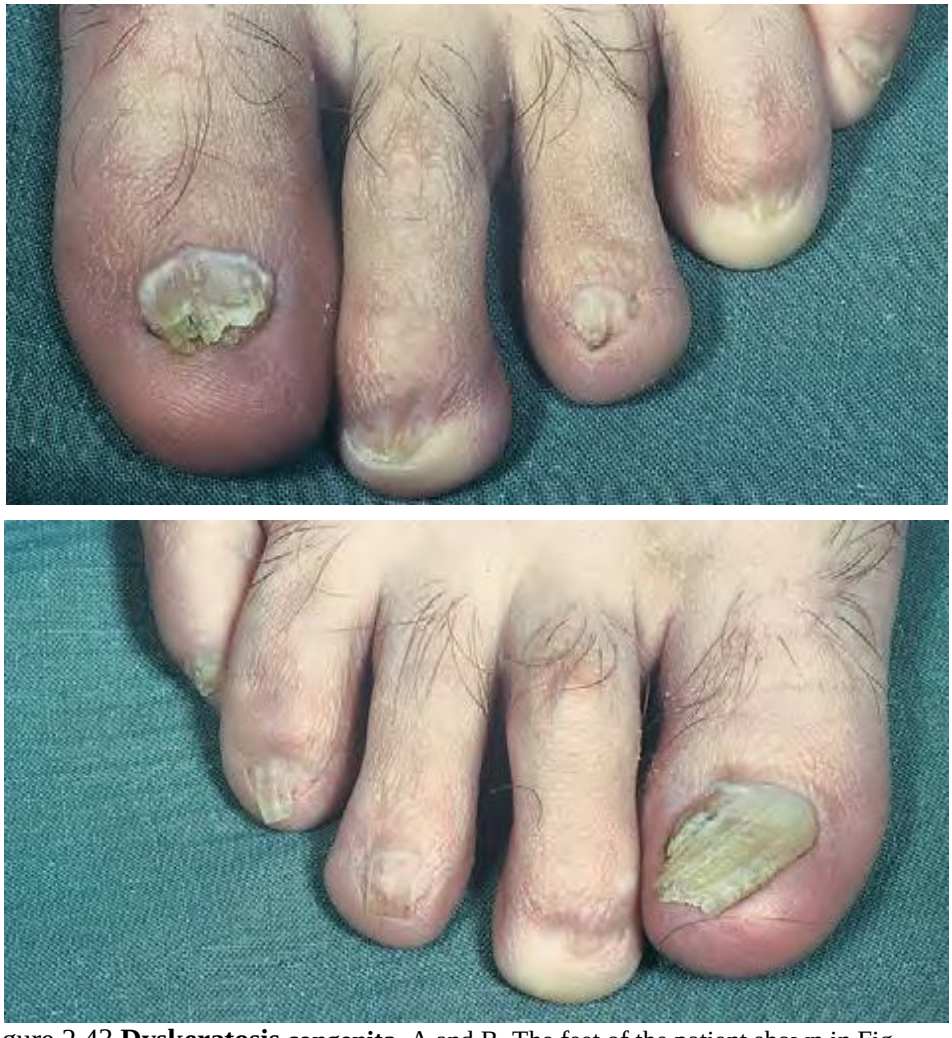


Figure 2.43 **Dyskeratosis congenita**. A and B, The feet of the patient shown in Fig. 17-14 show grossly abnormal nails and excessive hair in an abnormal distribution. (7)

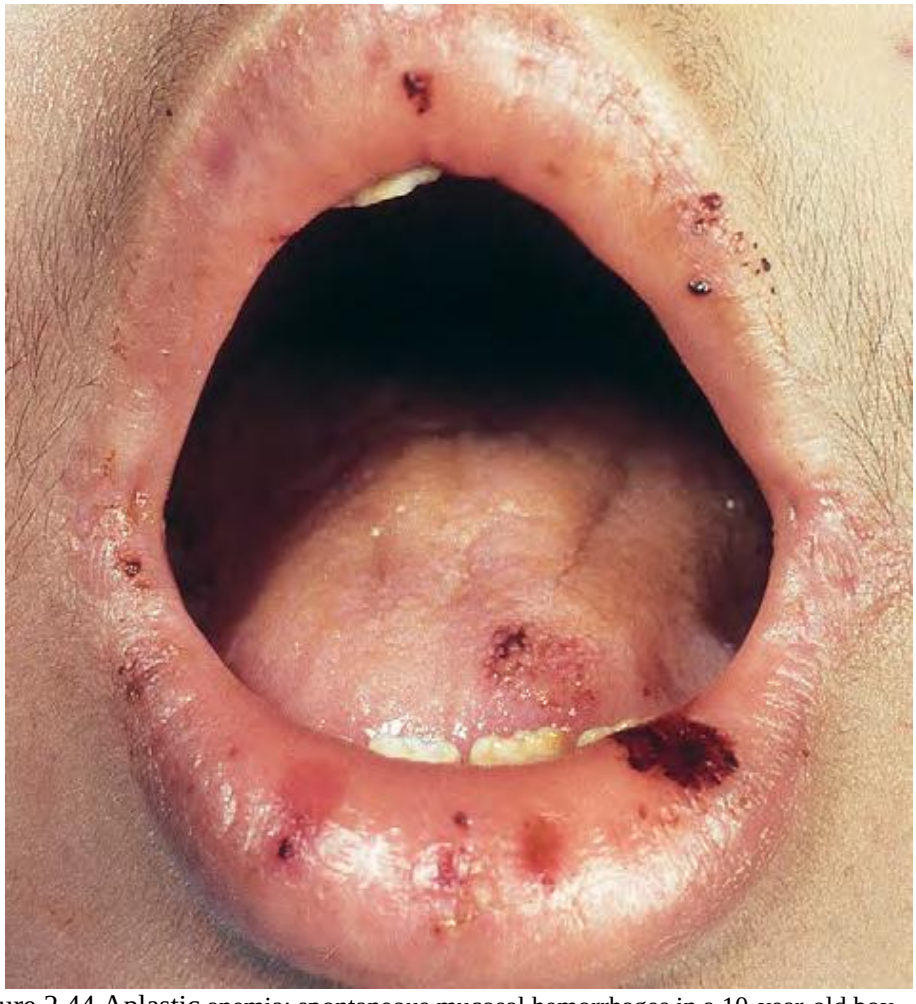


Figure 2.44 Aplastic anemia: spontaneous mucosal hemorrhages in a 10-year-old boy with severe congenital (Fanconi) anemia. (Hb, 7.3 g/dl; white blood cell count [WBC], 1.1 109/L [neutrophils, 21%; lymphocytes, 77%]; platelets 5.0 109/L.) [(7)]

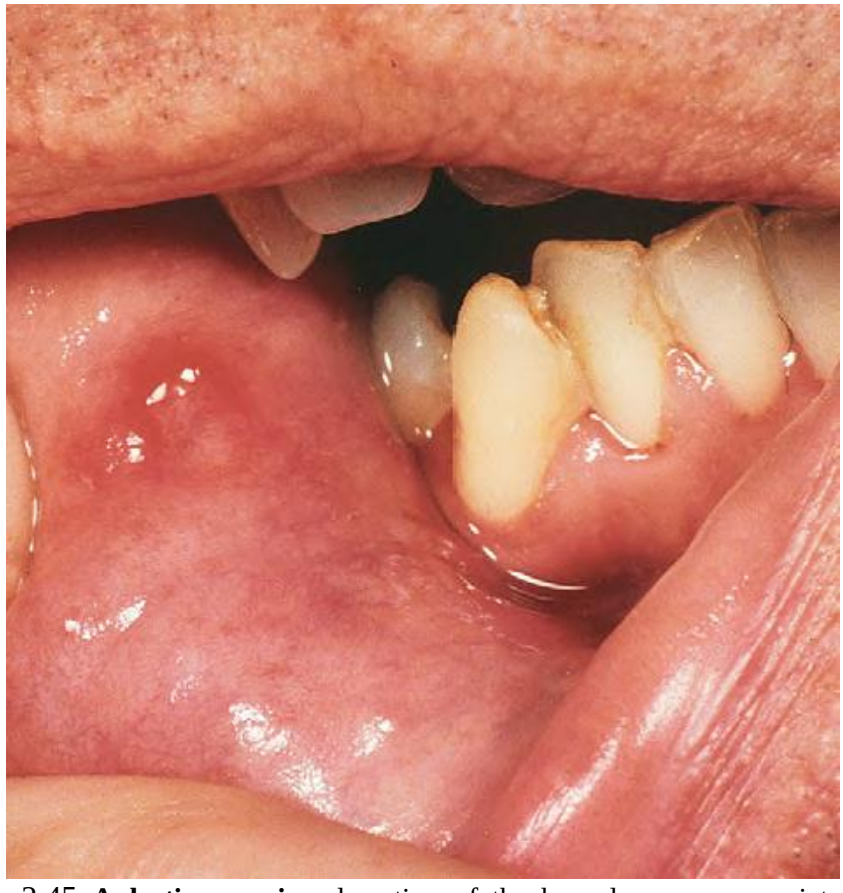


Figure 2.45 **Aplastic anemia**: ulceration of the buccal mucosa associated with severe neutropenia. Herpes simplex virus was grown from the ulcers. (Total leukocyte count, 0.8 109/L; neutrophils, 20%.) [7]

Laboratory investigation

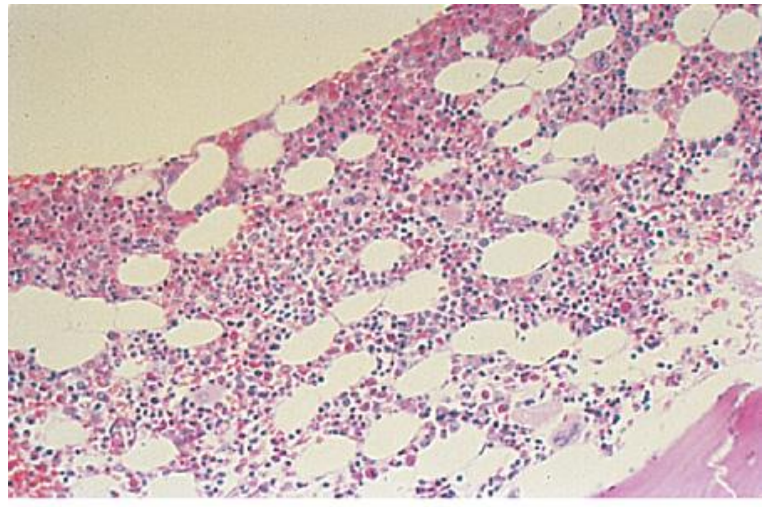
Blood

1. The smear shows large erythrocytes
2. A paucity of platelets and granulocytes
3. Mean corpuscular volume (MCV) is commonly increased
4. Reticulocytes are absent or few
5. Lymphocyte numbers may be normal or reduced

6. The presence of immature myeloid forms suggests leukemia or MDS
7. Nucleated RBCs suggest marrow fibrosis or tumor invasion
8. Abnormal platelets suggest either peripheral destruction or MDS [10]

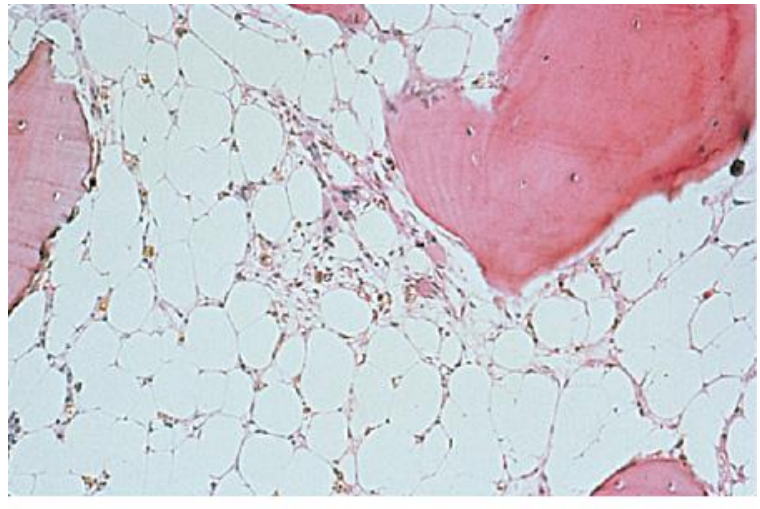
Bone Marrow

1. The fatty biopsy specimen may be grossly pale
2. A "dry tap" instead suggests fibrosis or myelophthisis
3. In severe aplasia the smear shows only red cells
4. Residual lymphocytes
5. Stromal cells
6. The biopsy (which should be >1 cm in length) shows:
   - Mainly fat
   - With hematopoietic cells (<25%)
   - In the most serious cases (100% fat)
   - Marrow cellularity declines physiologically with aging
   - "Hot spots" of hematopoiesis may be seen in severe cases
7. Residual hematopoietic cells are normal except for:
   - Mildly megaloblastic erythropoiesis
   - Megakaryocytes are greatly reduced and usually absent
8. Granulomas may indicate an infectious etiology of the marrow failure. [10]



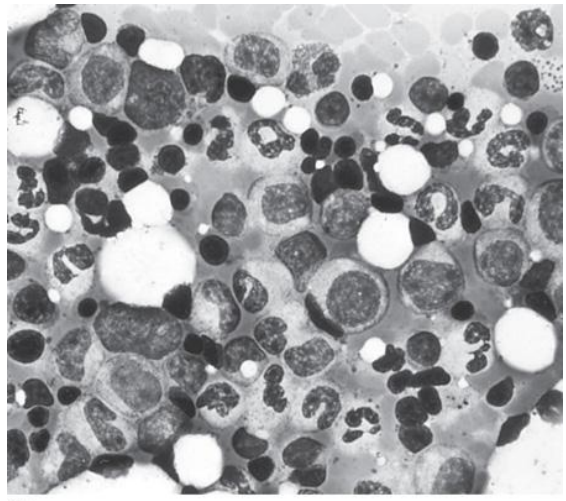
A

Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com



B

Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com



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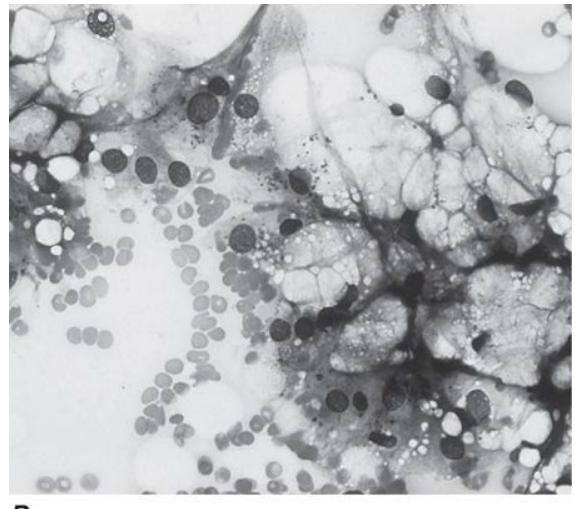

D

Figure 2.46 ***A.*** Normal bone marrow biopsy. ***B.*** Normal bone marrow aspirate smear. The marrow is normally 30–70% cellular, and there is a heterogeneous mix of myeloid, erythroid, and lymphoid cells. ***C.*** Aplastic anemia biopsy. ***D.*** Marrow smear in aplastic anemia. The marrow shows replacement of hematopoietic tissue by fat and only residual stromal and lymphoid cells. [(10)]

Ancillary Studies

1. Chromosome breakage studies for Fanconi's anemia
2. Very short telomere length suggests the presence of a telomerase or shelterin mutation
3. Chromosome studies of bone marrow cells:
   - Revealing in MDS
   - Negative in typical aplastic anemia
4. Flow cytometry offers a sensitive diagnostic test for PNH
5. Serologic studies for Epstein-Barr virus and HIV
6. Posthepatitis aplastic anemia is seronegative.
7. The spleen size by CT scanning or ultrasound
8. MRI may be helpful for the fat content of a few vertebrae [10]

Treatment

Severe acquired aplastic anemia can be cured by:

1. Replacement of the absent hematopoietic cells (and the immune system) by stem cell transplant, or
2. Suppression of the immune system to allow recovery of the patient's residual bone marrow function
3. Hematopoietic growth factors have limited usefulness
4. Glucocorticoids are of no value
5. Suspect exposures to drugs or chemicals should be discontinued
6. Spontaneous recovery of severe blood count depression is rare[4] [10]

[4] **Hematopoietic Stem Cell Transplantation**

This is the best therapy for the younger patient with a fully histocompatible sibling donor. Human leukocyte antigen (HLA) typing should be ordered as soon as the diagnosis of aplastic anemia is established in a child or younger adult.

For allogeneic transplant from fully matched siblings, long-term survival rates for children are approximately 90%. [10]

Immunosuppression

The standard regimen of antilymphocyte globulin (ALG) in combination with cyclosporine induces hematologic recovery (independence from transfusion and a leukocyte count adequate to prevent infection) in 60–60% of patients. (H)
Horse ATG and rabbit antilymphocyte globulin (ALG) are administered as intravenous infusions over 4 or 5 days, respectively. ATG binds to peripheral blood cells; therefore, platelet and granulocyte numbers may decrease further during active treatment. Serum sickness, a flulike illness with a characteristic cutaneous eruption and arthralgia, often develops approximately 10 days after initiating treatment. (10)
Methylprednisolone, 1 mg/kg per d for 2 weeks, can ameliorate the immune consequences of heterologous protein infusion.
Cyclosporine is administered orally at an initial high dose, with subsequent adjustment according to blood levels obtained every 2 weeks, rough levels should be between 150 and 200 ng/mL. The most important side effects are nephrotoxicity, hypertension, seizures, and opportunistic infections, especially *Pneumocystis carinii*. (10)

Most patients with aplastic anemia lack a suitable marrow donor, and immunosuppression is the treatment of choice. (10)

Other Therapies

The effectiveness of androgens has not been verified in controlled trials, but occasional patients will respond or even demonstrate blood count dependence on continued therapy. Sex hormones upregulate telomerase gene activity in vitro, possibly also their mechanism of action in improving marrow functions. For patients with moderate disease or those with severe pancytopenia in whom immunosuppression has failed, a 3–4-month trial is appropriate. Hematopoietic growth factors (HGFs) are not recommended as initial therapy for severe aplastic anemia, and even their roles as adjuncts to immunosuppression are not clear. (10)

Supportive Care

Meticulous medical attention is required so that the patient may survive to benefit from definitive therapy or, having failed treatment, to maintain a reasonable existence in the face of pancytopenia. First and most important, infection in the presence of severe neutropenia must be aggressively treated by prompt institution of parenteral, broad-spectrum antibiotics, usually ceftazidime or a combination of an aminoglycoside, cephalosporin, and semisynthetic penicillin. Therapy is empirical and must not await results of culture, although specific foci of infection such as oropharyngeal or anorectal abscesses, pneumonia, sinusitis, and typhlitis (necrotizing colitis) should be sought on physical examination and with radiographic studies. When indwelling plastic catheters become contaminated, vancomycin should be added. Persistent or recrudescent fever implies fungal disease: *Candida* and *Aspergillus* are common, especially after several courses of antibacterial antibiotics. A major reason for the improved prognosis in aplastic anemia has been

7. Methylprednisolone, 1 mg/kg per d for 2 weeks
8. Cyclosporine is administered orally
9. The effectiveness of androgens has not been verified
10. Hematopoietic growth factors (HGFs) are not recommended as initial therapy. (10)

Supportive Care

11. First and most important, infection in the presence of severe neutropenia must be aggressively treated by prompt institution of parenteral, broad-spectrum antibiotics, usually ceftazidime

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the development of better antifungal drugs and the timely institution of such therapy when infection is suspected. Granulocyte transfusions using granulocyte colony-stimulating factor (G-CSF)—mobilized peripheral blood may be effective in the treatment of overwhelming or refractory infections. Hand washing, the single best method of preventing the spread of infection, remains a neglected practice. Nonabsorbed antibiotics for gut decontamination are poorly tolerated and not of proven value. Total reverse isolation does not reduce mortality from infections. (10)

Both platelet and erythrocyte numbers can be maintained by transfusion. Alloimmunization historically limited the usefulness of platelet transfusions and is now minimized by several strategies, including use of single donors to reduce exposure and physical or chemical methods to diminish leukocytes in the product; HLA-matched platelets are often effective in patients refractory to random donor products. Inhibitors of fibrinolysis such as aminocaproic acid have not been shown to relieve mucosal oozing; the use of low-dose glucocorticoids to induce "vascular stability" is unproven and not recommended. Whether platelet transfusions are better used prophylactically or only as needed remains unclear. Any rational regimen of prophylaxis requires transfusions once or twice weekly to maintain the platelet count >10,000/L (oozing from the gut, and presumably also from other vascular beds, increases precipitously at counts <5000/L). Menstruation should be suppressed either by oral estrogens or nasal follicle-stimulating hormone/luteinizing hormone (FSH/LH) antagonists. Aspirin and other nonsteroidal anti-inflammatory agents inhibit platelet function and must be avoided.

Red blood cells should be transfused to maintain a normal level of activity, usually at a hemoglobin value of 70 g/L (90 g/L if there is underlying cardiac or pulmonary disease); a regimen of 2 units every 2 weeks will replace normal losses in a patient without a functioning bone marrow. In chronic anemia, the iron chelators, deferoxamine and deferasirox, should be added at approximately the fiftieth transfusion to avoid secondary hemochromatosis. (10)

or a combination of an aminoglycoside, cephalosporin, and semisynthetic penicillin.

12. Therapy is empirical and must not await results of culture,
13. Both platelet and erythrocyte numbers can be maintained by transfusion [10]

Prognosis

1. The natural history of severe aplastic anemia is rapid deterioration and death
2. Provision first of RBC and later of platelet transfusions and effective antibiotics are of some benefit, but few patients show spontaneous recovery
3. The major prognostic determinant is the blood count
4. Historically, severe disease was defined by the presence of two of three parameters:
   - Absolute neutrophil count <500/L
   - Platelet count <20,000/L
   - Corrected reticulocyte count <1% (or absolute reticulocyte count <60,000/L)
5. In the era of effective immunosuppressive therapies, absolute numbers of reticulocytes (>25,000/uL)
6. Lymphocytes (>1000/uL) may be a better predictor of response to treatment and long-term outcome [10]

Polycythemia

Definition

Polycythaemia (or erythrocytosis) is defined as an increase in haemoglobin, PCV and red cell count. (8)

The word *polycythemia* is derived from the Greek and literally translates as "too many blood cells (Fig. 2.47)."[1] However, in terminology, polycythemia refers to either:

1. Real (true polycythemia) (Fig. 2.48)
2. Spurious (apparent polycythemia) (5)

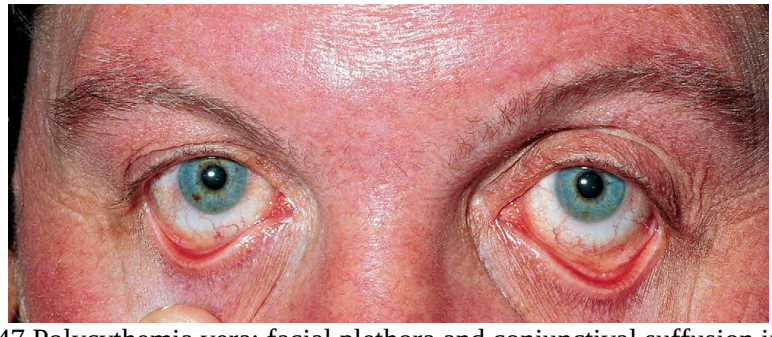

Figure 2.47 Polycythemia vera: facial plethora and conjunctival suffusion in a 40-year-old woman. (Hb, 19.5 g/dl.) (7)

[1] However, in conventional terminology, polycythemia refers to either a real (true polycythemia) or spurious (apparent polycythemia) perception of an increase in red blood cell mass. True polycythemia may represent either a clonal myeloproliferative disorder (polycythemia vera) or a nonclonal increase in red blood cell mass that is often mediated by erythropoietin (secondary polycythemia). Apparent polycythemia results from either a decrease in plasma volume (relative polycythemia) or a misperception of what constitutes the upper limit of normal values for either hemoglobin or hematocrit. Occasionally, a true increase in red blood cell mass may be masked by a normal-appearing hematocrit because of a concomitant increase in plasma volume, often accompanied by marked splenomegaly (inapparent polycythemia).(5)

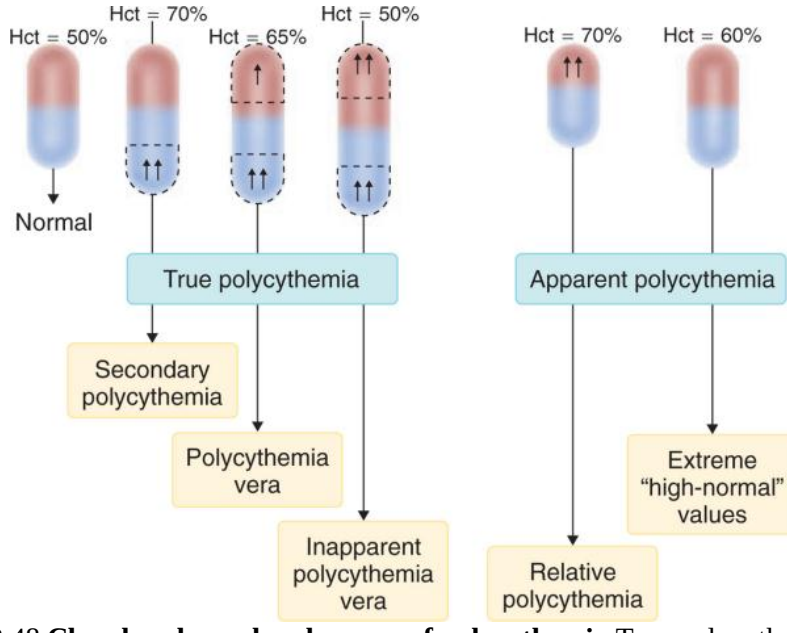

Figure 2.48 **Clonal and nonclonal causes of polycythemia** True polycythemia denotes an increase in red cell mass (denoted in blue). Apparent polycythemia is not associated with increased red cell mass and can be caused by contracted plasma volume (denoted in red). Hct = hematocrit. [5]

Aetiology

Primary

1. Polycythaemia vera
2. Mutations in erythropoietin receptor
3. High-oxygen-affinity haemoglobins

Secondary

1. Due to an appropriate increase in erythropoietin:
    - High altitude
    - Lung disease
    - Cardiovascular disease (right-to-left shunt)
    - Heavy smoking
    - Increased affinity of haemoglobin, e.g.

Familial polycythaemia

2. Due to an inappropriate increase in erythropoietin:
   - Renal cell carcinoma (Table 2.20)
   - Wilms' tumour
   - Hepatocellular carcinoma
   - Adrenal tumours
   - Cerebellar haemangioblastoma
   - Massive uterine fibroma
3. Relative:
   - Stress or spurious polycythaemia
   - Dehydration
   - Burns [8]

Table 2.20 **Classification of "Polycythemia"** [12]

1. Apparent Polycythemia

a. Relative polycythemia

b. Extreme "high normal" values

2. True Polycythemia

a. Polycythemia vera

b. Secondary polycythemia

i. Congenital

1. Associated with high or normal serum erythropoietin level

(a) Chuvash and other polycythemias associated with von values Hippel-Lindau (VHL) gene mutation (autosomal) recessive)

(b) High–oxygen affinity hemoglobinopathy (autosomal dominant)

(c) 2,3-Diphosphoglycerate mutase deficiency (autosomal recessive)

(d) Pathogenetically undefined cases

2. Associated with low serum erythropoietin level

(a) Activating mutation of the erythropoietin receptor (autosomal dominant)

ii. Acquired

1. Erythropoietin mediated

(a) Hypoxia-driven

(i) Central hypoxic process

1. Chronic lung disease

2. Right-to-left cardiopulmonary vascular shunts

3. High-altitude habitat

4. Carbon monoxide poisoning

5. Smoker's polycythemia (chronic carbon monoxide exposure)

6. Hypoventilation syndromes including sleep apnea

(ii) Peripheral hypoxic process

1. Localized

2. Renal artery stenosis

(b) Hypoxia-independent (pathologic erythropoietin production)

(i) Malignant tumors

1. Hepatocellular carcinoma

2. Renal cell cancer

3. Cerebellar hemangioblastoma

4. Parathyroid carcinoma

(ii) Nonmalignant conditions

1. Uterine leiomyomas
2. Renal cysts (polycystic kidney disease)
3. Pheochromocytoma
4. Meningioma

2. Drug associated
- **(a)** Erythropoietin doping
- **(b)** Treatment with androgen preparations

3. Unknown mechanisms
- **(a)** Post–renal transplant erythrocytosis

Polycythemia Vera (PV)

Definition

PV is a clonal stem cell disorder in which there is an alteration in the pluripotent progenitor cell leading to excessive proliferation of erythroid, myeloid and megakaryocytic progenitor cells.[2] (K)

Aetiology

Over 95% of patients with PV have acquired mutations of the gene Janus Kinase 2 (JAK2). (8)

[2] Polycythemia vera is currently classified with both essential thrombocythemia and myelofibrosis with myeloid metaplasia as the third *BCR/ABL*-negative, classic myeloproliferative disorder. Accordingly, polycythemia vera is a clonal stem cell disease with growth factor–independent erythroid proliferation that results not only in increased red blood cell mass, but also in thrombocytosis and leukocytosis in a substantial number of patients. (5)

Epidemiology

1. Prevalence is 2.3 per 100,000 population
2. A higher incidence is in Ashkenazi Jewish
3. Median age is 60 years
4. Slight male excess
5. Less than 40 years old has 7% of cases
6. In children rare

Pathobiology

1. Polycythemia vera is a clonal stem cell disease that is not only erythropoietin independent but also hypersensitive to:
   - Insulin-like growth factor-I
   - Interleukin-3
   - Granulocyte-monocyte colony-stimulating factor
   - Stem cell factor
   - Thrombopoietin
2. The *JAK2* kinase gene that has recently been described in 65 to 97% of patients with polycythemia vera[3] (5)

Clinical Manifestations

Asymptomatic stage based on routine blood counts

When symptoms are present, they fall into three major categories:[4]

Vasomotor symptoms

1. Headache
2. Light headedness
3. Transient neurologic or

[3] The *JAK2*^{V617F} mutation occurs within the autoinhibitory JH2 domain (pseudokinase domain) of the gene, so the oncogenic mechanism is thought to involve dysregulation of the kinase activity that resides in the catalytically intact JH1 domain. In mice, this mutant allele induces hypersensitivity to erythropoietin and, as a result, erythrocytosis. (5)

[4] When symptoms are present, they fall into three major categories vasomotor, thrombohemorrhagic, and other nonvascular symptoms.

4. Ocular disturbances
5. Tinnitus
6. Atypical chest discomfort
7. Paresthesias
8. Erythromelalgia (Fig. 2.49)[5]

It is sometimes difficult to distinguish vasomotor disturbances from symptoms of hyperviscosity that include

1. Head fullness
2. Dizziness
3. Flushing (Fig. 2.49-2.53)
4. Visual disturbances
5. Tinnitus
6. Epistaxis
7. Dyspnea
8. Increased blood pressure (5)

Table 2.21 **Polycythemia vera-related clinical and laboratory features**

1	Persistent leukocytosis
2	Persistent thrombocytosis
3	Microcytosis secondary to iron deficiency
4	Increased leukocyte alkaline phosphatase
5	Splenomegaly
6	Generalized pruritus (usually after bathing)
7	Unusual thrombosis (e.g., Budd-Chiari syndrome)
8	Erythromelalgia (acral dysesthesia and erythema; see Fig. 2.49)

[5] Erythromelalgia occurs in fewer than 5% of the patients and may represent small vessel platelet-endothelium interaction with associated inflammation and transient thrombotic occlusion.

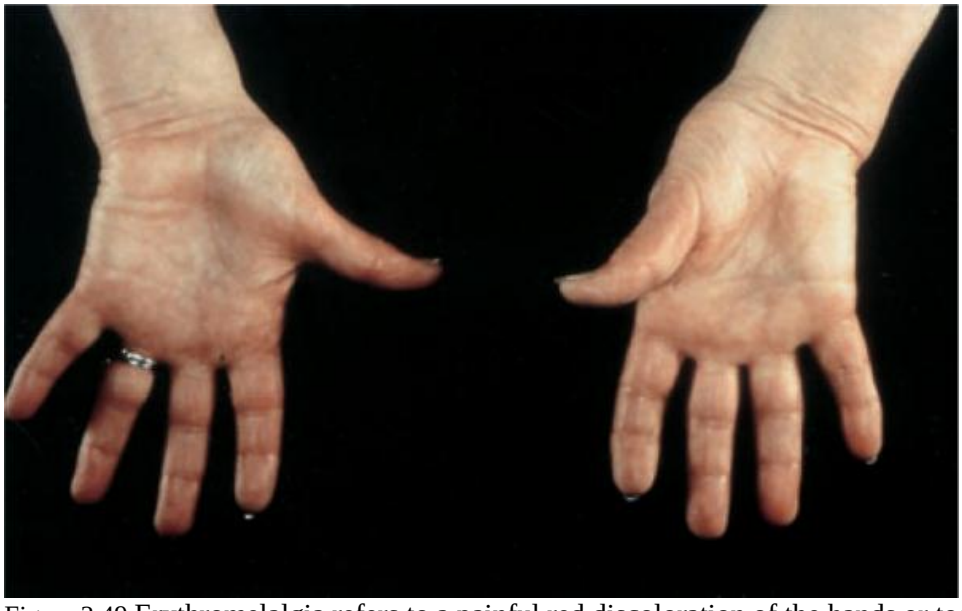

Figure 2.49 Erythromelalgia refers to a painful red discoloration of the hands or toes. (5)

Figure 2.49 **Polycythemia vera**: acne rosacea in a middle-aged woman after treatment by venesection. (7)

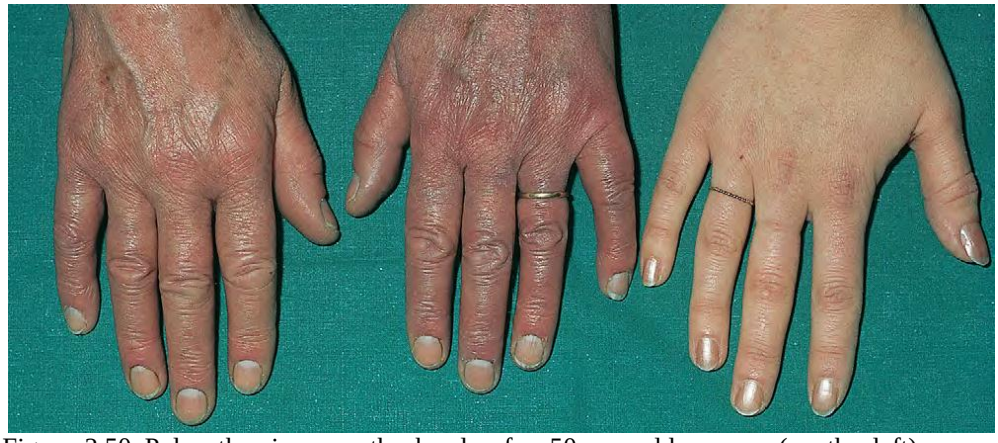

Figure 2.50 Polycythemia vera: the hands of a 50-year-old woman (on the left) appear congested and plethoric. (Hb, 20 g/dl; WBC, 15 109/L; platelets, 490 109/L.) The hand on the right is of a healthy 35-year-old woman. (Hb, 14.5 g/dl.) [7]

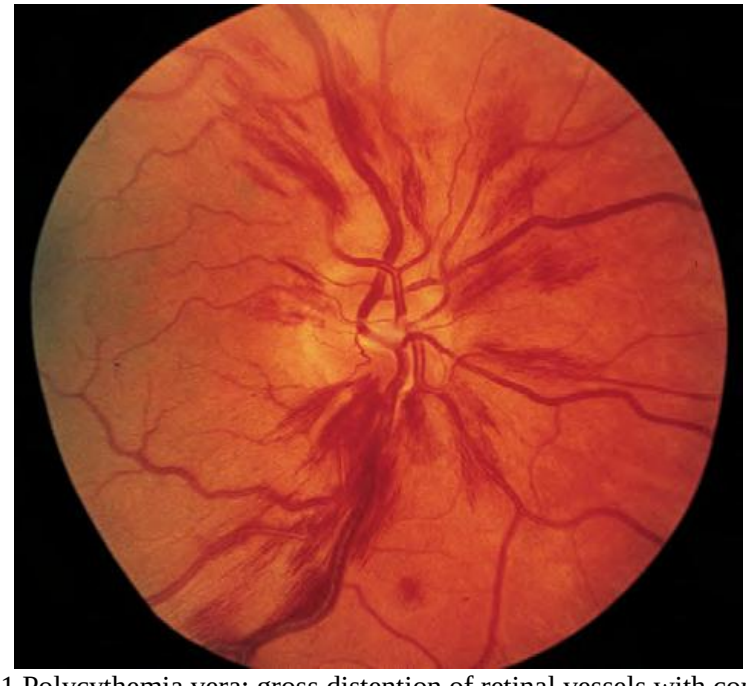

Figure 2.51 Polycythemia vera: gross distention of retinal vessels with conspicuous hemorrhage and mild swelling of the optic disc in hyperviscosity syndrome. The patient had headaches, lassitude, confusion, and blurred vision. (Hb, 23.5 g/dl; WBC, 35 109/L; platelets, 950 109/L.) (Courtesy of Professor J. C. Parr.) [7]

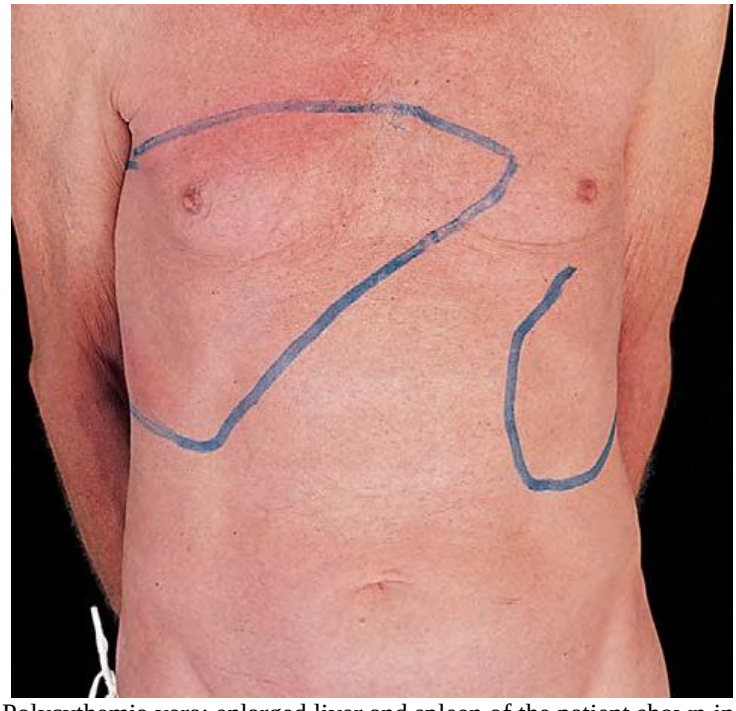

Figure 2.52 Polycythemia vera: enlarged liver and spleen of the patient shown in Fig. [7]

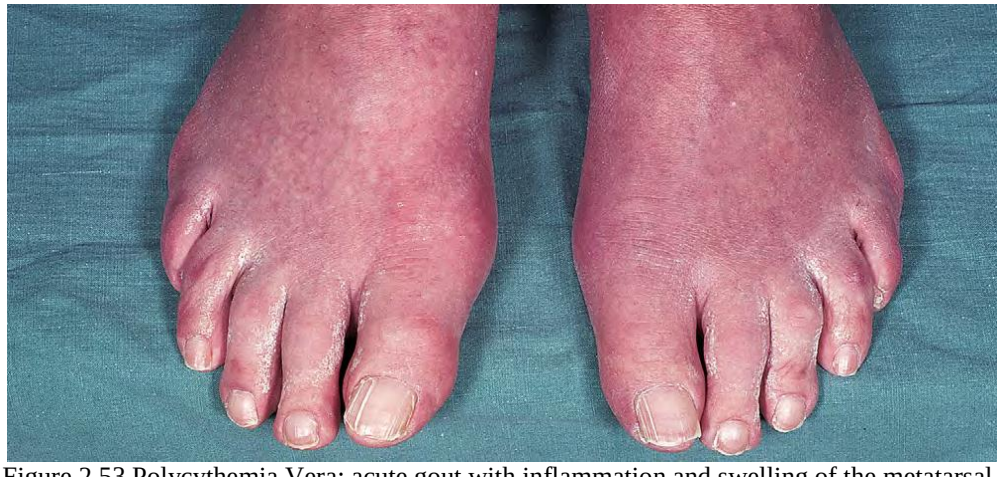

Figure 2.53 Polycythemia Vera: acute gout with inflammation and swelling of the metatarsal and interphalangeal joints of the right great toe. The skin also shows a dusky plethora. (Hb, 21.5 g/dl; total RCV, 53 ml/kg; serum uric acid, 0.9 mmol/L.) [7]

Thrombohemorrhagic symptoms

1. About 20% of patients present with thrombosis
2. Another 20 to 30% of patients have recurrent thrombosis
3. Arterial events:
   - Transient ischemic attack
   - Stroke
   - Myocardial infarction
   - Digital ischemia
4. Abdominal large vessel thrombosis including:
   - Budd-Chiari syndrome
   - Portal vein thrombosis
5. Cavernous sinus thrombosis
6. Pulmonary embolism
7. Major hemorrhage is much less frequent[6]
8. Minor mucocutaneous bleeding are:
   - Epistaxis
   - Gingival bleeding
   - Ecchymoses (5)

Nonvascular symptoms

1. Pruritus
2. Pruritus exacerbated by water contact (aquagenic)
3. Hypercatabolic symptoms
   - Weight loss
   - Fatigue
   - Diaphoresis
   - Night sweats
4. Peptic ulcer symptoms or
5. Toe or joint pain secondary to gouty arthritis
6. Plethora or ruddiness (a red and congested facial complexion)

[6] Major hemorrhage is much less frequent but may be precipitated by the use of aspirin or aspirin-like drugs

7. Palmar erythema
8. Sausage-shaped distention of retinal veins
9. Palpable splenomegaly occurs in 70% [5]

Laboratory findings

1. Leukocytosis (20 to 43%) (Table 2.21)
2. Thrombocytosis (48 to 63%)
3. Microcytosis (50 to 80%)

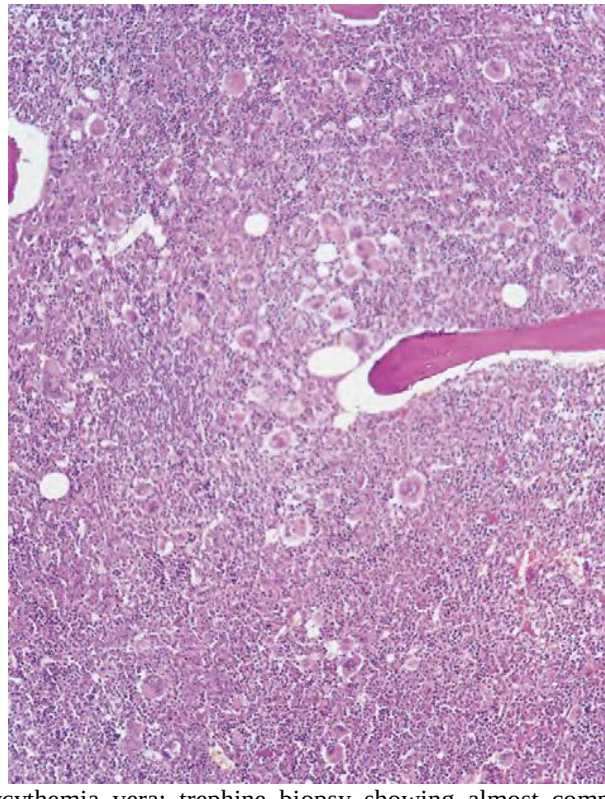

Figure 2.54 Polycythemia vera: trephine biopsy showing almost complete filling of the intertrabecular space with hyperplastic hematopoietic tissue. [7]

4. Increased serum levels of:
   - Uric acid
   - Lactate dehydrogenase
   - Vitamin B_{12}

- Vitamin B_{12} binding capacity
- Leukocyte alkaline phosphatase
- Low serum erythropoietin level (polycythemia vera)[7]

5. Bone marrow examination:
   - Morphologic abnormalities of megakaryocytes, including:
   - Cluster formation
   - Increased reticulin fibrosis
   - Bone marrow hypercellularity (Fig. 2.54, 2.55)
6. Screening for the *JAK2*^{V617F} mutation (5)

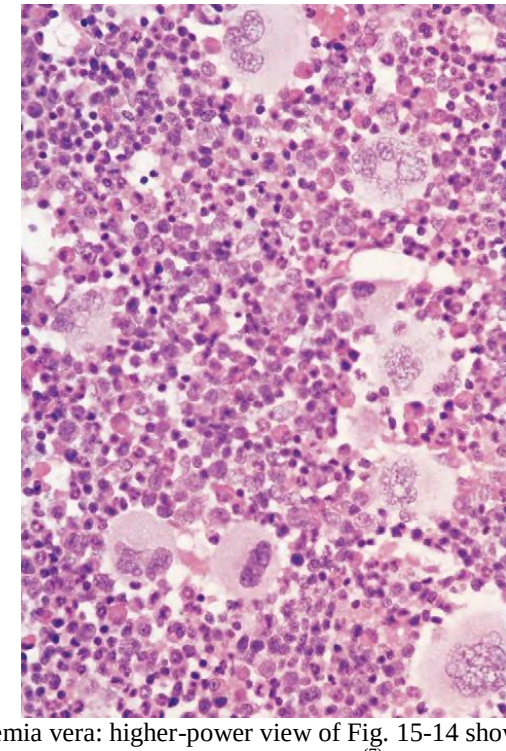

Figure 2.55 Polycythemia vera: higher-power view of Fig. 15-14 showing hyperplasia of erythropoiesis, granulopoiesis, and megakaryocytes. (7)

[7] This diagnosis is unlikely in the presence of an increased serum erythropoietin level, but a normal serum erythropoietin level does not exclude polycythemia vera. A bone marrow examination is indicated if the serum erythropoietin level is low.

Table 2.22 **Modified from proposed revised WHO criteria for polycythaemia vera (PV)** [8]

Major criteria

- Haemoglobin > 18.5 g/dL in men, 16.5 g/dL in women or other evidence of increased red cell volume
- Presence of JAK2 tyrosine kinase V617F or other functionally similar mutation such as JAK2 exon 12 mutation

Minor criteria

- Bone marrow biopsy, showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic and megakaryocytic proliferation
- Serum erythropoietin level below the reference range for normal
- Endogenous erythroid colony (EEC) formation in vitro*

Diagnosis requires the presence of both major criteria and one minor criterion or the presence of the first major criterion together with two minor criteria[8]

[8] **Diagnosis**

The diagnostic possibility of polycythemia vera (Fig.) may be entertained only if

(1) the hemoglobin and hematocrit levels are greater than the 95th percentile of the normal distribution adjusted for sex and race,

(2) the hemoglobin level is documented to be greater than a historical baseline for the individual patient, or

(3) polycythemia vera–related features are present. If any of these conditions is present, a serum erythropoietin level should be obtained. In the absence of all three criteria, a follow-up blood test in 3 months should be adequate. A rare exception is the patient with suspected polycythemia vera who presents with a normal or even low hemoglobin or hematocrit after bleeding from a peptic ulcer. [5]

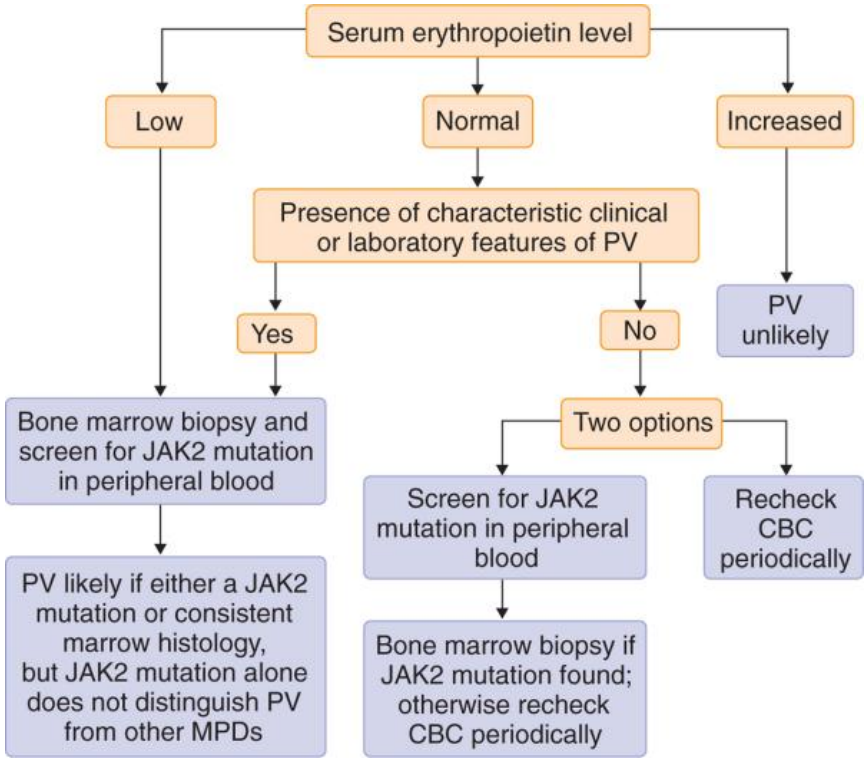

Figure 2.56 **A diagnostic algorithm for polycythemia vera (PV) that incorporates mutation screening for JAK2**[V617F]. A normal erythropoietin level is 536 μ/L. "Periodically" is usually interpreted clinically as approximately every 6 months. (See Table 172-2 for features related to polycythemia vera, including increased leukocyte alkaline phosphatase [LAP] score, thrombocytosis, leukocytosis, splenomegaly, thrombosis, pruritus, and erythromelalgia.) CBC = complete blood count; JAK = Janus kinase; MPD = myeloproliferative disorder. (5)

Treatment

Treatment of polycythemia vera is primarily directed at preventing thrombotic complications.

1. Phlebotomy
2. Aspirin[9]

[9] Phlebotomy is used to achieve a goal of keeping the hematocrit lower than 45% in white men and 42% in women and African American men. These levels reduce

3. Anticoagulants are only indicated when a thrombosis has occurred
4. Hydroxyurea
5. Oral chlorambucil or
6. Intravenous radioactive phosphorus
7. Busulfan
8. Interferon alfa (Table 2.23, 2.24)
9. Paroxetine is used to treat polycythemia vera–associated pruritus[10]

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blood hyperviscosity, improve cerebral blood flow, and lower the rate of thrombotic complications. Phlebotomy with hydroxyurea only in patients who are at high risk for thrombosis. Low-dose (81 mg/day) aspirin therapy has additional antithrombotic value without an increased risk of major hemorrhage. Aspirin therapy is also effective in controlling microvascular symptoms such as headaches and erythromelalgia. Whether any therapy modifies the risk of transformation into myelofibrosis with myeloid metaplasia is not clearly ascertained.

[10] The addition of oral chlorambucil or intravenous radioactive phosphorus to phlebotomy significantly reduces the rate of early thrombotic complications but also significantly lessens survival because of an increased incidence of therapy-related acute leukemia.

In patients with polycythemia vera who are at high risk for thrombosis, hydroxyurea appears to lower the risk of thrombosis compared with phlebotomy alone, with a lower risk of acute leukemia compared with either chlorambucil or radioactive phosphorus, but these data are not from randomized trials. Pipobroman (not available in the United States) is as effective as hydroxyurea in the treatment of polycythemia vera without being more or less leukemogenic.

In nonrandomized settings, both busulfan and interferon alfa have also shown activity against polycythemia vera, but they have not been compared rigorously with hydroxyurea. Anagrelide has been used to treat polycythemia vera, both in the presence and the absence of associated thrombocytosis, without any evidence of additional advantage over hydroxyurea. Finally, paroxetine, a selective serotonin reuptake inhibitor, is used to treat polycythemia vera–associated pruritus.

Future Directions

Studies are currently ongoing to define the precise pathogenetic role of *JAK2*^{V617F} in polycythemia vera and to design new therapies based on these discoveries.

10. Allogeneic bone marrow transplantation may be curative in young patients (5&10)

Table 2.23 **Risk stratification in polycythemia vera** (5)

Low risk
Age < 60 yr, *and* No history of thrombosis, and Platelet count <1.5 million/μL, and Absence of cardiovascular risk factors
Intermediate risk
Neither low-risk nor high-risk
High risk
Age ≥60 years, or A history of thrombosis

Table 2.24 **Treatment algorithm in Polycyemia vera** (5)

Risk Category	**Age <60 Yr**	**Age ≥60 Yr**	**Women of Childbearing Age**
Low risk	Phlebotomy and low-dose aspirin	Not applicable	Phlebotomy and low-dose aspirin
Indeterminate risk	Phlebotomy and low-dose aspirin[*]	Not applicable	Phlebotomy and low-dose aspirin[*]
High risk	Phlebotomy and low-dose aspirin and Hydroxyurea	Phlebotomy and low-dose aspirin and Hydroxyurea	Phlebotomy and low-dose aspirin and Interferon alfa[†]

*	The possibility of clinically significant acquired von Willebrand's syndrome must be excluded in the presence of a platelet count >1 million/μL before aspirin is given.
†	Based on anecdotal evidence of safety.

Prognosis

1. Life expectancy in polycythemia vera exceeds 10 years
2. The estimated rates of recurrent thrombosis
   - Low-risk patients is 5%

- High-risk patients is 30%

3. PV develops into myelofibrosis in 30% of cases
4. May develop acute myeloblastic leukaemia in 5%of cases [5&8]

Chapter three

Disorders of leukocytes

- *Acute Leukemia*
- *Chronic lymphocytic leukemia*
- *Chronic Myelogenous leukemia*
- *Hodgkin's disease*
- *The non-Hodgkin lymphomas*
- *Neutropenia*

Acute Leukemia

Definition

Acute leukemia is a malignancy of the hematopoietic progenitor cell. These cells proliferate in an uncontrolled fashion and replace normal bone marrow elements. (11)

Classification

Acute myeloid leukemia (AML)
Acute lymphoblastic leukemia (ALL) (Table 3.1) (11)

Table 3.1 **AML Classification Systems**
World Health Organization Classification[a]
AML with recurrent genetic abnormalities
AML with t(8;21)(q22;q22);*RUNX1-RUNX1T1*[b]
AML with inv(16)(pl3.1q22) or t(16;16)(p13.1;q22);*CBFB-MYH11*[b]
Acute promyelocytic leukemia with t(15;17)(q22;q12); *PML-RARA*[b]
AML with t(9;11)(p22;q23); *MLLT3-MLL*
AML with t(6;9)(p23;q34); *DEK-NUP214*
AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1* *Provisional entity: AML with mutated NPM1* *Provisional entity: AML with mutated CEBPA*
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML not otherwise specified

AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic and monocytic leukemia
Acute erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis
Myeloid leukemia associated with Down syndrome
Blastic plasmacytoid dendritic cell neoplasm
Acute leukemia of ambiguous lineage
Acute undifferentiated leukemia
Mixed phenotype acute leukemia with t(9;22)(q34;q11,20); *BCR-ABL11*
Mixed phenotype acute leukemia with t(v;11q23); *MLL* rearranged
Mixed phenotype acute leukemia, B/myeloid, NOS
Mixed phenotype acute leukemia, T/myeloid, NOS
Provisional entity: Natural killer (NK)-cell lymphoblastic leukemia/lymphoma
French-American-British (FAB) Classification[c]
MO: Minimally differentiated leukemia
Ml: Myeloblastic leukemia without maturation
M2: Myeloblastic leukemia with maturation
M3: Hypergranular promyelocytic leukemia

M4: Myelomonocytic leukemia
M4Eo: Variant: Increase in abnormal marrow eosinophils
M5: Monocytic leukemia
M6: Erythroleukemia (DiGuglielmo's disease)
M7: Megakaryoblastic leukemia

[a] From SH Swerdlow et al (eds): *World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, IARC Press, 2008.
[b] Diagnosis is AML regardless of blast count.
[c] From JM Bennett et al: Ann Intern Med 103:620, 1985.
Abbreviation: AML, acute myeloid leukemia.

Table 3.1 Acute Myeloid Leukemia (AML) Classification Systems

The "FAB," (French, American, British) classification was based on morphology and histochemistry as follows:

1. Acute undifferentiated leukemia (M0)
2. Acute myeloblastic leukemia (M1)
3. Acute myeloblastic leukemia with differentiation (M2)
4. Acute promyelocytic leukemia (APL) (M3)
5. Acute myelomonocytic leukemia (M4)
6. Acute monoblastic leukemia (M5)
7. Erythroleukemia (M6)
8. Megakaryoblastic leukemia (M7) (11)

Aetiology

1. No clear cause (In most cases)
2. Radiation
3. Chemotherapeutic agents:
    - Cyclophosphamide
    - Melphalan
    - Other alkylating agents
    - Etoposide

4. Toxin[1] (benzene)
5. Abnormalities in chromosomes 5 and 7 (11)

Epidemiology

1. Prevalence/Incidence:
   - The incidence of AML is 3.5 per 100,000 people per year
   - Incidence is higher in men than in women (4.3 vs 2.9)[2] (10)
2. Geographical variation:
3. Age group:
   - ALL comprises 80% of the acute leukemias of childhood
   - The peak incidence is between 3 and 7 years of age
   - In adults, causing 20% of adult acute leukemias
   - AML is primarily an adult disease
   - Median age of AML is 60 years and an increasing incidence with advanced age (11)
4. Seasonal variation:

Clinical Findings[3]

Symptoms

1. Most patients have been ill only for days or weeks.
2. Fatigue
3. Fever
4. Bleeding[4] occurs in the:

---

[1] The leukemias seen after toxin or chemotherapy exposure often develop from a myelodysplastic prodrome and are often associated with abnormalities in chromosomes 5 and 7, and those related to etoposide may have abnormalities in chromosome 11q23. (11)

[2] AML incidence increases with age; it is 1.7 in individuals aged <65 years and 15.9 in those aged >65 years. The median age at diagnosis is 67 years. (10)

[3] Most of the clinical findings in acute leukemia are due to replacement of normal bone marrow elements by the malignant cell. Less common manifestations result from organ infiltration (skin, gastrointestinal tract, meninges).

[4] Usually due to thrombocytopenia

- Skin
- Mucosal surfaces
- Gingival bleeding
- Epistaxis
- Menorrhagia

5. Disseminated intravascular coagulation (DIC)[5] (11)
6. Infection is due to neutropenia[6] include[7]:
   - Cellulitis (Fig. 3.1-3.4)
   - Pneumonia
   - Perirectal infections
7. Gum hypertrophy
8. Bone and joint pain
9. Hyperleukocytosis presenting as:
   - Headache
   - Confusion
   - Dyspnea (11)

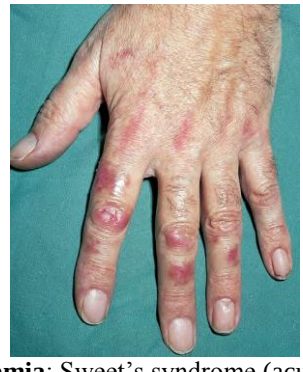

Figure 3.1 **Acute myeloid leukemia**: Sweet's syndrome (acute febrile neutrophilic dermatosis) in a 62-year-old man with AML M4: bullous pyoderma. (Courtesy of Professor H. G. Prentice.) (7)

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[5] Less commonly, widespread bleeding is seen in patients with disseminated intravascular coagulation (DIC) (in APL and monocytic leukemia).

[6] The most common pathogens are gram-negative bacteria *(Escherichia coli, Klebsiella, Pseudomonas*) or fungi (*Candida, Aspergillus*).

[7] death within a few hours may occur if treatment with appropriate antibiotics is delayed.

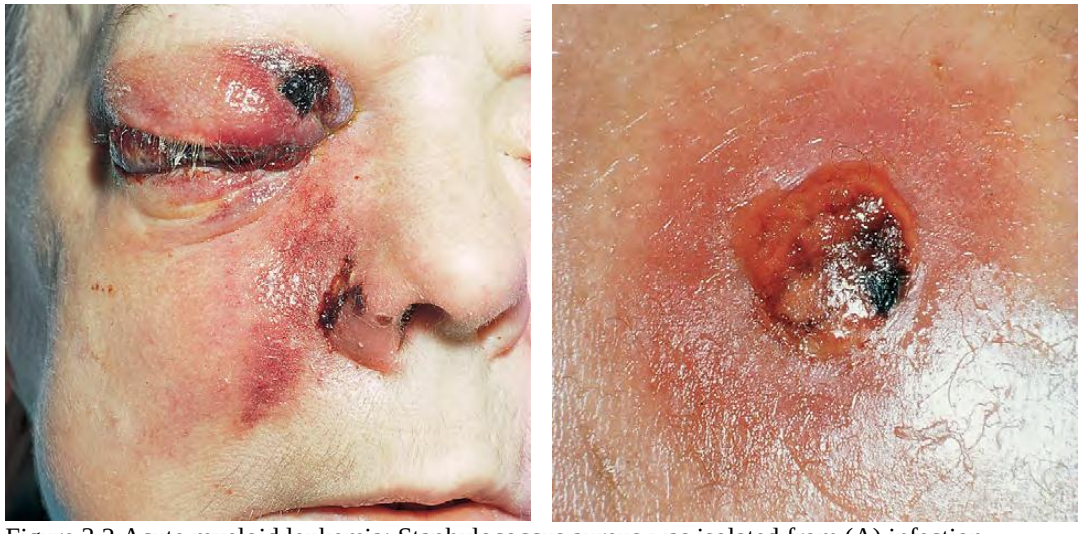


Figure 3.2 Acute myeloid leukemia: Staphylococcus aureus was isolated from (A) infection of the right orbit and surrounding tissue and (B) a necrotic erythematous skin ulcer. [7]

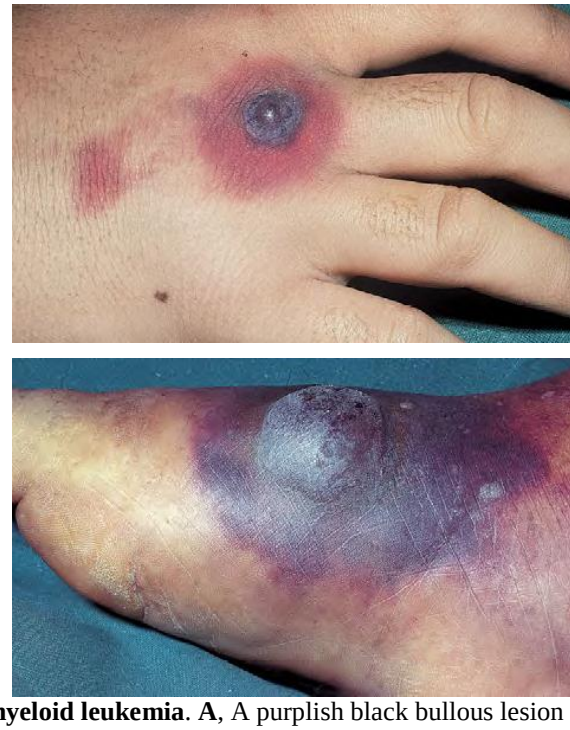


Figure 3.3 **Acute myeloid leukemia**. **A**, A purplish black bullous lesion with surrounding erythema caused by infection from Pseudomonas pyocyanea on the foot. **B**, Similar but less marked infection on the back of the hand. [7]

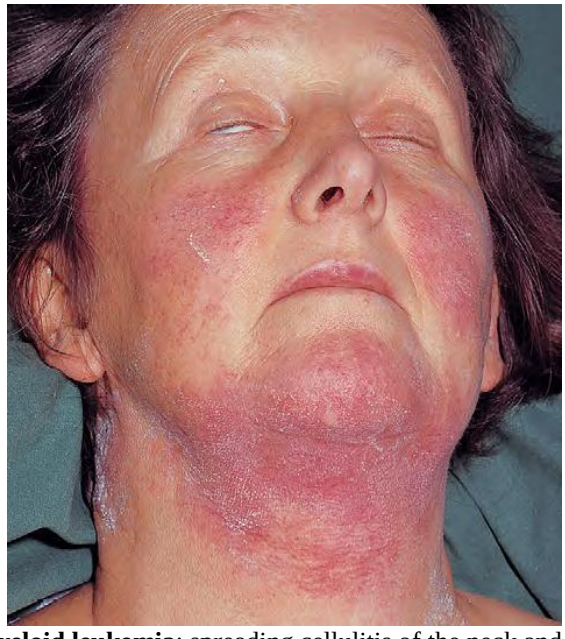


Figure 3.4 **Acute myeloid leukemia**: spreading cellulitis of the neck and chin resulting from mixed streptococcal and candidal infection, previous chemotherapy, and prolonged periods of neutropenia. (7)

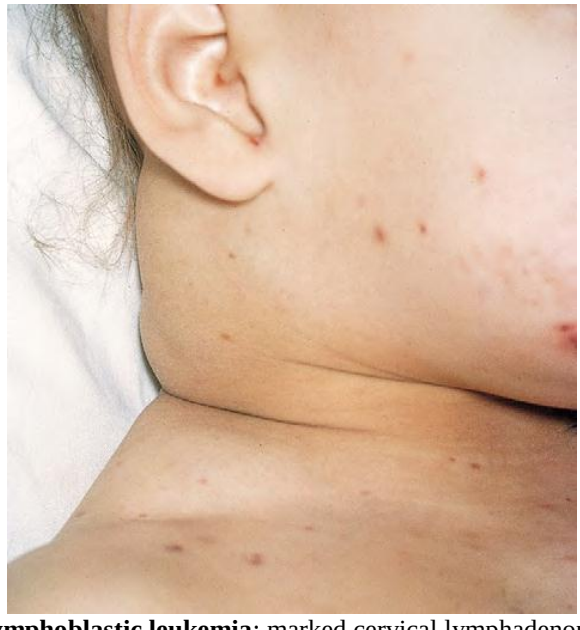


Figure 3.5 **Acute lymphoblastic leukemia**: marked cervical lymphadenopathy in a 4-year-old boy. (Courtesy of Professor J. M. Chessells.) (7)

Signs

1. Pallor
2. Purpura
3. Petechiae[8]
4. Stomatitis
5. Gum hypertrophy (Fig. 3.6)
6. Rectal fissures
7. Enlargement of the[9]:
   - Liver
   - Spleen
   - Lymph nodes (Fig. 3.5)
8. Bone tenderness may be present in the
   - Sternum
   - Tibia
   - Femur (11)

Figure 3.6 Acute myeloid leukemia, M5 subtype. A, B, Leukemic infi ltration of the gums results in their expansion and thickening, and partial covering of the teeth. (7)

[8] Signs of infection may not be present.

[9] There is variable enlargement of the liver, spleen, and lymph nodes.

Laboratory Findings

1. Pancytopenia
2. Circulating blasts (Fig. 3.7-3.8)
3. Blasts may be absent[10] ("aleukemic leukemia")
4. Bone marrow is:
   - Hypercellular
   - Dominated by blasts
5. More than 20% blasts are for diagnosis of acute leukemia (11)
6. Hyperuricemia
7. If DIC is present the:
   - Fibrinogen level will be reduced
   - Prothrombin time prolonged
   - Fibrin degradation products
8. The Auer rod cytoplasmic inclusion[11]
9. ALL is with Philadelphia chromosome (11)

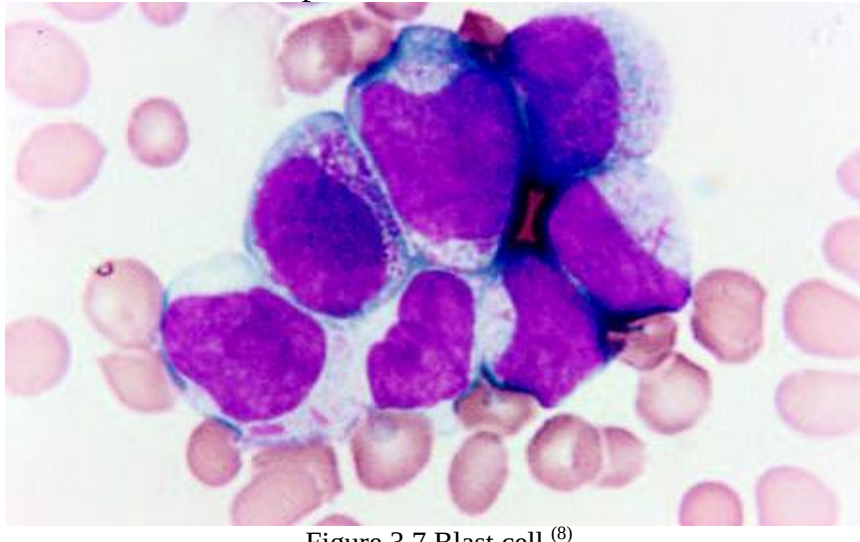
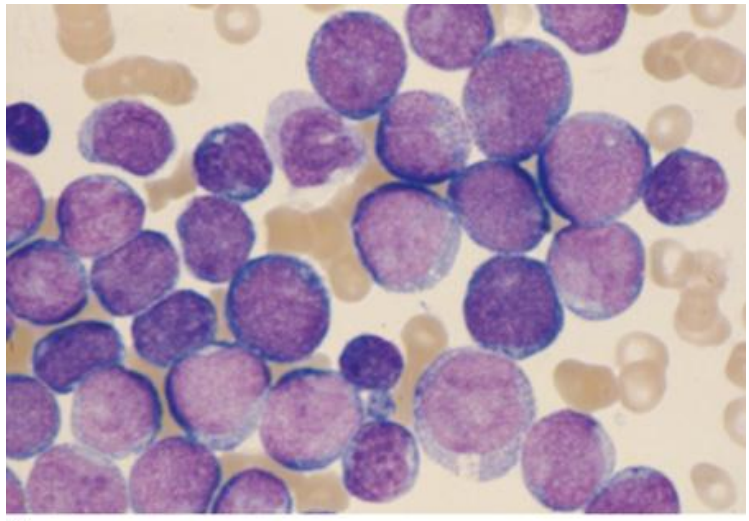


Figure 3.7 Blast cell (8)

[10] As many as 10% of cases

[11] The Auer rod, an eosinophilic needle-like inclusion in the cytoplasm, is pathognomonic of AML and, if seen, secures the diagnosis.



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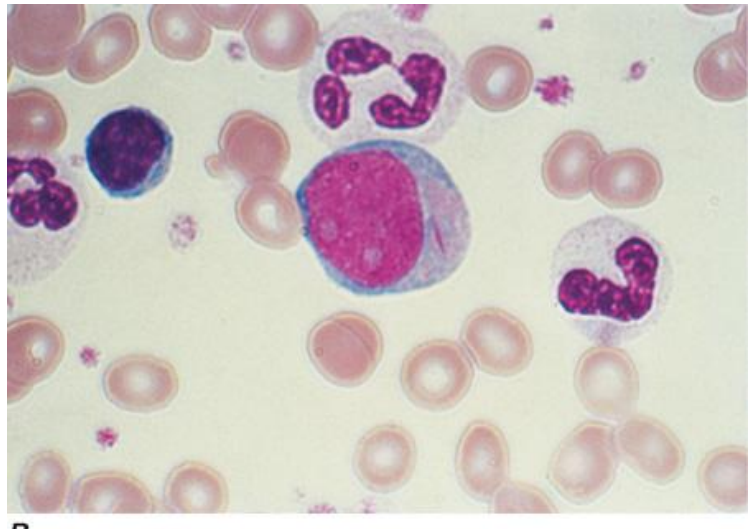

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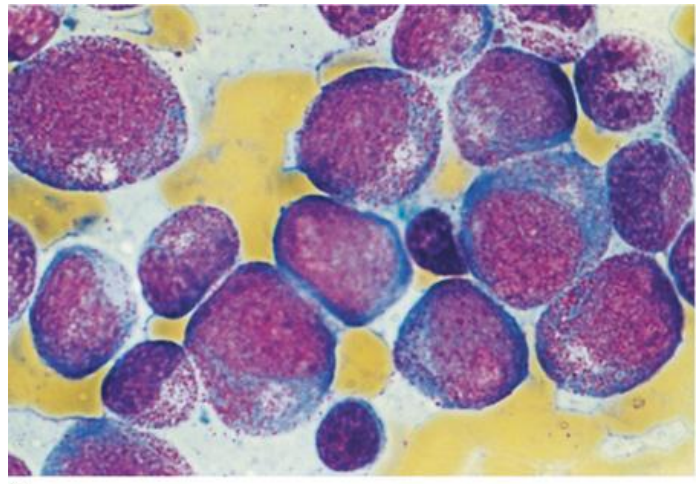

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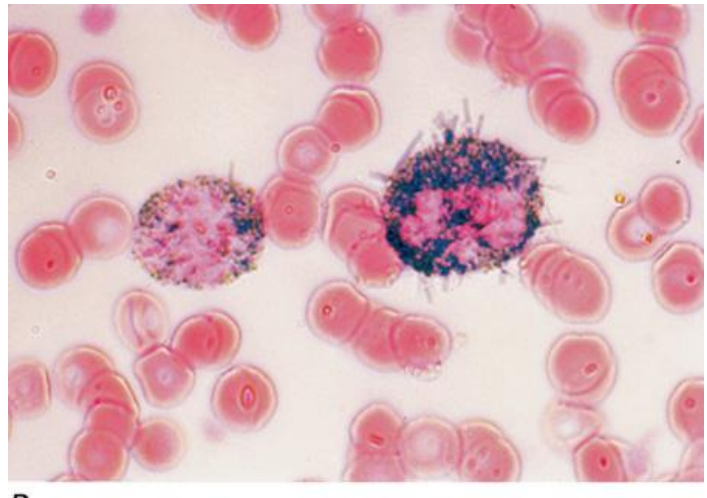


D

Figure 3.8 **Morphology of AML cells.** ***A.*** Uniform population of primitive myeloblasts with immature chromatin, nucleoli in some cells, and primary cytoplasmic granules. ***B.*** Leukemic myeloblast containing an Auer rod. ***C.*** Promyelocytic leukemia cells with prominent cytoplasmic primary granules. ***D.*** Peroxidase stain shows dark blue color characteristic of peroxidase in granules in AML. [(10)]

Differential Diagnosis

1. AML must be distinguished from:
    - Myeloproliferative disorders
    - Chronic myeloid leukemia
    - Myelodysplastic syndromes
2. ALL must be separated from:
    - Chronic lymphocytic leukemia
    - Lymphomas
    - Hairy cell leukemia
    - Atypical lymphocytosis of:
    a. Mononucleosis
    b. Pertussis (11)

Treatment

The type of initial chemotherapy depends on the subtype of leukemia.[12] (11)

AML

1. Combination of an anthracycline:
    - Daunorubicin or
    - Idarubicin plus
2. Cytarabine
3. Either alone or in combination with other agents
4. Transplantation (autologous or allogeneic) (11)

ALL

1. Combination chemotherapy, including[13]:
    - Daunorubicin
    - Vincristine
    - Prednisone
    - Asparaginase

[12] Acute leukemia is potentially curable with combination chemotherapy.
[13] This treatment produces complete remissions in 90% of patients.

2. ALL with Philadelphia chromosome:
   - Initial chemotherapy plus
   - Imatinib (or dasatinib)

3. High-dose chemotherapy plus bone marrow transplantation. (11)

Prognosis

Approximately 70–80% of adults with AML under age 60 years have complete remission.[14] (11)

[14] High-dose postremission chemotherapy leads to cure in 35–40% of these patients, and high-dose cytarabine has been shown to be superior to therapy with lower doses. Allogeneic bone marrow transplantation (for younger adults with HLA-matched siblings) is curative in 50–60% of cases. Autologous bone marrow transplantation may be superior to nonablative chemotherapy. Older adults with AML achieve complete remission in up to 50% of instances. The cure rates for older patients with AML have been very low (approximately 10–15%) even if they achieve remission and are able to receive postremission chemotherapy. The use of reduced-intensity allogeneic transplantation is being explored in order to improve on these outcomes.

Chronic lymphocytic leukemia

Definition

Chronic lymphocytic leukemia (CLL) is a clonal malignancy of B lymphocytes. (11)

Epidemiology

1. CLL is a disease of older patients
2. 90% of cases occurring after age 50 years
3. A median age at presentation is 70 years (11)

Pathophysiology

1. The disease is usually indolent, with slowly progression
2. Accumulation of long-lived small lymphocytes
3. These cells are responding poorly to antigenic stimulation
4. CLL is manifested clinically by:
    - Immunosuppression
    - Bone marrow failure
    - Organ infiltration with lymphocytes (11)

Staging

The long-standing Rai classification system remains prognostically useful:

- Stage 0, lymphocytosis only
- Stage I, lymphocytosis plus lymphadenopathy
- Stage II, organomegaly
- Stage III, anemia
- Stage IV, thrombocytopenia[1] (11)

[1] These stages can be collapsed in to low-risk (stages 0– CLL usually pursues an indolent course, but some subtypes behave more aggressively; a variant, prolymphocytic leukemia, is

Clinical Findings

Symptoms

Many patients

1. Asymptomatic with lymphocytosis
2. Enlarged lymph nodes (Fig. 4.9, 4.11)

Nonspecific

3. Fatigue
4. Lethargy
5. Loss of appetite
6. Weight loss
7. Reduced exercise tolerance

Rare

8. Fever
9. Night sweats
10. Weight loss
11. The most common infections are sinopulmonary (Fig. 4.10, 4.12, 4.14, 4.15) [5]

more aggressive. The morphology of the latter is different, characterized by larger and more immature cells.

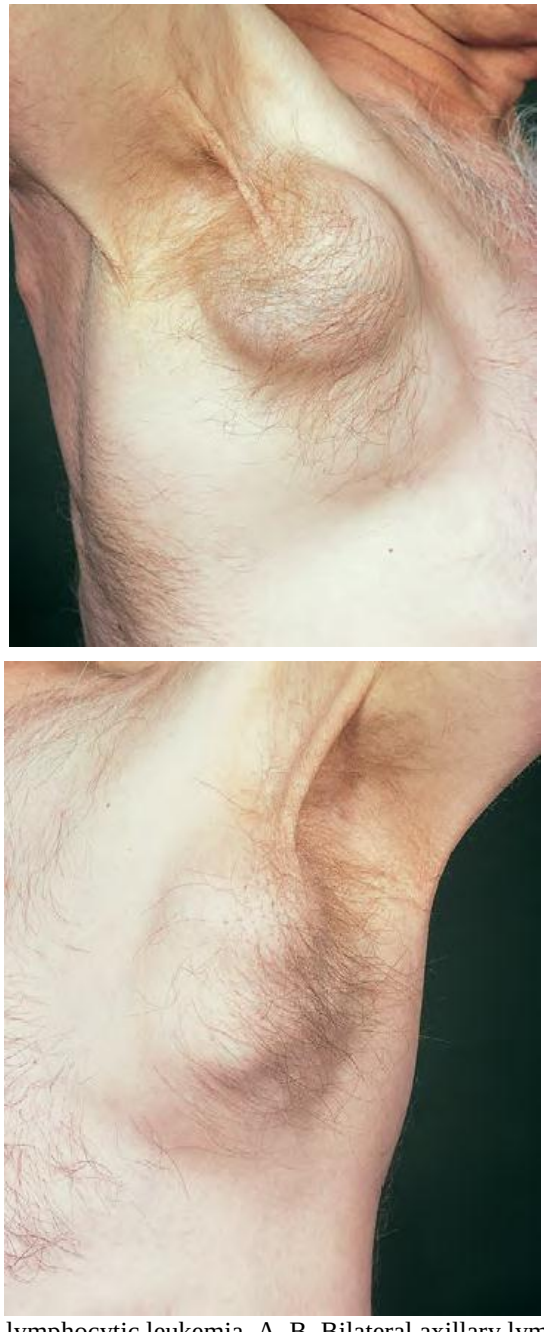


Figure 3.9 Chronic lymphocytic leukemia. A, B, Bilateral axillary lymphadenopathy (same patient as shown). [7]

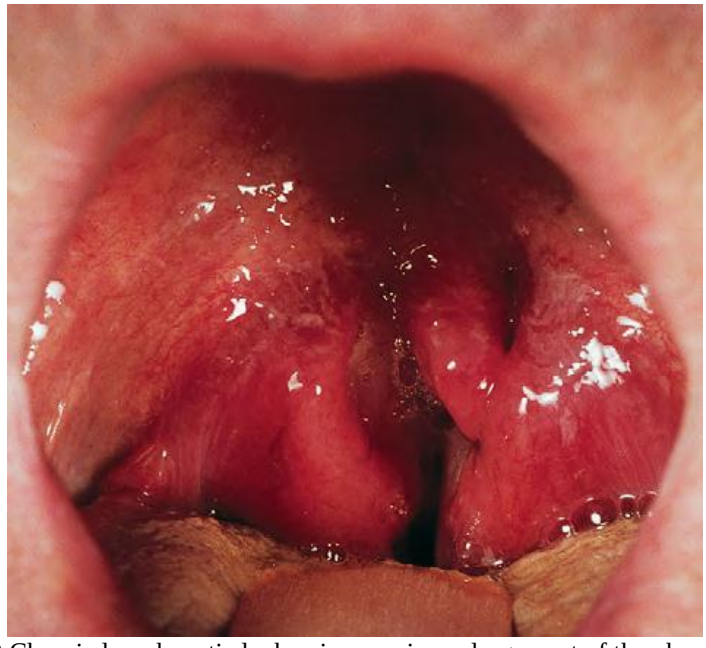


Figure 3.10 Chronic lymphocytic leukemia: massive enlargement of the pharyngeal tonsils (same patient as shown). [7]

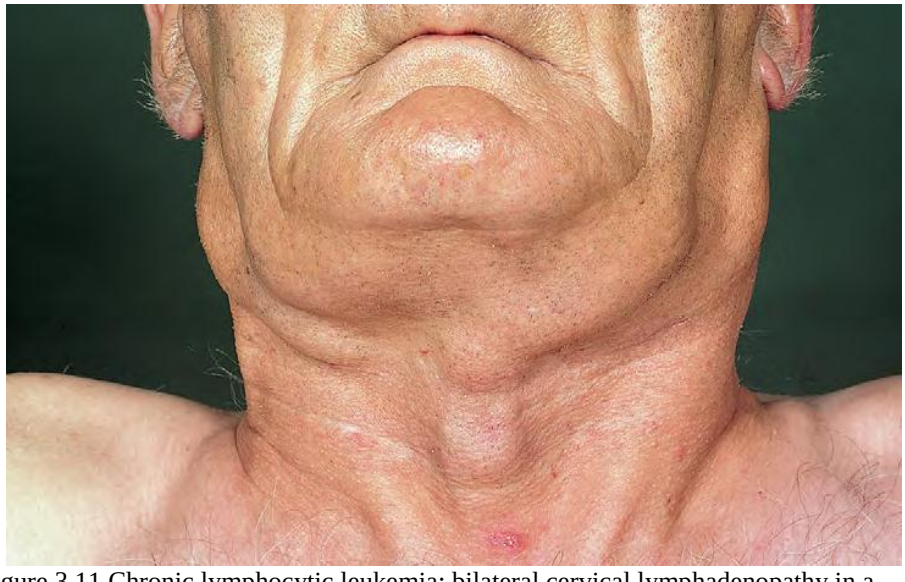


Figure 3.11 Chronic lymphocytic leukemia: bilateral cervical lymphadenopathy in a 65-year-old man. (Hb, 12.5 g/dl; WBC, 150 109/L [lymphocytes, 140 109/L]; platelets, 120 109/L.) [7]

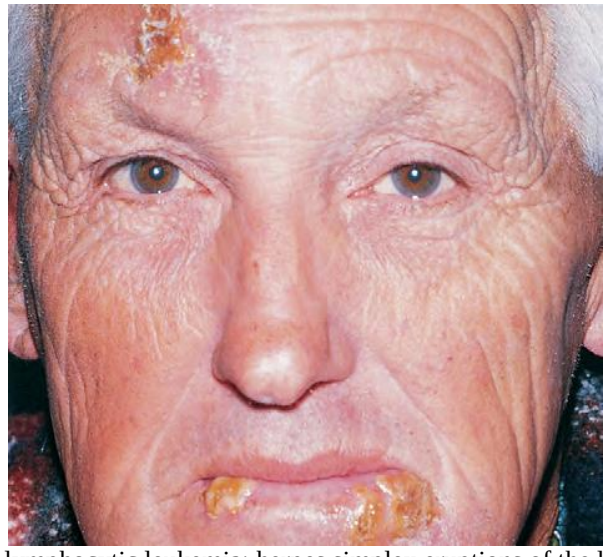


Figure 3.12 Chronic lymphocytic leukemia: herpes simplex eruptions of the lower lip and of the skin of the forehead. (7)

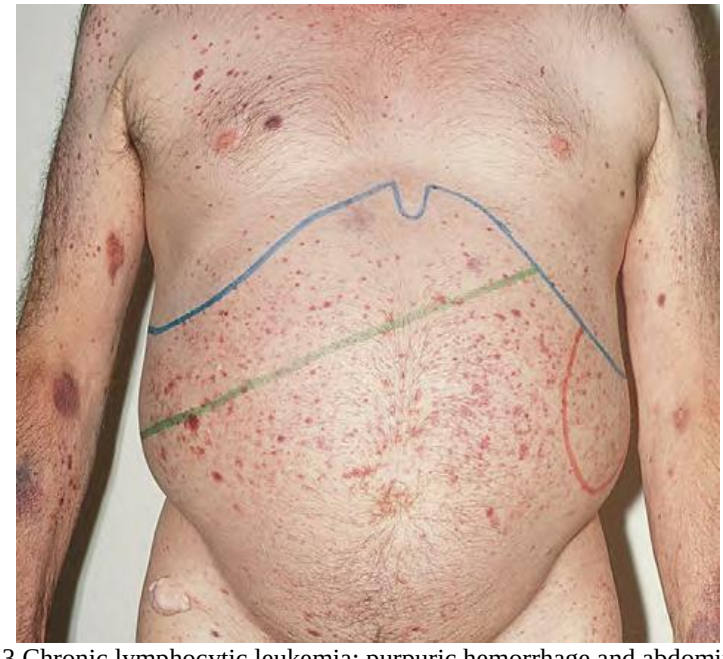


Figure 3.13 Chronic lymphocytic leukemia: purpuric hemorrhage and abdominal swelling in a 54-year-old man. The extent of liver and splenic enlargement is indicated. (Hb, 10.9 g/dl; WBC, 250 109/L [lymphocytes, 245 109/L]; platelets, 35 109/L.) (7)

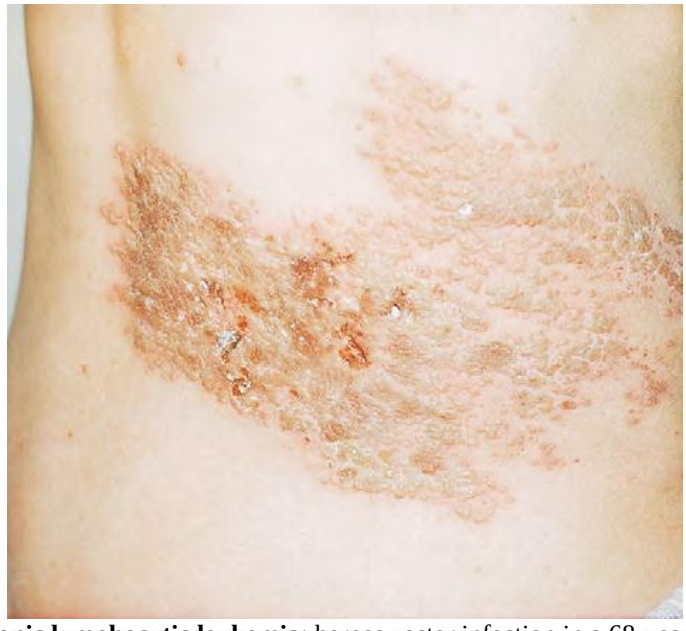


Figure 3.14 **Chronic lymphocytic leukemia**: herpes zoster infection in a 68-year-old woman. [7]

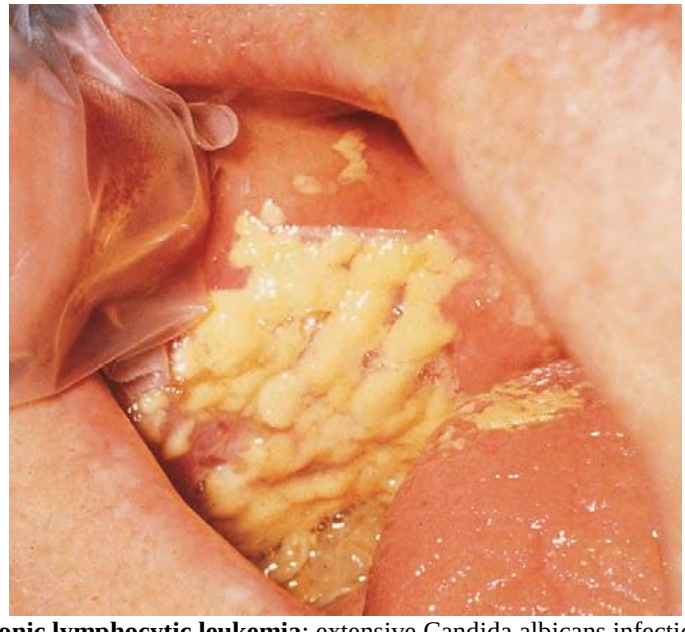


Figure 3.15 **Chronic lymphocytic leukemia**: extensive Candida albicans infection of the buccal mucosa of a 73-year-old woman. [7]

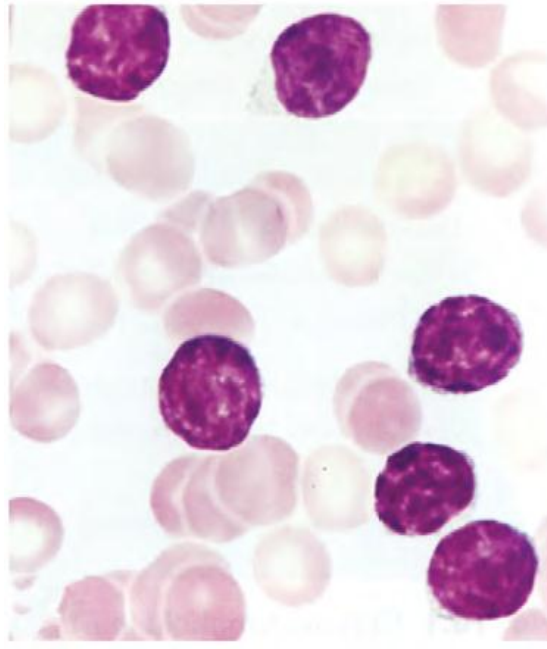
Signs:

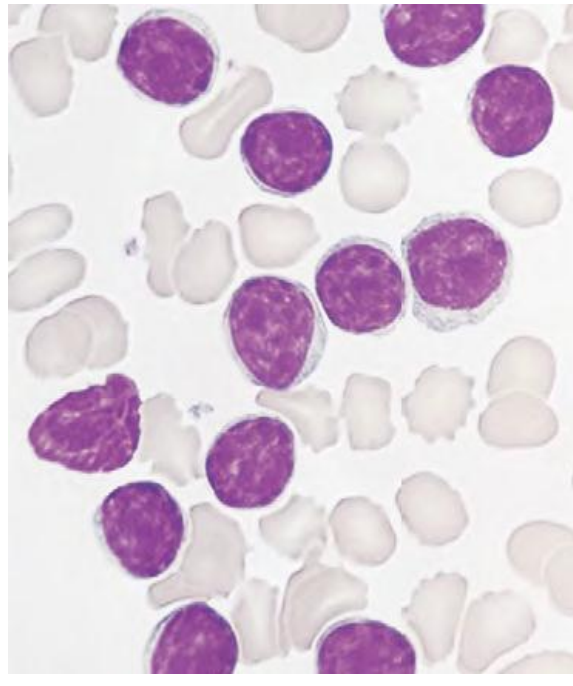
2. Lymphadenopathy in 80%
3. Enlargement of the liver or spleen in 50% (Fig. 4.13)
4. In 5–10% of cases, CLL may be complicated by:
   - Autoimmune hemolytic anemia
   - Autoimmune thrombocytopenia
5. In 5% of cases aggressive large cell lymphoma **(Richter syndrome)**[2] (11)
6. Organ failure from infiltration with CLL is uncommon (5)

Laboratory Findings

1. TLC: 20,000/mcL
2. DLC: Lymphocytosis (75–98% lymphocytes)
3. Lymphocytes appear:
   - Small
   - Mature
   - Condensed nuclear chromatin (Fig. 3.16, 3.17)
4. The hematocrit and platelet count are usually normal at presentation
5. The bone marrow is variably infiltrated with small lymphocytes
6. Hypogammaglobulinemia is present in 50% of patients (11)

[2] In approximately 5% of cases, while the systemic disease remains stable, an isolated lymph node transforms into an aggressive large cell lymphoma **(Richter syndrome)**. (11)





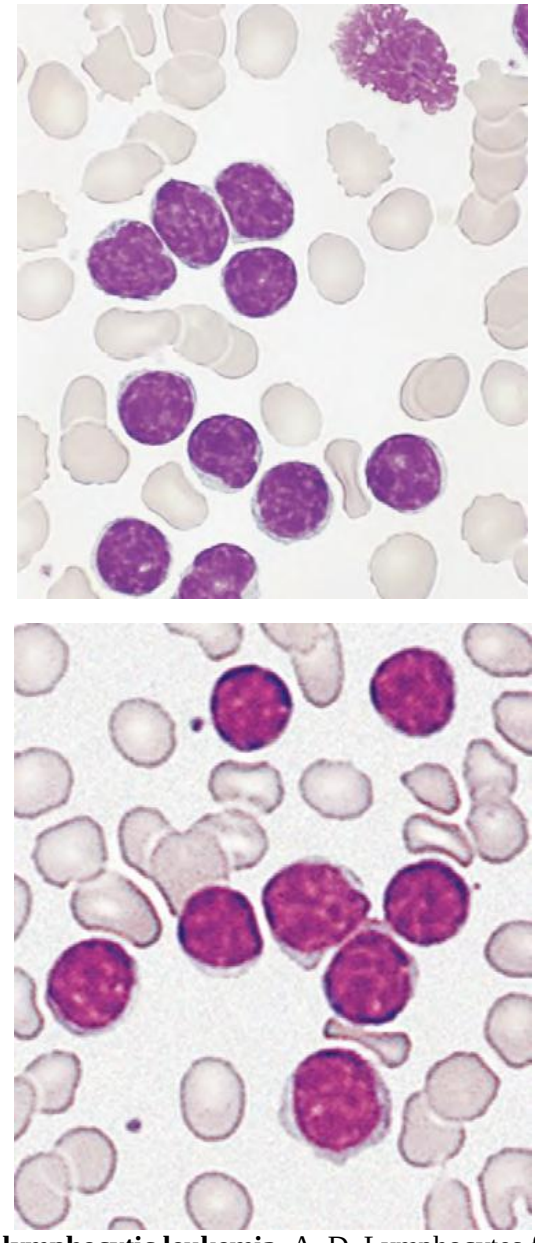


Figure 3.16 **Chronic lymphocytic leukemia.** A–D, Lymphocytes from the peripheral blood of four different patients show thin rims of cytoplasm, condensed coarse chromatin, and only rare nucleoli. [7]

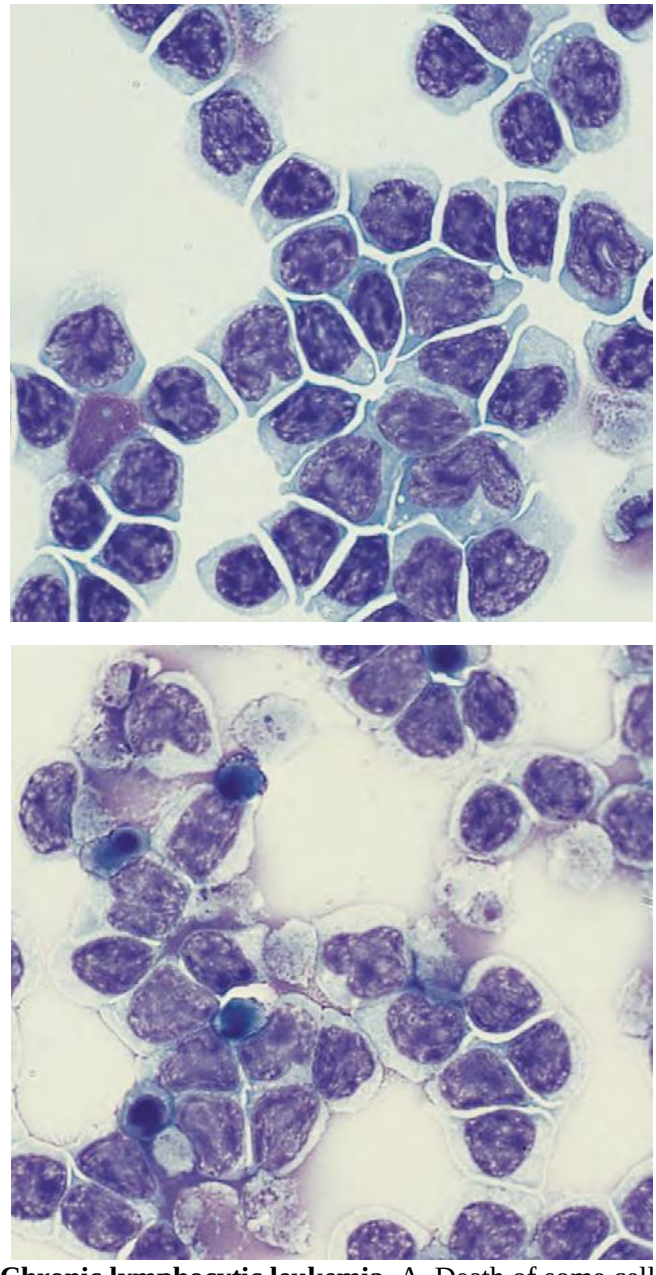


Figure 3.17 **Chronic lymphocytic leukemia.** A, Death of some cells (small, darkly stained chromatin) by apoptosis after 30 hours' culture in medium and plasma. B, Prevention of apoptotic cell death by IL-4 addition to culture (10 days). (Courtesy of Dr. P. Panayiotides.) [7]

Treatment

1. Most cases of early indolent CLL require no specific therapy
2. Indications for treatment include:
   - Progressive fatigue
   - Symptomatic lymphadenopathy
   - Anemia
   - Thrombocytopenia
3. The initial treatment:
   - Fludarabine plus
   - Antibody rituximab, with or without
   - Cyclophosphamide[3]
   - Chlorambucil, 0.6–1 mg/kg orally every 3 weeks for 6 months
4. For refractory CLL:
   - Antibody alemtuzumab
   - Lenalidomide
5. Autoimmune hemolytic anemia or immune thrombocytopenia:
   - Rituximab
   - Prednisone
   - Splenectomy[4]
6. Allogeneic transplantation (11)

Prognosis

1. New therapies are changing the prognosis of CLL
2. In the past, median survival was 6 years

---

[3] The addition of cyclophosphamide appears to increase the risk of treatment-related infection, and the question of whether this increase in toxicity is warranted by improved anti-leukemic effectiveness is currently being studied.

[4] Fludarabine should be avoided in patients with autoimmune hemolytic anemia since it may exacerbate this condition.

3. Only 25% of patients lived more than 10 years
4. Patients with stage 0 or stage I have a median survival of 10–15 years [11]

Chronic Myelogenous leukemia

Definition

CML is a disorder of proliferation which is uncontrolled and excessive [3]

Aetiology

1. Cytotoxic drugs
2. Radiation
3. Chromosomal abnormalities (Philadelphia chromosome)
4. Cigarette has role [10]

Epidemiology

1. Incidence: 1.5 per 100,000 people per year
2. Men women ratio (2:1.2) [10]
3. Age group: 55 years with range 30-80 years [3]

Phases

Has three phases:

1. Chronic phase
2. Accelerated phase
3. Blast crisis phase [3]

Clinical features

Symptoms

1. Tiredness
2. Weight loss
3. Breathlessness
4. Abdominal pain
5. Lethargy

6. Anorexia
7. Sweating
8. Abdominal fullness
9. Bruising
10. Vague ill health [3] 8
11. Organ infiltration (lymph nodes, skin, liver) is less common (Fig. 3.18-3.20) [5]

Figure 3.18 **Chronic myeloid leukemia:** ocular fundus in the hyperviscosity syndrome showing distended retinal veins and deep retinal hemorrhages at the macula. (Hb, 14 g/dl; white blood cell count [WBC], 590 109/L; platelets, 1050 109/L.) [7]

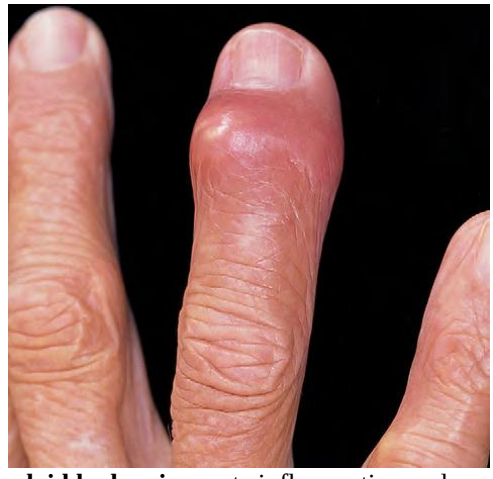


Figure 3.19 **Chronic myeloid leukemia:** acute inflammation and swelling of the fourth finger because of uric acid deposition. (Hb, 8.6 g/dl; WBC, 540 109/L; platelets, 850 109/L; serum uric acid, 0.85 mmol/L.) [7]

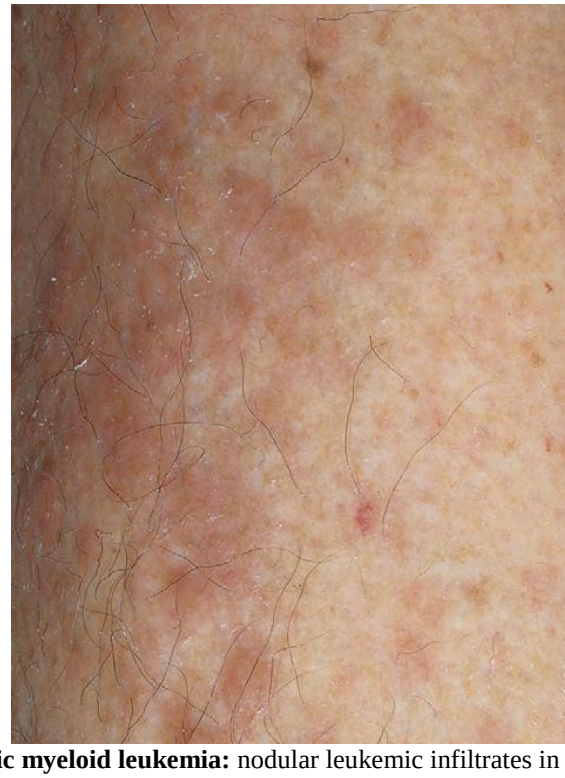


Figure 3.20 **Chronic myeloid leukemia:** nodular leukemic infiltrates in the skin over the anterior surface of the tibia in a 48-year-old woman with blast cell transformation. [7]

Signs

1. Splenomegaly90%
2. Hepatomegaly 50%
3. Lymphadenopathy is unusual [3]

Investigation

1. TLC: Elevated (Fig. 3.21)
2. DLC: Blast cells 5%
3. Platelets: Elevated
4. Bone marrow: Hypercellular [3]

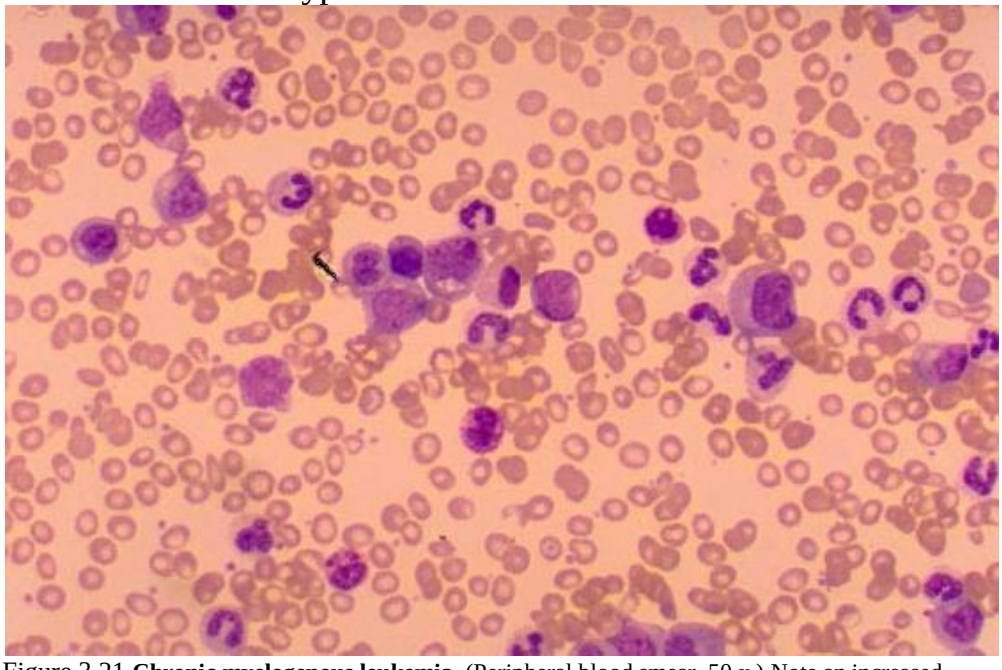


Figure 3.21 **Chronic myelogenous leukemia.** (Peripheral blood smear, 50 x.) Note an increased white blood cell count as well as the typical features of the disease, including the presence of a full range of myeloid maturation. Present are neutrophils, bands, metamyelocytes, myelocytes, and promyelocytes as well as increased numbers of eosinophils and basophils. The platelet count is normal in this patient.

Treatment

1. Chemotherapy
    - Busulphan

- Hydroxyurea
- Melphalan

2. Bone marrow transplantation [10]

Prognosis

1. Death was expected in:
   - 10% of patients within 2 years
   - In about 20% yearly thereafter
2. The median survival time was ~4 years [10]

Hodgkin's disease

Definition

Hodgkin's disease is a group of cancers characterized by Reed–Sternberg cells in an appropriate reactive cellular background. The malignant cell is derived from B lymphocytes of germinal center origin. (11)

Epidemiology

There is a bimodal age distribution, with one peak in the 20s and a second over age 50 years (Fig. 3.22). (11)

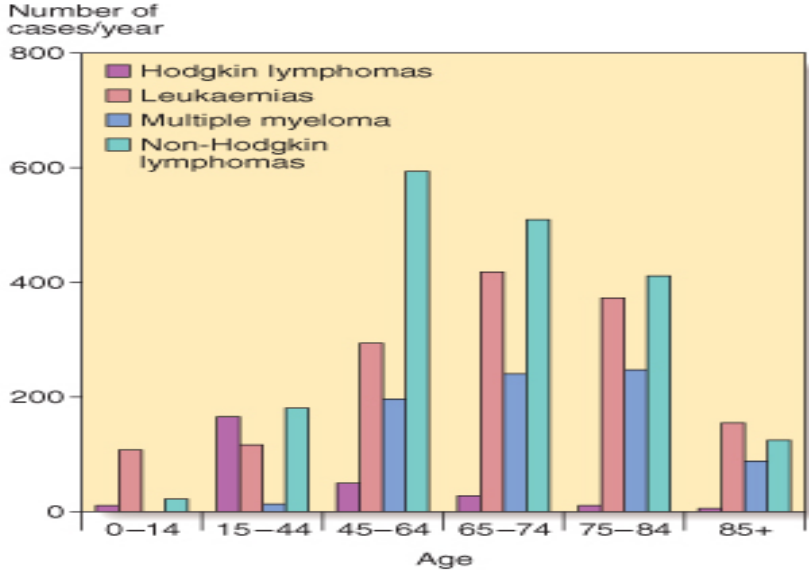


Figure 3.22 Variation in the incidence of different haematological malignancies in the UK by age. (3)

Staging

The staging nomenclature (Ann Arbor) is as follows:

- Stage I, one lymph node region involved (Fig. 3.23)
- Stage II, involvement of two lymph node areas on one side of the diaphragm
- Stage III, lymph node regions involved on both sides of the diaphragm
- Stage IV, disseminated disease with bone marrow or liver involvement [(11)]

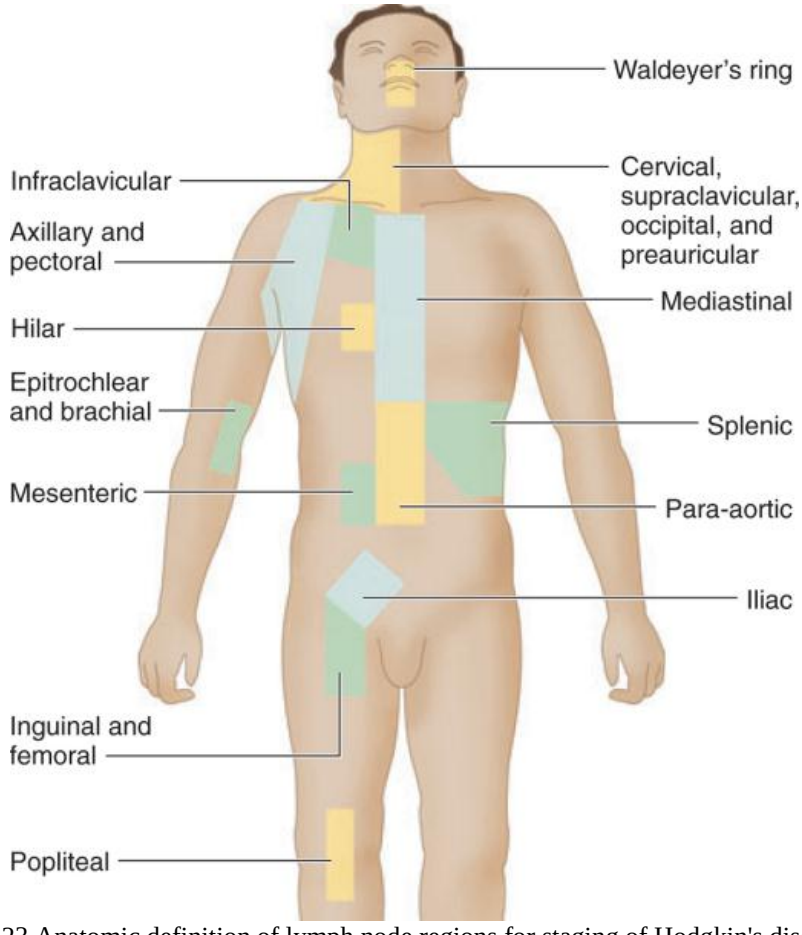


Figure 3.23 Anatomic definition of lymph node regions for staging of Hodgkin's disease. [(5)]

Types

Hodgkin disease is divided into several subtypes:

1. Lymphocyte predominance
2. Nodular sclerosis
3. Mixed cellularity
4. Lymphocyte depletion

Clinical Findings

1. Painless mass, commonly in the neck (Fig. 3.24-3.28) [1]
2. Fever
3. Weight loss
4. Night sweats
5. Generalized pruritus
6. Pain in an involved lymph node following alcohol ingestion[2]
7. Spread to contiguous areas of lymph nodes[3]
8. Widespread hematogenous dissemination[4] (11)

[1] Most patients present because of a painless mass, commonly in the neck.
[2] An unusual symptom of Hodgkin disease is pain in an involved lymph node following alcohol ingestion.
[3] An important feature of Hodgkin disease is its tendency to arise within single lymph node areas and spread in an orderly fashion to contiguous areas of lymph nodes.
[3] Only late in the course of the disease will vascular invasion lead to widespread hematogenous dissemination.

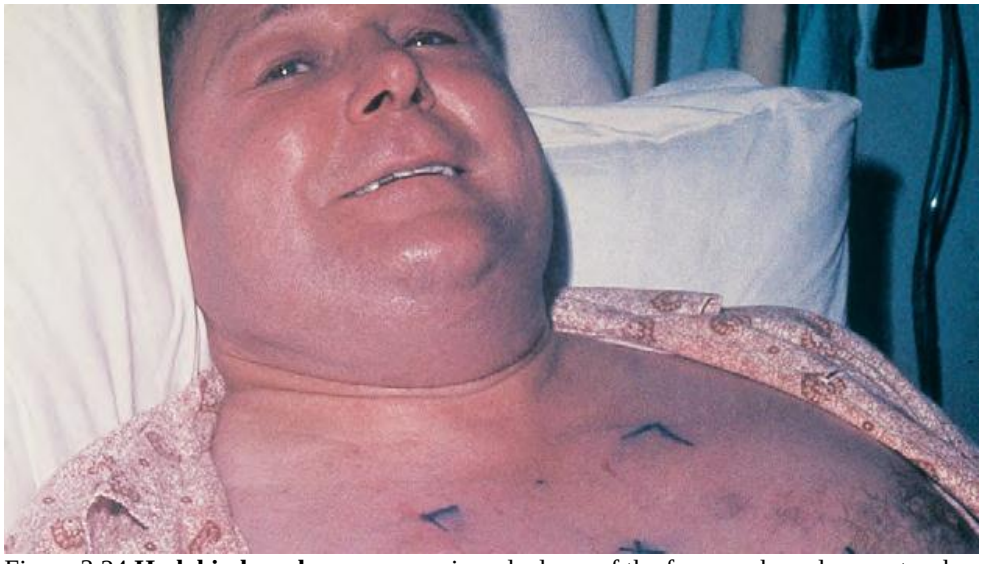


Figure 3.24 **Hodgkin lymphoma:** cyanosis and edema of the face, neck, and upper trunk result from superior vena cava obstruction caused by mediastinal node involvement. The skin markings over the anterior chest indicate the fi eld of radiotherapy. (7)

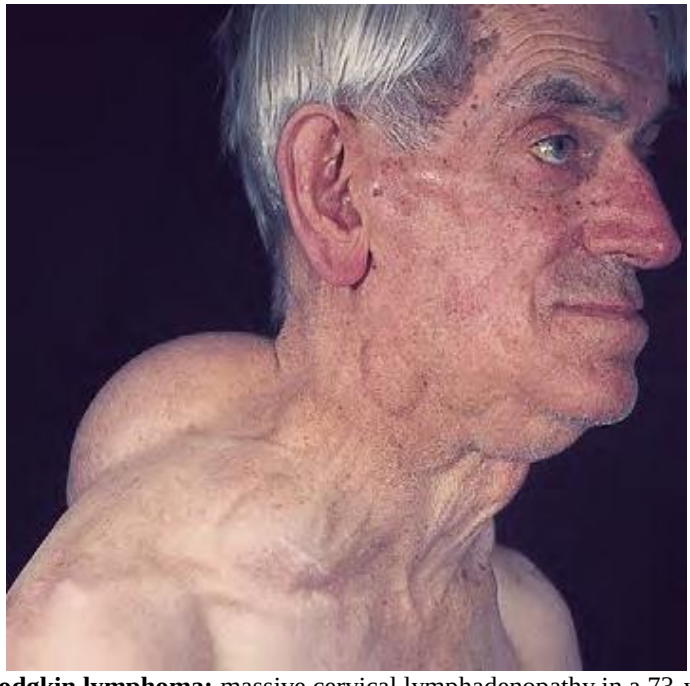


Figure 3.25 **Hodgkin lymphoma:** massive cervical lymphadenopathy in a 73-year-old man with extensive disease. (7)

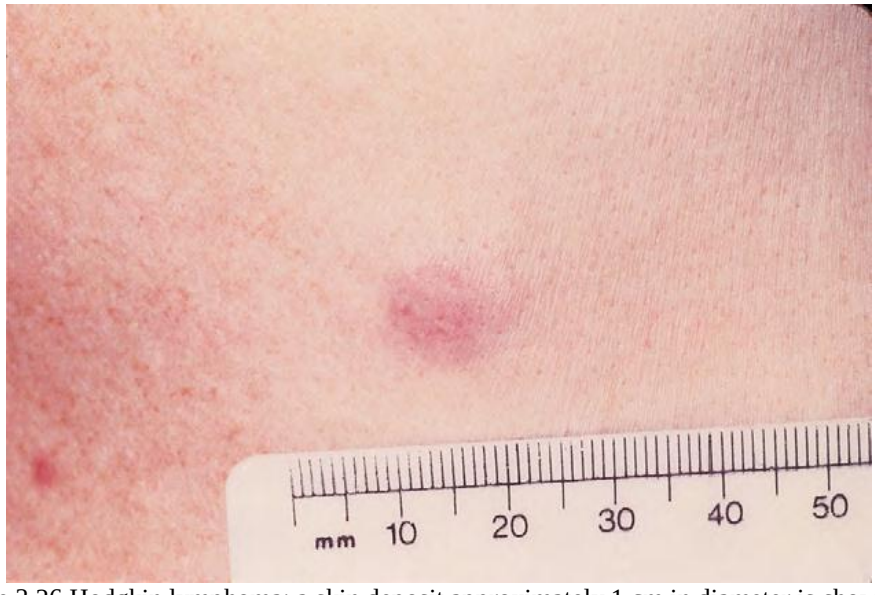


Figure 3.26 Hodgkin lymphoma: a skin deposit approximately 1 cm in diameter is shown. [7]

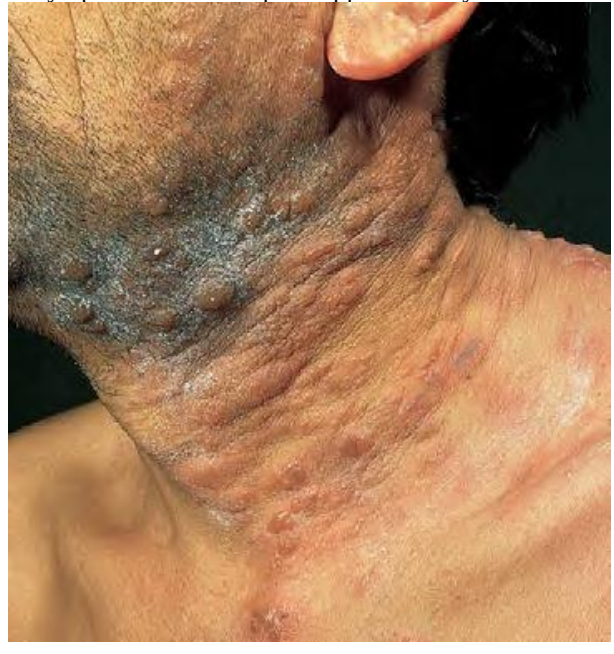


Figure 3.27 **Hodgkin lymphoma:** (**A**) vesicular cutaneous eruption of the neck caused by herpes zoster; (**B**) atypical herpetic eruption of the palmar surface of the hand. [7]

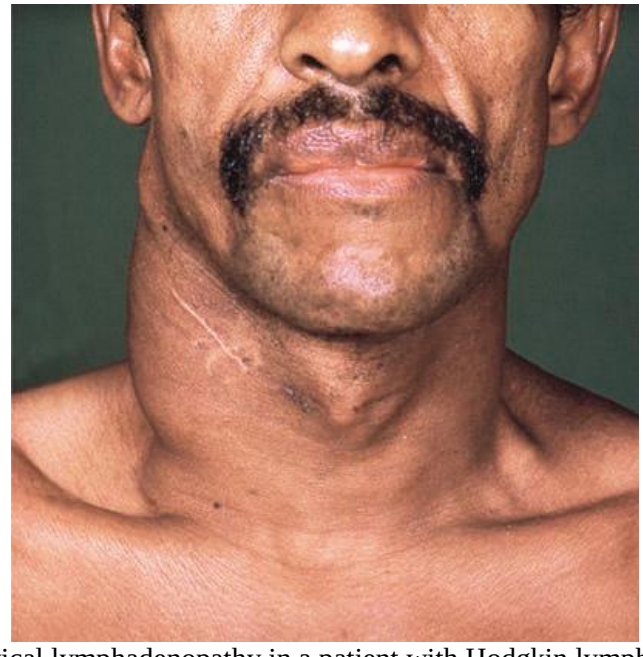


Figure 3.28 Cervical lymphadenopathy in a patient with Hodgkin lymphoma. [7]

Investigations

1. Blood count may be:
   - Normal
   - Normochromic,normocytic anaemia
   - Lymphopenia
2. Erythrocyte sedimentation rate (ESR)is usually raised
3. Liver biochemistry is often abnormal
4. Serum lactate dehydrogenase; raised level is adverse prognostic factor.
5. Uric acid is normal or raised.
6. Reed-Sternberg cell (a large cell with a bilobed nucleus) (Fig. 3.30-3.32)
7. Chest X-ray may show mediastinal widening (Fig. 3.29)
8. Lung involvement
9. CT scans show involvement of intrathoracic nodes in 70% of cases. (11)

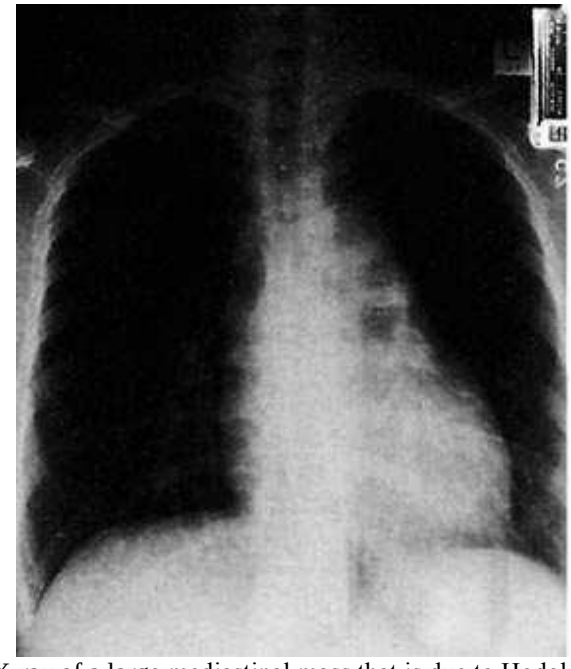


Figure 3.29 Chest X-ray of a large mediastinal mass that is due to Hodgkin's lymphoma.

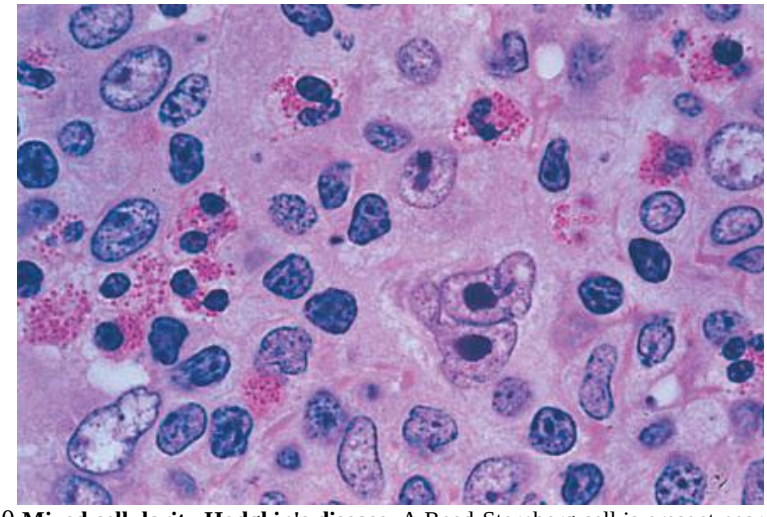


Figure 3.30 **Mixed cellularity Hodgkin's disease.** A Reed-Sternberg cell is present near the center of the field; a large cell with a bilobed nucleus and prominent nucleoli giving an "owl's eyes" appearance. The majority of the cells are normal lymphocytes, neutrophils, and eosinophils that form a pleiomorphic cellular infiltrate. [10]

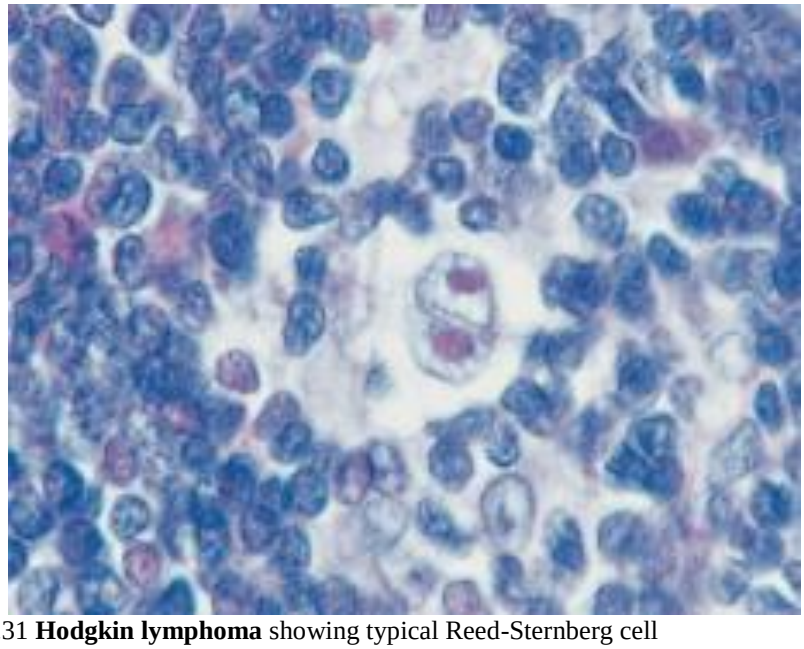


Figure 3.31 **Hodgkin lymphoma** showing typical Reed-Sternberg cell

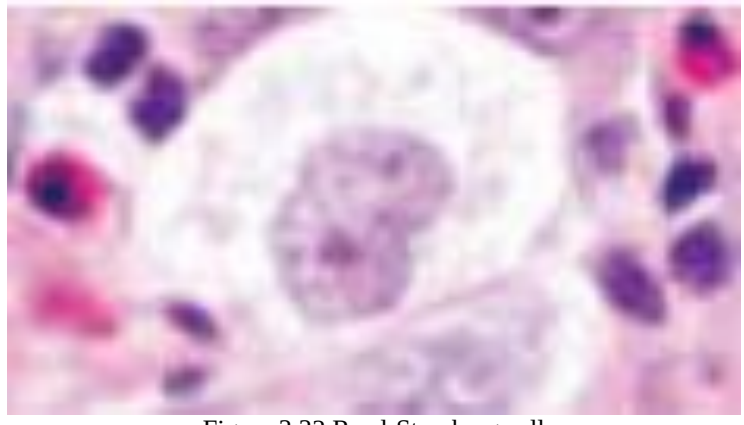


Figure 3.32 Reed-Sternberg cell

Treatment

1. Radiation therapy[5]
2. Combination chemotherapy using:

- Doxorubicin (Adriamycin)
- Bleomycin
- Vincristine
- Dacarbazine

3. Autologous hematopoietic stem cell transplantation (11)

Prognosis

1. Prognosis in advanced stage Hodgkin lymphoma is influenced by seven features[6]:
   - Stage
   - Age
   - Gender

[5] Radiation therapy used as initial treatment only for patients with low-risk stage IA and IIA disease.

[6] Poorer results are seen in patients who are older, those who have bulky disease, and those with lymphocyte depletion or mixed cellularity on histologic examination. Non-classic Hodgkin lymphoma (nodular lymphocyte predominant) is highly curable with radiotherapy alone for early-stage disease; however, for highstage disease, it is characterized by long survival with repetitive relapses after chemotherapy. (11)

- Hemoglobin
- Albumin
- White blood count
- Lymphocyte count

2. The cure rate is 75% if zero to two risk features are present
3. 55% when three or more risk features are present
4. The prognosis of patients with stage IA or IIA disease is excellent, with 10-year survival rates in excess of 90%.
5. Patients with advanced disease (stage III or IV) have 10-year survival rates of 50–60% [11]

The non-Hodgkin lymphomas

Definition

The non-Hodgkin lymphomas are a heterogeneous group of cancers of lymphocytes. The disorders vary in clinical presentation and course from indolent to rapidly progressive. [11]

Pathogenesis

Chromosomal abnormalitis

Classification

The classification of lymphomas is shown in Table 3.2.

Table 3.2 WHO proposed classification of non-Hodgkin lymphomas.[11]
Precursor B
B cell lymphoblastic lymphoma
Mature B
Diffuse large B cell lymphoma
Mediastinal large B cell lymphoma
Follicular lymphoma
Small lymphocytic lymphoma
Lymphoplasmacytic lymphoma
Mantle cell lymphoma
Burkitt lymphoma
Marginal zone lymphoma
MALT type
Nodal
Splenic
Mucosal tissue associated
Precursor T
T cell lymphoblastic lymphoma
Mature T (and NK cell)
Anaplastic T cell lymphoma
Peripheral T cell lymphoma

Clinical Features

Symptoms

1. Painless lymphadenopathy
2. Involved lymph nodes may be present in the retroperitoneum, mesentery, and pelvis
3. Patients with intermediate and high-grade lymphomas may have constitutional symptoms such as:
   - Fever

- Drenching night sweats
- Weight loss (Fig. 3.33-3.36) [11]

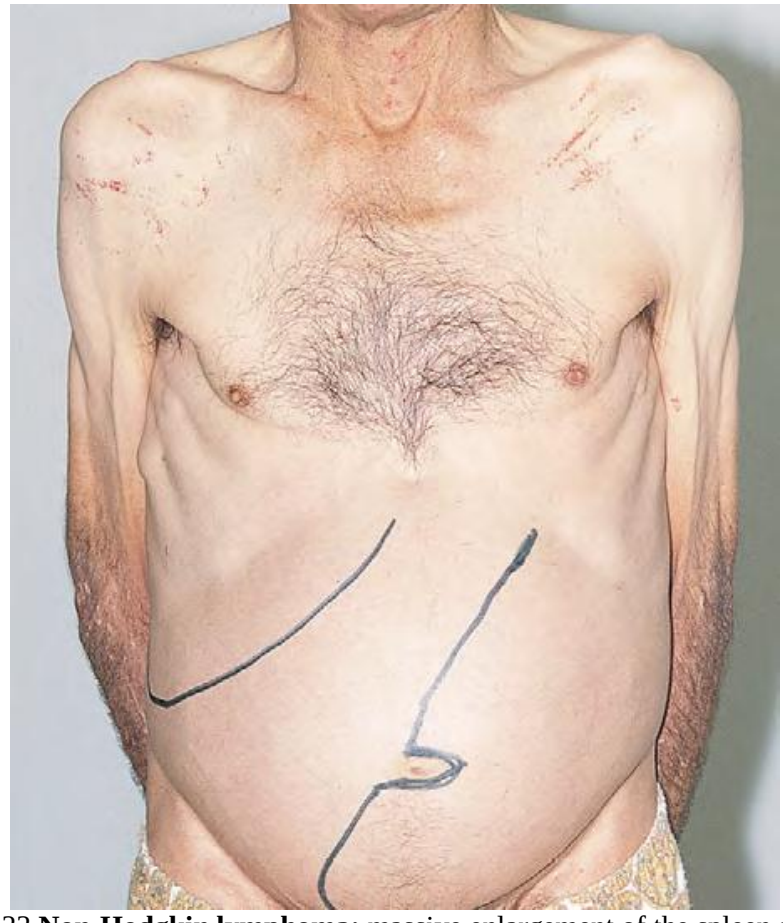


Figure 3.33 **Non-Hodgkin lymphoma:** massive enlargement of the spleen and hepatomegaly in CLL/small lymphocytic lymphoma. (Hb, 9.5 g/dl; WBC, 6.0 109/L; lymphocytes, 2.7 109/L; platelets, 80 109/L.) [7]

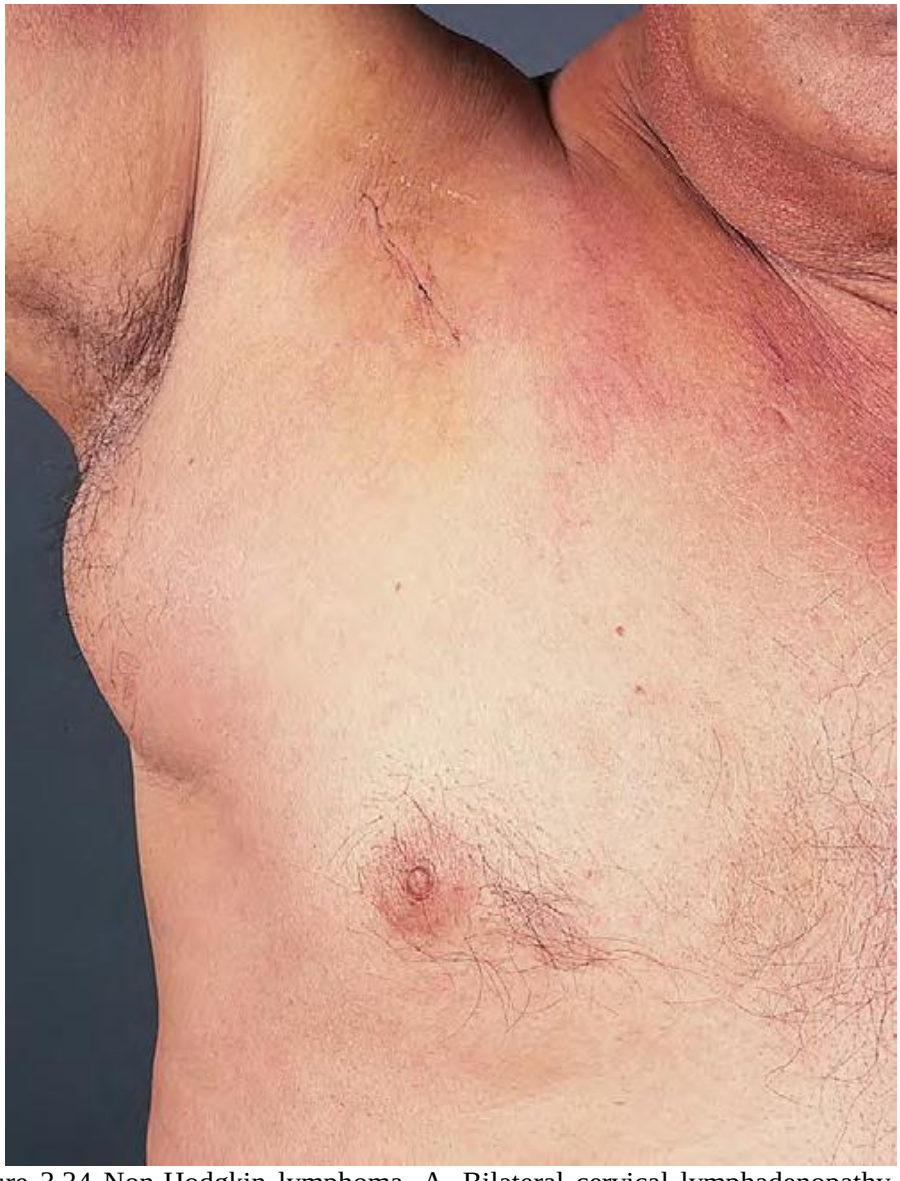


Figure 3.34 Non-Hodgkin lymphoma. A, Bilateral cervical lymphadenopathy in a patient with CLL/small lymphocytic lymphoma. B, Massive enlargement of lymph nodes in the left submandibular area, with extensive ulceration of the overlying skin in a patient with diffuse large B-cell lymphoma. C, Massive enlargement of axillary nodes with mass extending subcutaneously and also intramuscularly in the right infraclavicular and supraclavicular regions in a patient with diffuse large B-cell lymphoma. [7]

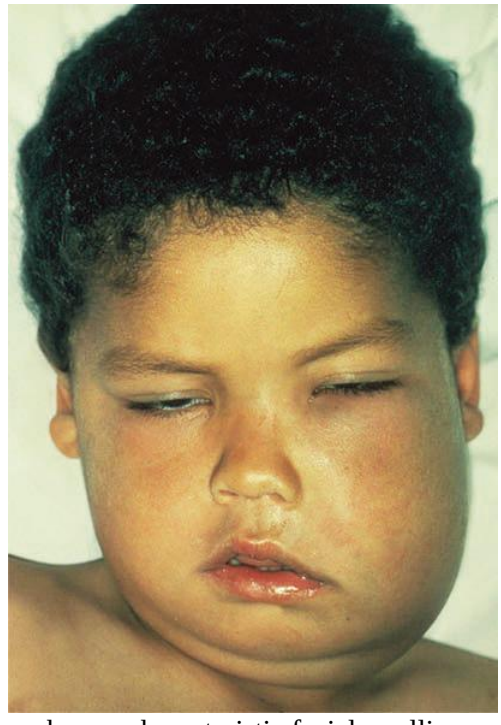


Figure 3.35 Burkitt lymphoma: characteristic facial swelling caused by extensive tumour involvement of the mandible and surrounding soft tissues. (7)

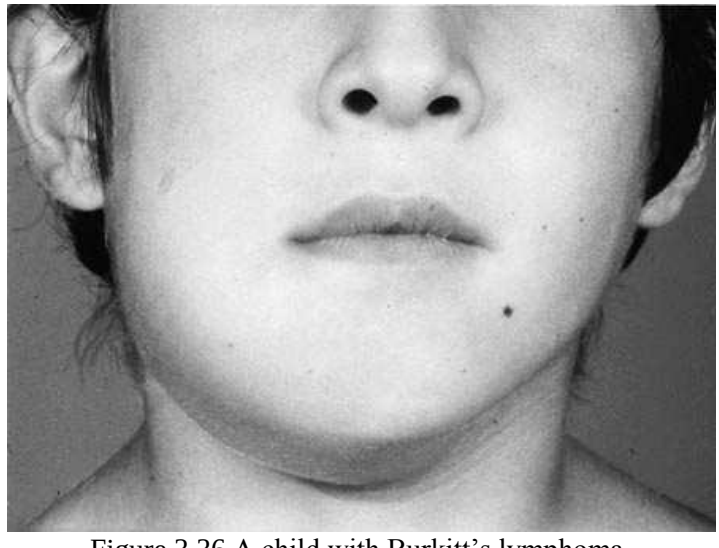


Figure 3.36 A child with Burkitt's lymphoma.

Signs

1. lymphadenopathy may be isolated, or extranodal sites of disease (skin, gastrointestinal tract) may be found
2. Patients with Burkitt lymphoma are noted to have abdominal pain or abdominal fullness because of the predilection of the disease for the abdomen
3. Once a pathologic diagnosis is established, the patient is staged [11]

Investigations

1. Chest radiograph
2. CT scan of the abdomen and pelvis
3. Bone marrow biopsy
4. The peripheral blood is usually normal
5. Lymph node biopsy [11]

Treatment

1. Antibody rituximab
2. Vincristine
3. Prednisone
4. Cyclophosphamide
5. Doxorubicin
6. Fludarabine [11]

Prognosis

The median survival of patients with indolent lymphomas has been 6–8 years. [11]

Neutropenia

Definition

Neutropenia is defined as a circulatory neutrophil count below 1.5 × 10_9/L. A virtual absence of neutrophils is called agranulocytosis.[1]

It should be noted that black patients may have somewhat lower neutrophil counts. (8)

Severity of neutropenia

The risk of infection is related to the severity of neutropenia.

1. The risk of serious infection (neutrophil counts below 500/mcL)
2. Profound neutropenia (Neutrophil counts < 100/mcL)
3. Chronic benign neutropenia (Free of infection for years)
4. Cyclic neutropenia (alternates between normal and low) (11)

Aetiology

Acquired

1. Viral infection
2. Severe bacterial infection, e.g. typhoid
3. Felty's syndrome
4. Immune neutropenia – autoimmune, autoimmune neonatal neutropenia
5. Pancytopenia from any cause, including drug-induced marrow aplasia
6. Pure white cell aplasia (8)

[1] **Definition:** The clinical consequences of prolonged phagocyte dysfunction can be life-threatening. Fortunately, acquired and inherited phagocyte dysfunction syndromes are rare. (5)

Inherited

1. Ethnic (neutropenia is common in black races)
2. Kostmann's syndrome (severe infantile agranulocytosis) due to mutation in elastane 2 (ELA 2) gene
3. Cyclical (genetic mutation in ELA2 gene with neutropenia every 2–3 weeks) [8]
4. Others:
   - Schwachman–Diamond syndrome
   - Dyskeratosis congenital
   - Chédiak–Higashi syndrome (Table 3.3) [8]

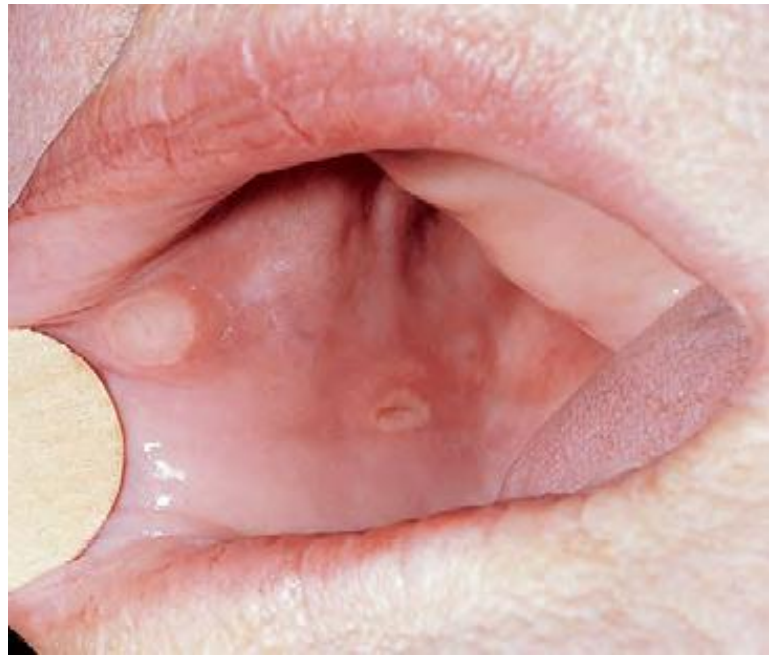
Table 3.3 **Causes of neutropenia.** [11]
Bone marrow disorders
Aplastic anemia
Pure white cell aplasia
Congenital
Cyclic neutropenia
Drugs: sulfonamides, chlorpromazine, procainamide, penicillin, cephalosporins, cimetidine, methimazole, phenytoin, chlorpropamide, antiretroviral medications
Benign chronic
Large granular lymphocytic leukemia
Peripheral disorders
Hypersplenism
Sepsis
Immune
Felty syndrome
HIV infection

Clinical features

1. Neutropenia results in stomatitis and in infections due to:
   - Gram-positive
   - Gram-negative
   - Aerobic bacteria or

- Fungi such as *Candida* or *Aspergillus*

2. The most common infections are:
   - Septicemia
   - Cellulitis
   - Pneumonia
   - Mouth and ulceration (Fig. 3.37, 3.38)
3. In the presence of severe neutropenia, the usual signs of inflammatory response to infection may be reduced or absent.
4. Nevertheless, fever in the neutropenic patient should always be initially assumed to be of infectious origin. (11&8)



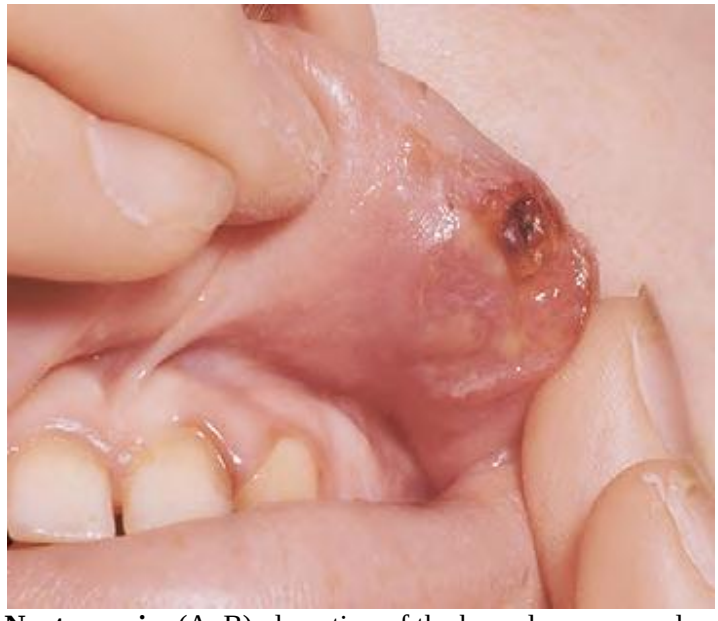


Figure 3.37 **Neutropenia:** (A, B) ulceration of the buccal mucosa and upper lip in two patients with severe neutropenia. [7]

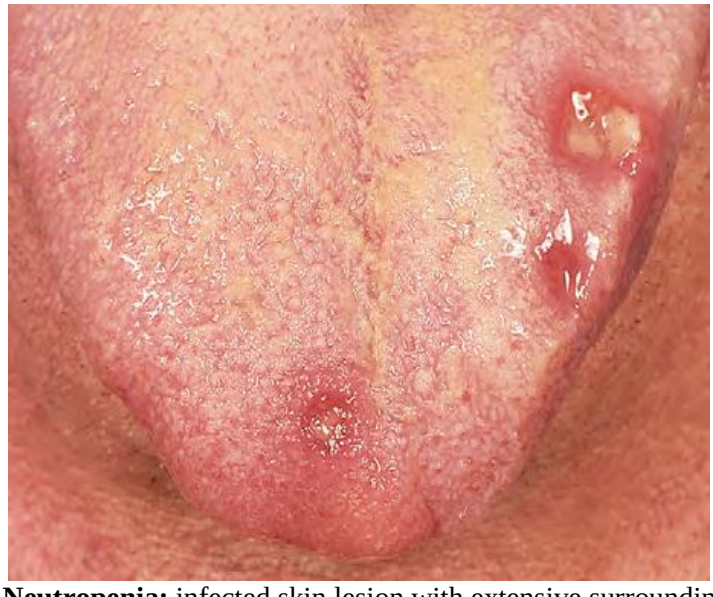


Figure 3.38 **Neutropenia:** infected skin lesion with extensive surrounding subcutaneous cellulitis in severe neutropenia. Cultures grew Staphylococcus aureus and Pseudomonas pyocyanea. [7]

Investigations

1. Peripheral blood: agranulocytosis (complete absence of neutrophils) is almost always due to a drug reaction.
2. bone marrow shows an almost complete absence of myeloid precursors, with other cell lines undisturbed (Fig. 3.39). [11]
3. Neutrophil antibody studies are performed if an immune mechanism is suspected. [8]

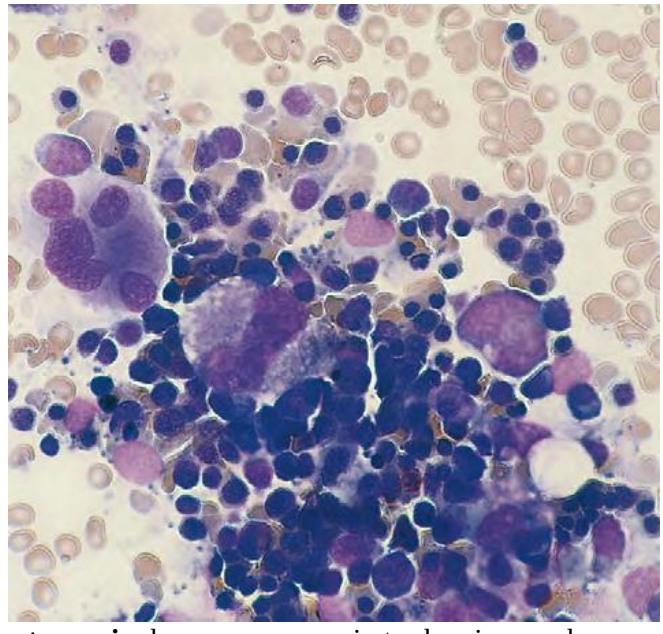


Figure 3.39 **Neutropenia:** bone marrow aspirate showing an absence of granulopoietic cells. The small fragment and cell trail contain mainly erythroblasts and megakaryocytes. [7]

Treatment

1. Potential causative drugs should be discontinued.
2. Infections are treated with broad-spectrum antibiotics, but particular attention should be paid to enteric gram-negative bacteria.

3. Effective antibiotics include the quinolones such as levofloxacin, 500 mg orally or intravenously daily, or new cephalosporins such as cefepime, 2 g intravenously every 8 hours.
4. The antifungal agent voriconazole (oral or intravenous) provides both better efficacy and reduced toxicity compared with amphotericin. (11)
5. Many cases of idiopathic or autoimmune neutropenia respond to myeloid growth factors such as granulocyte colony-stimulating factor (G-CSF). Once-weekly or twice-weekly dosage will often be sufficient to produce a protective neutrophil count. (11)
6. When Felty syndrome leads to repeated bacterial infections, splenectomy has been the treatment of choice, but it now appears that sustained use of G-CSF is effective and provides a nonsurgical alternative. (11)

Prognosis

1. The prognosis of patients with neutropenia depends on the underlying cause.
2. Most patients with drug-induced agranulocytosis can be supported with broad-spectrum antibiotics and will recover completely.
3. The myeloid growth factor G-CSF (filgrastim) may be useful in shortening the duration of neutropenia associated with chemotherapy.
4. The neutropenia associated with large granular lymphocytes may respond to therapy with either cyclosporine or low-dose methotrexate. (11)

Chapter fuor

Disorders of Thrombocyte & Coagulation

- *Immune Thrombocytopenic Purpura (ITP)*
- *Disseminated intravascular coagulation (DIC)*

Immune Thrombocytopenic Purpura (ITP)

Definition

Immune thrombocytopenic purpura (ITP; also termed *idiopathic thrombocytopenic purpura*) is an acquired disorder leading to immune-mediated destruction of platelets and possibly inhibition of platelet release from the megakaryocyte.

- In children it is usually an acute disease
- In adults it usually runs a more chronic course
- The exact nature is not known
- ITP is termed *secondary* if it is associated with an underlying disorder; SLE, HIV and hepatitis C
- ITP with *Helicobacter pylori* infection is unclear [(10)]

Aetiology

Secondary associations of immune thrombocytopenia

1. Infections
   - HIV
   - Varicella
   - Epstein–Barr virus
2. Collagen vascular disease
   - SLE
   - Rheumatoid arthritis
3. Lymphoproliferative disorders
   - Chronic lymphocytic leukaemia

- Hodgkin's disease
- Non-Hodgkin's lymphoma

4. Other
   - Antiphospholipid antibody syndrome
   - Autoimmune thyroid dysfunction
   - Sarcoidosis
   - Post bone marrow transplantation [12]

Clinical features

1. Mucocutaneous bleeding
   - Oral mucosa
   - Gastrointestinal
   - Heavy menstrual bleeding
2. Ecchymoses
3. Petechiae (Fig. 4.1, 4.2)
4. Thrombocytopenia incidentally found on a routine CBC
5. Rarely, life-threatening bleeding, including in the CNS
6. Wet purpura (blood blisters in the mouth)
7. Retinal hemorrhages [10]
8. Findings of secondary couses such as:
   - Collagen vascular disease (Fig. 4.3)
   - HIV
   - HCV infection
   - Lymphoproliferative malignancy [11]

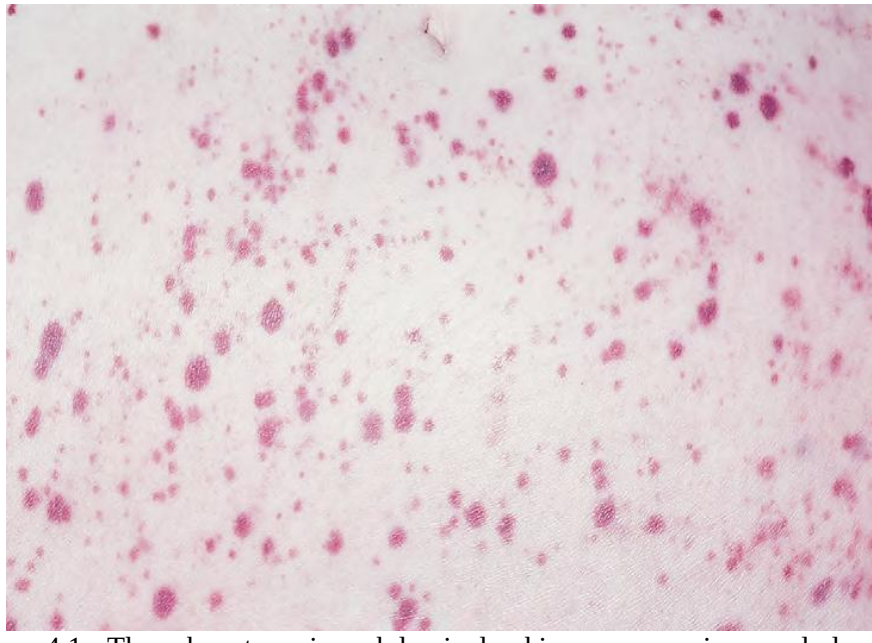


Figure 4.1 Thrombocytopenia: abdominal skin purpura in myelodysplastic syndrome. The platelets are often functionally abnormal, as well as reduced in number. (7)

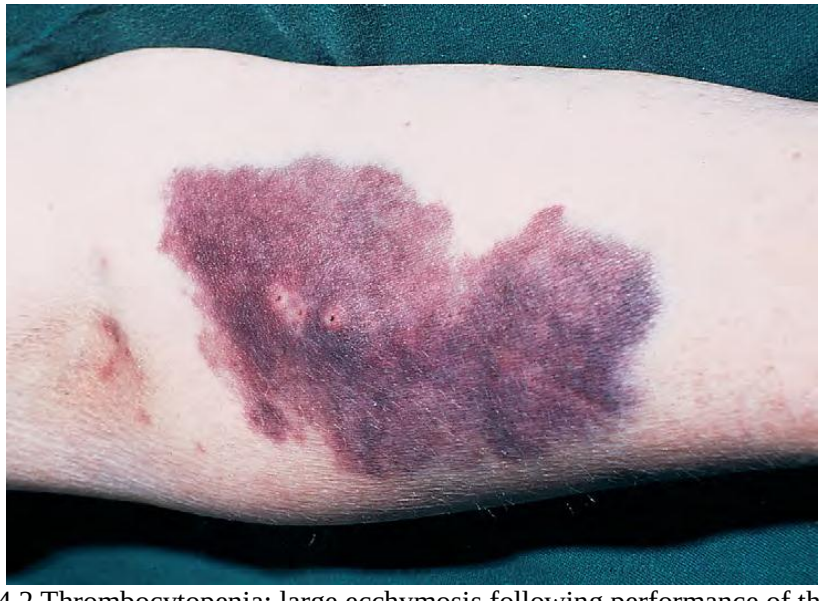


Figure 4.2 Thrombocytopenia: large ecchymosis following performance of the Ivy bleeding-time test. The puncture marks of the stylet cutter are clearly seen. (7)

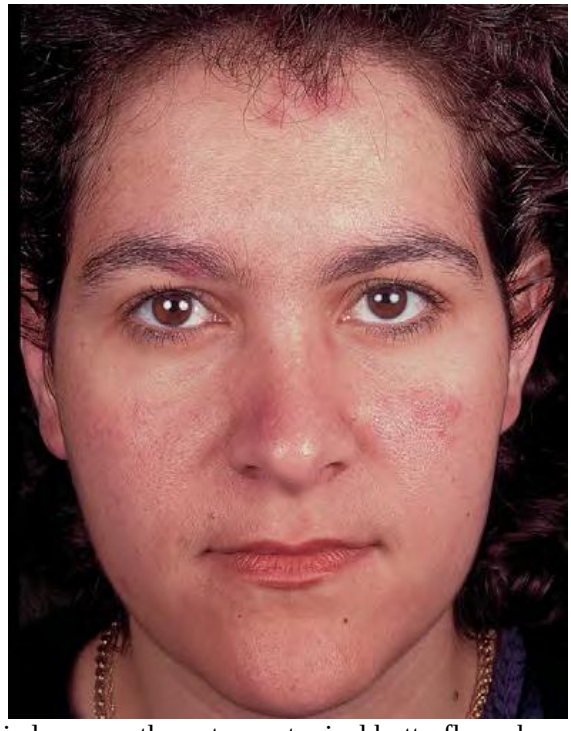


Figure 4.3 Systemic lupus erythematosus: typical butterfly rash and frontal alopecia in a woman who also suffered from immune thrombocytopenia. (7)

Laboratory Testing

1. Serologic teste is usually not helpful
2. Bone marrow examination (Fig. 4.4)
3. The peripheral blood smear may show large platelets (Fig. 4.5)
4. Iron deficiency anemia
5. Testes for secondary causes:
    - HIV infection
    - Hepatitis C
    - Serologic testing for SLE
    - Serum protein electrophoresis
    - Immunoglobulin levels
    - IgA deficiency (10)

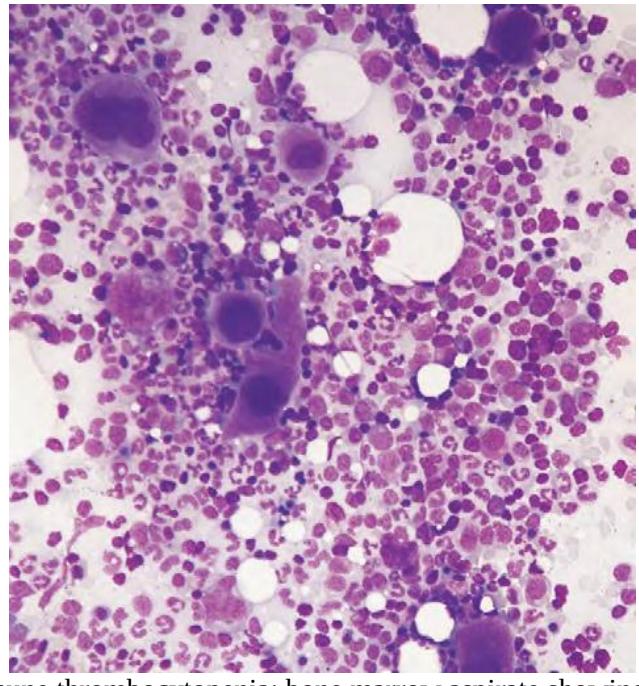


Figure 4.4 Immune thrombocytopenia: bone marrow aspirate showing increased numbers of megakaryocytes. [7]

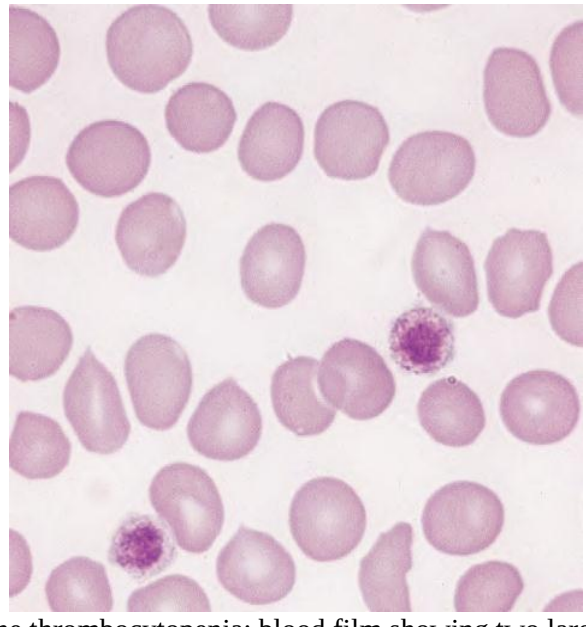


Figure 4.5 Immune thrombocytopenia: blood film showing two large platelets. [7]

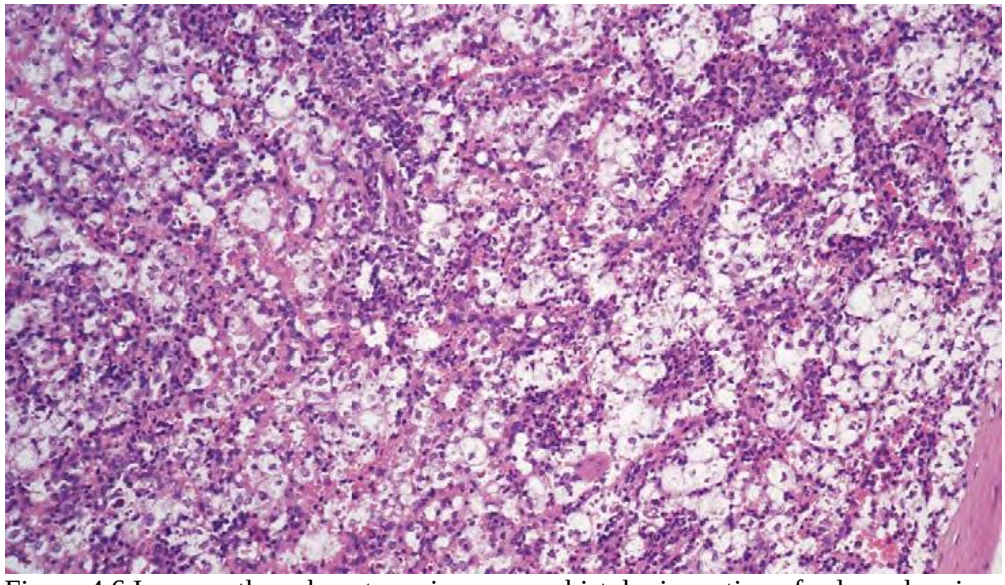


Figure 4.6 Immune thrombocytopenic purpura: histologic section of spleen showing prominent collections of lipid-filled macrophages caused by excessive breakdown of platelets in the splenic pulp. (7)

Treatment

1. Prednisone at 1 mg/kg
2. Rh_0(D) immune globulin 50–75 g/kg
3. Rituximab, an anti-CD20 (B cell) antibody
4. Splenectomy (10)

Prevention

Vaccination against encapsulated organisms especially:

1. Pneumococcus
2. Menningococcus
3. *Haemophilus influenzae* (10)

Disseminated intravascular coagulation (DIC)

Definition

Disseminated intravascular coagulation (DIC) also referred to as *consumptive coagulopathy* or *defibrination*.[1] (5)

Aetiology

DIC is caused by a wide variety of serious disorders:

Sepsis

1. Bacterial
   - Staphylococci
   - Streptococci
   - Pneumococci
   - Meningococci
   - Gram-negative bacilli
2. Viral
3. Mycotic
4. Parasitic
5. Rickettsial (10)

Trauma and tissue injury

1. Brain injury (gunshot)
2. Extensive burns
3. Fat embolism
4. Rhabdomyolysis

[1] DIC is a clinicopathologic syndrome characterized by widespread intravascular fibrin formation in response to excessive blood protease activity that overcomes the natural anticoagulant mechanisms. (10)

Vascular disorders
1. Giant hemangiomas (Kasabach-Merrit syndrome)
2. Large vessel aneurysms (e.g., aorta)

Obstetric complications
1. Abruptio placentae
2. Amniotic fluid embolism
3. Dead fetus syndrome
4. Septic abortion [10]

Cancer
1. Adenocarcinoma (prostate, pancreas, etc)
2. Hematologic malignancies (acute promyelocytic leukemia)

Immunologic disorders
1. Acute hemolytic transfusion reaction
2. Organ or tissue transplant rejection
3. Graft-versus-host disease

Drugs
1. Fibrinolytic agents
2. Aprotinin
3. Warfarin (especially in neonates with protein C deficiency)
4. Prothrombin complex concentrates
5. Recreational drugs (amphetamines) [10]

Envenomation
1. Snake
2. Insects

Liver disease
1. Fulminant hepatic failure
2. Cirrhosis
3. Fatty liver of pregnancy

Miscellaneous
1. Shock
2. Respiratory distress syndrome
3. Massive transfusion [10]

Pathophysiology

1. The exposure of blood to phospholipids from:
- Damaged tissue
- Hemolysis
- Endothelial damage

2. All contributing factors to the development of DIC
3. Purpura fulminans is a severe form of DIC
4. The central mechanism of DIC is the uncontrolled generation of thrombin by exposure of the blood to pathologic levels of tissue factor (Fig. 4.7). [10]

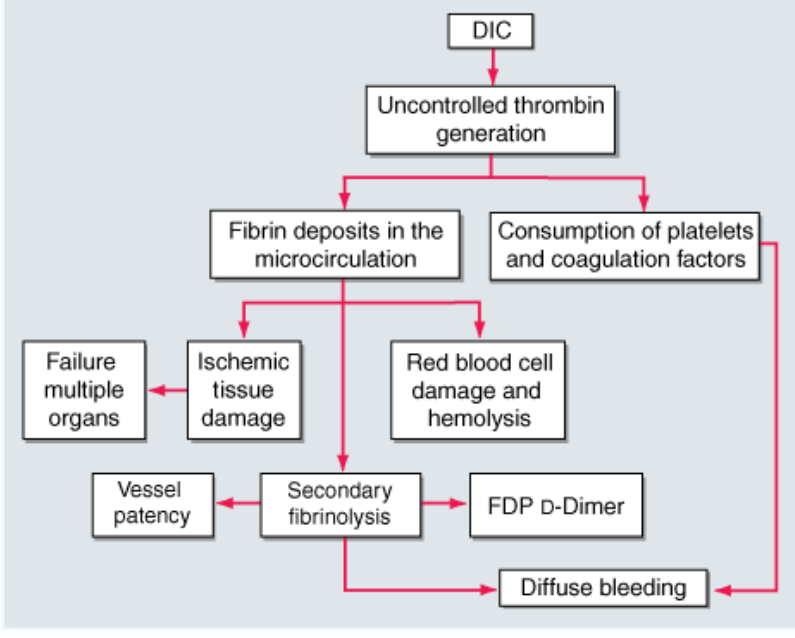


Figure 4.7 **The pathophysiology of disseminated intravascular coagulation (DIC).** Interactions between coagulation and fibrinolytic pathways result in bleeding and thrombosis in the microcirculation in patients with DIC. [10]

Clinical Findings

The most common findings are

1. Bleeding ranging from (Fig. 4.11)
   - Oozing from venipuncture sites
   - Petechiae
   - Ecchymoses (Fig. 4.10)
   - Severe hemorrhage from the gastrointestinal tract or lung or into the central nervous system
2. Widespread severe hemorrhage (**purpura fulminans**) (Fig. 4.8)
3. Malignancy-related DIC may manifest principally as thrombosis (Trousseau syndrome)
4. In chronic DIC the symptoms are discreet and restricted to skin or mucosal surfaces
5. The hypercoagulability of DIC manifests as the occlusion of vessels in the microcirculation and resulting organ failure
6. Thrombosis of large vessels

Cerebral embolism can also occur

7. Hemodynamic complications[2]
8. Shock (10 &11)

[2] Hemodynamic complications and shock are common among patients with acute DIC.

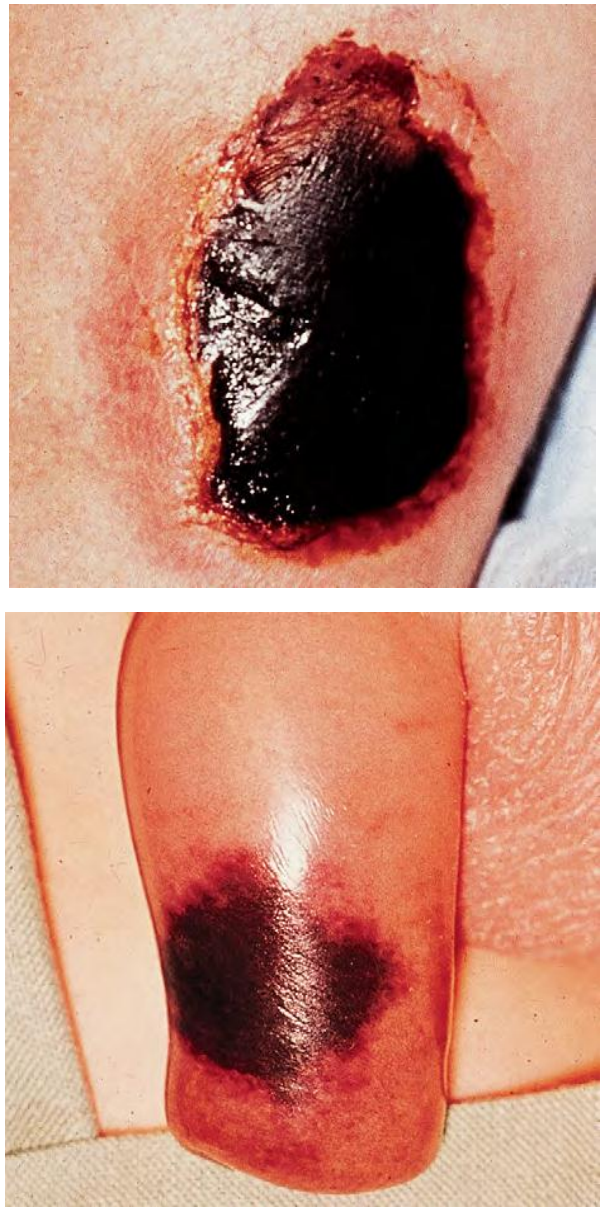


Figure 4.8 Purpura fulminans: large necrotic ecchymoses of skin of (A) the leg. (A, B, Courtesy of Dr. M. D. Holdaway.) [7]

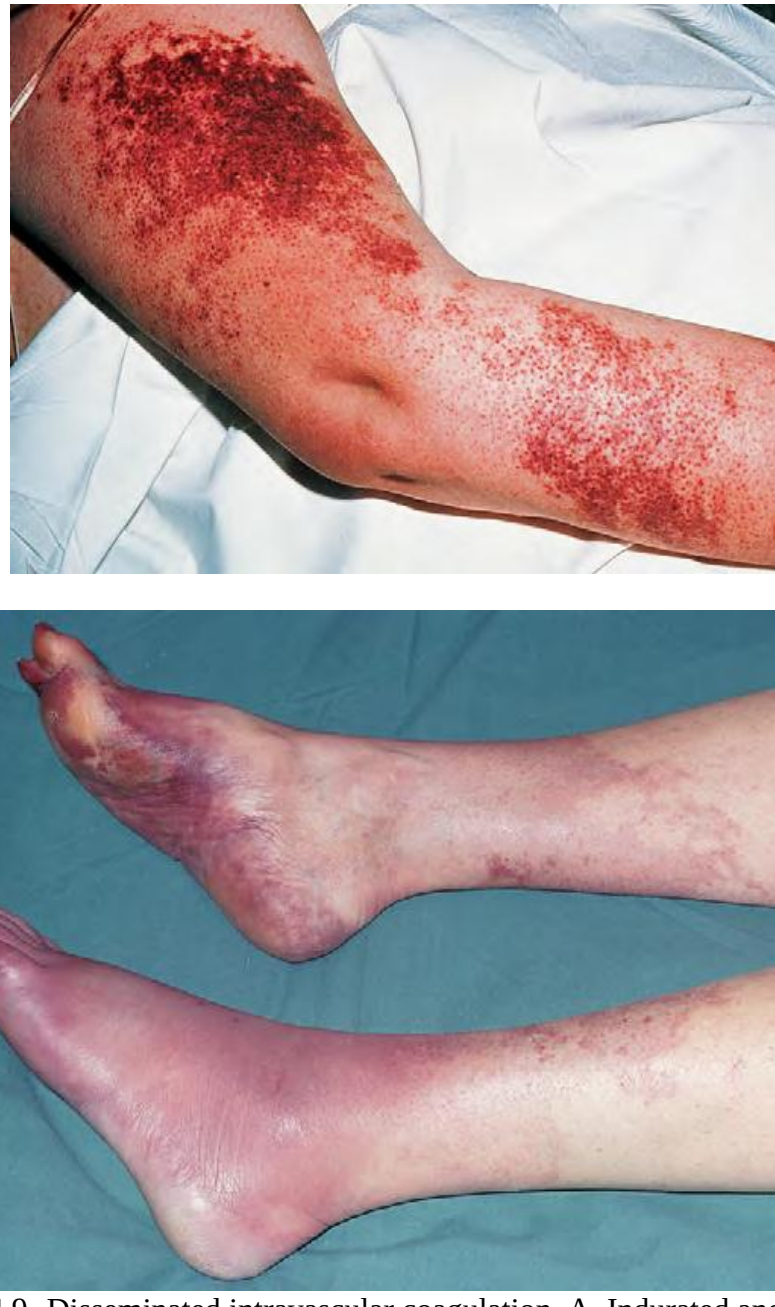


Figure 4.9 Disseminated intravascular coagulation. A, Indurated and confluent purpura of the arm. B, Peripheral gangrene with swelling and discoloration of the skin of the feet in fulminant disease. (7)

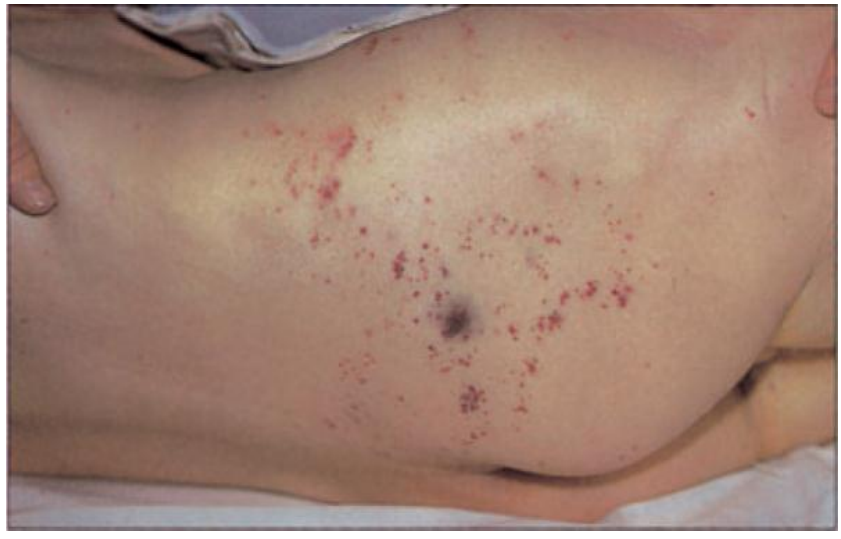


Figure 4.10 **Disseminated intravascular coagulation resulting from staphylococcal septicemia.** Note the characteristic skin hemorrhage ranging from small purpuric lesions to larger ecchymoses. [5]

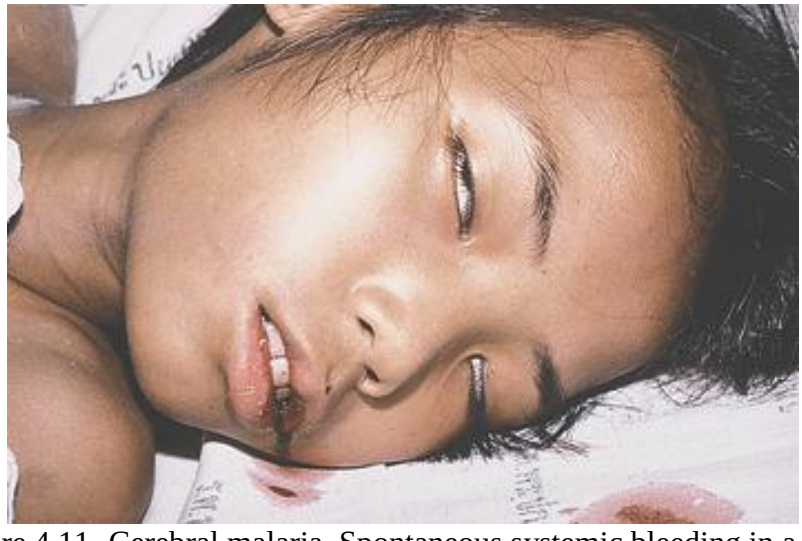


Figure 4.11 Cerebral malaria. Spontaneous systemic bleeding in a Thai patient with disseminated intravascular coagulation. (Copyright D.A. Warrell.) [12]

Laboratory Findings

1. In early DIC
   - The platelet count normal
   - Fibrinogen normal
2. There is progressive thrombocytopenia (rarely severe)
3. Prolongation of:
   - Activated partial thromboplastin time (aPTT)
   - Prothrombin time (PT)
4. Low levels of fibrinogen
5. D-dimer levels typically are elevated[3]
6. Schistocytes on the blood smear[4]
7. Laboratory abnormalities in the HELLP syndrome (hemolysis, elevated liver enzymes, low platelets)[5]
8. Malignancy-related DIC may feature normal platelet counts and coagulation studies (10&11)

Treatment

1. Identify and eliminate the underlying cause eg:
   - Antimicrobials
   - Chemotherapy
   - Surgery
   - Delivery of conceptus
2. No treatment if mild, asymptomatic, and self-limited
3. Hemodynamic support, as indicated, in severe cases
4. Blood component therapy

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[3] D-dimer levels typically are elevated due to the activation of coagulation and diffuse cross-linking of fibrin

[4] Schistocytes on the blood smear, due to shearing of red cells through the microvasculature, are present in 10-20% of patients.

[5] a severe form of DIC with a particularly high mortality rate that occurs in peripartum women, include elevated liver transaminases and (many cases) renal dysfunction due to gross hemoglobinuria and pigment nephropathy.

Indications: active bleeding or high risk for bleeding

- Fresh-frozen plasma
- Platelets
- Packed red blood cells
- In some cases, consider cryoprecipitate, antithrombin III

5. Drug therapy
   Indications: Heparin for DIC manifested by thrombosis or acrocyanosis; antifibrinolytic agents generally contraindicated except with life-threatening bleeding and failure of blood component therapy

If clinically significant bleeding is present, hemostasis must be achieved (Table 4.1). (11&5)

Table 4.1 Management of DIC.	
I. Assess for underlying cause of DIC and treat.	
II. Establish baseline platelet count, PT, aPTT, D-dimer, fibrinogen.	
III. Transfuse blood products only if ongoing bleeding or high risk of bleeding:	Platelets: goal > 20,000/mcL (most patients) or > 50,000/mcL (severe bleeding, eg, intracranial hemorrhage)
	Cryoprecipitate: goal fibrinogen level < 80–100 mg/dL
	Fresh frozen plasma: goal PT and aPTT < 1.5 x normal
	Packed red blood cells: goal hemoglobin > 8 g/dL or improvement in symptomatic anemia
IV. Follow platelets, aPTT/PT, fibrinogen every 4–6 hours or as clinically indicated.	
V. If persistent bleeding, consider use of heparin[1] (initial infusion, 5–10 units/kg/h); do not administer bolus.	
VI. Follow laboratory parameters every 4–6 hours until DIC resolved and underlying condition successfully treated	

[1]Contraindicated if platelets cannot be maintained at > 50,000/mcL, in cases of gastrointestinal or central nervous system bleeding, in conditions that may require surgical management, or placental abruption. DIC, disseminated intravascular coagulation; PT, prothrombin time; aPTT, activated partial thromboplastin time.(11)

Prognosis

The mortality ranges from 30 to >80% depending on:

1. The underlying disease
2. The severity of the DIC
3. The age of the patient [11]

Chapter five

Blood transfusion

- *Blood transfusion*

Blood transfusion

Blood transfusion is used to treat patients with severe anaemia, heamorrhage, thrombocytopenia, and coagulation disorders. (12)
Worldwide, more than 75 million units of whole blood are estimated to be donated every year. In the United States, the yearly transfusion of more than 13 million units corresponds to transfusion of 1 unit of blood every 0.39 second. (5)
Greater understandings of red cell, platelet, and leucocyte antigen structure have greatly improved transfusion therapy. (12)

Erythrocyte Antigen and Antibodies

The study of red blood cell (RBC) antigens and antibodies forms the foundation of transfusion medicine. (10)

Definition

The ABO blood group antigens are oligosaccharide chains that project from the red cell surface. These chains are attached to proteins and lipids that lie in the red cell membrane.[1] (3)

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[1] The study of red blood cell (RBC) antigens and antibodies forms the foundation of transfusion medicine. Serologic studies initially characterized these antigens, but now the molecular composition and structure of many are known. Antigens, either carbohydrate or protein, are assigned to a blood group system based on the structure and similarity of the determinant epitopes. Other cellular blood elements and plasma proteins are also antigenic and can result in *alloimmunization*, the production of antibodies directed against the blood group antigens of another individual. These antibodies are called *alloantibodies*.
Antibodies directed against RBC antigens may result from "natural" exposure, particularly to carbohydrates that mimic some blood group antigens. Those antibodies that occur via natural stimuli are usually produced by a T cell—independent response (thus, generating no memory) and are IgM isotype. *Autoantibodies* (antibodies against autologous blood group antigens) arise spontaneously or as the result of infectious sequelae (e.g., from *Mycoplasma*

The first blood group antigen system, recognized in 1900, was ABO, the most important in transfusion medicine. The major blood groups of this system are A, B, AB, and O. O type RBCs lack A or B antigens. These antigens are carbohydrates attached to a precursor backbone, may be found on the cellular membrane either as glycosphingolipids or glycoproteins, and are secreted into plasma and body fluids as glycoproteins. H substance is the immediate precursor on which the A and B antigens are added. This H substance is formed by the addition of fucose to the glycolipid or glycoprotein backbone. The subsequent addition of *N*-acetylgalactosamine creates the A antigen, while the addition of galactose produces the B antigen. (10)

The ABO blood group system is important because essentially all individuals produce antibodies to the ABH carbohydrate antigen that they lack. The naturally occurring anti-A and anti-B antibodies are termed *isoagglutinins*. Thus, type A individuals produce anti-B, while type B individuals make anti-A. Neither isoagglutinin is found in type AB individuals, while type O individuals produce both anti-A and anti-B. Thus, persons with type AB are "universal recipients" because they do not have antibodies against any ABO phenotype, while persons with type O blood can donate to essentially all recipients because their cells

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pneumoniae) and are also often IgM. These antibodies are often clinically insignificant due to their low affinity for antigen at body temperature. However, IgM antibodies can activate the complement cascade and result in hemolysis. Antibodies that result from allogeneic exposure, such as transfusion or pregnancy, are usually IgG. IgG antibodies commonly bind to antigen at warmer temperatures and may hemolyze RBCs. Unlike IgM antibodies, IgG antibodies can cross the placenta and bind fetal erythrocytes bearing the corresponding antigen, resulting in hemolytic disease of the newborn, or *hydrops fetalis*. (10)

Alloimmunization to leukocytes, platelets, and plasma proteins may also result in transfusion complications such as fevers and urticaria but generally does not cause hemolysis. Assay for these other alloantibodies is not routinely performed; however, they may be detected using special assays. (10)

are not recognized by any ABO isoagglutinins. The rare individuals with Bombay phenotype produce antibodies to H substance (which is present on all red cells except those of hh phenotype) as well as to both A and B antigens and are therefore compatible only with other hh donors. (10)

In most people, A and B antigens are secreted by the cells and are present in the circulation. Nonsecretors are susceptible to a variety of infections (e.g., *Candida albicans*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Haemophilus influenzae*) as many organisms may bind to polysaccharides on cells. Soluble blood group antigens may block this binding. (10)

Rh System

The Rh system is the second most important blood group system in pretransfusion testing. The Rh antigens are found on a 30- to 32-kDa RBC membrane protein that has no defined function. Although >40 different antigens in the Rh system have been described, five determinants account for the vast majority of phenotypes. The presence of the D antigen confers Rh "positivity," while persons who lack the D antigen are Rh negative. Two allelic antigen pairs, E/e and C/c, are also found on the Rh protein. The three Rh genes, E/e, D, and C/c, are arranged in tandem on chromosome 1 and inherited as a haplotype, i.e., cDE or Cde. Two haplotypes can result in the phenotypic expression of two to five Rh antigens.[2] (10)

Other Blood Group Systems and Alloantibodies

More than 100 blood group systems are recognized, composed of more than 500 antigens. The presence or absence of certain antigens has been associated with various diseases and anomalies;

[2] The D antigen is a potent alloantigen. About 15% of individuals lack this antigen. Exposure of these Rh-negative people to even small amounts of Rh-positive cells, by either transfusion or pregnancy, can result in the production of anti-D alloantibody. (10)

antigens also act as receptors for infectious agents. Alloantibodies of importance in routine clinical practice are listed in Table 5.1.[3] (10)

[3] Antibodies to *Lewis system* carbohydrate antigens are the most common cause of incompatibility during pretransfusion screening. The Lewis gene product is a fucosyl transferase and maps to chromosome 19. The antigen is not an integral membrane structure but is adsorbed to the RBC membrane from the plasma. Antibodies to Lewis antigens are usually IgM and cannot cross the placenta. Lewis antigens may be adsorbed onto tumor cells and may be targets of therapy.

I system antigens are also oligosaccharides related to H, A, B, and Le. I and i are not allelic pairs but are carbohydrate antigens that differ only in the extent of branching. The i antigen is an unbranched chain that is converted by the I gene product, a glycosyltransferase, into a branched chain. The branching process affects all the ABH antigens, which become progressively more branched in the first 2 years of life. Some patients with cold agglutinin disease or lymphomas can produce anti-I autoantibodies that cause RBC destruction. Occasional patients with mononucleosis or *Mycoplasma* pneumonia may develop cold agglutinins of either anti-I or anti-i specificity. Most adults lack i expression; thus, finding a donor for patients with anti-i is not difficult. Even though most adults express I antigen, binding is generally low at body temperature. Thus, administration of warm blood prevents isoagglutination.

The *P system* is another group of carbohydrate antigens controlled by specific glycosyltransferases. Its clinical significance is in rare cases of syphilis and viral infection that lead to paroxysmal cold hemoglobinuria. In these cases, an unusual autoantibody to P is produced that binds to RBCs in the cold and fixes complement upon warming. Antibodies with these biphasic properties are called *Donath-Landsteiner antibodies*. The P antigen is the cellular receptor of parvovirus B19 and also may be a receptor for *Escherichia coli* binding to urothelial cells.

The *MNSsU system* is regulated by genes on chromosome 4. M and N are determinants on glycophorin A, an RBC membrane protein, and S and s are determinants on glycophorin B. Anti-S and anti-s IgG antibodies may develop after pregnancy or transfusion and lead to hemolysis. Anti-U antibodies are rare but problematic; virtually every donor is incompatible because nearly all persons express U.

The *Kell* protein is very large (720 amino acids), and its secondary structure contains many different antigenic epitopes. The immunogenicity of Kell is third behind the ABO and Rh systems. The absence of the Kell precursor protein (controlled by a gene on X) is associated with acanthocytosis, shortened RBC survival, and a progressive form of muscular dystrophy that includes cardiac defects. This rare

Pretransfusion Testing

Pretransfusion testing of a potential recipient consists of the "type and screen." The "forward type" determines the ABO and Rh phenotype of the recipient's RBC by using antisera directed against the A, B, and D antigens.

The "reverse type" detects isoagglutinins in the patient's serum and should correlate with the ABO phenotype, or forward type.

The alloantibody screen identifies antibodies directed against other RBC antigens. The alloantibody screen is performed by mixing patient serum with type O RBCs that contain the major antigens of most blood group systems and whose extended phenotype is known. The specificity of the alloantibody is identified by correlating the presence or absence of antigen with the results of the agglutination.

Cross-matching is ordered when there is a high probability that the patient will require a packed RBC (PRBC) transfusion. Blood selected for cross-matching must be ABO compatible and lack antigens for which the patient has alloantibodies. Nonreactive cross-matching confirms the absence of any major incompatibility and reserves that unit for the patient.

In the case of Rh-negative patients, every attempt must be made to provide Rh-negative blood components to prevent alloimmunization to the D antigen. In an emergency, Rh-positive

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condition is called the *McLeod phenotype*. The K_x gene is linked to the 91-kDa component of the NADPH-oxidase on the X chromosome, deletion or mutation of which accounts for about 60% of cases of chronic granulomatous disease.

The *Duffy* antigens are codominant alleles, Fy^a and Fy^b, that also serve as receptors for *Plasmodium vivax*. More than 70% of persons in malaria-endemic areas lack these antigens, probably from selective influences of the infection on the population.

The *Kidd* antigens, Jk^a and Jk^b, may elicit antibodies transiently. A delayed hemolytic transfusion reaction that occurs with blood tested as compatible is often related to delayed appearance of anti-Jk^a.

blood can be safely transfused to an Rh-negative patient who lacks anti-D; however, the recipient is likely to become alloimmunized and produce anti-D. Rh-negative women of childbearing age who are transfused with products containing Rh-positive RBCs should receive passive immunization with anti-D (RhoGam or WinRho) to reduce or prevent sensitization. [10]

Compatibility Testing

1. Before transfusion, the recipient's and the donor's blood are typed (Table 5.1)
2. Cross-matched to avoid hemolytic transfusion reactions
3. Although many antigen systems are present on red blood cells
4. Only the ABO and Rh systems are specifically tested prior to all transfusions
5. The A and B antigens are the most important, because everyone who lacks one or both red cell antigens has IgM isoantibodies (called isoagglutinins) against the missing antigen or antigens in his or her plasma
6. The isoagglutinins activate complement and can cause rapid intravascular lysis of the incompatible red blood cells.
7. In emergencies, type O-negative blood can be given to any recipient, but only packed cells should be given to avoid transfusion of donor plasma containing anti-A or anti-B antibodies
8. The other important antigen routinely tested for is the D antigen of the Rh system
9. Approximately 15% of the population lack this antigen. In patients lacking the antigen, anti-D antibodies are not naturally present, but the antigen is highly immunogenic
10. A recipient whose red cells lack D and who receives D-positive blood may develop anti-D antibodies that can cause severe lysis of subsequent transfusions of D-positive red cells

11. Blood typing includes a crossmatch assay of recipient serum for unusual alloantibodies directed against donor red blood cells by mixing recipient serum with panels of red blood cells representing commonly occurring minor antigens
12. The screening is particularly important if the recipient has had previous transfusions or pregnancy [11]

Table 5.1 **RBC Blood Group Systems and Alloantigens**

Blood Group System	**Antigen**	**Alloantibody**	**Clinical Significance**
Rh (D, C/c, E/e)	RBC protein	IgG	HTR, HDN
Lewis (Le^a, *Leactivity="italic">*^{b})	Oligosaccharide	IgM/IgG	Rare HTR
Kell (K/k)	RBC protein	IgG	HTR, HDN
Duffy (Fy^a/Fy^b)	RBC protein	IgG	HTR, HDN
Kidd (Jk^a/Jk^b)	RBC protein	IgG	HTR (often delayed), HDN (mild)
I/i	Carbohydrate	IgM	None
MNSsU	RBC protein	IgM/IgG	Anti-M rare HDN, anti-S, -s, and -U HDN, HTR

Abbreviation: RBC, red blood cell; HDN, hemolytic disease of the newborn; HTR, hemolytic transfusion reaction. [10]

Blood Components

Blood products intended for transfusion are routinely collected as whole blood (450 mL) in various anticoagulants. Most donated blood is processed into components:

1. PRBCs (Fig. 5.1, 5.2)
2. Platelets
3. Fresh-frozen plasma (FFP)

4. Cryoprecipitate (Table 5.1)

Whole blood is first separated into PRBCs and platelet-rich plasma by slow centrifugation

The platelet-rich plasma is then centrifuged at high speed to yield one unit of random donor (RD) platelets and one unit of FFP

Cryoprecipitate is produced by thawing FFP to precipitate the plasma proteins, and then separated by centrifugation [10]

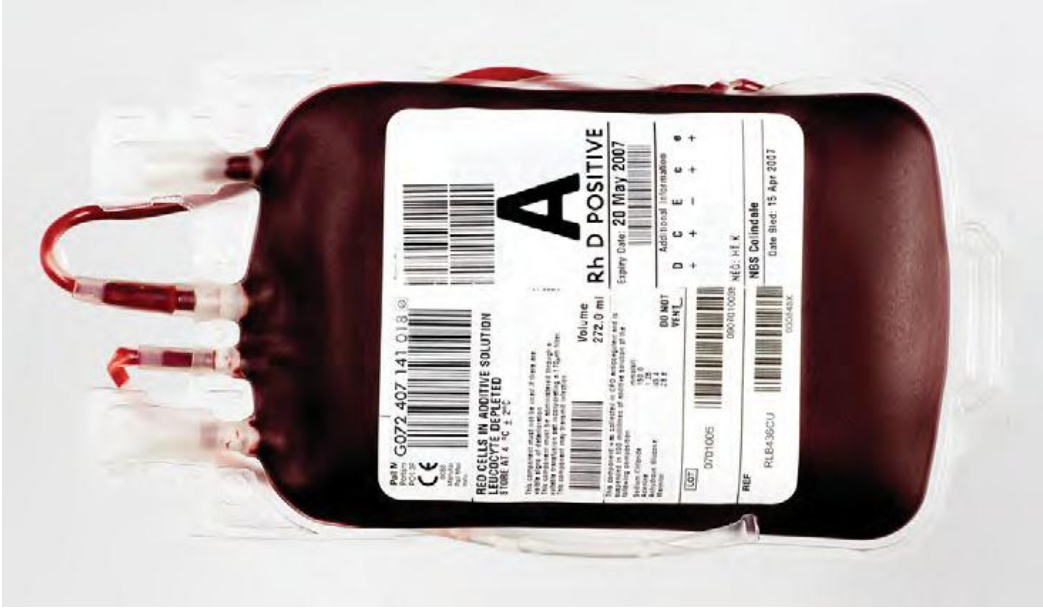


Figure 5.1 Leukocyte-depleted red cells: the average volume of this product, which contains CPDA-1 as the anticoagulant, is 280 ml. SAG-M additive solution (100 ml) is added to give a fi nal hematocrit of 50% to 70%. The storage temperature of these red cells is 4° ± 2° C and the shelf life is 35 days. Depletion in the white cell content of red cell products to less than 5 106/unit reduces the incidence of reactions caused by human leukocyte antigen (HLA) alloimmunization and of transfusion of CMV or prions. (Courtesy of G. Hazlehurst.) [7]

Figure 5.2 Small volume neonatal transfusions: it is usually supplied as group O RhDnegative blood, which should be stored at 4° C for up to 35 days. It may be anti-CMV antibody negative, HbS negative, and have up to seven attached satellite bags. This enables a single unit of blood to be dedicated to an individual infant so that donor exposure is limited. Dosage is 10 to 20 ml/kg. (Courtesy of G. Hazlehurst.) (7)

Table 5.2 **Characteristics of Selected Blood Components**

Component	**Volume, mL**	**Content**	**Clinical Response**
PRBC	180–200	RBCs with variable leukocyte content and small amount of plasma	Increase hemoglobin 10 g/L and hematocrit 3%
Platelets	50–70	5.5×10^{10}/RD unit	Increase platelet count 5000–10,000/Micro L
	200–400	$\geq 3 \times 10^{11}$/SDAP product	CCI $\geq 10 \times 10^{9}$/L within 1 h and $\geq 7.5 \times$

			10^9/L within 24 h posttransfusion
FFP	200–250	Plasma proteins—coagulation factors, proteins C and S, antithrombin	Increases coagulation factors about 2%
Cryoprecipitate	10–15	Cold-insoluble plasma proteins, fibrinogen, factor VIII, Vwf	Topical fibrin glue, also 80 IU factor VIII

Abbreviation: CCI, corrected count increment; FFP, fresh-frozen plasma; PRBC, packed red blood cells; RBC, red blood cell; RD, random donor; SDAP, single-donor apheresis platelets; vWF, von Willebrand factor. (10)

Apheresis technology is used for the collection of multiple units of platelets from a single donor. These single-donor apheresis platelets (SDAP) contain the equivalent of at least six units of RD platelets and have fewer contaminating leukocytes than pooled RD platelets.

Plasma may also be collected by apheresis. Plasma derivatives such as albumin, intravenous immunoglobulin, antithrombin, and coagulation factor concentrates are prepared from pooled plasma from many donors and are treated to eliminate infectious agents. (10)

Whole Blood

1. It provides both oxygen-carrying capacity and volume expansion. (Table 5.2)
2. It is the ideal component for patients who have sustained acute hemorrhage of ≥25% total blood volume loss.
3. Whole blood is stored at 4°C to maintain erythrocyte viability, but platelet dysfunction and degradation of some coagulation factors occurs.
4. In addition, 2,3-bisphosphoglycerate levels fall over time, leading to an increase in the oxygen affinity of the hemoglobin

and a decreased capacity to deliver oxygen to the tissues, a problem with all red cell storage.
5. Fresh whole blood avoids these problems, but it is typically used only in emergency settings (i.e., military).
6. Whole blood is not readily available, since it is routinely processed into components. (10)

Packed Red Blood Cells

1. This product increases oxygen-carrying capacity in the anemic patient. Adequate oxygenation can be maintained with a hemoglobin content of 70 g/L in the normovolemic patient without cardiac disease; however, comorbid factors may necessitate transfusion at a higher threshold.
2. The decision to transfuse should be guided by the clinical situation and not by an arbitrary laboratory value.
3. In the critical care setting, liberal use of transfusions to maintain near-normal levels of hemoglobin has not proven advantageous.
4. In most patients requiring transfusion, levels of hemoglobin of 100 g/L are sufficient to keep oxygen supply from being critically low.
5. PRBCs may be modified to prevent certain adverse reactions.
6. The majority of cellular blood products are now leukocyte reduced and universal prestorage leukocyte reduction has been recommended. Prestorage filtration appears superior to bedside filtration as smaller amounts of cytokines are generated in the stored product.
7. These PRBC units contain $<5 \times 10^6$ donor white blood cells (WBCs), and their use lowers the incidence of posttransfusion fever, cytomegalovirus (CMV) infections, and alloimmunization.
8. Other theoretical benefits include less immunosuppression in the recipient and lower risk of infections. Plasma, which may

cause allergic reactions, can be removed from cellular blood components by washing. (10)

Platelets

Thrombocytopenia is a risk factor for hemorrhage, and platelet transfusion reduces the incidence of bleeding. The threshold for prophylactic platelet transfusion is 10,000/Micro.L. In patients without fever or infections, a threshold of 5000/ Micro.L may be sufficient to prevent spontaneous hemorrhage. For invasive procedures, 50,000/ Micro.L platelets is the usual target level.

Platelets are given either as pools prepared RDs or as SDAPs from a single donor. In an unsensitized patient without increased platelet consumption [splenomegaly, fever, disseminated intravascular coagulation (DIC)], two units of transfused RD per square-meter body surface area (BSA) is anticipated to increase the platelet count by approximately 10,000/uL. Patients who have received multiple transfusions may be alloimmunized to many HLA- and platelet-specific antigens and have little or no increase in their posttransfusion platelet counts. Patients who may require multiple transfusions are best served by receiving SDAP and leukocyte-reduced components to lower the risk of alloimmunization. (10)

Refractoriness to platelet transfusion may be evaluated using the corrected count increment (CCI):

$$\text{CCI} = \frac{\text{posttransfusion count (/}\mu\text{L)} - \text{pretransfusion count (/}\mu\text{L)}}{\text{number of platelets transfused} \times 10^{-11}} \times \text{BSA (m}^2\text{)}$$

where BSA is body surface area measured in square meters. The platelet count performed 1 h after the transfusion is acceptable if the CCI is 10 x 10^9/mL, and after 18—24 h an increment of 7.5 x 10^9/mL is expected. Patients who have suboptimal responses are likely to have received multiple transfusions and have antibodies directed against class I HLA antigens. Refractoriness can be investigated by detecting anti-HLA antibodies in the recipient's serum. Patients who are sensitized will often react with 100% of

the lymphocytes used for the HLA-antibody screen, and HLA-matched SDAPs should be considered for those patients who require transfusion. Although ABO-identical HLA-matched SDAPs provide the best chance for increasing the platelet count, locating these products is difficult. Platelet cross-matching is available in some centers. Additional clinical causes for a low platelet CCI include fever, bleeding, splenomegaly, DIC, or medications in the recipient (Fig. 5.3, 5.4). [10]

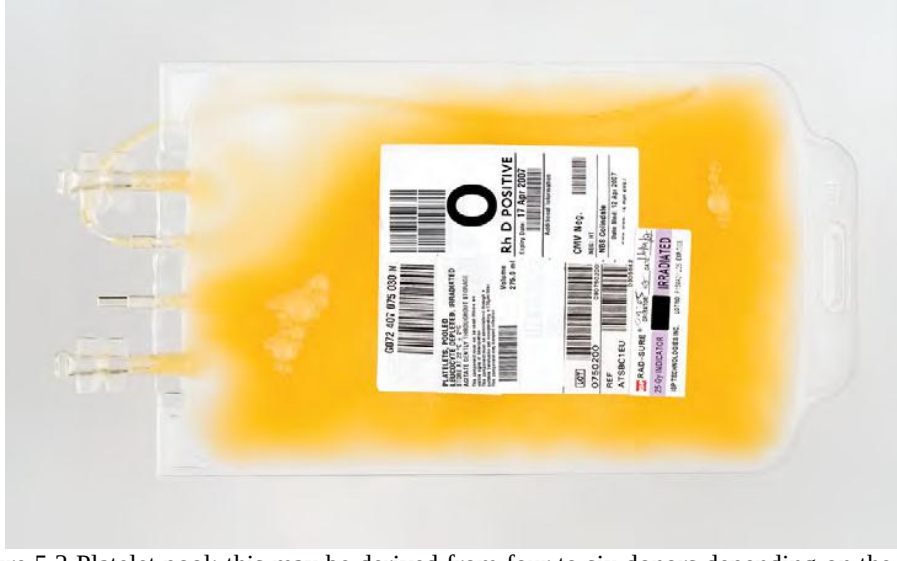


Figure 5.3 Platelet pool: this may be derived from four to six donors depending on the place of manufacture. When produced by the buffy coat method a pool of platelets contains more than 250 109/L platelets per pool. [7]

Figure 5.4 Platelet concentrates (A) from a single donor. These platelet concentrates are derived from a single blood donation and are approximately 50 ml in volume. The concentrate is derived from CPDA-1 plasma and contains platelets 55 109 and leukocytes 0.05 109 per unit. Platelets should be maintained at 22 C, and they have a shelf life of 5 days. (B) Platelet concentrates can also be made by apheresis from a single donor. These platelet concentrates are approximately 215 ml in volume and contain about 290 109 platelets and 0.3 106 white cells per pack. In addition, they may be HLA-matched or cross-matched to be compatible with recipient serum in cases of refractory patients. (Courtesy of G. Hazlehurst.) [7]

Fresh-Frozen Plasma

FFP contains stable coagulation factors and plasma proteins: fibrinogen, antithrombin, albumin, as well as proteins C and S. Indications for FFP include correction of coagulopathies, including the rapid reversal of warfarin; supplying deficient plasma proteins; and treatment of thrombotic thrombocytopenic purpura. FFP should not be routinely used to expand blood volume. FFP is an acellular component and does not transmit intracellular infections, e.g., CMV. Patients who are IgA-deficient and require plasma support should receive FFP from IgA-deficient donors to prevent anaphylaxis (Fig. 5.5). [10]

Figure 5.5 Fresh frozen plasma (FFP): this is provided in 240 to 300 ml volumes including adult and pediatric doses. It can be supplied from volunteer plasmapheresis donors or recovered from routine blood donations. Illustrated here is a 240 ml FFP pack anticoagulated with CPDA-1. It should be stored at 30° C, and will then keep for up to 2 years. It should not be used as a plasma expander. Its use should be monitored with tests of coagulation when administered to correct a documented coagulation abnormality. (Courtesy of G. Hazlehurst.) [7]

Cryoprecipitate

Cryoprecipitate is a source of fibrinogen, factor VIII, and von Willebrand factor (vWF). It is ideal for supplying fibrinogen to the volume-sensitive patient. When factor VIII concentrates are not available, cryoprecipitate may be used since each unit contains approximately 80 units of factor VIII. Cryoprecipitate may also supply vWF to patients with dysfunctional (type II) or absent (type III) von Willebrand disease (Fig. 5.6). [10]

Figure 5.6 Batched cryoprecipitate: individual cryoprecipitates from different donors may be batched together in groups of six to represent an adult dose. Each unit contains approximately 20 ml and is derived from CPDA-1 plasma. It contains fi brinogen greater than 140 mg per unit and factor VIII greater than 70 IU per unit. It should be stored at 30° C and has a shelf life of 1 year. In general, group A donors have higher levels of factor VIII than group O donors. (Courtesy of G. Hazlehurst.) [7]

Plasma Derivatives

Plasma from thousands of donors may be pooled to derive specific protein concentrates, including:

1. Albumin
2. Intravenous immunoglobulin
3. Antithrombin

4. Coagulation factors

In addition, donors who have high-titer antibodies to specific agents or antigens provide hyperimmune globulins, such as anti-D (RhoGam, WinRho), and antisera to hepatitis B virus (HBV), varicella-zoster virus, CMV, and other infectious agents.

Uses of blood transfusion components

Red blood cells

Indication: Symptomatic acute and chronic anaemias and acute blood loss

1. Red blood cells frozen and deglycerolized

Indication: Symptomatic anaemia, storage of red cells of rare antigen composition for up to 10 years

2. Leucocyte-reduced components (red blood cells and platelets)

Indication: Symptomatic anaemia, reduce febrile reactions from leucocyte antibodies, alternative to CMV-seronegative components, prevent HLA alloimmunization

3. Washed components (red blood cells and platelets)

Indication: Remove harmful plasma antibodies

4. Platelet components (pooled platelets and pheresis platelets)

Indication: Thrombocytopenia with bleeding, prophylactic transfusion, platelet function abnormalities

5. HLA matched/selected platelets and crossmatch-compatible platelets

Indication: HLA-alloimmunized thrombocytopenic patients with decreased platelet survival

6. Fresh frozen plasma

Indication: Replacement of plasma coagulation factors for which specific factor concentrates are not available, liver disease, DIC (disseminated intravascular coagulation), hypofibrinogenaemia, TTP(thrombcytopenic purpura)

7. Cryoprecipitate

Indication: Fibrinogen and factor XIII replacement, factor VIII and vWF (von Willebrand factor) replacement when recombinant and virus-inactivated concentrates are not available

8. ***Granulocytes by apheresis***

Indication: Neutropenic patient with infection unresponsive to antibiotics (Table 5.3) (12 & 3)

Table 5.3 **Blood components and their use**

	Major haemorrhage	**Other indications**
Red cell concentrate[1] Most of the plasma is removed and replaced with a solution of glucose and adenine in saline to maintain viability of red cells ABO compatibility with recipient essential	Replace acute blood loss: increase circulating red cell mass to relieve clinical features caused by insufficient oxygen delivery	**Severe anaemia** If no cardiovascular disease, transfuse to maintain Hb at 70-90 g/L If known or likely to have cardiovascular disease, maintain Hb 90-100 g/L
Platelet concentrate One adult dose is made from 4-5 donations of whole blood, or from a single platelet apheresis ABO compatibility with recipient preferable	Maintain platelet count > 50×10^9/L, or in multiple or CNS trauma > 100×10^9/L Each adult dose has ~2.5-3 $\times 10^{11}$ platelets, which raises platelet count by ~50×10^9/L unless there is consumptive coagulopathy, e.g. disseminated intravascular coagulation (DIC)	**Thrombocytopenia**, e.g. in acute leukaemia Maintain platelet count >10×10^9/L if not bleeding Maintain platelet count > 20×10^9/L if bleeding or at risk (sepsis, concurrent use of antibiotics, abnormal clotting) Increase platelet count > 50×10^9/L for minor invasive procedure (e.g. lumbar puncture, gastroscopy and biopsy, insertion of indwelling lines, liver biopsy, laparotomy)

		Increase platelet count > 100×10^9/L for operations in critical sites such as brain or eyes
Fresh frozen plasma (FFP)[2] 150-300 mL plasma from one donation of whole blood ABO compatibility with recipient recommended	Dilutional coagulopathy with a prothrombin time prolonged > 50% is likely after replacement of 1-1.5 blood volumes with red cell concentrate Initial dose of FFP 15 mL/kg Further doses only if bleeding continues and guided by PT and APTT	**Replacement of coagulation factor deficiency** If no virally inactivated or recombinant product is available **Thrombotic thrombocytopenic purpura (TTP)** Plasma exchange (or plasma infusion in HIV-related TTP) is frequently effective
Cryoprecipitate[2] Fibrinogen and coagulation factor concentrated from plasma by controlled thawing 10-20 mL pack contains fibrinogen 150-300 mg, factor VIII 80-120 U, von Willebrand factor 80-120 U In UK supplied as pools of 5 units	May be indicated if fibrinogen < 1 g/L due to dilution and DIC Pooled units containing 3-6 g fibrinogen in 200-500 mL raise fibrinogen by ~1 g/L	**von Willebrand disease and haemophilia** If virus-inactivated or recombinant products are not available

[1]**Whole blood** is an alternative to red cell concentrate. ABO compatibility with recipient essential.
[2]Use only if an alternative virus-inactivated plasma is unavailable: Pooled plasma can be treated with solvent and detergent or single units treated with methylene blue. (3)

Adverse Reactions to Blood Transfusion

Adverse reactions to transfused blood components occur despite multiple tests, inspections, and checks. Fortunately, the most common reactions are not life threatening, although serious reactions can present with mild symptoms and signs. Some reactions can be reduced or prevented by modified (filtered, washed, or irradiated) blood components. When an adverse reaction is suspected, the transfusion should be stopped and reported to the blood bank for investigation. (10)

Transfusion reactions may result from immune and nonimmune mechanisms. Immune-mediated reactions are often due to preformed donor or recipient antibody; however, cellular elements may also cause adverse effects. Nonimmune causes of reactions are due to the chemical and physical properties of the stored blood component and its additives.

Transfusion-transmitted viral infections are increasingly rare due to improved screening and testing. As the risk of viral infection is reduced, the relative risk of other reactions increases, such as hemolytic transfusion reactions and sepsis from bacterially contaminated components. Pretransfusion quality assurance improvements further increase the safety of transfusion therapy. Infections, like any adverse transfusion reaction, must be brought to the attention of the blood bank for appropriate studies (Table 5.4).

Table 5.4 Risks of Transfusion Complications

	Frequency, Episodes: Unit
Reactions	
Febrile (FNHTR)	1–4:100
Allergic	1–4:100
Delayed hemolytic	1:1000
TRALI	1:5000
Acute hemolytic	1:12,000
Fatal hemolytic	1:100,000
Anaphylactic	1:150,000

Infections[a]	
Hepatitis B	1:220,000
Hepatitis C	1:1,800,000
HIV-1, -2	1:2,300,000
HTLV-I and -II	1:2,993,000
Malaria	1:4,000,000
Other complications	
RBC allosensitization	1:100
HLA allosensitization	1:10
Graft-versus-host disease	Rare

[a]Infectious agents rarely associated with transfusion, theoretically possible or of unknown risk include West Nile virus, hepatitis A virus, parvovirus B-19, *Babesia microti* (babesiosis), *Borrelia burgdorferi* (Lyme disease), *Anaplasma phagocytophilum* (human granulocytic ehrlichiosis), *Trypanosoma cruzi* (Chagas disease), *Treponema pallidum*, and human herpesvirus-8.

Abbreviations: FNHTR, febrile nonhemolytic transfusion reaction; TRALI, transfusion-related acute lung injury; HTLV, human T lymphotropic virus; RBC, red blood cell. (10)

Immune-Mediated Reactions

Acute Hemolytic Transfusion Reactions

Immune-mediated hemolysis occurs when the recipient has preformed antibodies that lyse donor erythrocytes. The ABO isoagglutinins are responsible for the majority of these reactions, although alloantibodies directed against other RBC antigens, i.e., Rh, Kell, and Duffy, may result in hemolysis. (10)

Acute hemolytic reactions may present with hypotension, tachypnea, tachycardia, fever, chills, hemoglobinemia, hemoglobinuria, chest and/or flank pain, and discomfort at the infusion site. Monitoring the patient's vital signs before and during the transfusion is important to identify reactions promptly. When acute hemolysis is suspected, the transfusion must be stopped immediately, intravenous access maintained, and the reaction reported to the blood bank. A correctly labeled posttransfusion

blood sample and any untransfused blood should be sent to the blood bank for analysis. The laboratory evaluation for hemolysis includes the measurement of serum haptoglobin, lactate dehydrogenase (LDH), and indirect bilirubin levels. (10)

The immune complexes that result in RBC lysis can cause renal dysfunction and failure. Diuresis should be induced with intravenous fluids and furosemide or mannitol. Tissue factor released from the lysed erythrocytes may initiate DIC. Coagulation studies including prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, and platelet count should be monitored in patients with hemolytic reactions.

Errors at the patient's bedside, such as mislabeling the sample or transfusing the wrong patient, are responsible for the majority of these reactions. The blood bank investigation of these reactions includes examination of the pre- and posttransfusion samples for hemolysis and repeat typing of the patient samples; direct antiglobulin test (DAT), sometimes called the *direct Coombs test*, of the posttransfusion sample; repeating the cross-matching of the blood component; and checking all clerical records for errors. DAT detects the presence of antibody or complement bound to RBCs in vivo.

Delayed Hemolytic and Serologic Transfusion Reactions

Delayed hemolytic transfusion reactions (DHTRs) are not completely preventable. These reactions occur in patients previously sensitized to RBC alloantigens who have a negative alloantibody screen due to low antibody levels. When the patient is transfused with antigen-positive blood, an anamnestic response results in the early production of alloantibody that binds donor RBCs. The alloantibody is detectable 1–2 weeks following the transfusion, and the posttransfusion DAT may become positive due to circulating donor RBCs coated with antibody or complement. The transfused, alloantibody-coated erythrocytes are

cleared by the reticuloendothelial system. These reactions are detected most commonly in the blood bank when a subsequent patient sample reveals a positive alloantibody screen or a new alloantibody in a recently transfused recipient. (10)

No specific therapy is usually required, although additional RBC transfusions may be necessary. Delayed serologic transfusion reactions are similar to DHTR, as the DAT is positive and alloantibody is detected; however, RBC clearance is not increased.

Febrile Nonhemolytic Transfusion Reaction

1. The most frequent reaction associated with the transfusion of cellular blood components is a febrile nonhemolytic transfusion reaction (FNHTR).
2. These reactions are characterized by:
3. Chills
4. Rigors
5. A $\geq$1°C rise in temperature
6. FNHTR is diagnosed when other causes of fever in the transfused patient are ruled out.
7. Antibodies directed against donor leukocyte and HLA antigens may mediate these reactions; thus, multiply transfused patients and multiparous women are felt to be at increased risk.
8. Although anti-HLA antibodies may be demonstrated in the recipient's serum, investigation is not routinely done because of the mild nature of most FNHTR.
9. The use of leukocyte-reduced blood products may prevent or delay sensitization to leukocyte antigens and thereby reduce the incidence of these febrile episodes.
10. Cytokines released from cells within stored blood components may mediate FNHTR; thus, leukoreduction before storage may prevent these reactions. (10)

Allergic Reactions

1. Urticarial reactions are related to plasma proteins found in transfused components.
2. Mild reactions may be treated symptomatically by temporarily stopping the transfusion and administering antihistamines (diphenhydramine, 50 mg orally or intramuscularly).
3. The transfusion may be completed after the signs and/or symptoms resolve.
4. Patients with a history of allergic transfusion reaction should be premedicated with an antihistamine.
5. Cellular components can be washed to remove residual plasma for the extremely sensitized patient. [10]

Anaphylactic Reaction

1. This severe reaction presents after transfusion of only a few milliliters of the blood component.
2. Symptoms and signs include:
   - Difficulty breathing
   - Coughing
   - Nausea
   - Vomiting
   - Hypotension
   - Bronchospasm
   - Loss of consciousness
   - Respiratory arrest
   - Shock
3. Treatment includes stopping the transfusion, maintaining vascular access, and administering epinephrine (0.5–1 mL of 1:1000 dilution subcutaneously). Glucocorticoids may be required in severe cases.
4. Patients who are IgA-deficient, <1% of the population, may be sensitized to this Ig class and are at risk for anaphylactic reactions associated with plasma transfusion.

5. Individuals with severe IgA deficiency should therefore receive only IgA-deficient plasma and washed cellular blood components.
6. Patients who have anaphylactic or repeated allergic reactions to blood components should be tested for IgA deficiency. (10)

Graft-versus-Host Disease (GVHD)

GVHD is a frequent complication of allogeneic stem cell transplantation, in which lymphocytes from the donor attack and cannot be eliminated by an immunodeficient host. Transfusion-related GVHD is mediated by donor T lymphocytes that recognize host HLA antigens as foreign and mount an immune response, which is manifested clinically by the development of fever, a characteristic cutaneous eruption, diarrhea, and liver function abnormalities. GVHD can also occur when blood components that contain viable T lymphocytes are transfused to immunodeficient recipients or to immunocompetent recipients who share HLA antigens with the donor (e.g., a family donor). In addition to the aforementioned clinical features of GVHD, transfusion-associated GVHD (TA-GVHD) is characterized by marrow aplasia and pancytopenia. TA-GVHD is highly resistant to treatment with immunosuppressive therapies, including glucocorticoids, cyclosporine, antithymocyte globulin, and ablative therapy followed by allogeneic bone marrow transplantation. Clinical manifestations appear at 8–10 days, and death occurs at 3–4 weeks posttransfusion.

TA-GVHD can be prevented by irradiation of cellular components (minimum of 2500 cGy) before transfusion to patients at risk. Patients at risk for TA-GVHD include fetuses receiving intrauterine transfusions, selected immunocompetent (e.g., lymphoma patients) or immunocompromised recipients, recipients of donor units known to be from a blood relative, and recipients who have undergone marrow transplantation. Directed donations

by family members should be discouraged (they are not less likely to transmit infection); lacking other options, the blood products from family members should always be irradiated. (10)

Transfusion-Related Acute Lung Injury

1. Transfusion-related acute lung injury (TRALI) presents as acute respiratory distress, either during or within 6 h of transfusing the patient.
2. The recipient develops symptoms of respiratory compromise and signs of noncardiogenic pulmonary edema, including bilateral interstitial infiltrates on chest x-ray.
3. Treatment is supportive, and patients usually recover without sequelae.
4. TRALI usually results from the transfusion of donor plasma that contains high-titer anti-HLA antibodies that bind recipient leukocytes.
5. The leukocytes aggregate in the pulmonary vasculature and release mediators that increase capillary permeability.
6. Testing the donor's plasma for anti-HLA antibodies can support this diagnosis.
7. The implicated donors are frequently multiparous women, and transfusion of their plasma component should be avoided. (10)

Posttransfusion Purpura

This reaction presents as thrombocytopenia 7–10 days after platelet transfusion and occurs predominantly in women. Platelet-specific antibodies are found in the recipient's serum, and the most frequently recognized antigen is HPA-1a found on the platelet glycoprotein IIIa receptor. The delayed thrombocytopenia is due to the production of antibodies that react to both donor and recipient platelets. Additional platelet transfusions can worsen the thrombocytopenia and should be avoided. Treatment with intravenous immunoglobulin may neutralize the effector

antibodies, or plasmapheresis can be used to remove the antibodies. (10)

Alloimmunization

A recipient may become alloimmunized to a number of antigens on cellular blood elements and plasma proteins. Alloantibodies to RBC antigens are detected during pretransfusion testing, and their presence may delay finding antigen-negative cross-match-compatible products for transfusion. Women of childbearing age who are sensitized to certain RBC antigens (i.e., D, c, E, Kell, or Duffy) are at risk for bearing a fetus with hemolytic disease of the newborn. Matching for D antigen is the only pretransfusion selection test to prevent RBC alloimmunization.

Alloimmunization to antigens on leukocytes and platelets can result in refractoriness to platelet transfusions. Once alloimmunization has developed, HLA-compatible platelets from donors who share similar antigens with the recipient may be difficult to find. Hence, prudent transfusion practice is directed at preventing sensitization through the use of leukocyte-reduced cellular components, as well as limiting antigenic exposure by the judicious use of transfusions and use of SDAPs. (10)

Nonimmunologic Reactions

Fluid Overload

Blood components are excellent volume expanders, and transfusion may quickly lead to volume overload. Monitoring the rate and volume of the transfusion and using a diuretic can minimize this problem.

Hypothermia

Refrigerated (4°C) or frozen (–18°C or below) blood components can result in hypothermia when rapidly infused. Cardiac dysrhythmias can result from exposing the sinoatrial node to cold fluid. Use of an in-line warmer will prevent this complication. (10)

Electrolyte Toxicity

RBC leakage during storage increases the concentration of potassium in the unit. Neonates and patients in renal failure are at risk for hyperkalemia. Preventive measures, such as using fresh or washed RBCs, are warranted for neonatal transfusions because this complication can be fatal.

Citrate, commonly used to anticoagulate blood components, chelates calcium and thereby inhibits the coagulation cascade. Hypocalcemia, manifested by circumoral numbness and/or tingling sensation of the fingers and toes, may result from multiple rapid transfusions. Because citrate is quickly metabolized to bicarbonate, calcium infusion is seldom required in this setting. If calcium or any other intravenous infusion is necessary, it must be given through a separate line. (10)

Iron Overload

Each unit of RBCs contains 200–250 mg of iron. Symptoms and signs of iron overload affecting endocrine, hepatic, and cardiac function are common after 100 units of RBCs have been transfused (total-body iron load of 20 g). Preventing this complication by using alternative therapies (e.g., erythropoietin) and judicious transfusion is preferable and cost effective. Chelating agents, such as deferoxamine and deferasirox, are available, but the response though is often suboptimal. (10)

Hypotensive Reactions

Transient hypotension may be noted among transfused patients who take angiotensin-converting enzyme (ACE) inhibitors. Since blood products contain bradykinin that is normally degraded by ACE, patients on ACE inhibitors may have increased bradykinin levels that cause hypotension in the recipient. The blood pressure typically returns to normal without intervention. (10)

Immunomodulation

Transfusion of allogeneic blood is immunosuppressive. Multiply transfused renal transplant recipients are less likely to reject the

graft, and transfusion may result in poorer outcomes in cancer patients and increase the risk of infections. Transfusion-related immunomodulation is thought to be mediated by transfused leukocytes. Leukocyte-depleted cellular products may cause less immunosuppression, though controlled data have not been obtained and are unlikely to be obtained as the blood supply becomes universally leukocyte-depleted. (10)

Infectious Complications

The blood supply is initially screened by selecting healthy donors without high-risk lifestyles, medical conditions, or exposure to transmissible pathogens, such as intravenous drug use or visiting malaria endemic areas. Multiple tests performed on donated blood to detect the presence of infectious agents using nucleic acid amplification testing (NAT) or evidence of prior infections by testing for antibodies to pathogens further reduce the risk of transfusion-acquired infections. (10)

Viral Infections

Hepatitis C virus Blood donations are tested for antibodies to HCV and HCV RNA. The risk of acquiring HCV through transfusion is now calculated to be approximately 1 in 2,000,000 units. Infection with HCV may be asymptomatic or lead to chronic active hepatitis, cirrhosis, and liver failure.

Human immunodeficiency virus type 1 Donated blood is tested for antibodies to HIV-1, HIV-1 p24 antigen, and HIV RNA using NAT. Approximately a dozen seronegative donors have been shown to harbor HIV RNA. The risk of HIV-1 infection per transfusion episode is 1 in 2 million. Antibodies to HIV-2 are also measured in donated blood. No cases of HIV-2 infection have been reported in the United States since 1992. (10)

Hepatitis B virus

Donated blood is screened for HBV using assays for hepatitis B surface antigen (HbsAg). NAT testing is not practical because of

slow viral replication and lower levels of viremia. The risk of transfusion-associated HBV infection is several times greater than for HCV. Vaccination of individuals who require long-term transfusion therapy can prevent this complication.

Other hepatitis viruses

Hepatitis A virus is rarely transmitted by transfusion; infection is typically asymptomatic and does not lead to chronic disease. Other transfusion-transmitted viruses—TTV, SEN-V, and GBV-C—do not cause chronic hepatitis or other disease states. Routine testing does not appear to be warranted. (10)

West nile virus

Transfusion-transmitted WNV infections were documented in 2002. This RNA virus can be detected using NAT; routine screening began in 2003. WNV infections range in severity from asymptomatic to fatal, with the older population at greater risk. (H)

Cytomegalovirus

This ubiquitous virus infects $\geq$ 50% of the general population and is transmitted by the infected "passenger" WBCs found in transfused PRBCs or platelet components. Cellular components that are leukocyte-reduced have a decreased risk of transmitting CMV, regardless of the serologic status of the donor. Groups at risk for CMV infections include immunosuppressed patients, CMV-seronegative transplant recipients, and neonates; these patients should receive leukocyte-depleted components or CMV seronegative products. (10)

Human T lymphotropic virus (HTLV) type I

Assays to detect HTLV-I and -II are used to screen all donated blood. HTLV-I is associated with adult T cell leukemia/lymphoma and tropical spastic paraparesis in a small percentage of infected persons (Chap. 188). The risk of HTLV-I infection via transfusion is 1 in 641,000 transfusion episodes. HTLV-II is not clearly associated with any disease. (10)

Parvovirus B-19

Blood components and pooled plasma products can transmit this virus, the etiologic agent of erythema infectiosum, or fifth disease, in children. Parvovirus B-19 shows tropism for erythroid precursors and inhibits both erythrocyte production and maturation. Pure red cell aplasia, presenting either as acute aplastic crisis or chronic anemia with shortened RBC survival, may occur in individuals with an underlying hematologic disease, such as sickle cell disease or thalassemia (Chap. 107). The fetus of a seronegative woman is at risk for developing hydrops from this virus. [(10)]

Bacterial Contamination

The relative risk of transfusion-transmitted bacterial infection has increased as the absolute risk of viral infections has dramatically decreased.

Most bacteria do not grow well at cold temperatures; thus, PRBCs and FFP are not common sources of bacterial contamination. However, some gram-negative bacteria can grow at 1° to 6°C. *Yersinia*, *Pseudomonas*, *Serratia*, *Acinetobacter*, and *Escherichia* species have all been implicated in infections related to PRBC transfusion. Platelet concentrates, which are stored at room temperature, are more likely to contain skin contaminants such as gram-positive organisms, including coagulase-negative staphylococci. It is estimated that 1 in 1000—2000 platelet components is contaminated with bacteria. The risk of death due to transfusion-associated sepsis has been calculated at 1 in 17,000 for single-unit platelets derived from whole blood donation and 1 in 61,000 for apheresis product. Since 2004, blood banks have instituted methods to detect contaminated platelet components.

Recipients of transfusion contaminated with bacteria may develop fever and chills, which can progress to septic shock and DIC. These reactions may occur abruptly, within minutes of initiating

the transfusion, or after several hours. The onset of symptoms and signs is often sudden and fulminant, which distinguishes bacterial contamination from an FNHTR. The reactions, particularly those related to gram-negative contaminants, are the result of infused endotoxins formed within the contaminated stored component.

When these reactions are suspected, the transfusion must be stopped immediately. Therapy is directed at reversing any signs of shock, and broad-spectrum antibiotics should be given. The blood bank should be notified to identify any clerical or serologic error. The blood component bag should be sent for culture and Gram stain. (10)

Other Infectious Agents

Various parasites, including those causing malaria, babesiosis, and Chagas disease, can be transmitted by blood transfusion. Geographic migration and travel of donors shift the incidence of these rare infections. Other agents implicated in transfusion transmission include dengue, chikungunya virus, variant Creutzfeldt-Jakob disease, *Anaplasma phagocytophilum*, and yellow fever vaccine virus and the list will grow. Tests for some pathogens are available, such as *Trypanosoma cruzi*, but not universally required. These infections should be considered in the transfused patient in the appropriate clinical setting. (10)

Alternatives to Transfusion

Alternatives to allogeneic blood transfusions that avoid homologous donor exposures with attendant immunologic and infectious risks remain attractive. Autologous blood is the best option when transfusion is anticipated. However, the cost-benefit ratio of autologous transfusion remains high. No transfusion is a zero-risk event; clerical errors and bacterial contamination remain potential complications even with autologous transfusions. Additional methods of autologous transfusion in the surgical patient include preoperative hemodilution, recovery of shed blood

from sterile surgical sites, and postoperative drainage collection. Directed or designated donation from friends and family of the potential recipient has not been safer than volunteer donor component transfusions. Such directed donations may in fact place the recipient at higher risk for complications such as GVHD and alloimmunization. (10)

Granulocyte and granulocyte-macrophage colony-stimulating factors are clinically useful to hasten leukocyte recovery in patients with leukopenia related to high-dose chemotherapy. Erythropoietin stimulates erythrocyte production in patients with anemia of chronic renal failure and other conditions, thus avoiding or reducing the need for transfusion. This hormone can also stimulate erythropoiesis in the autologous donor to enable additional donation. (10)

Chapter six

Immune disorders

- *Disorder of immune System*
- *Human immunodeficiency virus (HIV) and AIDS*

Disorder of immune System

General consideration

Immune system

The immune system consists of a linked network of cells, proteins and lymphoid organs which are strategically placed to ensure maximal protection against infection. (3)

Immune defences are categorised into the innate immune response, and the adaptive or acquired immune response. (3)

The innate immune system

Innate defences against infection include:

1. Anatomical barriers
2. Phagocytes ('eating cells') (Figure 6.1)
3. Soluble molecules
   a. Complement system, has three pathways:
      - *The alternative pathway*
      - *The classical pathway*
      - *The lectin pathway*
   b. Acute phase proteins (3)
4. Natural killer cells
5. Dendritic cells

Adaptive or acquired immune

1. Humoral immunity

B lymphocytes: Humoral immunity involves antibodies that are produced by B lymphocytes which are 5 types (Table 6.1):
IgG, IgA, IgM, IgE or IgD (Figure 6.2) (3)

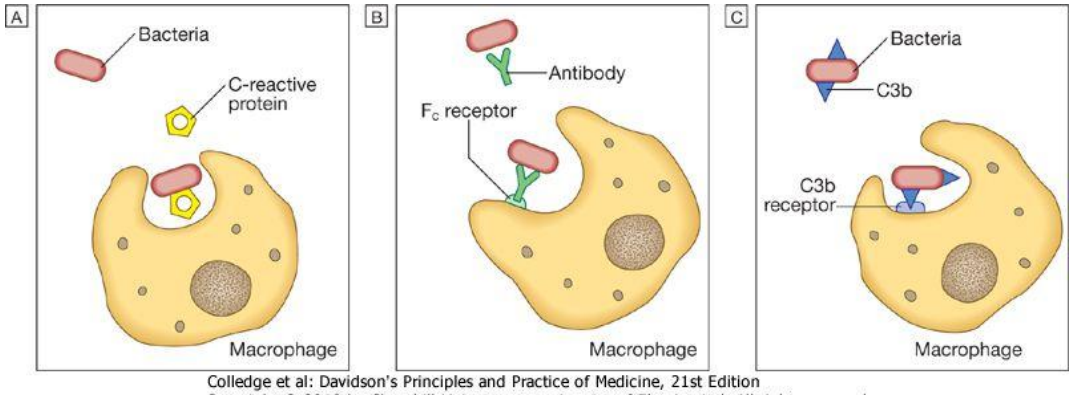


Figure 6.1 **Opsonisation.** Phagocytosis of microbial products may be augmented by several opsonins. A C-reactive protein, B Antibody, C Complement fragments.

Table 6.1 **Classes and properties of antibody** [3]

Antibody	Concentration in adult serum	Complement activation*	Opsonisation	Presence in external secretions	Other properties
IgG	8.0-16.0 g/L	IgG1 +++	IgG1 ++	++	4 subclasses: IgG1, IgG2, IgG3, IgG4
		IgG2 + IgG3 +++	IgG3 ++		Distributed equally between blood and extracellular fluid, and transported across placenta
					IgG2 is particularly important in making antibodies against polysaccharides
IgA	1.5-4.0 g/L	-	-	++++	2 subclasses: IgA1, IgA2
					Highly effective at neutralising toxins
					Particularly important at mucosal surfaces
IgM	0.5-2.0 g/L	++++	-	+	Highly effective at agglutinating pathogens
IgE	0.003-0.04 g/L	-	-	-	Majority of IgE is bound to mast cells, basophils and eosinophils
					Important in allergic disease and defence against parasite infection
IgD	Not detected	-	-	-	Function unknown

*I.e. activation of the classical pathway, also called 'complement fixation'.[3]

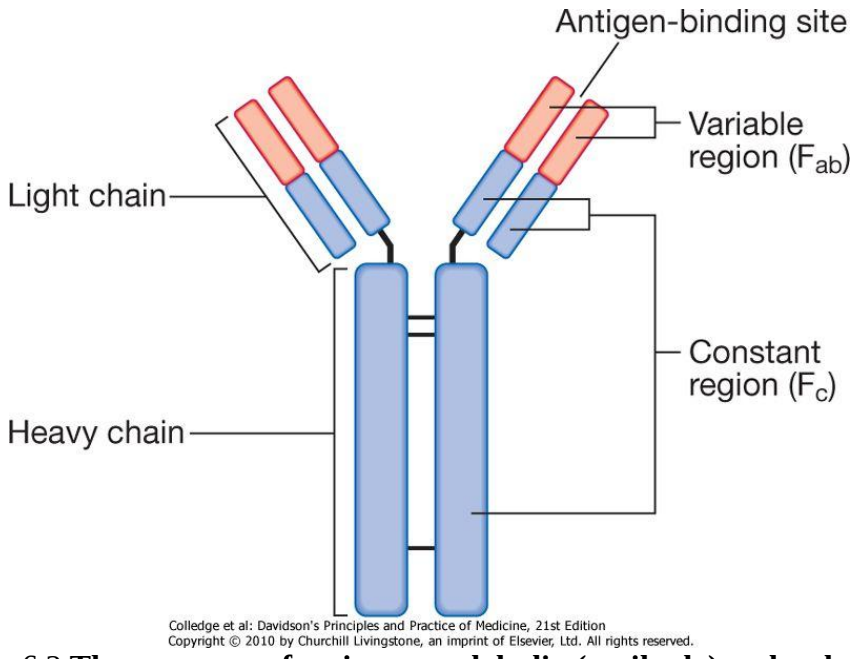


Figure 6.2 **The structure of an immunoglobulin (antibody) molecule.**

2. Cellular immunity[1]

[1] **$CD8^+$ ('cytotoxic') T lymphocytes**

These cells recognise antigenic peptides in association with HLA class I molecules (HLA-A, HLA-B, HLA-C). They kill infected cells directly through the production of pore-forming molecules such as perforin, or by triggering apoptosis of the target cell.

$CD4^+$ ('helper') T lymphocytes

These cells recognise peptides presented on HLA class II molecules (HLA-DR, HLA-DP and HLA-DQ) and have mainly immunoregulatory functions. They produce cytokines and provide co-stimulatory signals that support the activation of $CD8^+$ T lymphocytes and assist the production of mature antibody by B cells. In addition, their close interaction with phagocytes determines cytokine production by both cell types.

$CD4^+$ lymphocytes can be further subdivided into subsets on the basis of the cytokines they produce:

- Typically, Th1 cells produce IL-2, IFN-γ and TNF-α, and support the development of delayed type hypersensitivity responses.
- Th2 cells typically secrete IL-4, IL-5 and IL-10, and promote allergic responses.
- A further subset of specialised $CD4^+$ lymphocytes known as regulatory cells are important in the regulation of other $CD4^+$ cells and the prevention of autoimmune disease. (D)

Cellular immunity is mediated by T lymphocytes, which synthesise and release cytokines that affect other cells.
T lymphocytes:

- $CD8^+$ ('cytotoxic') T lymphocytes
- $CD4^+$ ('helper') T lymphocytes [3]

Cytokines

Cytokines are small soluble proteins that act as multipurpose chemical messengers. (Table 6.2) [3]

Table 6.2 **Cytokines and cytokine function** [5]

Cytokines	Major Producer Cells	Principal Action
Hematopoietin family		
IL-2	T cells	Proliferation of T cells, B cells, and NK cells
IL-3	T cells	Early hematopoiesis
IL-4	T cells, mast cells	B-cell activation, IgE switch, inhibition of T_H1 cells
IL-5	T cells, mast cells	Eosinophil growth and differentiation
IL-6	Macrophages, endothelial cells	T-cell and B-cell growth and differentiation, induction of acute phase proteins
IL-7	Bone marrow, thymic epithelium	Growth of pre-B cells and pre-T cells
IL-9	T cells	Stimulates mast cells and T_H2 cells
IL-11	Stromal fibroblasts	Hematopoiesis
IL-13	T cells	B-cell growth and differentiation, inhibition of T_H1 cells and macrophages
G-CSF	Fibroblasts and monocytes	Neutrophil development and differentiation

Cytokines	Major Producer Cells	Principal Action
IL-15	Non-T cells	Growth of T cells and NK cells
GM-CSF	Macrophages, T cells	Growth and differentiation of myelomonocytic lineage cells
Interferon family		
IFN-α	Leukocytes	Antiviral, increases MHC class I expression
IFN-β	Fibroblasts	Antiviral, increases MHC class I expression
IFN-γ	T cells, NK cells	Macrophage activation, increases expression of MHC molecules, Ig class switching, inhibition of T_H2 cells
TNF family		
TNF-α	Macrophages, NK cells, T cells	Induction of proinflammatory cytokines, endothelial cell activation, apoptosis
TNF-β (LT-α)	T cells, B cells	Cell death, endothelial activation, lymphoid organ development
LT-β	T cells, B cells	Cell death, lymphoid organ development
Others		
TGF-β	Monocytes, T cells	Anti-inflammatory, inhibits cell growth, induces IgA secretion
IL-1α, IL-1β	Macrophages, endothelial cells	Acute phase response, fever, macrophage activation, costimulation
IL-10, IL-1β	T cells, macrophages	Suppression of macrophage functions
IL-12	Macrophages, dendritic cells	NK cell activation, T_H1 cell differentiation
IL-16	T cells, mast cells, eosinophils	Chemoattractant for CD4 T cells, monocytes, and eosinophils

Cytokines	Major Producer Cells	Principal Action
IL-17	CD4 memory cells	Cytokine production by epithelia, endothelia, and fibroblasts
IL-18	Macrophages	IFN-γ production by T cells and NK cells

CD = cluster of differentiation; G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; IFN = interferon; Ig = immunoglobulin; IL = interleukin; LT = lymphotoxin; MHC = major histocompatibility complex; NK = natural killer; TGF = transforming growth factor; T_H = helper T lymphocyte; TNF = tumor necrosis factor. (5)

Lymphoid organs

Primary lymphoid organs

- Bone marrow
- Thymus

Secondary lymphoid organs

- Spleen
- Lymph nodes (Figure 6.3)
- Mucosa-associated lymphoid tissue (3)

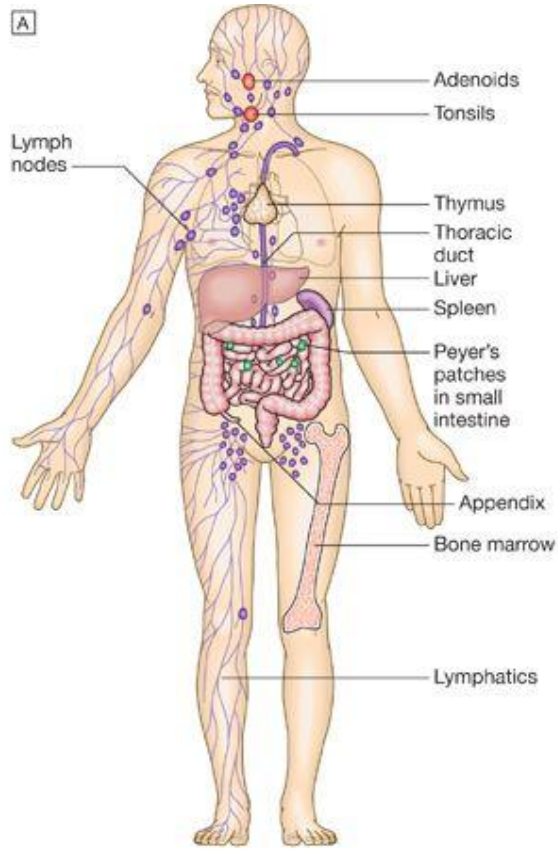


Figure 6.3 **Anatomy of the adaptive immune system.**
A Macroanatomy, B Anatomy of a lymph node

Immunologic disorders

Dysfunction or deficiency of the immune response leads to a wide variety of diseases, involving every organ system in the body. (3)

Epidemiology

Diseases related to disordered immune function (immunodeficiency) are far less common than allergic disorders.

1. The most frequent is IgA deficiency[2]
2. Disorders of B and T lymphocytes such as common variable hypogammaglobulinemia (5)

Diagnosis

Evaluation of recurrent, persistent, severe, and otherwise unexplained infections

History

The most important historical information includes the following:

1. Age of onset
2. Family history of frequent infection
3. Death at an early age from infection
4. The number, sites, and type of infection
5. Physical abnormalities (5)

In a patient with a T-cell disorder, viral, fungal, mycobacterial, and other opportunistic infections (*Pneumocystis jirovecii, Toxoplasma gondii*) are most commonly noted.

In B-cell or antibody deficiency, pyogenic bacterial infections predominate (Table 6.3).

[2] Which occurs in approximately 1 in 1000 individuals and is often asymptomatic.

Table 6.3 **Key points regarding immunologic disorders** [(5)]

Antibody deficiency disorders
1. Onset after 6 mo of age 2. Recurrent respiratory infection 3. Infection with bacteria, especially encapsulated organisms 4. Absence of isohemagglutinins 5. Evaluation of B-cell function, not numbers
Cellular immune defects
1. Onset before 6 mo of age 2. Recurrent viral, fungal, or parasitic (opportunistic) infection 3. Defective delayed hypersensitivity skin responses 4. Malabsorption or diarrhea
Complement deficiencies
1. Recurrent bacterial infection 2. Recurrent neisserial infection (deficiency of late components) 3. Associated rheumatic disorder (systemic lupus erythematosus)
Factors suggesting neutrophils dysfunction
1. Late separation of umbilical cord 2. Persistent neutrophilic leukocytosis 3. Recurrent or persistent gingivitis or periodontitis 4. Recurrent bacterial infection with granuloma formation

Classification of immunodeficiency disease

Primary immunodeficiency diseases [3]

.The main categories of primary immunodeficiency diseases are:[4]

[3] Primary immunodeficiency diseases:

- Are heritable disorders
- Result from defects in an intrinsic component of the immune system
- Caused by single-gene defects
- May represent the end result eg infections
- Primary immunodeficiencies are rare
- Occur in 1 in 2000 to 1 in 10 000 live births

[4] The International Union of Immunological Societies (IUIS) convenes a committee which meets biannually to review the classification of primary immunodeficiency diseases [(12)]

1. Antibody deficiencies
2. T-cell deficiencies
3. Both B- and T-cell function is defective
4. Defects in phagocyte function
5. Complement deficiencies (Table 6.4)

Secondary immunodeficiencies

Causes of secondary immune deficiency[5]

1. Physiological

- Ageing
- Prematurity
- Pregnancy

2. Infection

- HIV
- Measles
- Mycobacterial infection

3. Iatrogenic

- Antineoplastic agents
- Corticosteroids
- Stem cell transplantation
- Radiation injury
- Anti-epileptic agents

4. Malignancy

- B-cell malignancies including leukaemia, lymphoma and myeloma
- Solid tumours
- Thymoma

[5] Secondary immune deficiencies are much more common than primary immune deficiencies and occur if the immune system is compromised by external factors. Common causes include infections, such as HIV and measles, and cytotoxic and immunosuppressive drugs, particularly those used in the management of transplantation, autoimmunity and cancer. Physiological immune deficiency occurs at the extremes of life; the decline of the immune response in the elderly is known as immune senescence.

5. Biochemical and nutritional disorders

- Malnutrition
- Renal insufficiency/dialysis
- Diabetes mellitus
- Specific mineral deficiencies, e.g. iron, zinc

6. Other conditions

- Burns
- Asplenia/hyposplenism (Table 6.5) [3]

Table 6.4 **Classification of primary immunodeficiency states**

Antibody deficiency diseases	**Mutated gene/pathogenesis**	**Associated features**
X-linked agammaglobulinaemia	*BTK*	Antibody deficiency and B lymphopenia
Autosomal recessive agammaglobulinaemia	Mutations in genes for μ, Igα, Igβ, λ5, or BLNK	Antibody deficiency and B lymphopenia
Thymoma with antibody deficiency	Unknown	Antibody deficiency and B lymphopenia
Hyper IgM syndome (autosomal recessive)	UNG or AICDA which encodes for AID or mutation in gene encoding the PMS2 component of the mismatch repair machinery	Low IgG and IgA, raised IgM
Common variable immunodeficiency	Unknown in most; *TACI* in *c.*10%, rarely *ICOS, CD19*, or *BAFFR*	Antibody deficiency; may have autoimmunity, lymphoproliferation, systemic granulomata
Selective IgA deficiency	Most unknown; few due to *TACI* mutations	Most remain healthy; increase in autommunity, atopy, coeliac disease
IgG subclass deficiency	Unknown	If associated with selective antibody deficiency may have recurrent sinopulmonary infections

Specific antibody deficiency with normal serum immunoglobulins	Unknown	Deficient antibody responses to some antigens. Anti-polysaccharide antibody deficiency may be associated with recurrent sinopulmonary infections
Transient antibody deficiency of infancy	Unknown	Reduced IgA and IgG; recovery by 3 years of age
Combined T-and B-cell deficiency		
Severe combined immunodeficiency (SCID)		Lymphopenia, low serum Igs, failure to thrive, severe recurrent infections by viruses, bacteria, and parasites; fatal without BMT
SCID due to failure of cytokine receptor signaling	*IL2RG* (common γ-chain), *IL2RA, IL7RA, JAK3*	T lymphopenia; B cell number normal (T-B+ SCID)
SCID due to defective VDJ gene recombination	*RAG 1, RAG2, DCLRE1C* (*Artemis)*	T-B-SCID
SCID due to defective nucleotide salvage	*ADA, PNP*	ADA deficiency gives rise to T-and B-cell lymphopenia (T-B-SCID); PNP deficiency give rise to T-lymphopenia and neurologic defects
SCID due to defective T-cell receptor function	*CD3D, ZAP70, CD45*	
SCID due to defective calcium entry into T-cells	Mutation in the *ORAI1* gene which encodes a subunit of the plasma membrane calcium channel CRAC. T-cell function is impaired	

SCID due to lack of T-cell egress from thymus	Mutation in *CORO1A* which encodes the actin regulator Coronin A, which is required for normal T-cell migration	Causes a T-B+NK+ SCID
SCID due to defective MHC class II transcription	*MHC2TA, RFXANK, RFX5, RFXAP*	Lack of MHC class II expression resulting in CD4 lymphopenia and severe failure of T-cell and B-cell function
Omenn's syndrome	hypomorphic mutation of *RAG1, RAG2*, DCLRE1C (*Artemis)*, or IL7Ra	Variant of SCID. some T and B cells may develop but are oligoclonal. Features include erythroderma, lymphadenopathy, hepatosplenomegaly, eosinophilia. Outcome poor without BMT
MHC class I deficiency	*TAP1* or *TAP2*	Lack of MHC class I expression on cells; CD8 lymphopenia; present with bronchiectasis or vasculitis
X-linked hyper IgM syndrome	*CD40L*	Lack of CD40-ligand on activated T cells. Failure of Ig class- switching and affinity maturation; low IgG/IgA, raised or normal IgM; may develop neutropenia, autoimmune cytopenias, opportunistic infections and gastrointestinal and liver pathologies
CD40 deficiency (a type of autosomal recessive hyper IgM	*CD40*	Lack of CD40 expression on B cells. Other features similar to CD40L

syndrome)		deficiency
X-Linked lymphoproliferative disorder (XLP)	Mutation of *SAP* or *XIAP* genes. (see text for explanantion)	Clinical manifestations precipitated by EBV infection: hepaptitis, haemophagocytosis, aplastic anaemia, hypogammaglobulinaemia, Non-hodgkins lymphoma
DOCK 8 deficiency	Mutation in gene encoding dedicator of cytokinensis 8 (DOCK 8)	Recurrent sino-pulmonary infections and cutaneous viral infections (*Molluscum contagiosum* and HPV); low serum IgM and variable IgG responses
T cell deficiency		
MHC class I deficiency	*TAP1, TAP2 or TAPBP* (which encodes for the TAP binding protein Tapasin)	Lack of MHC class I expression on cells; CD8 Lymphopenia; present with bronchiectasis or vasculitis
Phagocyte deficiencies (excluding congenital neutropenias)		
Chronic granulomatous disease	Mutations in components of the phagocye oxidase (see text for details)	Pyogenic and fungal infections; lymph node and visceral abscesses; chronic granulomata (see text for details)
Leucocyte adhesin deficiency Type 1	Mutation of gene encoding CD18, which is a component of leucocyte adhesins (see text for details)	Delayed umbilical cord separating, omphalitis, pyoderma, periodontitis, leukocytosis
Leucocyte adhesin deficiency Type 2	Mutation of gene encoding GDP-fucose transporter (see text for details)	As above plus mental retardation
Leucocyte adhesin deficiency Type 3	Mutation in calcium and diacylglycerol-regulated guanine nucleotide exchange factor	Omphalitis, pyogenic infections, mulberry haematomas, bleeding tendency due to defective

		platelet activation, defect in leucocyte adhesion to endothelial surface due to defect in β1,2,3 integrin activation
Rac 2 deficiency	Mutation in RAC2 leading to impaired actin polymerization/cytoskeletal function	Poor wound healing, leucocytosis, Clinical picture similar to Type1 Leucocyte Adhesin deficiency
Mendelian susceptibility to mycobacterial infection	Defects in the production or response to IFNγ: Mutated genes include *IL12B, IL12RB1, IFNGR1, IFNGR2, STAT 1, TYK2*. Intact response to IFNγ is essential for control of intracellular bacterial infection. Some *NEMO* mutations cause X-linked susceptibility to mycobacterial infection. See text for full explanation	Recurrent disseminated infections with poorly pathogenic mycobacteria (NTM or *BCG*) and systemic infections with non-typhi salmonella species
Miscellaneous well-characterized immunodeficiencies		
Wiskott–Aldrich syndrome	*WASP*: Lack or dysfunctional protein results in cytoskeletal defect affecting myeloid and lymphoid cells	Thrombocytopenia, small platelets, eczema, combined immunodeficiency, lymphomas, autoimmune disease
DNA repair defects		
Ataxia telangiectasia	*ATM:* Mutation causes dysfunction of cell cycle check point pathway leading to chromosomal instability	Ataxia, oculocutaneous telangiectasia, raised serum α-fetoprotein, increased malignancies (especially lymphoma), IgA/IgG

		subclass deficiencies with poor anti-polysaccharide responses, sino-pulmonary infections, Radiation sensitivity
Ataxia-like syndrome	*Mre 11*	
Nijmegen breakage syndrome	Mutation in NBS1: impaired DNA double strand break repair	Microcephaly, radiation sensitivity, lymphomas
DNA-ligase IV deficiency	Mutation in DNA ligase IV: defective DNA repair	Microcephaly, facial dysmorphism, radiation sensitivity
DiGeorge anomaly	Heterozygous deletion of 22q11 in 90%; mutation in *TBOX-1* genein a few	Conotruncal defects, facial dysmorphism, hypoparathyroidism, velo-pharyngeal defects, thymic hypoplasia
Immunodeficiency with partial albinism		
Chediak–Higashi syndrome	Mutation in *LYST* gene. Impaired lysosomal function and defect in sorting cytolytic proteins into secretaory granules; poor NK-cell and T-cell mediated cytolysis	Partial albinism, giant lysosomes, recurrent pyogenic infections, mild mental retardation, peripheral neuropathy, eventually 85% develop accelerated phase with syndrome resembling haemophagocytic lymphohistiocytosis
Griscelli syndrome (type 2)	Deficiency of the RAB27A GTPase required for secretory vesicle function. Exocytosis of cytolytic granules deficient leading to poor NK-cell and T-cell mediated cytolysis	Partial albinism, giant lysosomes, recurrent pyogenic infections, encephalopathy, eventually 85% develop accelerated phase as in Chediak–Higashi syndrome

Disorders of homeostasis of immune function		
Syndromes with autoimmunity		
Autoimmune lymphoproliferative syndrome (ALPS)	Defects of components in the apoptosis pathway in lymphocytes: mutations in genes encoding CD95 (*TNFRSF6*), CD95 ligand (*TNFSF6*), caspase 10 or caspase 8	Lymphadenopathy, hepatosplenomegaly, hypergammaglobulinaemia, deficient lymphocyte apoptosis, autoimmune diseases, increased CD4-CD8- T cells
Autoimmune polyendocrinopathy, candidiasis, ectodermal dysplasia syndrome (APCED)	Mutation in autoimmune regulator gene (*AIRE*) encoding protein required for expression of ectopic antigens in the thymic epithelial cells. This is required for induction of tolerance to autoantigens	Multiple endocrine autoimmunity; chronic mucocutaneous candidiasis
Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX)	Mutation of *FOXP3* gene whose product is expressed by and is required for function of T-regulatory cells	Childhood onset autoimmune endocrinopathy, enteropathy, eczema
Familial haemophagocytic lymphohistiocytosis	Defective T-cell and NK-cell mediated cytotoxicity due to mutations in genes encoding perforin (PRF1), or MUNC protein (MUNK13-4) needed for fusion of intracellular vesicles	Viral infection triggers haemophagocytosis
X-linked lymphoproliferative syndrome	*SAP* (see text for explanation of function)	Clinical manifestations precipitated by EBV infection: hepatitis, haemophagocytosis, aplastic anaemia, hypogammaglobulinaemia,

		lymphomas
Disorders of homeostasis of inflammation (autoinflammatory syndromes)		
Familial Mediterranean fever	*MEFV*	Periodic fever, amyloidosis
Hyper-IgD syndrome	Partial mevalonate kinase deficiency; mechanism of disease uncertain	Periodic fever
TNF receptor associated periodic fever	*TNFRSF*1: results in decreased availability of soluble TNF receptor for mopping up TNF	Periodic fever, amyloidosis
Familial cold autoinflammatory syndrome	CIAS1: defect in cryopyrin required for leukocyte apoptosis NF-κB signalling and IL-1 processing	Cold induced urticaria, fever
Neonatal onset multisystem inflammatory disease (NOMID)	CIAS1: as above	Neonatal onset rash, fever, chronic meningitis, arthropathy
Muckle–Wells syndrome	CIAS1: as above	Urticaria, deafness, amyloidosis
Defects in innate immunity		
Anhydrotic ectodermal dysplasia with immunodeficiency	Hypomorphic mutation in *NEMO* which is a component in the NF-κB signalling pathway; results in defective NF-κB activation	Ectodermal dysplasia in some but not all patients, lack of anti-polysaccharide antibodies, failure to switch to IgG, pyogenic and mycobacterial infection; inheritance is X-linked recessive
Anhydrotic ectodermal dysplasia with immunodeficiency	Mutation in *IKBA* encoding a regulatory component of the NF-κB pathway	Ectodermal dysplasia and T-cell deficiency
IL-1 receptor associated kinase	*IRAK4* encoding a component of the	Recurrent pyogenic infections especially with

(IRAK4) deficiency	signalling pathway utilized by Toll-like receptors	*S. pneumonia*
WHIM syndrome	Gain of function mutation in gene encoding chemokine receptor CXCR4	Warts, hypogammaglobulinaemia, neutropenia with myelokathexis, bacterial infections
Severe herpes viral infections in childhood	Impaired production of IFNα and β in response to viral nucleic acids due to deficiency in UNC93B, a protein involved with Toll receptor activation. Similar syndrome produced by a mutation of gene encoding Toll Receptor 3 (TLR3)	Herpes simplex encephalitis in childhood
Severe herpes viral infections in childhood	Impaired response to IFNα and β due to lack of type-1 interferon receptor function. Caused by homozygous mutation of *STAT1*	Herpes simplex encephalitis in childhood; these patients also develop mycobacterial infections as STAT1 is required for signalling via IFNγ receptors
Hyper IgE syndrome	Heterozygous mutation in gene encoding signal transducing factor STAT 3	Recurrent bacterial and fungal infections, staphylococcal pneumonia with pneumatocoele formation, pyogenic infection causing cold abscess formation, poor acute phase responses, delayed shedding of primary dentition, facial dysmorphism, dermatitis, elevated serum IgE
CARD9 deficiency	Homozygous mutation in gene encoding Caspase	Recurrent mucocutaneous fungal infection and fatal

	recruitment domain containing protein 9 required for effective antifungal immune response (see text)	invasive brain infection with Candida
Dectin-1 deficiency	Mutation in gene encoding Dectin-1, which is a pattern recognition receptor for fungal cell wall b glucan	Recurrent vulvo-vaginal candidiasis and fungal nail infection
Early onset Crohn's disease	Mutation in *IL10RA* and *IL10RB* genes encoding for the IL10R1 and IL10R2 components of the IL10-receptor. This abrogates responses to IL10, resulting in an increase in pro-inflammatory cytokine production, suggesting that IL10-dependent homesostasis of inflammatory pathways is abnormal in these patients.	Early onset severe, treatment refractory Crohn's disease. In one patient a cure was obtained by haemopietic stem cell transplantation.
Miscellaneous primary immunodeficiencies of unknown pathogenesis		
Idiopathic CD4 cell lymphopenia	CD4 lymphopenia of unknown cause	Infections typical of T-cell deficiency
Chronic mucocutaneous candidiasis without endocrinopathy	Unknown Aetiology	Autosomal recessive and autosomal dominant cases of chronic mucocutaneous candida infection have been documented

JOHNSTON, R. B., JR. (1984) Recurrent bacterial infections in children. N Engl J Med, 310, 1237–43.) Copyright © [1984] Massachusetts Medical Society. All rights reserved. [12]

Table 6.5 **Causes of secondary immunodeficiency**

	Defect
Defects in anatomical and physical barriers to infection	Various (see text for explanation)

Malignancies of the B-cell system	Antibody
Myelomatosis	
Non Hodgkin's lymphoma	
Chronic lymphocytic leukaemia	
Therapeutic agents	
Biological agents—	
Anti-B-cell antibodies (e.g. rituximab)	Antibody
Anti-TNF agents	Innate immunity and CMI
Cytotoxic drugs—alkylating agents, cytotoxic antibiotics, antimetabolites, vinca alkaloids, etoposide, etc.	Myelosupression and CMI
Immunosupressive drugs—corticosteroids, calcineurin inhibitors, antiproliferative immunosuppressants (azathioprine, mycophenelate)	CMI
Drugs causing antibody deficiency—gold, penicillamine, sulphasalazine, carbamazepine, valproate	Antibody
Radiotherapy	CMI
Metabolic/nutritional deficiencies	
Renal failure	CMI and innate immunity
Liver failure	CMI and innate immunity
Protein–calorie malnutrition	CMI
Vitamin A deficiency	CMI
Transcobolamine-II deficiency	Antibody
Increased loss of immunoglobulin	
Nephrotic syndrome	
Protein-losing enteropathy	
Dystrophia myotonica	
Virus infections	
HIV	CMI
Congenital rubella	Antibody
Congenital CMV	Antibody

CMI, cell-mediated immunity; CMV, cytomegalovirus;TNF, tumour necrosis factor. [12]

Hypersensitivity Immune Responses

The Gell and Coombs classification of hypersensitivity is the most widely used and distinguishes four types of immune response which result in bystander tissue damage:

1. **Type I hypersensitivity**

Type I is relevant in allergy but is not associated with autoimmune disease

Anaphylactic or immediate hypersensitivity reactions occur after binding of antigen to IgE antibodies attached to the surface of the mast cell or basophil and result in the release of preformed and newly generated inflammatory mediators that produce the clinical manifestations. (Figure 6.4) [3]

Common allergic diseases

1. Urticaria
2. Angioedema
3. Atopic dermatitis
4. Allergic conjunctivitis
5. Allergic rhinitis (hayfever)
6. Allergic asthma
7. Food allergy
8. Drug allergy
9. Allergy to insect venom
10. Anaphylaxis (Figure 6.5) [3]

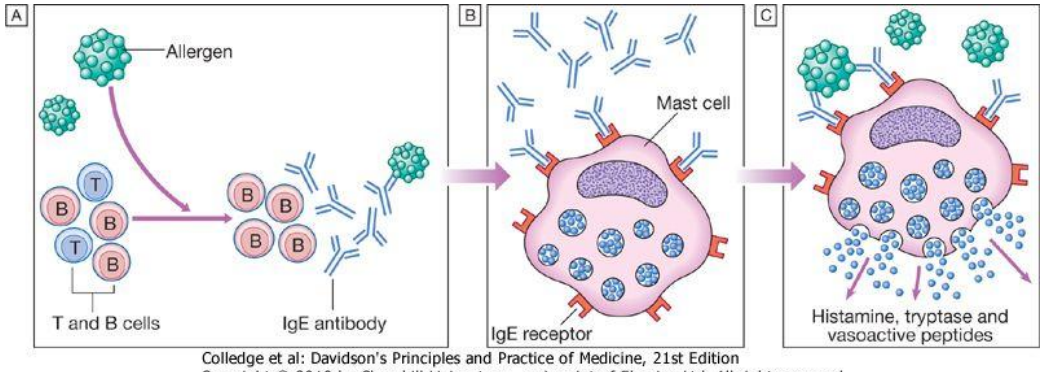


Figure 6.4 **Type I (immediate) hypersensitivity response.**
After an encounter with allergen, B cells produce IgE antibody against the allergen.
Specific IgE antibodies bind to circulating mast cells via high-affinity IgE cell surface receptors.
On re-encounter with allergen, the allergen binds to the IgE antibody-coated mast cells. This triggers mast cell activation with release of vasoactive mediators.

2. **Type II hypersensitivity**

In this type injury is localised to a single tissue or organ

3. **Type III hypersensitivity**

Type III is a generalised reaction resulting from immune complex deposition in blood vessel walls, skin, joints and glomeruli, where they cause a chronic inflammatory response. This triggers the classical complement cascade[6]

4. **Type IV hypersensitivity**

---

1. *Type III hypersensitivity* is a generalised reaction resulting from immune complex deposition in blood vessel walls, skin, joints and glomeruli, where they cause a chronic inflammatory response. This triggers the classical complement cascade as well as recruitment and activation of phagocytes and $CD4^+$ lymphocytes. The site of immune complex deposition is determined by the relative amount of antibody, size of the immune complexes, nature of the antigen and local haemodynamics. Generalised deposition of immune complexes gives rise to systemic diseases such as SLE. (3)

In this type activated T cells and macrophages mediate phagocytosis and NK cell recruitment (Table 6.7) [3]

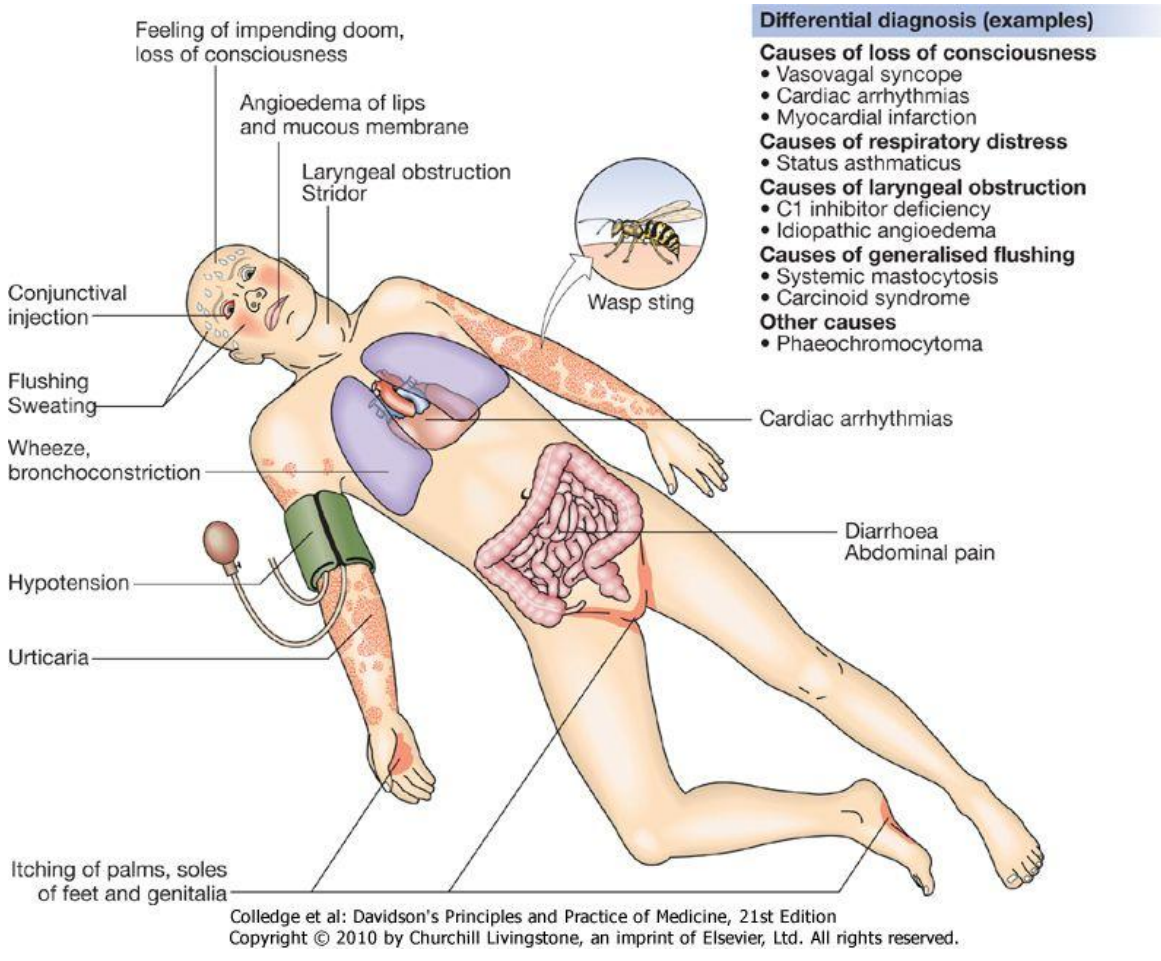


Figure 6.5 **Clinical manifestations of anaphylaxis.** In this example, the response is to an insect sting containing venom to which the patient is allergic.

Table 6.7 **Gell and Coombs classification of hypersensitivity diseases** [3]

Type	**Mechanism**	**Example of disease in response to exogenous agent**	**Example of autoimmune disease**
Type I Immediate hypersensitivity	IgE-mediated mast cell degranulation	Allergic disease	None described
Type II Antibody-mediated	Binding of cytotoxic IgG or IgM antibodies to antigens on cell surface causes cell killing	ABO blood transfusion reaction Hyperacute transplant rejection	Autoimmune haemolytic anaemia Idiopathic thrombocytopenic purpura Goodpasture's disease
Type III Immune complex-mediated	IgG or IgM antibodies bind soluble antigen to form immune complexes which trigger classical complement pathway activation	Serum sickness Farmer's lung	SLE
Type IV Delayed type	Activated T cells, phagocytes and NK cells	Acute cellular transplant rejection Nickel hypersensitivity	Type 1 diabetes Hashimoto's thyroiditis

Autoimmune disease

Autoimmunity can be defined as the presence of immune responses against self tissue

Physiology and pathology of autoimmunity

Immunological tolerance

This is the process by which the immune system distinguishes self from foreign tissue, failure of which may result in autoimmune disease.

Classification of autoimmune diseases

The spectrum of autoimmune diseases is broad. These diseases be classified as organ-specific or multisystem, or by the predominant mechanism responsible for tissue damage (Table 6.8). [3]

Table 6.8 **The spectrum of autoimmune disease** [3]

Type	**Disease**
Organ-specific	
Immune response directed against localised antigens	Graves' disease
	Hashimoto's thyroiditis
	Addison's disease
	Pernicious anaemia
	Type 1 diabetes
	Sympathetic ophthalmoplegia
	Goodpasture's syndrome
	Pemphigus vulgaris
	Bullous pemphigoid
	Idiopathic thrombocytopenic purpura
	Autoimmune haemolytic anaemia
	Myasthenia gravis
	Primary antiphospholipid syndrome

	Rheumatoid arthritis
	Dermatomyositis
	Primary biliary cirrhosis
	Autoimmune hepatitis
	Sjögren's syndrome
Multisystem	
Immune response directed to widespread target antigens	Systemic sclerosis
	Mixed connective tissue disease
	SLE

Human immunodeficiency virus (HIV) and AIDS

Definition

The definition of AIDS is complex, however we should not focus on whether AIDS is present but should view HIV disease as a spectrum ranging from primary infection with or without the acute symptom to the asymptomatic stage and to the advanced disease. (10)

Origin of the HIV

- AIDS was first recognized in USA in the summer of 1981 in five homosexual men in Los Angeles with pneumocystis carinii pneumonia
- And 26 homosexual in New York and Los Angeles with Kaposi's sarcoma. (10)
- In 1983 HIV was isolated from a patient with lymphadenopathy (10)
- In 1984 HIV was demonstrated to be the causative agent of AIDS (10)
- In 1986 HIV-2 was first identified in West Africa (10)

Etiology

1. The causative agent: The etiologic agent is HIV (Figure 6.6)
2. The characteristics of agent:
   - RNA virus
   - Belong to the family of retrovirus
   - Has two groups:
     a. HIV-1

b. HIV-2

3. The common cause of HIV disease is HIV-1
4. HIV-2 was first identified in West Africa [10]

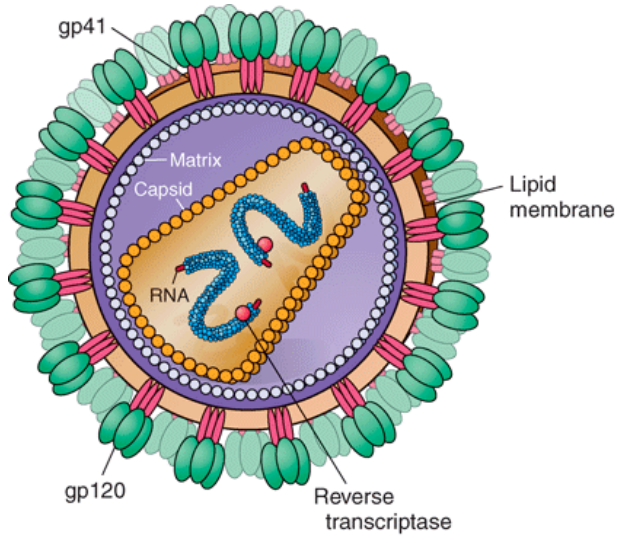


Figure 6.6 Structure of HIV-1, including the gp120 envelope, gp41 transmembrane components of the envelope, genomic RNA, enzymereverse transcriptase, p18(17) inner membrane (matrix), and p24 core protein (capsid). [10]

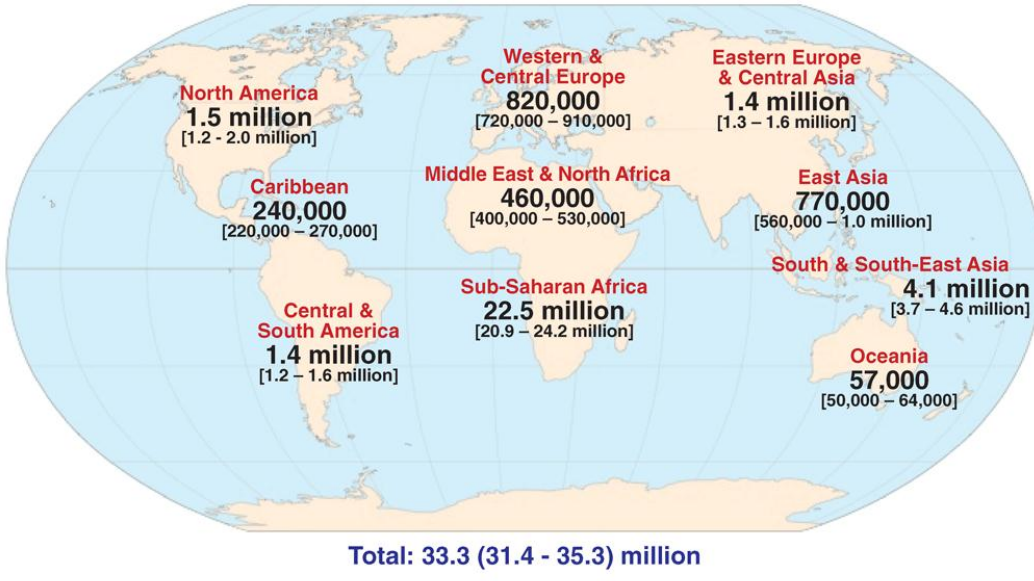
Epidemiology

1. Prevalence/Incidence:
   - Pandemic disease
   - Epidemic disease
   - Has sporadic cases
   - Global total: At the end of 2009, an estimated 33,3 million[1]
   - More than 95% of HIV/AIDS are in low- & middle-income

[1] Global total: 40 million in 2003
In 2003 newly infected cases are 5 million
Has >14000 new infection each day [10]

- 50% are female
- 2.5 million are children <15 years [10]

2. Geographical variation: Worldwide disease, in 2009 worldwide estimation are:
   - Sub Sahara Africa 22,5million
   - South and southeast Asia 4,1 million
   - North America 1,5 million
   - Central and south America 1,4 million
   - Eastern Europe and central Asia 1,4 million
   - Global total 33,3 million (Figure 6.7) [3]



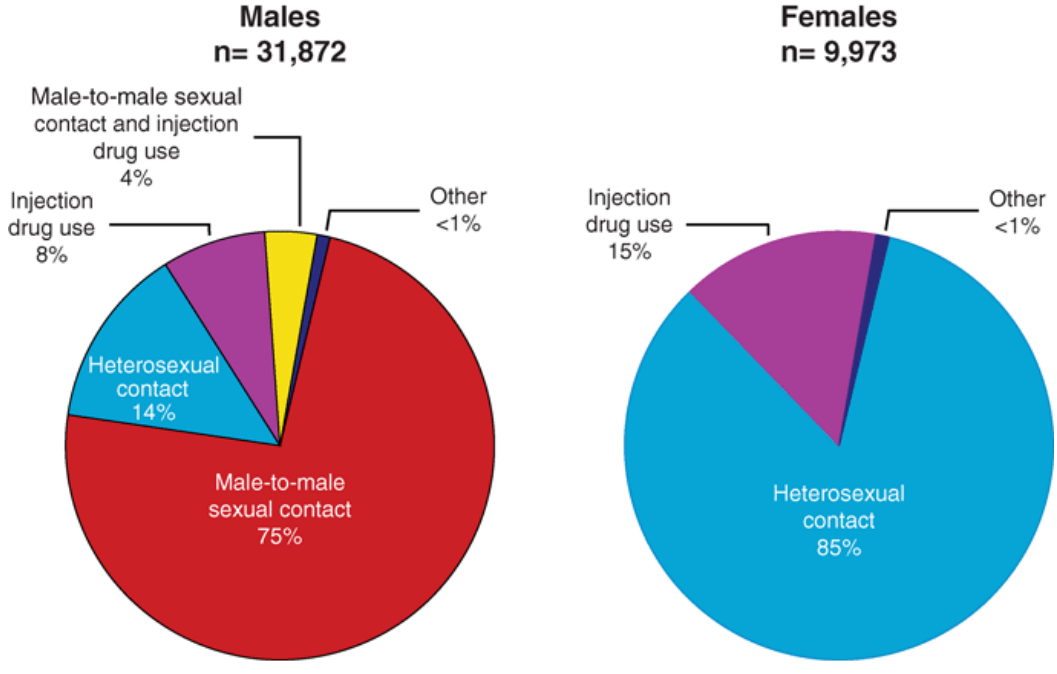
Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com

Figure 6.7 **Estimated number of adults and children living with HIV infection** as of December, 2009. Total: 33.3 (31.4–35.3) (31.1–35.8) million. *[From Joint United Nations Programme on HIV/AIDS (UNAIDS).]* [10]

3. Age group:
   - Any age but common in adult
   - Worldwide 30,8 million adult cases
   - In Sub Sahara Africa 50% cases are women

- 2,5 million cases are children [10]

4. Seasonal variation: No any seasonal variation
5. Route of transmission:
   - Homosexual route
   - Heterosexual route
   - Through blood and blood products
   - Transplacental route
   - Via breast milk (Figure 6.8) [10]
6. Reservoir of infection: Human
7. Incubation period: 2-4 weeks [8]
8. Infectivity period: Lifelong



Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com

Figure 6.8 **Transmission categories of adults and adolescents with HIV/AIDS** diagnosed during 2009 in the United States. Estimates from 40 states with confidential, name-based HIV infection reporting. Data include persons with a diagnosis of HIV infection regardless of AIDS status at diagnosis. *(From CDC.)* [10]

Pathogenesis

1. Entry of virus into the bloodstream
2. Cleared to the spleen and other lymphoid organs
3. Replication in spleen and lymphoid organs
4. Then lead to viremia and dissemination of viruses
5. Dendritic cells play role in the initiation of HIV infection
6. Dendritic cells increase infectivity of HIV to target cells
7. Target cells (CD4 T cells or helper T cells) infection
8. Virus replication in CD4 T cells leads to a burst of viremia
9. Rapid dissemination of viruses to other lymphoid organs and brain
10. Appearance of acute HIV syndromes [10]
11. Infection of T4 (CD4) and B lymphocytes causes immunodeficiency
12. Macrophages act as reservoir for HIV and serve to disseminate
13. HIV can directly infect brain, renal tubular cells and GI epithelium [11]

Pathophysiology

Clinically, the syndromes caused by HIV infection are usually by one of three known mechanism:

1. Immunodeficiency:
   - This is a result of the direct effect of HIV upon immune cells.
   - Tow features of HIV immunodeficiency are:
     The low incidence of certain infection such as listeriosis and aspergillosis.
     The frequent occurrence of certain neoplasm such as lymphoma or Kaposi's sarcoma.
   - The latter complication is seen primarily in bisexual men.

2. Autoimmunity/allergy & hypersensitivity reaction:
   - This is a result of disordered cellular immune function or B lymphocyte dysfunction, the example of both are:
     Lymphocytic infiltration of organ e.g. lymphocytic interstitial pneumonia
     Autoantibody production e.g. immunologic thrombocytopenia
   - These phenomena may be the only clinically apparent disease or may be coexist with obvious immunodeficiency
   - HIV infected patients have higher rate of allergic reaction to unknown allergen.
3. Direct HIV infection of some organs:
   - May cause neurologic, renal and gastrointestinal dysfunction.
   - See discussion in pathogenesis (10)

Clinical features

1. Asymptomatic for years
2. Systemic complaints:
   - Fever
   - Night sweat
   - Weight loss
   - Anorexia
   - Nausea
   - Vomiting
3. Sinopulmonary diseases:

a. Pneumocystis pneumonia

b. Other infectious pulmonary diseases:
   - Viral pneumonia
   - Mycobacterial tuberculosis
   - Haemophilus influenza

- Pseudomonas aerogenosa

c. Non infectious pulmonary diseases:
   - Kaposi's sarcoma
   - Non Hodgkin's lymphoma
   - Interstitial pneumonitis

d. Sinusitis:
   - Sinus congestion
   - Discharge
   - Headache
   - Fever

4. Central nervous system diseases:
   - Toxoplasmosis
   - CNS lymphoma
   - AIDS dementia complex
   - Cryptococcal meningitis
   - HIV myelopathy
   - Progressive multifocal leukoencephalopathy
5. Peripheral nervous system syndromes:
   - Inflammatory polyneuropathies
   - Sensory neuropathies
   - Mononeuropathies
6. Rheumatologic manifestations:
   - Arthritis:
     Suppurative
     Fungal
     Mycobacterial
   - Reiter's syndrome (Reactive arthritis):
   - Psoriatic arthritis
   - Sicca syndrome
   - Lupus erythematosis
7. Myopathy:

- Proximal muscle weakness
- Muscle tenderness due to Zidovudine (Antiviral)

8. Retinitis:
   - Visual changes
   - Cytomegalovirus retinitis
   - Herpes virus infection
   - Toxoplasmosis
9. Oral lesion:
   - Oral candidiasis
   - Hairy leukoplakia (Figure 6.9)
   - Angular cheilitis
   - Angular fissures
   - Gingival diseases
   - Kaposi's sarcoma (Figure 6.10)
   - Aphthous ulcer (Figure 6.11)
   - Warts [11]

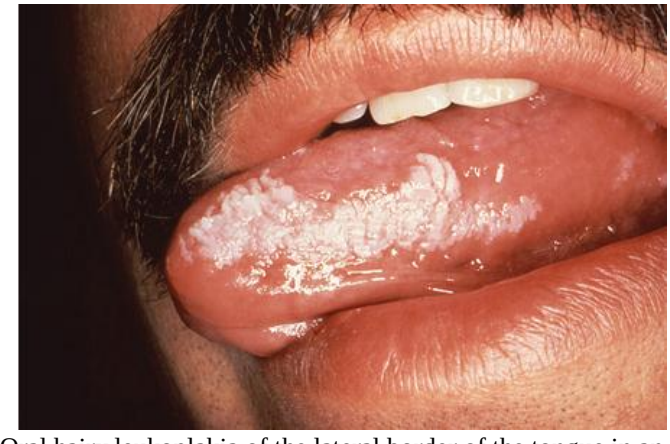


Figure 6.9 Oral hairy leukoplakia of the lateral border of the tongue in an HIV-infected patient. [11]

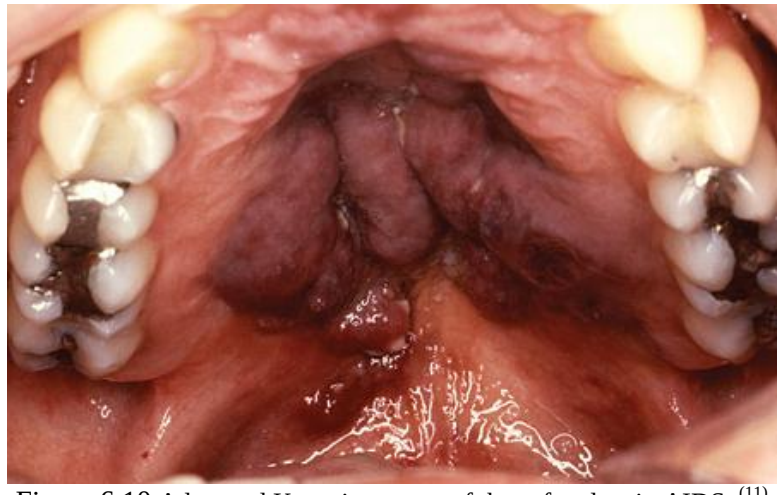
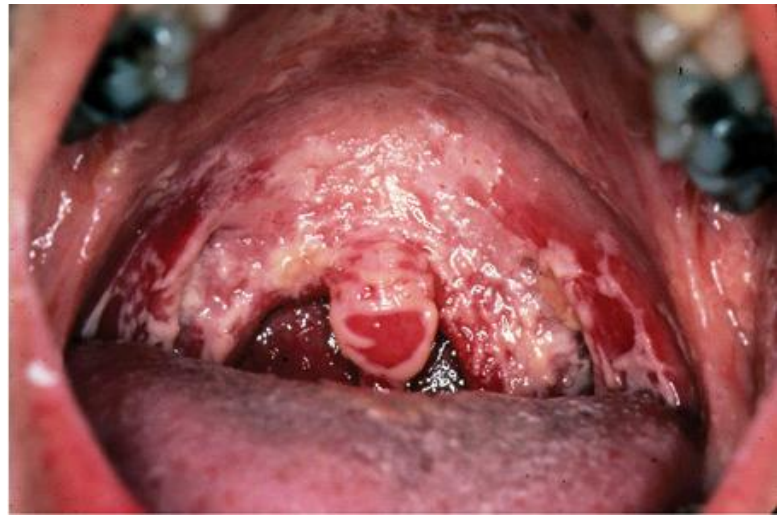


Figure 6.10 Advanced Kaposi sarcoma of the soft palate in AIDS. [11]



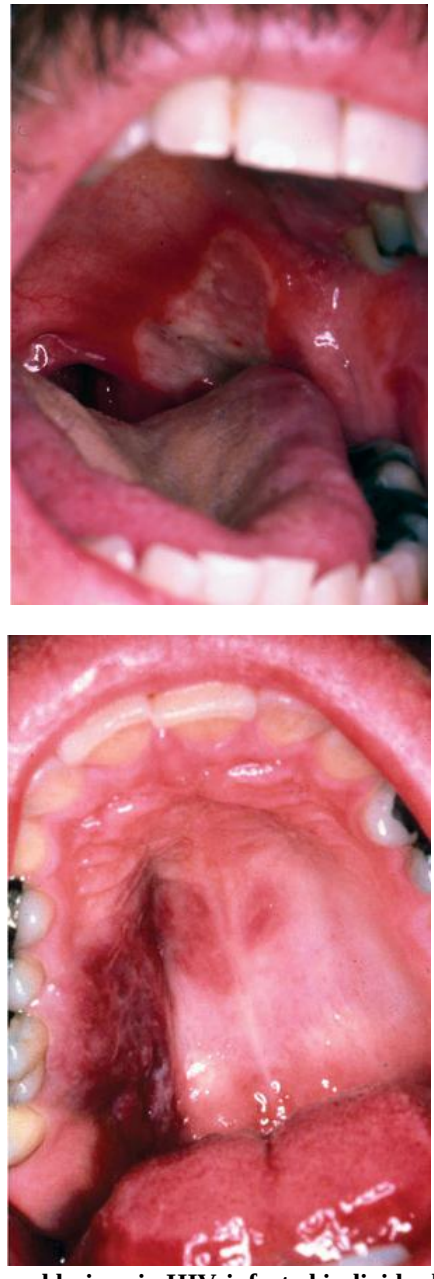


Figure 6.11 **Various oral lesions in HIV-infected individuals. *A.*** Thrush. ***B.*** Hairy leukoplakia. ***C.*** Aphthous ulcer. ***D.*** Kaposi's sarcoma. [11]

10.Gastrointestinal manifestations:

a. Esophageal diseases:
- Candidal esophagitis
- Herpes simplex
- Cytomegalovirus infection

b. Hepatic diseases:
- Mycobacterial diseases
- Cytomegalovirus diseases
- Hepatitis B virus
- Hepatitis C virus
- Lymphoma
- Toxicity of drugs in HIV diseases

c. Biliary diseases:
- Cholecystitis
- Sclerosing cholangitis
- Papillary stenosis
- Syndrome with
  - Nausea
  - Vomiting
  - Right upper quadrant pain

e. Enterocolitis:

May be due to:
- Bacteria
  - Campylobacter
  - Salmonella
  - Shigella
- Viruses:
  - Cytomegalovirus
  - Adenovirus
- Protozoan:
  - Cryptosporidium
  - Entamoeba histolytica

- Giardia
- Isospora
- Microsporidium

Common manifestation:

- Watery diarrhea
- Fever
- Abdominal pain

f. Other disorders:

- Gastropathy
- Malabsorption
- Infections:
  - Campylobacter
  - Salmonella
  - Shigella
- Helicobacter pylori is common

11. Endocrinologic manifestations:

- Mild adrenal abnormalities
- Mild abnormalities of thyroid function

12. Skin manifestations:

- Herpes simplex infection
- Herpes zoster
- Molluscum contagiosum
- Bacillary angiomatosis: due to:
  a. Bartonella henselea
  b. Bartonella Quintana
- Kaposi's sarcoma (Figure 6.12)
- Seboric dermatitis
- Xerosis
- Psoriasis

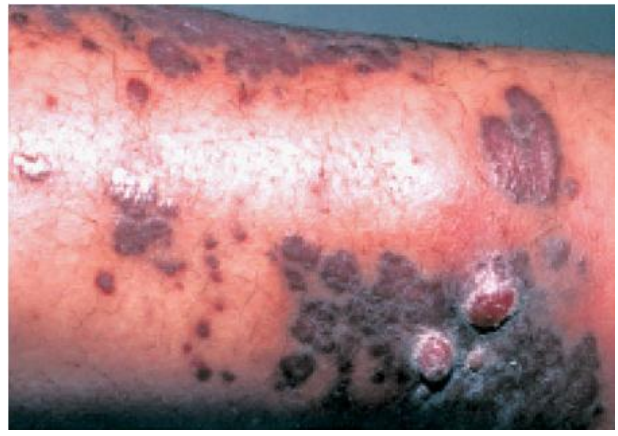


Figure 6.12 **Kaposi's sarcoma in a patient with AIDS** demonstrating patch, plaque, and tumor stages. (11)

13.HIV related malignancies:

- Kaposi's sarcoma
- Non Hodgkin's lymphoma
- Hodgkin's diseases
- Anal dysplasia and squamous cell carcinoma
- Cervical dysplasia and neoplasia

14.Gynecologic manifestations:

- Vaginal candidiasis
- Cervical dysplasia and neoplasia
- Pelvic inflammatory diseases

15.Inflammatory reactions:

- Cytomegalovirus retinitis
- Focal lymphadenitis
- Granulomatus masses
- Tuberculosis
- Cryptococcal meningitis (11)

16.Haematological conditions

a. Thrombocytopenia

- Thrombocytopenia is relatively common (5–15%)
- It is associated with antiplatelet antibodies

- Symptomatic thrombocytopenia is uncommon
- But more likely in the later stages of HIV infection
- Life-threatening bleeding is rare
- Thrombocytopenia is not a marker for HIV progression
- Spontaneous remissions are frequent
- Treatment include the use of prednisolone, intravenous immunoglobulin, and splenectomy [12]

b. Anaemia
- Anaemia is common in patients with advanced HIV infection
- Frequently related to medications (such as zidovudine)
- Human (B19) parvovirus infection is a reversible cause of chronic anaemia in HIV infection
- Bone marrow shows an absence of erythroid cells
- Giant pronormoblasts, and B19 parvovirus is detected
- The anaemia may respond to intravenous immunoglobulin

c. Neutropenia
- Mild neutropenia is common in HIV-positive patients at all stages of infection
- May be partly responsible for the increased risk of pyogenic bacterial infections
- Antineutrophil antibodies may be present
- Drugs (co-trimoxazole, ganciclovir, and antiretrovirals) may increase the incidence and severity of neutropenia
- Recombinant human granulocyte colony-stimulating factor is the treatment [12]

Investigations

1. HIV RNA by PCR
2. Immune complex–dissociated p24 antigen capture assay
3. HIV RNA by bDNA
4. HIV RNA by NucliSens [10]

5. Complete blood count(In advanced HIV infection):
   - Anemia
   - Neutropenia
   - Thrombocytopenia
6. Absolute CD4 lymphocyte count
7. CD4 lymphocyte percentage
8. Western blot
9. HIV rapid antibody test
10. HIV viral load tests (Table 6.9)
11. HIV enzyme-linked immunosorbent assay (ELISA) [11]

Table 6.9 Characteristics of Tests for Direct Detection of HIV

Test	**Technique**	**Sensitivity**[a]	**Cost/Test**[b]
Immune complex–dissociated p24 antigen capture assay	Measurement of levels of HIV-1 core protein in an EIA-based format following dissociation of antigen-antibody complexes by weak acid treatment	Positive in 50% of patients; detects down to 15 pg/mL of p24 protein	$1–2
HIV RNA by PCR	PCR amplification of cDNA generated from viral RNA (target amplification)	Reliable to 40 copies/mL of HIV RNA	$75–150
HIV RNA by bDNA	Measurement of levels of particle-associated HIV RNA in a nucleic acid capture assay employing signal amplification	Reliable to 50 copies/mL of HIV RNA	$75–150
HIV RNA by NucliSens	Isothermic nucleic acid amplification with internal controls	Reliable to 80 copies/mL of HIV RNA	$75–150

[a]Sensitivity figures refer to those approved by the US FDA. [b]Prices may be lower in large volume settings. ***Note:*** EIA, enzyme immunoassay; PCR, polymerase chain reaction. [10]

Treatment

Treatment for HIV infection can be divided into four categories:

1. Therapy for opportunistic infections and malignancies (Table 10)
2. Antiretroviral treatment (Table 6.11)

3. Hematopoietic stimulating factors
4. Prophylaxis of opportunistic infections. (11)

Table 6.10 Treatment of AIDS-related opportunistic infections and malignancies.[1]

Infection or Malignancy	**Treatment**	**Complications**
Pneumocystis jiroveci infection[2]	Trimethoprim-sulfamethoxazole, 15 mg/kg/d (based on trimethoprim component) orally or intravenously for 14–21 days.	Nausea, neutropenia, anemia, hepatitis, drug rash, Stevens-Johnson syndrome.
	Pentamidine, 3–4 mg/kg/d intravenously for 14–21 days.	Hypotension, hypoglycemia, anemia, neutropenia, pancreatitis, hepatitis.
	Trimethoprim, 15 mg/kg/d orally, with dapsone, 100 mg/d orally, for 14–21 days.[3]	Nausea, rash, hemolytic anemia in G6PD[3]-deficient patients. Methemoglobinemia (weekly levels should be < 10% of total hemoglobin).
	Primaquine, 15–30 mg/d orally, and clindamycin, 600 mg every 8 hours orally, for 14–21 days.	Hemolytic anemia in G6PD-deficient patients. Methemoglobinemia, neutropenia, colitis.
	Atovaquone, 750 mg orally three times daily for 14–21 days.	Rash, elevated aminotransferases, anemia, neutropenia.
	Trimetrexate, 45 mg/m^2 intravenously for 21 days (given with leucovorin calcium) if intolerant of all other regimens.	Leukopenia, rash, mucositis.
Mycobacterium avium complex infection	Clarithromycin, 500 mg orally twice daily with ethambutol, 15 mg/kg/d orally (maximum, 1 g). May also add:	Clarithromycin: hepatitis, nausea, diarrhea; ethambutol: hepatitis, optic neuritis.
	Rifabutin, 300 mg orally daily.	Rash, hepatitis, uveitis.
Toxoplasmosis	Pyrimethamine, 100–200 mg orally as loading dose, followed by 50–75 mg/d, combined with sulfadiazine, 4–6 g orally daily in four divided doses, and folinic acid, 10 mg daily for 4–8 weeks; then pyrimethamine, 25–50 mg/d, with clindamycin, 2–2.7 g/d in three or four divided doses, and folinic acid, 5 mg/d, until clinical and radiographic resolution is achieved.	Leukopenia, rash.
Lymphoma	Combination chemotherapy (eg, modified CHOP, M-BACOD, with or without G-CSF or GM-CSF). Central nervous system disease: radiation treatment with dexamethasone for edema.	Nausea, vomiting, anemia, leukopenia, cardiac toxicity (with doxorubicin).
Cryptococcal meningitis	Amphotericin B, 0.6 mg/kg/d intravenously, with or without flucytosine, 100 mg/kg/d orally in four divided doses for 2 weeks, followed by:	Fever, anemia, hypokalemia, azotemia.

	Fluconazole, 400 mg orally daily for 6 weeks, then 200 mg orally daily.	Hepatitis.
Cytomegalovirus infection	Valganciclovir, 900 mg orally twice a day for 21 days with food (induction), followed by 900 mg daily with food (maintenance).	Neutropenia, anemia, thrombocytopenia.
	Ganciclovir, 10 mg/kg/d intravenously in two divided doses for 10 days, followed by 6 mg/kg 5 days a week indefinitely. (Decrease dose for renal impairment.) May use ganciclovir as maintenance therapy (1 g orally with fatty foods three times a day).	Neutropenia (especially when used concurrently with zidovudine), anemia, thrombocytopenia.
	Foscarnet, 60 mg/kg intravenously every 8 hours for 10–14 days (induction), followed by 90 mg/kg once daily. (Adjust for changes in renal function.)	Nausea, hypokalemia, hypocalcemia, hyperphosphatemia, azotemia.
Esophageal candidiasis or recurrent vaginal candidiasis	Fluconazole, 100–200 mg orally daily for 10–14 days.	Hepatitis, development of imidazole resistance.
Herpes simplex infection	Acyclovir, 400 mg orally three times daily until healed; or acyclovir, 5 mg/kg intravenously every 8 hours for severe cases.	Resistant herpes simplex with chronic therapy.
	Famciclovir, 500 mg orally twice daily until healed.	Nausea.
	Valacyclovir, 500 mg orally twice daily until healed.	Nausea.
	Foscarnet, 40 mg/kg intravenously every 8 hours, for acyclovir-resistant cases. (Adjust for changes in renal function.)	See above.
Herpes zoster	Acyclovir, 800 mg orally four or five times daily for 7 days. Intravenous therapy at 10 mg/kg every 8 hours for ocular involvement, disseminated disease.	See above.
	Famciclovir, 500 mg orally three times daily for 7 days.	Nausea.
	Valacyclovir, 500 mg orally three times daily for 7 days.	Nausea.
	Foscarnet, 40 mg/kg intravenously every 8 hours for acyclovir-resistant cases. (Adjust for changes in renal function.)	See above.
Kaposi sarcoma		
Limited cutaneous disease	Observation, intralesional vinblastine.	Inflammation, pain at site of injection.
Extensive or aggressive cutaneous disease	Systemic chemotherapy (eg, liposomal doxorubicin). Interferon-alpha (for patients with CD4 > 200 cells/mcL and no constitutional symptoms). Radiation (amelioration of edema).	Bone marrow suppression, peripheral neuritis, flu-like syndrome.
Visceral disease (eg, pulmonary)	Combination chemotherapy (eg, daunorubicin, bleomycin, vinblastine).	Bone marrow suppression, cardiac toxicity, fever.

For treatment of *Mycobacterium tuberculosis* infection, , methotrexate, bleomycin, doxorubicin (Adriamycin), cyclophosphamide, vincristine (Oncovin), and dexamethasone; G-CSF, granulocyte-colony stimulating factor (filgrastim); GM-CSF, granulocyte-macrophage colony-stimulating factor (sargramostim). [11]

Table 6.11 Antiretroviral therapy.

Drug	Dose	Common Side Effects	Special Monitoring [1]	Cost [2]	Cost/Month
Nucleoside reverse transcriptase inhibitors					
Zidovudine (AZT) (Retrovir)	600 mg orally daily in two divided doses	Anemia, neutropenia, nausea, malaise, headache, insomnia, myopathy	No special monitoring	$6.08/300 mg	$365.09
Didanosine (ddI) (Videx)	400 mg orally daily (enteric-coated capsule) for persons ≥ 60 kg	Peripheral neuropathy, pancreatitis, dry mouth, hepatitis	Bimonthly neurologic questionnaire for neuropathy, K^+, amylase, bilirubin, triglycerides	$11.50/400 mg	$344.92
Zalcitabine (ddC) (Hivid)	0.375–0.75 mg orally three times daily	Peripheral neuropathy, aphthous ulcers, hepatitis	Monthly neurologic questionnaire for neuropathy	$2.73/0.75 mg	$245.70
Stavudine (d4T) (Zerit)	40 mg orally twice daily for persons ≥ 60 kg	Peripheral neuropathy, hepatitis, pancreatitis	Monthly neurologic questionnaire for neuropathy, amylase	$7.31/40 mg	$438.61
Lamivudine (3TC) (Epivir)	150 mg orally twice daily	Rash, peripheral neuropathy	No special monitoring	$6.45/150 mg	$386.93
Emtricitabine (Emtriva)	200 mg orally once daily	Skin discoloration palms/soles (mild)	No special monitoring	$12.30/200 mg	$368.93
Abacavir (Ziagen)	300 mg orally twice daily	Rash, fever—if occur, rechallenge may be fatal	No special monitoring	$8.67/300 mg	$519.92
Nucleotide reverse transcriptase inhibitors					
Tenofovir (Viread)	300 mg orally once daily	Gastrointestinal distress	Renal function	$20.47/300 mg	$614.18
Protease inhibitors (PIs)					
Indinavir (Crixivan)	800 mg orally three times daily	Kidney stones	Cholesterol, triglycerides, bilirubin level	$3.05/400 mg	$548.12
Saquinavir hard gel (Invirase)	1000 mg orally twice daily with 100 mg ritonavir orally twice daily	Gastrointestinal distress	Cholesterol, triglycerides	$6.58/500 mg	$789.70 (plus cost of ritonavir)
Ritonavir (Norvir)	600 mg orally twice	Gastrointestinal	Cholesterol	$10.29/100	$3,703.20

	daily or in lower doses (eg, 100 mg orally once or twice daily) for boosting other PIs	distress, peripheral Paresthesias	triglycerides	mg	($617.20 in lower doses)
Nelfinavir (Viracept)	750 mg orally three times daily or 1250 mg twice daily	Diarrhea	Cholesterol, triglycerides	$2.42/250 mg	$680.99
				$6.05/625 mg	$726.40
Amprenavir (Agenerase)	1200 mg orally twice daily	Gastrointestinal, rash	Cholesterol, triglycerides	$0.60/50 mg	$862.20
Fosamprenavir (Lexiva)	For PI-experienced patients: 700 mg orally twice daily and 100 mg of ritonavir 100 orally twice daily. For PI-naïve patients: above or 1400 mg orally twice daily or 1400 mg orally once daily and 200 mg of ritonavir orally once daily	Same as amprenavir	Same as amprenavir	$12.24/700 mg	$734.56–$1469.12(plus cost of ritonavir for lower dose)
Lopinavir/ritonavir (Kaletra)	400 mg/100 mg orally twice daily	Diarrhea	Cholesterol, triglycerides	$7.02/200 mg (lopinavir)	$841.90
Atazanavir (Reyataz)	400 mg orally once daily	Hyperbilirubinemia	Bilirubin level; when used with ritonavir: cholesterol and triglycerides	$16.46/200 mg	$987.41
Tipranavir/ritonavir (Aptivus/Norvir)	500 mg of tipranavir and 200 mg of ritonavir orally twice daily	Gastrointestinal, rash	Cholesterol, triglycerides	$8.94/250 mg (tipranavir)	$2307.20 (for combination)
				$10.29/100 mg (ritonavir)	
Darunavir/ritonavir (Prezista/Norvir)	600 mg of darunavir and 100 mg of ritonavir orally twice daily	Rash	Cholesterol, triglycerides	$ 7.50/300 mg (darunavir)	$1517.20 (for combination)
				$10.29/100 mg (ritonavir)	
Nonnucleoside reverse transcriptase inhibitors (NNRTIs)					
Nevirapine (Viramune)	200 mg orally daily for 2 weeks, then 200 mg orally twice daily	Rash	No special monitoring	$7.73/200 mg	$463.85
Delavirdine	400 mg orally three	Rash	No special	$1.69/200	$303.70

(Rescriptor)	times daily		monitoring	mg	
Efavirenz (Sustiva)	600 mg orally daily	Neurologic disturbances	No special monitoring	$17.70/600 mg	$531.04
Entry inhibitors					
Enfuvirtide (Fuzeon)	90 mg subcutaneously twice daily	Injection site pain and allergic reaction	No special monitoring	$38.90/90 mg	$2333.93
Maraviroc (Selzentry)	150–300 mg orally daily	Cough, fever, rash	No special monitoring	$17.40/150 mg or 300 mg	$1044.00
Integrase inhibitor					
Raltegravir (Isentress)	400 mg orally twice daily	Diarrhea, nausea, headache	No special monitoring	$16.20/400 mg	$972.00

[1]Standard monitoring is complete blood count (CBC) and differential, and serum aminotransferases. [2]Average wholesale price (AWP, for AB-rated generic when available) for quantity listed. Source: *Red Book Update, Vol. 27, No. 2*, February 2008. AWP may not accurately represent the actual pharmacy cost because wide contractual variations exist among institutions. (11)

Prevention

Primary prevention

1. Notification
2. Effective precautions regarding sexual practices
3. Precautions regarding injection drug use
4. Use of perinatal HIV prophylaxis
5. Screening of blood products
6. Infection control practices in the health care setting.
7. Primary care clinicians should routinely obtain a sexual history and provide risk factor assessment of their patients.
8. Because approximately one-fourth of the HIV-infected persons in the United States do not know they are infected, the CDC recommends all adults be routinely tested for HIV.
9. Prior to testing, clinicians should obtain informed consent and review the risk factors for HIV infection with the patient and discuss safer sex and safer needle use as well as the meaning of a positive test.
10. For persons whose test results are positive, information on available medical and mental health services should be

provided as well as guidance for contacting sexual or needle-sharing partners. [11]

Secondary prevention

In the era prior to the development of highly effective antiretroviral treatment, cohort studies of individuals with documented dates of seroconversion demonstrate that AIDS develops within 10 years in approximately 50% of untreated seropositive persons. With currently available treatment, progression of disease has been markedly decreased. In addition to antiretroviral treatment, prophylactic regimens can prevent opportunistic infections and improve survival. Prophylaxis and early intervention prevent several infectious diseases, including tuberculosis and syphilis, which are transmissible to others. Recommendations for screening tests, vaccinations, and prophylaxis are listed in Table 6.12.

Table 6.12 Health care maintenance of HIV-infected individuals. [11]
For all HIV-infected individuals:
CD4 counts every 3–6 months
Viral load tests every 3–6 months and 1 month following a change in therapy
PPD
INH for those with positive PPD and normal chest radiograph
RPR or VDRL
Toxoplasma IgG serology
Hepatitis serologies: hepatitis A antibody, hepatitis B surface antigen, hepatitis B surface antibody, hepatitis B core antibody, hepatitis C antibody.
Pneumococcal vaccine
Inactivated influenza vaccine in season
Hepatitis A vaccine for those without immunity to hepatitis A.
Hepatitis B vaccine for those who are hepatitis B surface antigen and antibody negative. (Use 40 mcg formulation at 0, 1, and 6 months; repeat if no immunity 1 month after three-shot series.)
Tetanus/diphtheria vaccine
Human papillomavirus vaccine for HIV-infected women age 26 years or less.
Haemophilus influenzae type b vaccination
Papanicolaou smears every 6 months for women
Consider anal swabs for cytologic evaluation.

For HIV-infected individuals with CD4 < 200 cells/mcL:
Pneumocystis jiroveci prophylaxis (see Prophylaxis of Opportunistic Infections section under Treatment and Table 31–6)
For HIV-infected individuals with CD4 < 75 cells/mcL:
Mycobacterium avium complex prophylaxis (see Prophylaxis of Opportunistic Infections section under Treatment)
For HIV-infected individuals with CD4 < 50 cells/mcL:
Consider CMV prophylaxis

PPD, purified protein derivative; INH, isoniazid; RPR, rapid plasma reagin; VDRL, Venereal Disease Research Laboratories; IgG, immunoglobulin G.

HIV Risk for Health Care Professionals:

Epidemiologic studies show that needle sticks occur commonly among health care professionals, especially among surgeons performing invasive procedures, inexperienced hospital house staff, and medical students. Efforts to reduce needle sticks should focus on avoiding recapping needles and use of safety needles whenever doing invasive procedures under controlled circumstances. The risk of HIV transmission from a needle stick with blood from an HIV-infected patient is about 1:300. The risk is higher with deep punctures, large inocula, and source patients with high viral loads. (11)

Prognosis

With improvements in therapy, patients are living longer after the diagnosis of AIDS. A population-based study conducted in Denmark found that HIV-infected persons at age 25 years without hepatitis C had a life expectancy of 39 additional years. Unfortunately, not all HIV-infected persons have access to treatment. Studies consistently show less access to treatment for blacks, the homeless, and injection drug users. In addition to access to treatment, sustaining lower mortality will require developing new treatments for patients in whom resistance to existing agents develops. For patients whose disease progresses even though they are receiving appropriate treatment, meticulous palliative care must be provided. (11)

Chapter Seven

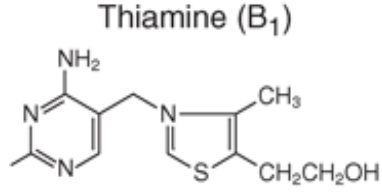
Vitamines Deficiency

- *Thiamine (Vitamin B_1)*
- *Niacin (Vitamin B_3)*
- *Pyridoxine (Vitamin B_6)*
- *Vitamin C (ascorbic acid)*

Thiamine (Vitamin B_1)

Definition

Thiamine was the first B vitamin to be identified and therefore is referred to as vitamin B1. (10)



Sources

Primary food sources for thiamine include:

1. Yeast
2. Organ meat
3. Pork
4. Legumes
5. Beef
6. Whole grains
7. Nuts
8. Milled rice and grains contain little thiamine (10) [1]

Requirements

Daily requirements

[1] Thiamine deficiency is therefore more common in cultures that rely heavily on a rice-based diet. Tea, coffee (regular and decaffeinated), raw fish, and shellfish contain thiaminases, which can destroy the vitamin. Thus, drinking large amounts of tea or coffee can theoretically lower thiamine body stores. (10)

1. Adult 0,5mg for each 1000 calories
2. Children
   - Infants 0,4mg
   - Preadolescents 1,3mg [(2)]
3. The median intake of thiamine in the United States from food alone is 2 mg/d. [(10)]

Absorption

Free thiamine is absorbed readily from the small intestine, but the pyrophosphate (ester-forme) is not. [(2)]

Metabolism

1. In tissues it is actively phosphorylated to form thiamine pyrophosphate (TPP) in the:
   - Liver
   - Muscle
   - Brain
   - Nucleated RBC
2. In plasma and CSF in the free forme 1 Mic.g/100 ml, blood cells contain 6 to 12 Mic.g/100ml as TPP.
3. Total amount of thiamine in the body is approx. 25mg.
4. If normal amount of thiamine is taken, it is excretd in urine. [(2)]

Metabolic role

1. Decarboxylation of alfi -ketoacids, such as pyruvate & alfa-ketoglutarate
2. Decarboxylation of branched-chain amino acids
3. Essential for energy
4. Acts as a coenzyme for a transketolase reaction that mediates the conversion of hexose and pentose phosphates
5. Thiamine plays a role in peripheral nerve conduction [(10)]

Deficiency

Most dietary deficiency of thiamine worldwide is the result of

1. Poor dietary intake
   In Western countries
2. Alcoholism
3. Chronic illnesses such as cancer
4. Tea
5. Coffee can theoretically lower thiamine body stores [(10)] [2]

Clinical features

Thiamine deficiency in its early stage induces:

1. Anorexia
2. Nonspecific symptoms (e.g., irritability, decrease in short-term memory).

Prolonged thiamine deficiency causes beriberi, which is classically categorized as wet or dry.

In either form of beriberi, patients may complain of pain and paresthesia.

Wet beriberi presents primarily with cardiovascular symptoms:

1. Enlarged heart
2. Tachycardia
3. Congestive heart failure
4. Peripheral edema
5. Peripheral neuritis [(10)]

Dry beriberi present with a symmetric peripheral neuropathy of the motor and sensory systems:

1. Diminished reflexes

[2] Thiamine should always be replenished when a patient with alcoholism is being refed, as carbohydrate repletion without adequate thiamine can precipitate acute thiamine deficiency with lactic acidosis. Other at-risk populations are women with prolonged hyperemesis gravidarum and anorexia, patients with overall poor nutritional status on parenteral glucose, patients after bariatric bypass surgery, and patients on chronic diuretic therapy due to increased urinary thiamine losses. Maternal thiamine deficiency can lead to infantile beriberi in breast-fed children. Thiamine deficiency should be considered in the setting of motor vehicle accidents associated with head injury. [(10)]

2. Difficulty in rising [10]

Alcoholic patients with chronic thiamine deficiency also may have CNS manifestations known as *Wernicke's encephalopathy*.[3]

When there is an additional loss of memory and a confabulatory psychosis, the syndrome is known as Wernicke-Korsakoff syndrome. [10]

Laboratory diagnosis

1. Empiric thiamine therapy is used to support a diagnosis [11]
2. A functional enzymatic assay of transketolase activity [4] [10]
3. Thiamine concentrations in blood or urine are also used [5]

Treatment

In acute thiamine deficiency with either cardiovascular or neurologic signs, 100 mg/d of thiamine should be given parenterally for 7 days, followed by 10 mg/d orally until there is complete recovery. Cardiovascular and ophthalmoplegic improvement occurs within 24 h. Other manifestations gradually clear, although psychosis in Wernicke-Korsakoff syndrome may be permanent or persist for several months. [10]

Toxicity

Anaphylaxis has been reported. [10]

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[3] *Wernicke's encephalopathy*, consisting of horizontal nystagmus, ophthalmoplegia (due to weakness of one or more extraocular muscles), cerebellar ataxia, and mental impairment. When there is an additional loss of memory and a confabulatory psychosis, the syndrome is known as *Wernicke-Korsakoff syndrome*. [10]

[4] The most effective measure of vitamin B_1 status is the RBC transketolase activity coefficient, which measures enzyme activity before and after addition of exogenous thiamine pyrophosphate: RBCs from a deficient individual express a substantial increase in enzyme activity with addition of thiamine pyrophosphate. [5]

Niacin (Vitamin B_3)

Definition

The term *niacin* refers to nicotinic acid and nicotinamide and their biologically active derivatives. (10)

Sources

Niacin bioavailability is high from:

1. Beans
2. Milk
3. Meat
4. Eggs (10)

Requirements

Daily requirements

1. Adult 17 to 21 mg daily
2. Infants 6 mg
3. Pre-adolescents 17 mg (2)

Absorption

Nicotinic acid and nicotinamide are absorbed well from the stomach and small intestine. (10)

Metabolism

1. Blood and plasma levesl

- Whole blood 0,2-0,9mg/100 ml
- RBC 1,3 mg %
- Plasma total activity 0,075mg %

2. It is excretd in urine [(2)]

Metabolic role

1. The coenzymes, NAD and NADP which are important in oxidation and reduction
2. Involved in DNA repair
3. Calcium mobilization [(10)] [1]

Deficiency

1. Niacin deficiency causes *pellagra*,
2. Among people eating corn-based diets in:
   - China
   - Africa
   - India
3. In North America is found mainly among alcoholics
4. Congenital defects of intestinal and kidney absorption of tryptophan (Hartnup disease)
5. Carcinoid syndrome (increased conversion of tryptophan to serotonin) [(10)]

Clinical features

The early symptoms of pellagra

1. Loss of appetite
2. Generalized weakness
3. Irritability
4. Abdominal pain

[1] Nicotinic acid and nicotinamide serve as precursors of two coenzymes, nicotinamide adenine dinucleotide (NAD) and NAD phosphate (NADP), which are important in numerous oxidation and reduction reactions in the body. In addition, NAD and NADP are active in adenine diphosphate–ribose transfer reactions involved in DNA repair and calcium mobilization. [(10)]

5. Vomiting
6. Bright red glossitis [H]

Followed by

7. Characteristic skin rash that is: (Figure 7.3)
    - Pigmented
    - Scaling
    - Particularly in skin areas exposed to sunlight
8. *Casal's necklace* (a ring around the neck) (Figure 7.2)
9. Vaginitis
10. Esophagitis
11. Diarrhea[2]
12. Depression
13. Seizures
14. Dementia[3] [10]

Pellagra syndrome (4Ds)

Pellagra (*pellis*, "skin"; *agra*, "rough") (Figure 7.1) [9]

1. Dermatitis
2. Diarrhea
3. Dementia
4. Death (leading to) [10]

[2] In part due to proctitis and in part due to malabsorption
[3] Are also part of the pellagra syndrome

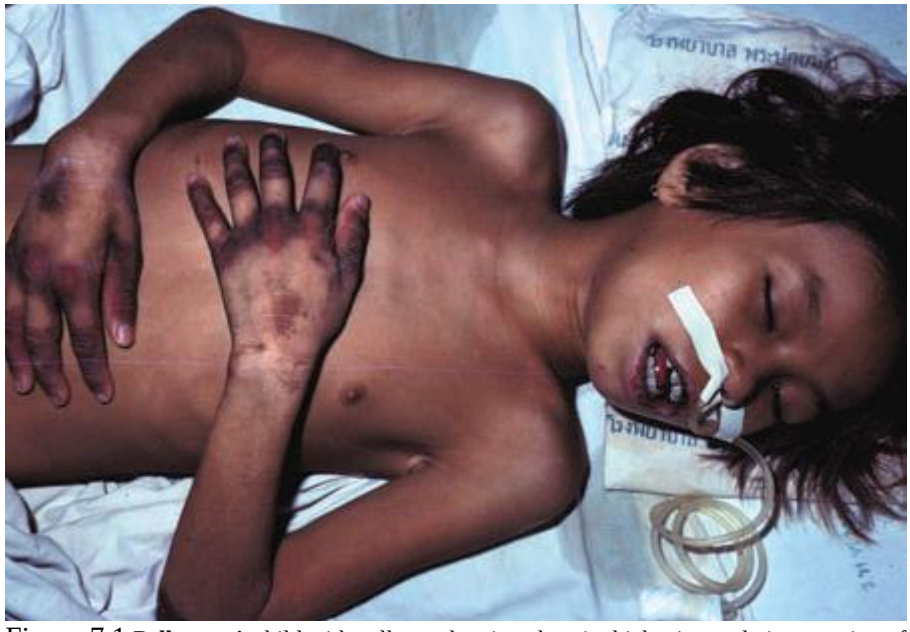


Figure 7.1 **Pellagra.** A child with pellagra showing chronic thickening and pigmentation of the skin, particularly on the backs of hands. Courtesy of Professor David Warrell. (8)

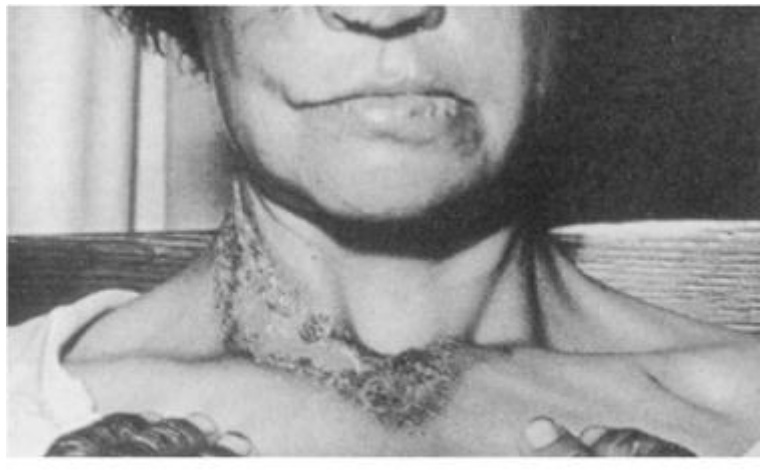


Figure 7.2 **Pellagra** showing an early lesion on the neck (Casal necklace). [9]

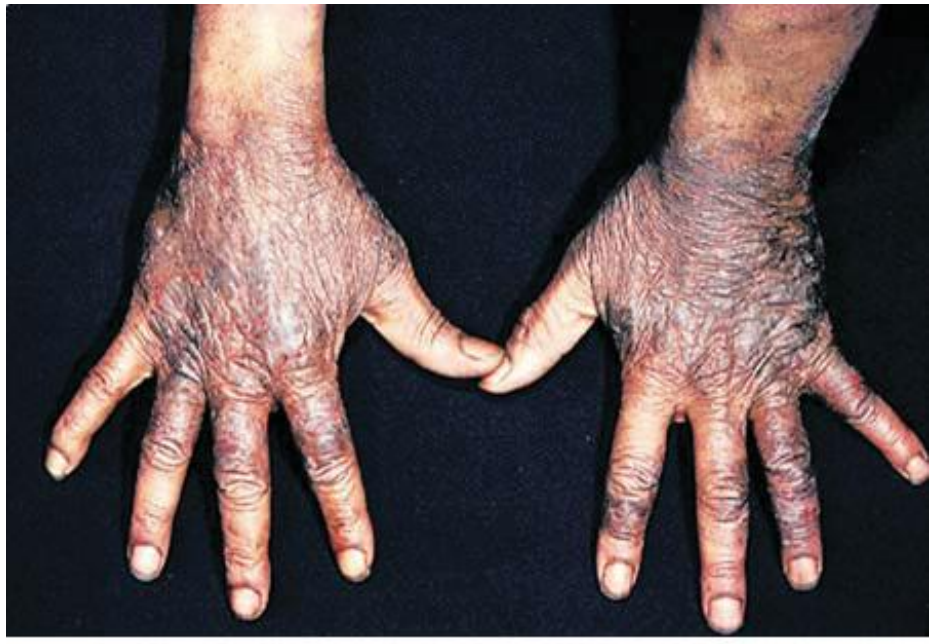


Figure 7.3 **"Gauntlet" of pellagra(niacin deficiency).** Indurated, lichenified, pigmented, and scaly skin on the dorsa of the hands. (***Source: K Wolff, RA Johnson, D Suurmond: Fitzpatrick's Color Atlas & Synopsis of Clinical Dermatology, 5th ed. New York, McGraw-Hill, 2005, www.accessmedicine.com.***) [(10)]

Laboratory Investigations

The urinary excretion products of niacin include 2-pyridone and 2-methyl nicotinamide, measurements of which are used in the diagnosis of niacin deficiency. [(10)]

Treatment

1. 100–200 mg of nicotinamide TID for 5 days
2. High doses of nicotinic acid (2 g/d in a time-release form) are used for the treatment of elevated cholesterol and triglyceride

levels and/or a low high-density lipoprotein (HDL) cholesterol level. [10]

Toxicity

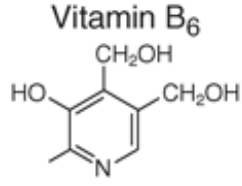
1. Flushing always of face and may be accompanied by:
   - Skin dryness
   - Itching
   - Paresthesia
   - Headache
2. Nausea
3. Vomiting
4. Abdominal pain [10]

Pyridoxine (Vitamin B_6)

Definition

Vitamin B6 refers to a family of compounds that include:

- Pyridoxine
- Pyridoxal
- Pyridoxamine
- Their 5'-phosphate derivatives (10)



Sources

1. Plants contain vitamin B6 in the form of pyridoxine
2. Animal tissues contain PLP and pyridoxamine phosphate[1]
3. Rich food sources:
   - Legumes
   - Nuts
   - Wheat bran
   - Meat (10)

Requirements

Daily requirements

1. Adult 2 mg daily
2. Infants 0.3-0,4 mg daily
3. During second half of pregnancy 2,5 mg daily (2) (Table 7.1)

[1] The vitamin B_6 contained in plants is less bioavailable than that in animal tissues. (H)

Table 7.1 Dietary Reference Intakes: Recommended Intakes for Individuals—Vitamins												
Life-Stage Group	**Vitamin, Mg/d M(Micro)**					**Thiamine, mg/d**	**Riboflavin, mg/d**	**Niacin, mg/d[e]**	**Vitamin B_6, mg/d**	**Folate, Mg/d[f]**	**Vitamin B_{12}, Mg/d**	**Pantothenic Acid, mg/d**
	A[a]	**C**	**D[b,c]**	**E[d]**	**K**							
Infants												
0–6 mo	400	40	5	4	2.0	0.2	0.3	2	0.1	65	0.4	1.7
712 mo	500	50	5	5	2.5	0.3	0.4	4	0.3	80	0.5	1.8
Children												
1–3 y	**300**	**15**	5	**6**	30	**0.5**	**0.5**	**6**	**0.5**	**150**	**0.9**	2
4–8 y	**400**	**25**	5	**7**	55	**0.6**	**0.6**	**8**	**0.6**	**200**	**1.2**	3
Males												
9–13 y	**600**	**45**	5	**11**	60	**0.9**	**0.9**	**12**	**1.0**	**300**	**1.8**	4
14–18 y	**900**	**75**	5	**15**	75	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**	5
19–30 y	**900**	**90**	5	**15**	120	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**	5
31–50 y	**900**	**90**	5	**15**	**120**	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**	5
51–70 y	**900**	**90**	10	**15**	120	**1.2**	**1.3**	**16**	**1.7**	**400**	**2.4[h]**	5
>70 y	**900**	90	**15**	**15**	120	**1.2**	**1.3**	**16**	**1.7**	**400**	**2.4[h]**	5
Females												
9–13 y	**600**	**45**	5	**11**	60	**0.9**	**0.9**	**12**	**1.0**	**300**	**1.8**	4
14–18 y	**700**	**65**	5	**15**	75	**1.0**	**1.0**	**14**	**1.2**	**400[i]**	**2.4**	5
19–30 y	**700**	**75**	5	**15**	90	**1.1**	**1.1**	**14**	**1.3**	**400[i]**	**2.4**	5
31–50 y	**700**	**75**	5	**15**	90	**1.1**	**1.1**	**14**	**1.3**	**400[i]**	**2.4**	5
51–70 y	**700**	**75**	10	**15**	90	**1.1**	**1.1**	**14**	**1.5**	**400**	**2.4[h]**	5
>70 y	**700**	**75**	15	**15**	90	**1.1**	**1.1**	**14**	**1.5**	**400**	**2.4[h]**	5
Pregnancy												
18 y	**750**	**80**	5	**15**	75	**1.4**	**1.4**	**18**	**1.6**	**600[j]**	**2.6**	6
19–30 y	**770**	**85**	5	**15**	90	**1.4**	**1.4**	**18**	**1.9**	**600[j]**	**2.6**	6
31–50 y	**770**	**85**	5	**15**	90	**1.4**	**1.4**	**18**	**1.9**	**600[j]**	**2.6**	6
Lactation												
18 y	**1200**	**115**	5	**19**	75	**1.4**	**1.6**	**17**	**2.0**	**500**	**2.8**	7
19–30 y	**1300**	**120**	5	**19**	90	**1.4**	**1.6**	**17**	**2.0**	**500**	**2.8**	7
31–50 y	**1300**	**120**	5	**19**	90	**1.4**	**1.6**	**17**	**2.0**	**500**	**2.8**	7

Note: This table presents recommended dietary allowances (RDAs) in **bold type** and adequate intakes (AIs) in ordinary type. RDAs and AIs may both be used as goals for individual intake. RDAs are set to meet the needs of almost all individuals (97 to 98%) in a group. For healthy breastfed infants, the AI is the mean intake. The AI for other life stage and gender groups is believed to cover needs of all individuals in the group, but lack of data or uncertainty in the data prevent being able to specify with confidence the percentage of individuals covered by this intake. [(10)]

Absorption

Dietery vitamin B_6 is readily absorbed by the intestine. [2]

Metabolism

Pyridoxine and pyridoxamine are excreted in urin in small amounts 0,5 to 0,7 mg daily

Majority urinary metabolite (3mg/d) is the inactive form 4-pyridoxic acid. [2]

Metabolic role

5'-Pyridoxal phosphate (PLP) is a cofactor for more than 100 enzymes involved in amino acid metabolism.

Vitamin B_6 also is involved in:

2. The synthesis of:
    - Heme
    - Neurotransmitter
3. Metabolism of:
    - Glycogen
    - Lipids
    - Steroids
    - Sphingoid bases
    - Several vitamins, including the conversion of tryptophan to niacin. [10]

Deficiency

Certain medications interact with PLP such as:

1. Isoniazid
2. l-dopa
3. Penicillamine
4. Cycloserine [10]

Severe vitamin B_6 deficiency can lead to:

1. Peripheral neuropathy

2. Abnormal electroencephalograms
3. Personality changes that include depression and confusion
4. Microcytic hypochromic anemia
5. Platelet dysfunction [10]

Clinical features

1. Seborrhea
2. Glossitis
3. Convulsions
4. Neuropathy
5. Depression
6. Confusion
7. Microcytic anemia [10]

Laboratory diagnosis

Low plasma PLP values (<20 nmol/L). [10]

Many useful laboratory methods of assessment exist. The plasma or RBC PLP levels are most common. Urinary excretion of xanthurenic acid after an oral tryptophan load and activity indices of RBC alanine or aspartic acid transaminases are functional measures of vitamin B_6–dependent enzyme activity. [5]

Treatment

Treatment of vitamin B_6 deficiency is done with 50 mg/d; higher doses of 100–200 mg/d. [10]

Toxicity

When toxicity occurs, it causes:

1. Severe sensory neuropathy
2. Patients unable to walk
3. Photosensitivity
4. Dermatitis [10]

Vitamin C

Definition

Both ascorbic acid and its oxidized product dehydroascorbic acid are biologically active. [10]

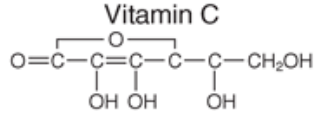


Figure 7.4 **Citrus fruits**

Dietary Sources

Good dietary sources:

1. Citrus fruits (Figure 7.4, 7.5)
2. Green vegetables
3. Tomatoes

4. Potatoes (10)

Figure 7.5 **Sources of vitamin C**

Requirements

Daily requirements

1. Adult 75 mg daily
2. Infants 30 mg daily
3. Adolescents 80 mg daily
4. Pregnant women 100 mg daily
5. Lactating women 150 mg daily [(2)]

Absorption

Almost complete absorption of vitamin C occurs if <100 mg is administered in a single dose; however, only 50% or less is absorbed at doses >1 g. [(10)]

Ascorbic acid is rapidly absorbed from the small intestine. [(12)]

Metabolism

1. The plasma concentration (5% as dehydroascorbate)
2. Dietary intake are in 80 μmol/litre or 100 mg/day which plateaus at 1000 mg/day intake
3. The body pool size is 900 mg
4. 3%, of pool size, is degraded each day and excreted in the urine as:
   - Free ascorbic acid
   - Dehydroascorbate
   - Diketogulonate [(12)]

Metabolic role and function

Actions of vitamin C include:

1. Antioxidant activity
2. Promotion of nonheme iron absorption
3. Carnitine biosynthesis
4. Conversion of dopamine to norepinephrine
5. Synthesis of many peptide hormones
6. Connective tissue metabolism
7. Cross-linking (proline hydroxylation)

8. Many drug-metabolizing enzyme systems (10)

Deficiency

Vitamin C deficiency causes scurvy.

Causes of scurvy:

1. Poverty
2. Elder age
3. Alcoholics
4. Severely unbalanced diets

Clinical features

Symptoms of scurvy:

1. Generalized fatigue
2. Bleeding into skin[1]:
   - Petechiae
   - Ecchymoses
3. Inflamed and bleeding gums
4. Bleeding into:
   - joints
   - Peritoneal cavity
   - Pericardium
   - Adrenal glands (10)

[1] Impaired to mature connective tissue and causes bleeding into skin

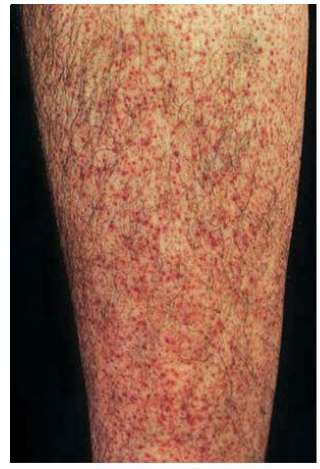


Figure 7.6 **Scurvy (vitamin C deficiency)** Perifollicular hemorrhage on the leg. The follicles are often plugged by keratin (perifollicular hyperkeratosis). This eruption occurred in a 46-year-old alcoholic, homeless male who also had bleeding gums and loose teeth. (10)

Laboratory diagnosis

On the basis of low plasma or leukocyte levels (10)

Plasma ascorbic acid concentration reflects recent dietary intake, whereas WBC levels more closely reflect tissue stores. Women's plasma levels are approximately 20% higher than men's for any given dietary intake. (5)

Treatment

Administration of vitamin C (200 mg/d) improves the symptoms of scurvy within a matter of several days.

High-dose vitamin C supplementation (e.g., 1–2 g/d) may slightly decrease the symptoms and duration of upper respiratory tract infections. (10)

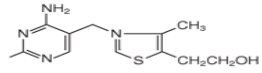
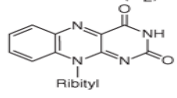
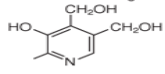
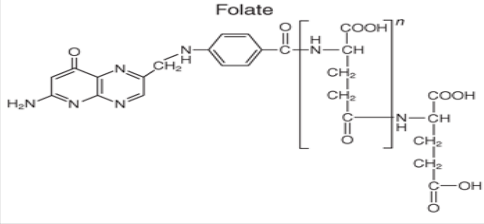
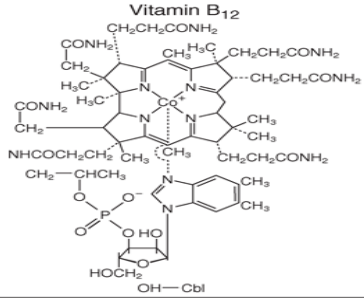
Toxicity

Taking >2 g of vitamin C in a single dose may result in:

1. Abdominal pain
2. Diarrhea

3. Nausea
4. Kidney stones [10]

Table 7.2 water soluble vitamins and their function

Vitamin	Active derivative or cofactor form	Principal function
Thiamine (B_1) 	Thiamine pyrophosphate	Coenzyme for cleavage of carbon-carbon bonds; amino acid and carbohydrate metabolism
Riboflavin (B_2) 	Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD)	Cofactor for oxidation, reduction reactions, and covalently attached prosthetic groups for some enzymes
Niacin 	Nicotinamide adenine dinucleotide phosphate (NADP) and nicotinamide adenine dinucleotide (NAD)	Coenzymes for oxidation and reduction reactions
Vitamin B_6 	Pyridoxal phosphate	Cofactor for enzymes of amino acid metabolism
Folate 	Polyglutamate forms of (5, 6, 7, 8) tetrahydrofolate with carbon unit attachments	Coenzyme for one carbon transfer in nucleic acid and amino acid metabolism
Vitamin B_{12} 	Methylcobalamine Adenosylcobalamin	Coenzyme for methionine synthase and L-methylmalonyl-CoA mutase

Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com

Chapter Eight

Hemoglobinopathies

- *Hemoglobinopathies*
- *a Thalassemia (4 Gene deletion)*
- *a Thalassemia (3 Gene deletion)*
- *or Hemoglobin H disease*
- *a Thalassemia minor and silent carrier*
- *b Thalassemia*
- *Hemoglobin C*
- *Hemoglobin constant spring syndrome*
- *Hemoglobin D*
- *Hemoglobin E*
- *Hemoglobin E/b Thalassemia*
- *Hemoglobin lepore syndrome*
- *Hereditary persistence fetal hemoglobin*
- *Sickle-cell (Hemoglobin S) disease*
- *Hemoglobin S/b Thalassemia*
- *Hemoglobin S/C disease*
- *Unstable hemoglobins*

Hemoglobinopathies

These diseases are caused by dysfunction of the genes encoding the globin chains of the haemoglobin molecule. (Figure 8.1) [3]

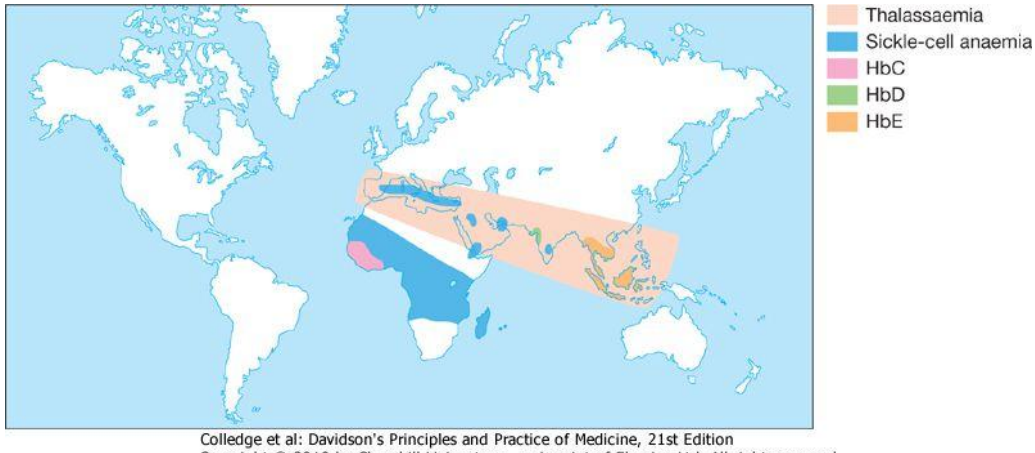


Figure 8.1 **The geographical distribution of the haemoglobinopathies.**

a Thalassemia (4 Gene deletion)

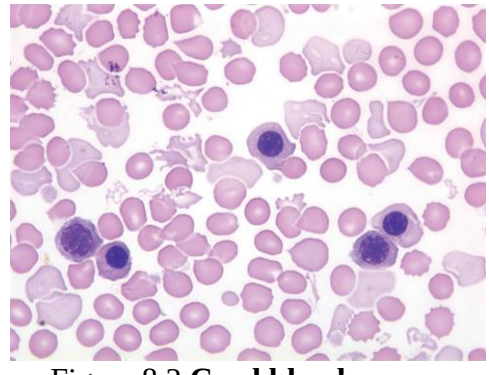


Figure 8.2 **Cord blood smear.**

Pathology

1. Parents are usually heterozygous for a thalassemia (– –/aa)
2. No a chains are produced (– –/– –)
3. Free g chains form tetrads, producing hemoglobin
4. Bart's hemoglobin
5. Excess b chains form tetramers and inclusions in red blood cells, which shortens survival [1]

Clinical Features

1. Produces hydrops fetalis, and affected infants are either stillborn or die within hours of birth
2. Marked hepatosplenomegaly
3. Marked anemia [1]

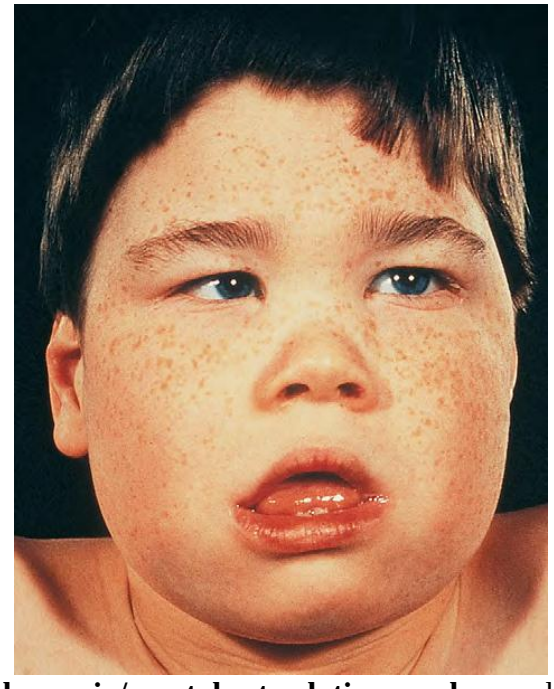


Figure 8.3 ***a*-Thalassemia/mental retardation syndrome**: boy with characteristic dysmorphic facies. (Courtesy of Professor D. R. Higgs.) [7]

Laboratory Features

White Blood Cells

Not remarkable

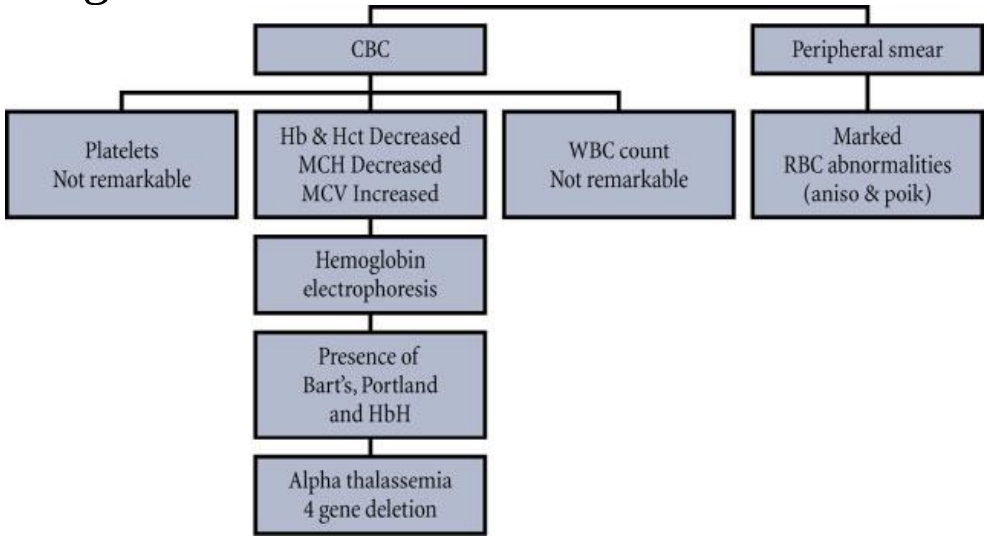
Platelets

Not remarkable

Red Blood Cells

1. Macrocytic/hypochromic anemia (Figure 8.2)
2. Mean corpuscular hemoglobin level decreased
3. Mean corpuscular volume increased
4. Increased nucleated red blood cells
5. 80–90% Bart's hemoglobin
6. 10–20% Portland hemoglobin
7. Trace of hemoglobin H [1]

Diagnostic Scheme



a Thalassemia (3 Gene deletion) or Hemoglobin H disease

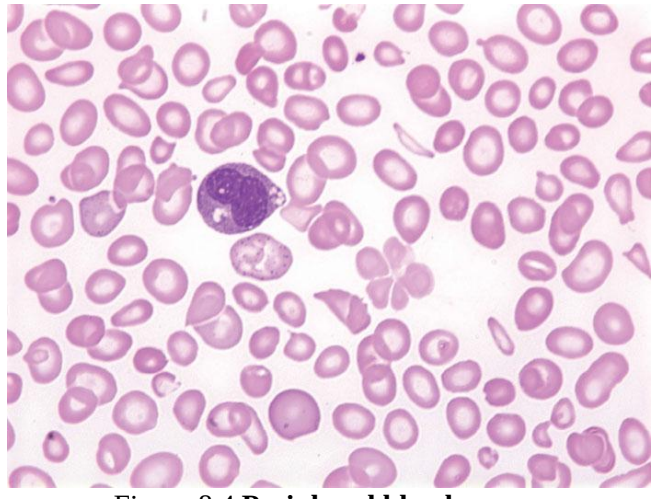


Figure 8.4 **Peripheral blood smear.**

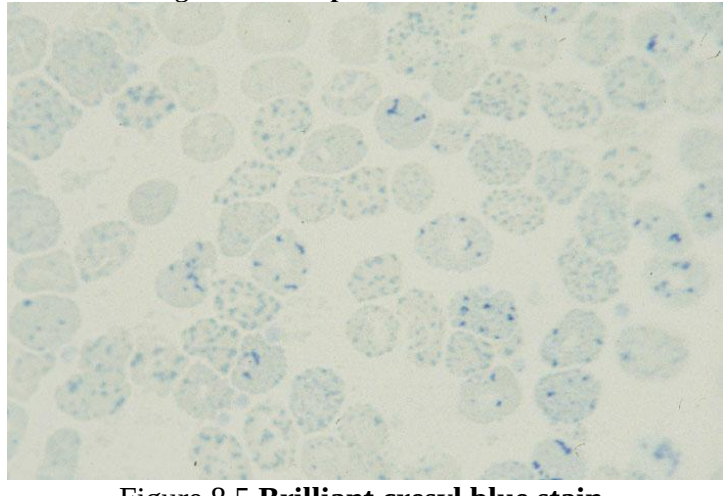


Figure 8.5 **Brilliant cresyl blue stain.**

Pathology

1. Deletion of three a genes (– –/–a)
2. Reduced hemoglobin A and thus oxygen delivery
3. Excess unpaired b chains are present and form unstable tetramers (b4)
4. (hemoglobin H)
5. Hemoglobin H has high oxygen affinity, resulting in decreased oxygen
6. delivery
7. Tetramers can cause disturbances in red blood cell metabolism, membrane
8. function, and deformability, resulting in chronic hemolytic anemia [(1)]

Clinical Features

1. Splenomegaly
2. Variable anemia, more severe during pregnancy, infections, and exposure to oxidant drugs [(1)]

Laboratory Features

White Blood Cells

Not remarkable

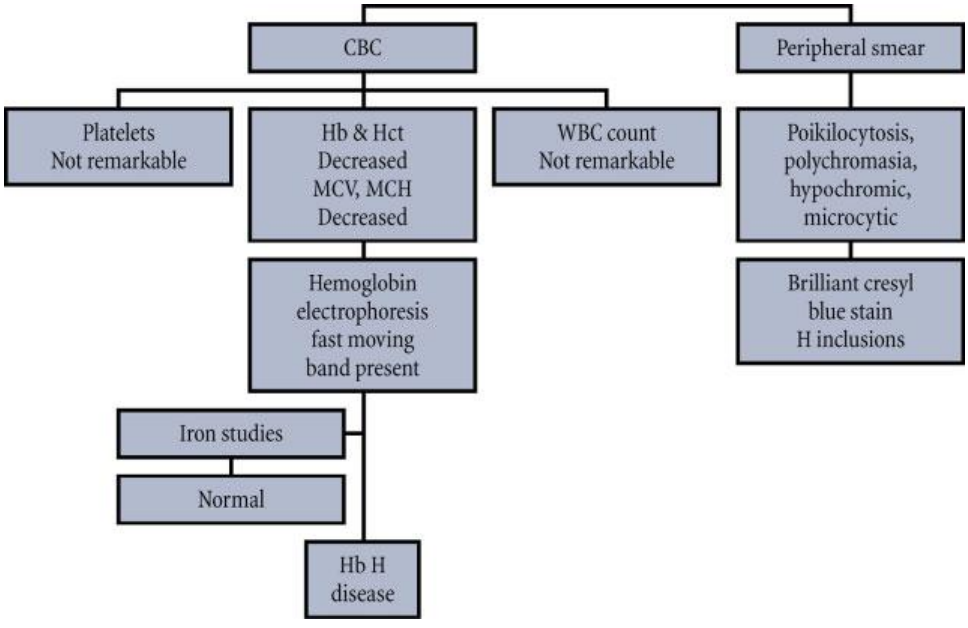
Platelets

Not remarkable

Red Blood Cells

1. Hemoglobin level, 8.0–10.0 g/dL
2. Reticulocyte count, 5–10% of red blood cells
3. Microcytic/hypochromic anemia
4. Increased red blood cell distribution width
5. Poikilocytosis
6. Polychromasia (Figure 8.4) [(1)]

Diagnostic Scheme



Treatment

1. Folic acid supplementation
2. Transfusion if required
3. Avoidance of iron therapy [3]

a Thalassemia minor and silent carrier

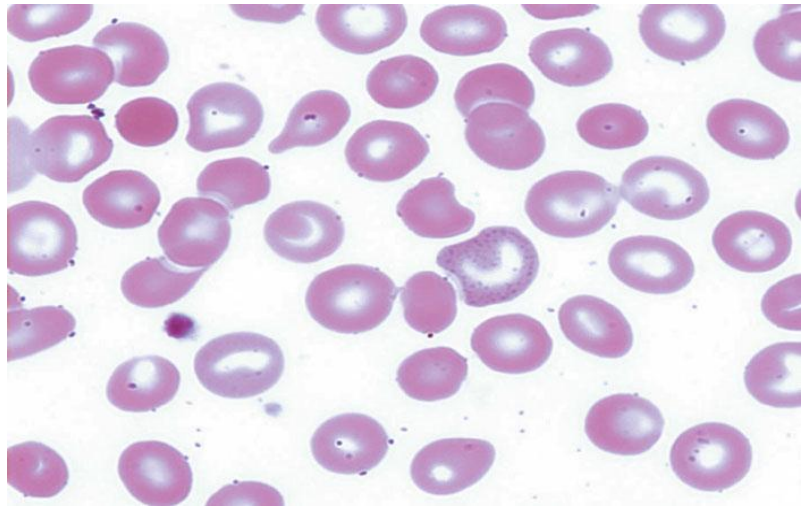


Figure 8.6 **Peripheral blood smear.**

Pathology

Minor or Trait

1. Decreased production of a-globin chains
2. Exists in two forms:
3. Heterozygous a0 thalassemia (– –/aa)
4. Homozygous a+ thalassemia (-a/-a)
5. Both forms are common in Southeast Asians, Chinese, and Filipinos
6. Homozygous form is common in African Americans (about 3%)

Silent Carrier

1. Heterozygous a+ (-a/aa) is common in Southeast Asians, Chinese, and Filipinos

2. About 28% of African Americans have the heterozygous a+ thalassemia [1]

Clinical Features

No clinical disease is seen with either the trait or the silent carrier [1]

Laboratory Features

Minor or Trait

White Blood Cells

Not remarkable

Platelets

Not remarkable

Red Blood Cells

1. Microcytic and slightly hypochromic anemia
2. Poikilocytosis
3. Codocytes (Figure 8.6)
4. Normal or slightly increased red blood cell distribution width
5. Hemoglobin H inclusions may occasionally be found in brilliant cresyl blue
6. (BCB) prep
7. 5–15% Bart's hemoglobin in cord blood (normal after about age 3 months) [1]

Silent Carrier

White Blood Cells

Not remarkable

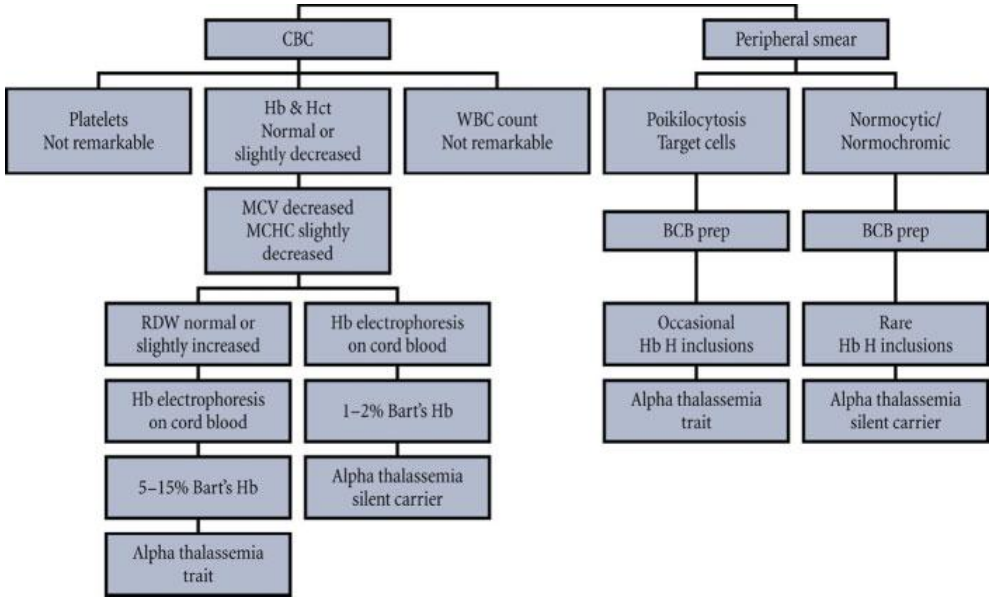
Platelets

Not remarkable

Red Blood Cells

1. No hematologic manifestations
2. 1–2% Bart's hemoglobin found at birth
3. Rare hemoglobin H inclusion found in BCB prep [1]

Diagnostic Scheme



b Thalassemia

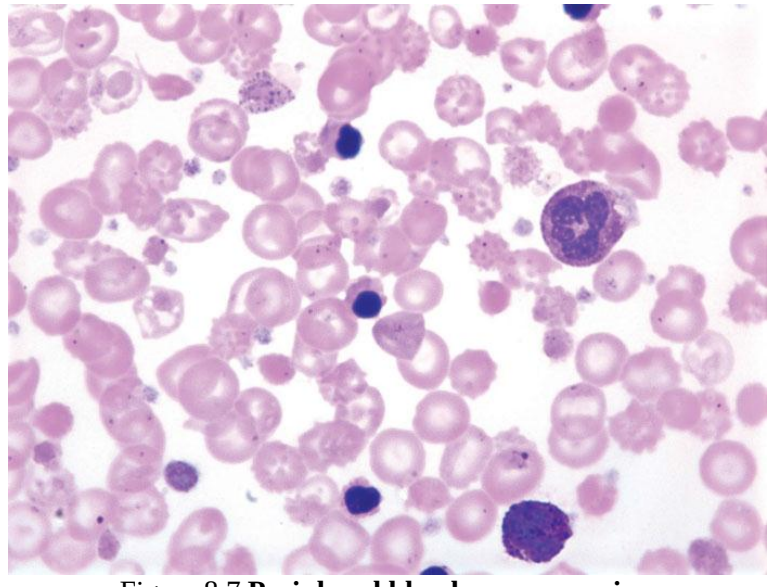


Figure 8.7 **Peripheral blood smear—major.**

Figure 8.8 **Peripheral blood smear—intermedia.**

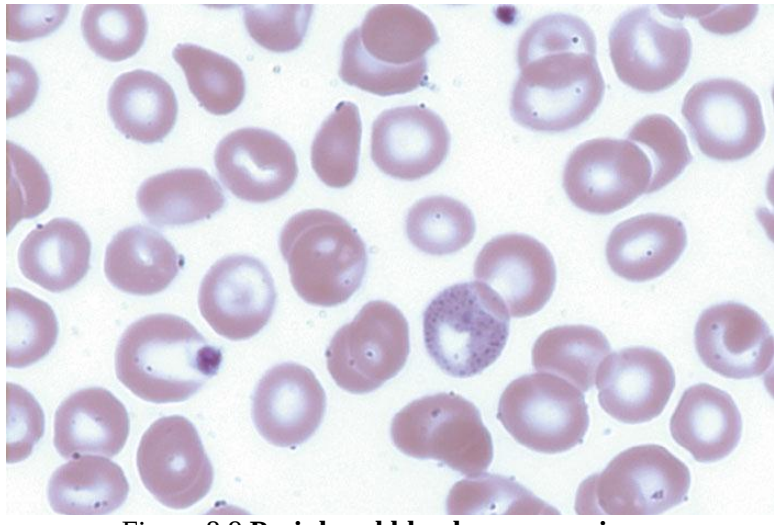


Figure 8.9 **Peripheral blood smear—minor.**

Pathology

1. *b* chain production is absent or diminished—the more b chain produced, the less severe the disease
2. Unmatched a chains accumulate and aggregate
3. Ineffective erythropoiesis
4. Chronic hemolytic process
5. Decreased erythrocyte hemoglobin production [(1)]

Clinical Features

Major variant

1. Severe anemia
2. Transfusion dependent
3. Growth retardation
4. Massive hepatosplenomegaly (Figure 8.13, 8.14)
5. Severe ineffective erythropoiesis
6. Early death with iron overload

Intermedia variant

7. Moderate anemia
8. Splenomegaly

9. Moderate, ineffective erythropoiesis

Minor variant

10. Mild anemia
11. Usually no symptoms [1]

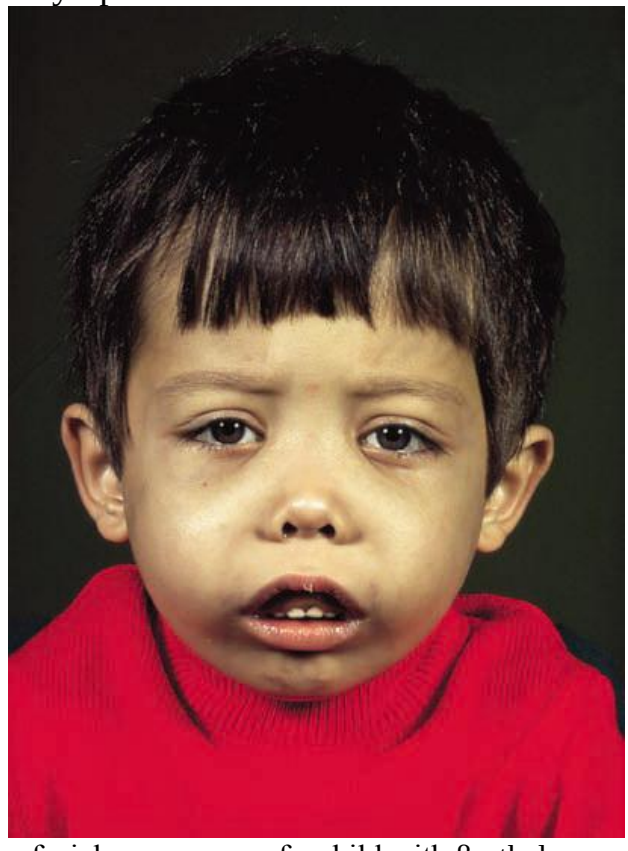


Figure 8.10 The facial appearance of a child with β - thalassaemia major. The skull is bossed with prominent frontal and parietal bones; the maxilla is enlarged. [7]

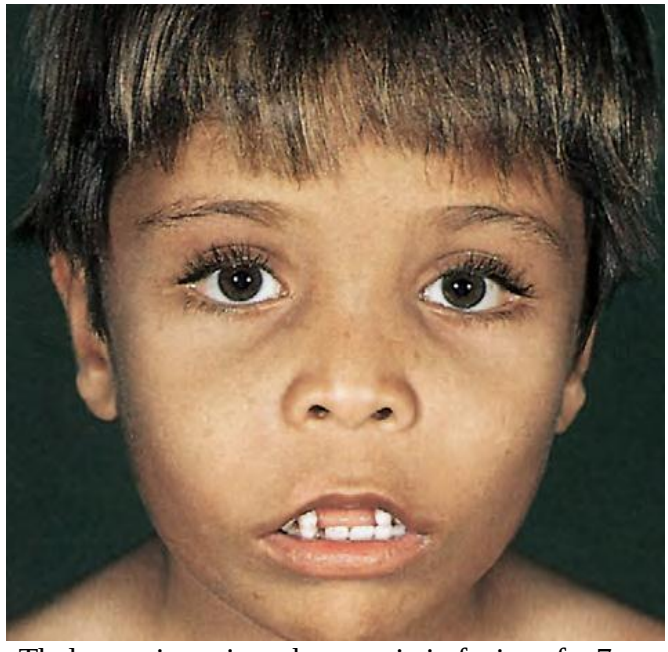


Figure 8.11 *b*-Thalassemia major: characteristic facies of a 7-year-old Middle Eastern boy include prominent maxilla and widening of the bridge of the nose. There is also marked bossing of the frontal and parietal bones and zygomata, giving a mongoloid appearance. (7)

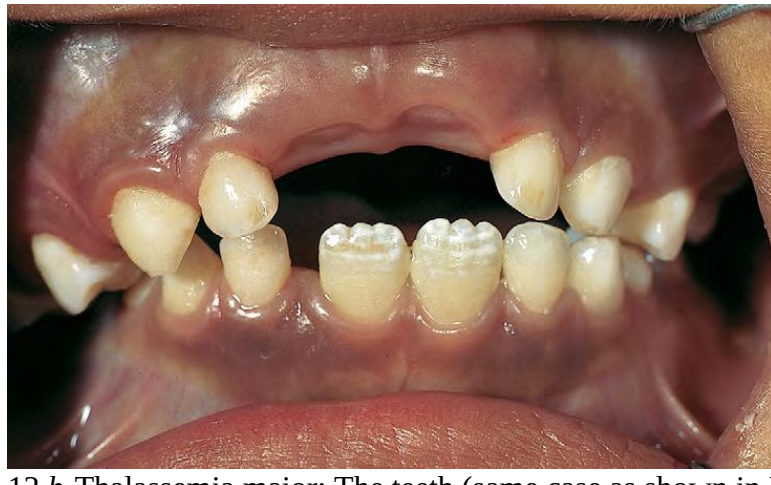


Figure 8.12 *b*-Thalassemia major: The teeth (same case as shown in Fig. 9-9) are splayed because of widening of the maxilla and mandible. (7)

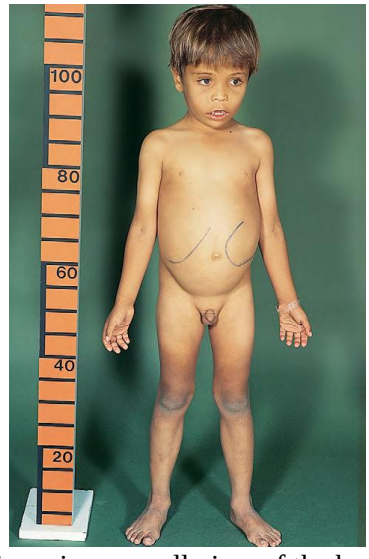


Figure 8.13 *b*-Thalassemia major: overall view of the boy shown in Fig. 9-9, showing enlargement of the liver and spleen, and stunted growth. The child had been inadequately transfused since presenting with anemia at age 4 months. [7]

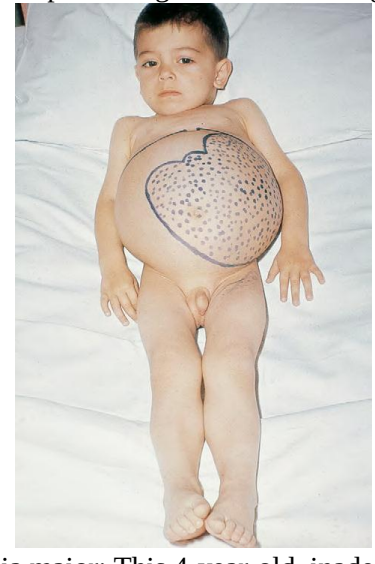


Figure 8.14 *b*-Thalassemia major: This 4-year-old, inadequately transfused Cypriot boy has enlargement of the spleen to an unusual degree, which may be partly reversed by adequate transfusion. Splenectomy is usually required but should usually be delayed until the child is older than 6 years of age to reduce the incidence of postoperative fatal infection. [7]

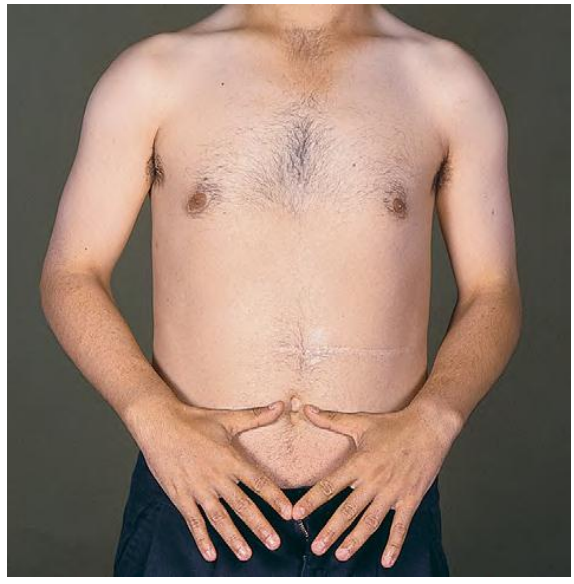


Figure 8.15 *b*-Thalassemia major: shortening of the upper arms because of premature epiphyseal closure of the humeral heads. [7]

Figure 8.16 *b*-Thalassemia major: tetany (Trousseau's sign) as a result of hypoparathyroidism caused by transfusional iron overload. An infusion of calcium is in progress. (Courtesy of Dr. B. Wonke.) [7]

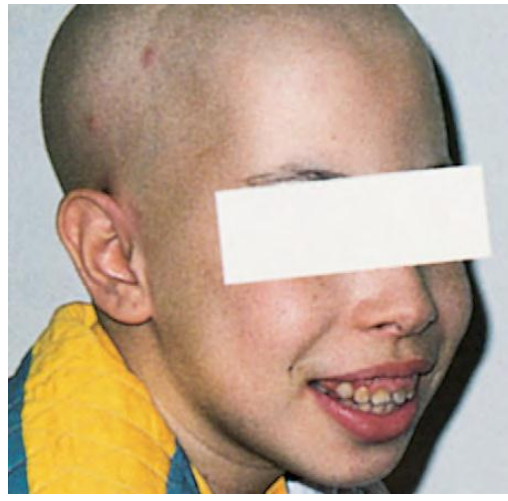
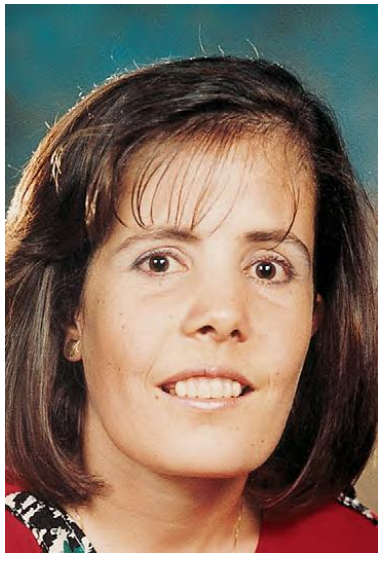


Figure 8.17 *b*-Thalassemia major: A 14-year-old girl, after marrow transplantation from a human leukocyte antigen (HLA)–matched sibling, shows hair loss as a result of chemotherapy and bossing of the skull. (Courtesy of Professor C. Luccarelli.) [7]



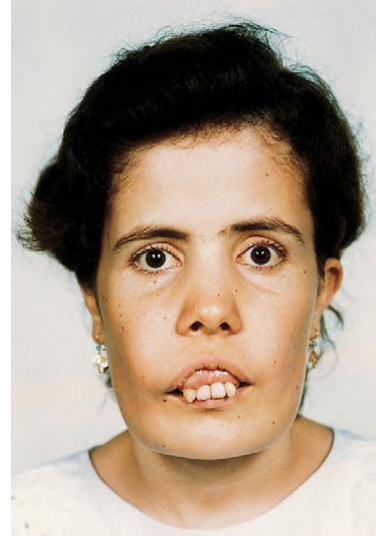


Figure 8.18 *b*-Thalassemia intermedia: facial bone deformities in a 20-year-old woman. A, Before surgery. B, After surgical correction. (A, B, Courtesy Dr. B. Wonke.) [7]

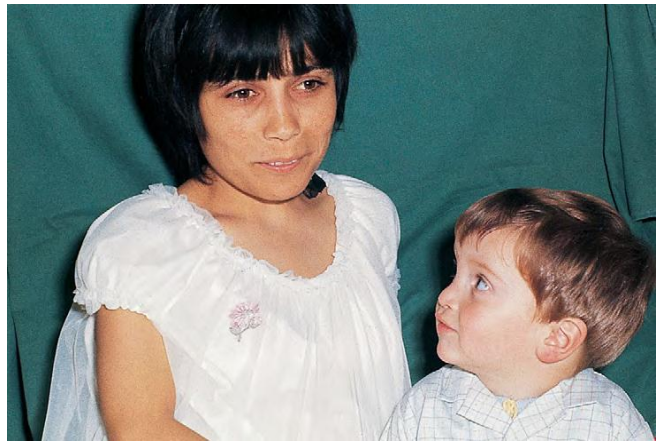


Figure 8.19 *b*-Thalassemia intermedia: This 29-year-old Cypriot patient received occasional blood transfusions, with her hemoglobin ranging between 6.5 and 9.0 g/dl. She displays a thalassemic facies with marked maxillary expansion and also developed pigment gallstones. She has normal sexual development and fertility, as shown by her 2-year-old son. [7]

Laboratory Features

White Blood Cells

Not remarkable

Platelets

Not remarkable

Red Blood Cells

1. Nucleated red blood cells (found in major and intermedia)
2. Hypochromic/microcytic anemia
3. Target cells present
4. Distorted cells
5. Basophilic stippling(Figure 8.7)
6. Normal to increased red blood cell distribution width
7. Increased red blood cell count relative to hemoglobin and hematocrit

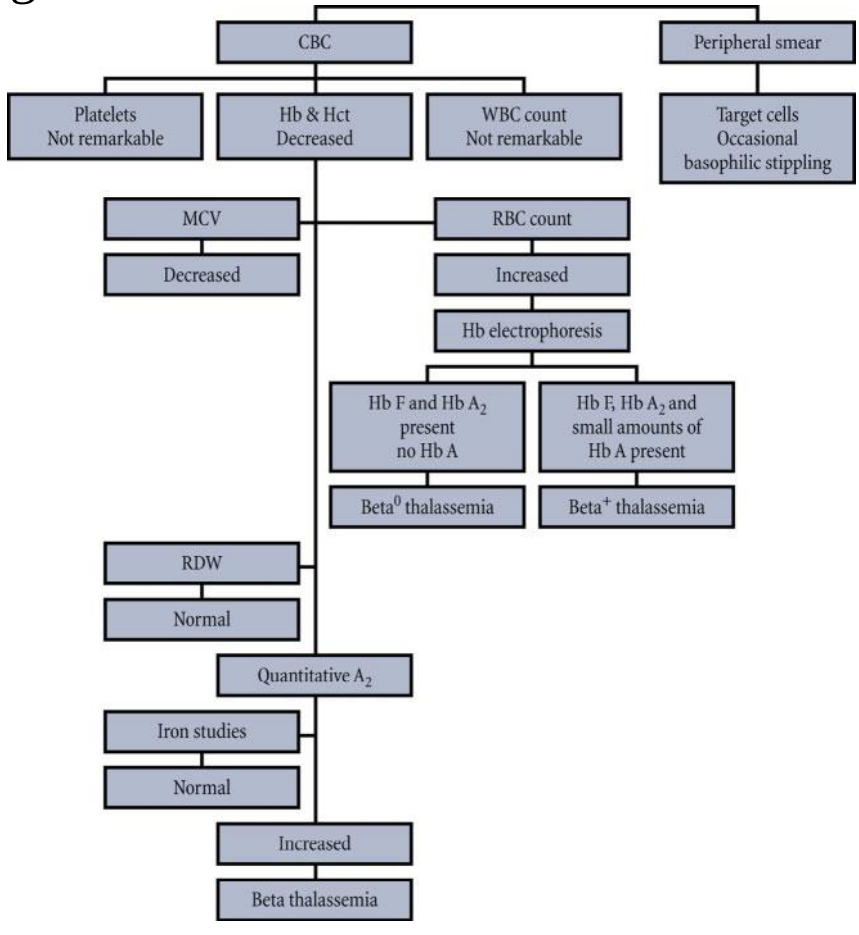
Bone Marrow

1. Ineffective erythropoiesis because of the accumulation of a-globulin chains
2. Extremely hyperplastic

Hemoglobin Electrophoresis

1. Only hemoglobin F and hemoglobin A2 are found in b0 thalassemia
2. Small amounts of hemoglobin A may be found in b+ thalassemia
3. Increased hemoglobin A2 is indicative of b thalassemia [1]

Diagnostic Scheme



Treatment

1. Allogeneic bone marrow transplantation
2. Transfusion
3. Folic acid 5 mg daily
4. Iron therapy forbidden
5. Iron chelation therapy
6. Splenectomy (Table 8.1) [3]

Table 8.1 **Treatment of beta-thalassaemia major** [3]

Problem	**Management**
Erythropoietic failure	Allogeneic bone marrow transplantation from human leucocyte antigen (HLA)-compatible sibling
	Transfusion to maintain Hb > 100 g/L
	Folic acid 5 mg daily
Iron overload	Iron therapy forbidden
	Iron chelation therapy
Splenomegaly causing mechanical problems, excessive transfusion needs	Splenectomy

Hemoglobin C

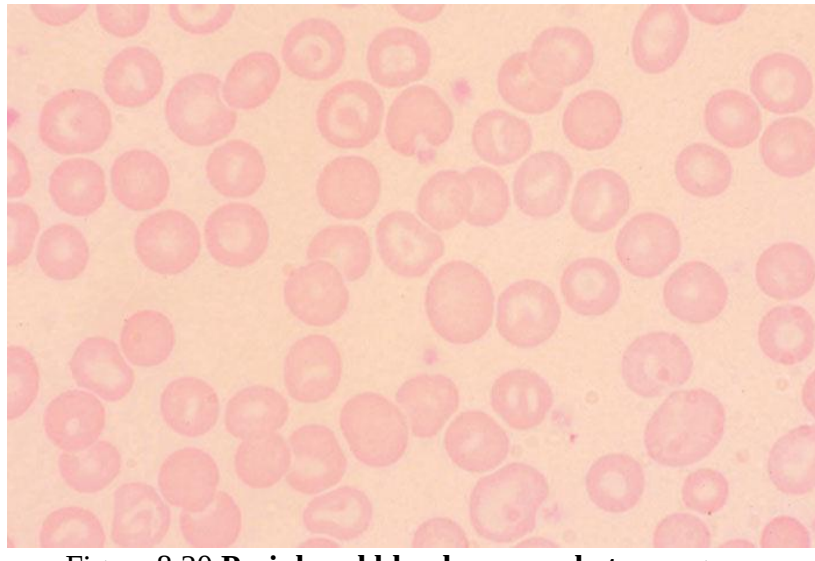


Figure 8.20 **Peripheral blood smear—heterozygous.**

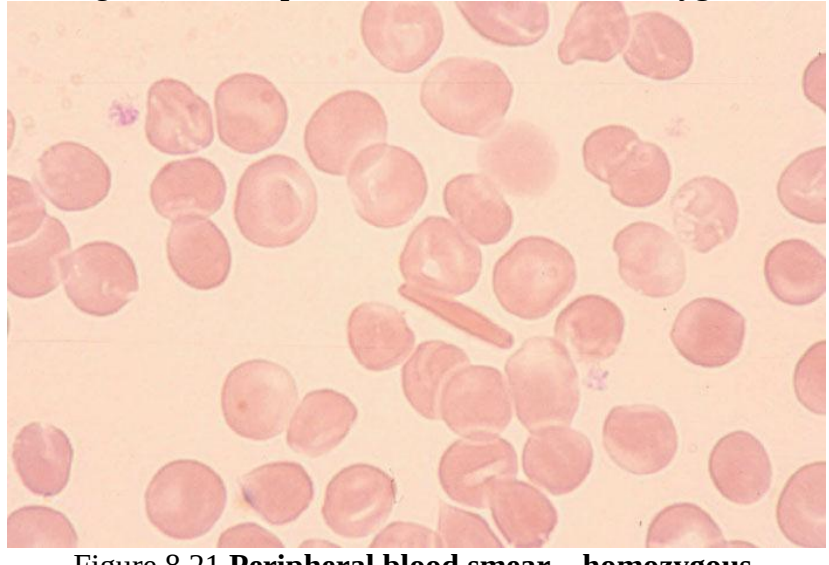


Figure 8.21 **Peripheral blood smear—homozygous.**

Pathology

1. a2/b2 (glutamic acid replaced by lysine on amino acid 6)
2. Relative insolubility of hemoglobin C causes red blood cells to become rigid
3. Loss of potassium and cell hydration
4. Cell is subject to fragmentation and loss of membrane material, resulting in microspherocytes [(1)]

Clinical Features

1. Mild to moderate hemolysis
2. Palpable splenomegaly
3. Cholelithiasis and aplastic crises may occur
4. Arthralgia is common
5. May be abdominal pain
6. Hemoglobin C trait has no clinical manifestations [(1)]

Laboratory Features

White Blood Cells

Not remarkable

Platelets

Not remarkable

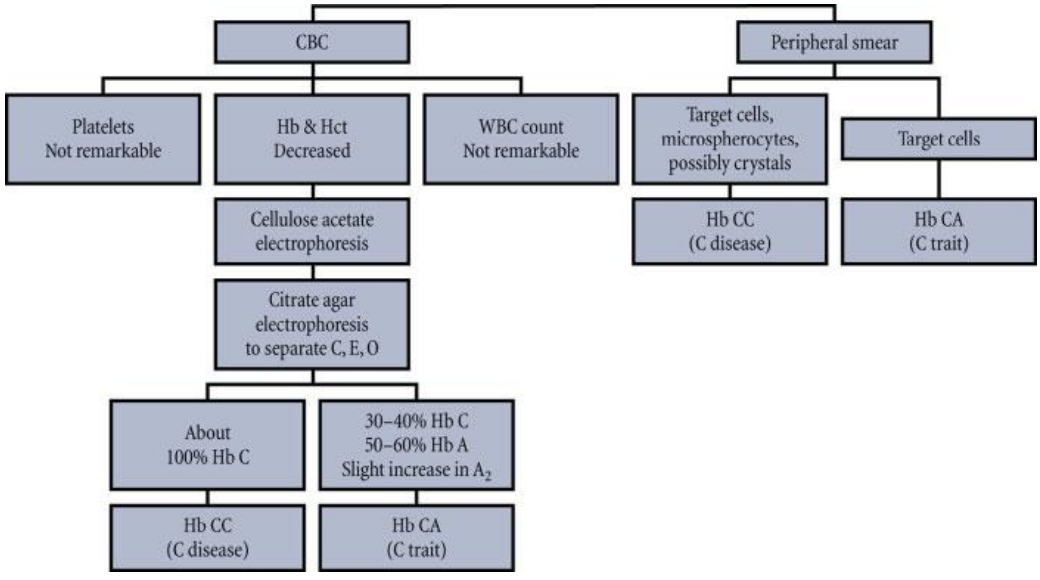
Red Blood Cells

1. Microspherocytes present
2. Reticulocyte count 4–8%
3. Hematocrit level approximately 0.25-0.37 L/L
4. Approximately 30–100% target cells (Figure 8.20, 8.21)
5. C crystals seen in oxyhemoglobin state

Hemoglobin Electrophoresis

1. Homozygous: about 100% hemoglobin C
2. Heterozygous: 30–40% C, 50–60% A, slight increase in A2 [(1)]

Diagnostic Scheme



Hemoglobin constant spring syndrome

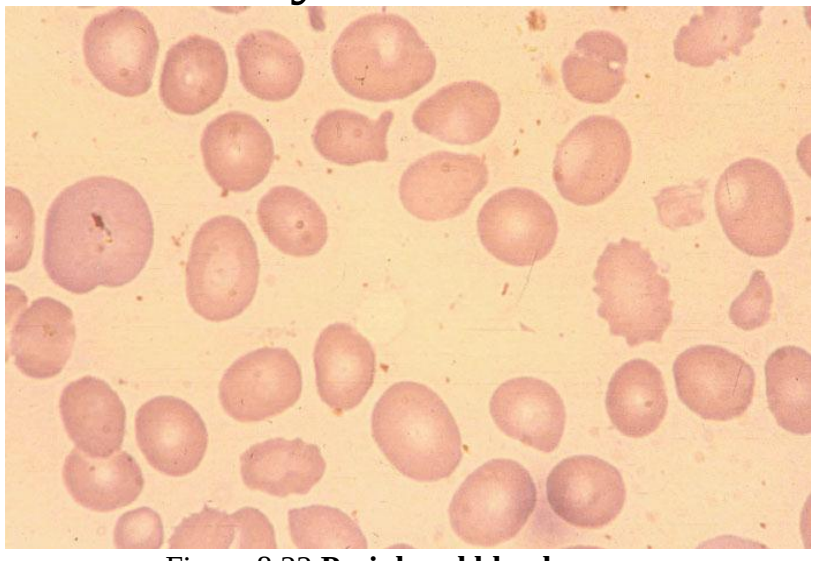


Figure 8.22 **Peripheral blood smear.**

Pathology

1. Four different types of hemoglobins
2. Hemoglobin Constant Spring is formed from the combination of two structurally abnormal a chains, each elongated by 31 amino acids at the C-terminal end and two normal b chains
3. The abnormal a chains are inefficiently synthesized owing to reduced stability of the messenger RNA translation apparatus
4. The deficiency of a chain synthesis produces an a thalassemia–like syndrome [(1)]

Clinical Features

1. Homozygotes have a condition similar to mild a thalassemia—mild anemia, mild jaundice, splenomegaly

2. Heterozygotes usually have no clinical abnormalities
3. Common in Thailand, Chinese, and Greek ancestry with a thalassemia
4. syndromes
5. May occur in about 40% of hemoglobin H disease in Southeast Asians [(1)]

Laboratory Features

White Blood Cells

Not remarkable

Platelets

Not remarkable

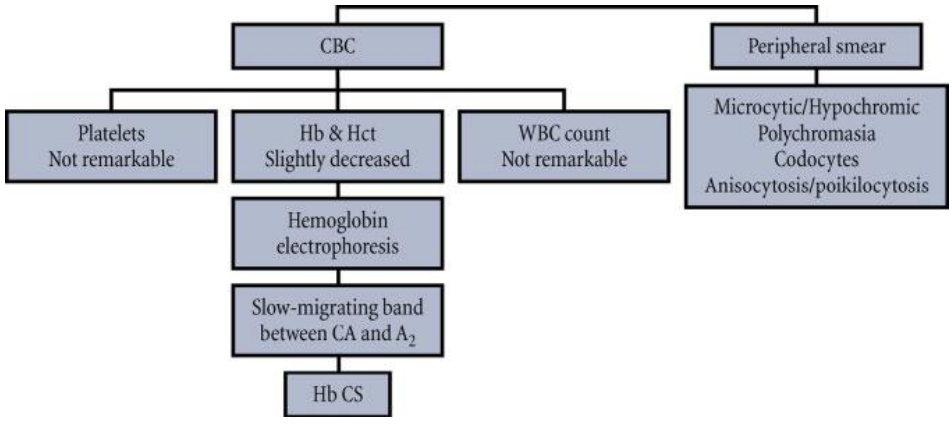
Red Blood Cells

1. Mild microcytic, hypochromic anemia
2. Hemoglobin level usually 9.0–11.0 g/dL
3. Reticulocyte count, 3.5–7.5%
4. Anisocytosis, poikilocytosis
5. Codocytes (Figure 8.22)

Hemoglobin Electrophoresis (Cellulose Acetate, pH 8.4)

1. Migrates on the cathode side of hemoglobin A2
2. In homozygotes
3. Bart's hemoglobin present at birth
4. Hemoglobin Constant Spring 5–7%
5. Hemoglobins A2 and F normal
6. In heterozygotes
7. Hemoglobin Constant Spring 0.2–1.7% [(A)]

Diagnostic Scheme



Hemoglobin D

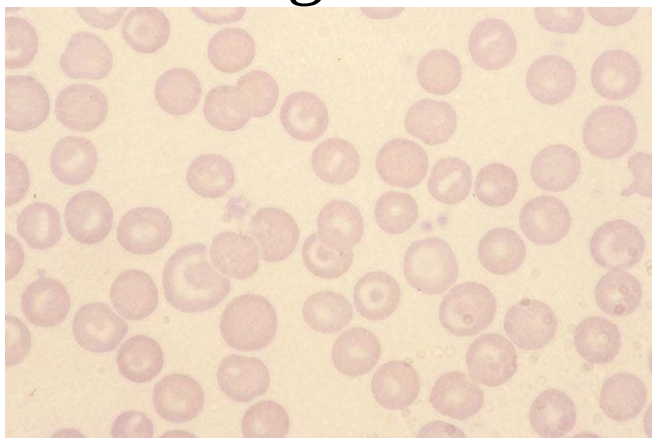


Figure 8.24 **Peripheral blood smear.**

Pathology

1. a/b2 (glutamic acid replaced by glycine on amino acid 121)
2. Many variants are found
3. Hemoglobin D-Punjab and hemoglobin D Los Angeles are the most commonly encountered of the D hemoglobins in American Blacks (0.02%) [(1)]

Clinical Features

1. Disorder is rare
2. Homozygous individuals may have a mild anemia
3. Both homozygous and heterozygous individuals are asymptomatic [(1)]

Laboratory Features

White Blood Cells

Not remarkable

Platelets

Not remarkable

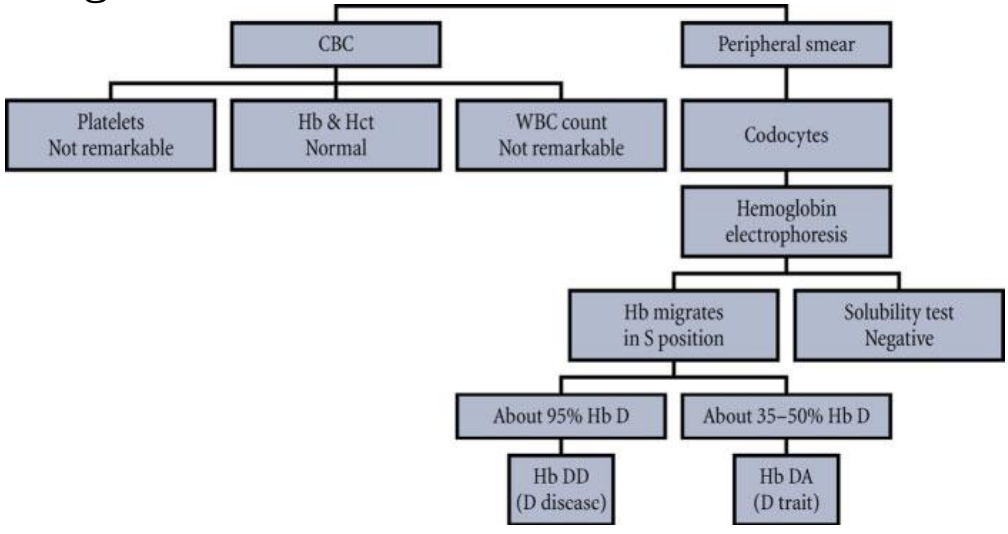
Red Blood Cells

1. Homozygotes may have a normal hemoglobin level and no evidence of
2. hemolysis
3. Indices normal
4. May see target cells
5. May see decreased osmotic fragility (Figure 8.24)

Hemoglobin Electrophoresis

1. Homozygous
2. 95% D and normal A2
3. Hemoglobin D migrates with S at pH 8.6 but does not sickle
4. Hemoglobin D migrates with A on acid electrophoresis [1]

Diagnostic Scheme



Hemoglobin E

HbE (i.e., $a_2b_2^{26Glu \rightarrow Lys}$) is extremely common in Cambodia, Thailand, and Vietnam. The gene has become far more prevalent in the United States as a result of immigration of Asian persons, especially in California, where HbE is the most common variant detected. HbE is mildly unstable but not enough to affect RBC life span significantly. (10)

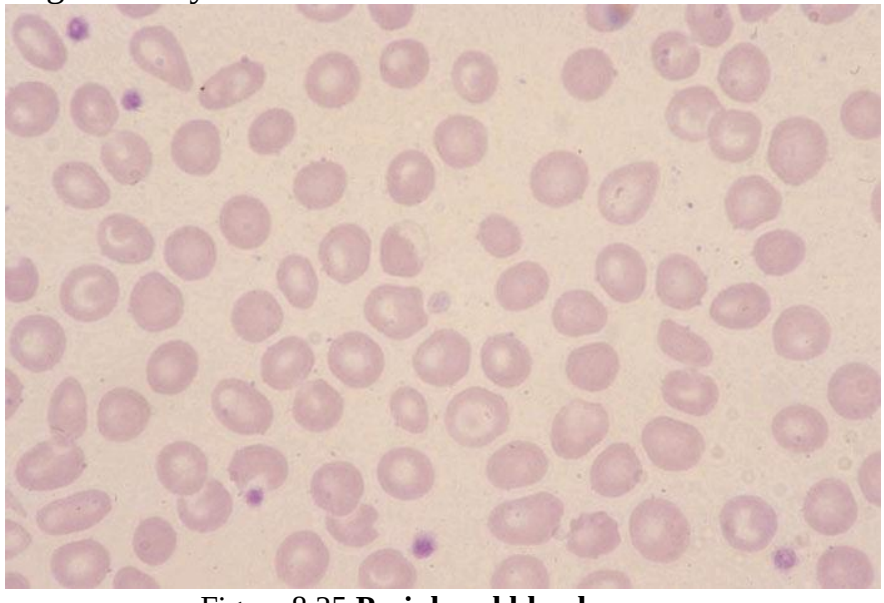


Figure 8.25 **Peripheral blood smear.**

Pathology

1. Substitution of lysine for glutamic acid in the b chain
2. Hemoglobin is slightly unstable with oxidant stress
3. Oxygen dissociation curve is shifted to the right, indicating that hemoglobin E has decreased oxygen affinity (1)

Clinical Features

1. Homozygous: mild or asymptomatic, microcytic anemia with decreased
   erythrocyte survival
2. Heterozygous: symptomless, microcytosis [1]

Laboratory Features

White Blood Cells

Not remarkable

Platelets

Not remarkable

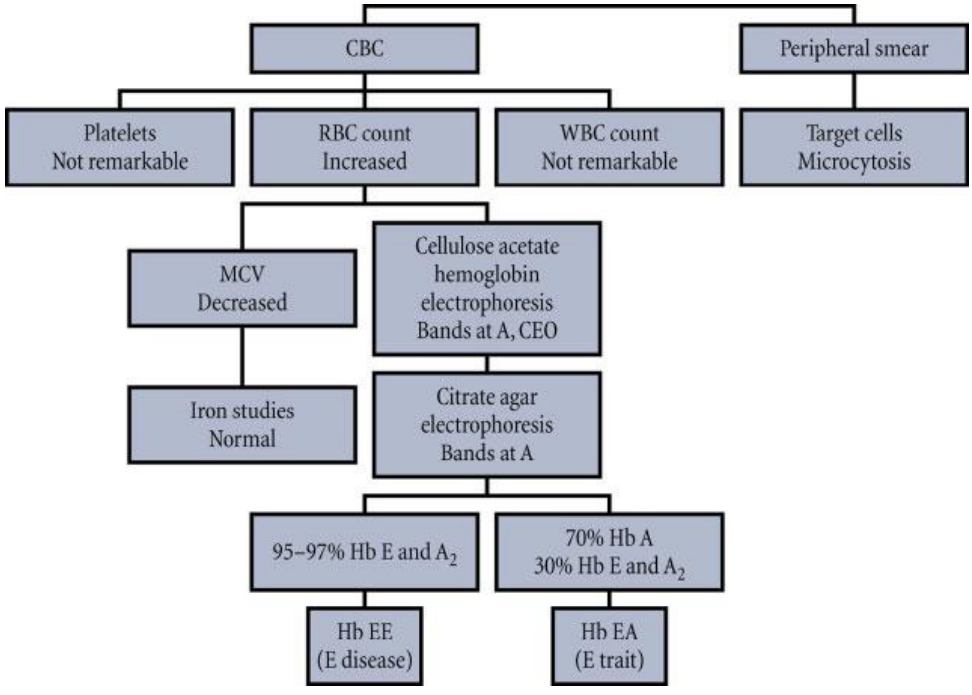
Red Blood Cells

1. Hemoglobin level is 12.0–13.0 g/dL
2. Decreased mean corpuscular volume
3. Target cells present
4. Increased red blood cells
5. Normal or decreased reticulocyte count (Figure 8.25)

Hemoglobin Electrophoresis

Presence of hemoglobin E [1]

Diagnostic Scheme



Hemoglobin E/*b* Thalassemia

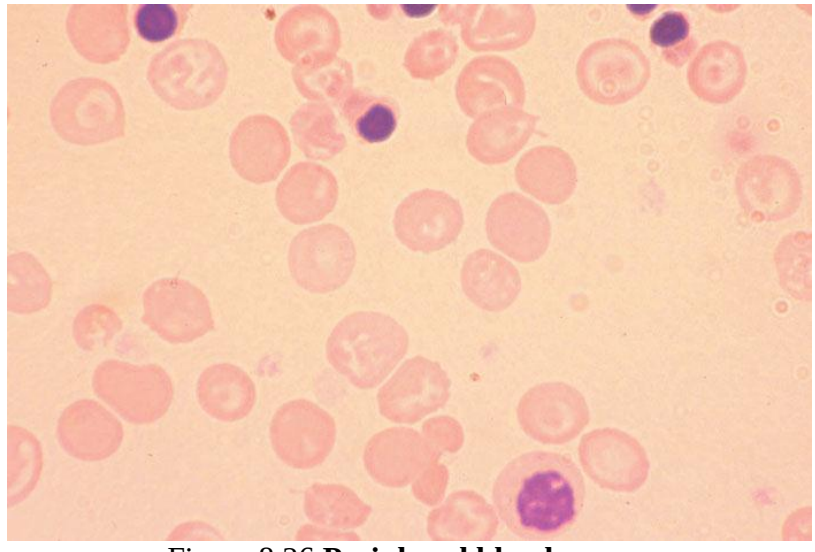


Figure 8.26 **Peripheral blood smear.**

Pathology

1. This is the most common combination in Southeast Asians
2. Double heterozygous for hemoglobin E and b thalassemia [(1)]

Clinical Features

1. Moderate to severe anemia
2. The most severe type is E/b0
3. Anemia is generally more severe than in patients with hemoglobin S/b
4. Anemia is more severe than in E trait
5. Splenomegaly [(1)]

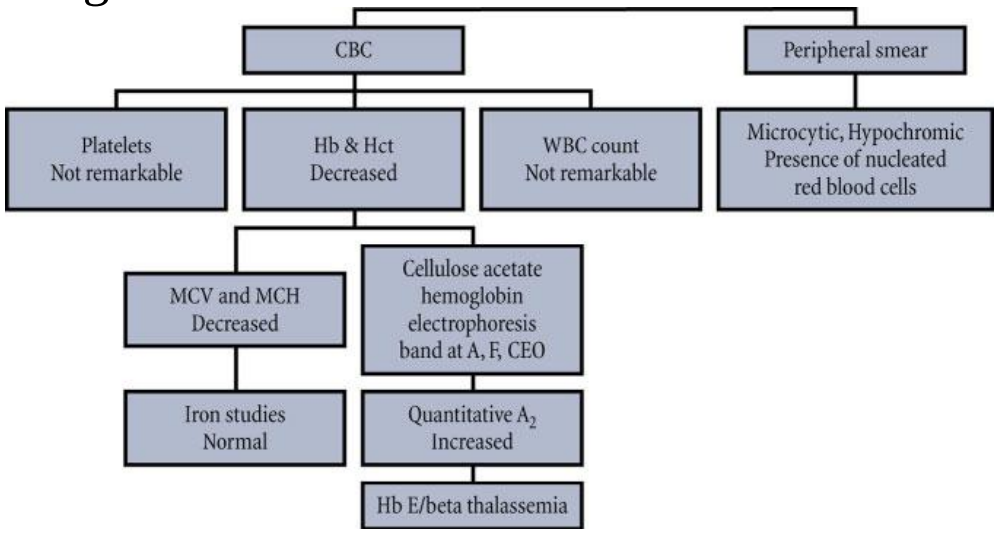
Laboratory Features

White Blood Cells

Not remarkable

Platelets
Not remarkable
Red Blood Cells
Decreased hemoglobin and hematocrit levels
Microcytic, hypochromic anemia
Presence of nucleated red blood cells (Figure 8.26)
Hemoglobin Electrophoresis (Cellulose Acetate, pH 8.4)
Bands at A, F, CEO [1]

Diagnostic Scheme



Hemoglobin lepore syndrome

Hb Lepore [$a_2(db)_2$] arises by an unequal crossover and recombination event that fuses the proximal end of the d-gene with the distal end of the closely linked *b*-gene. [10]

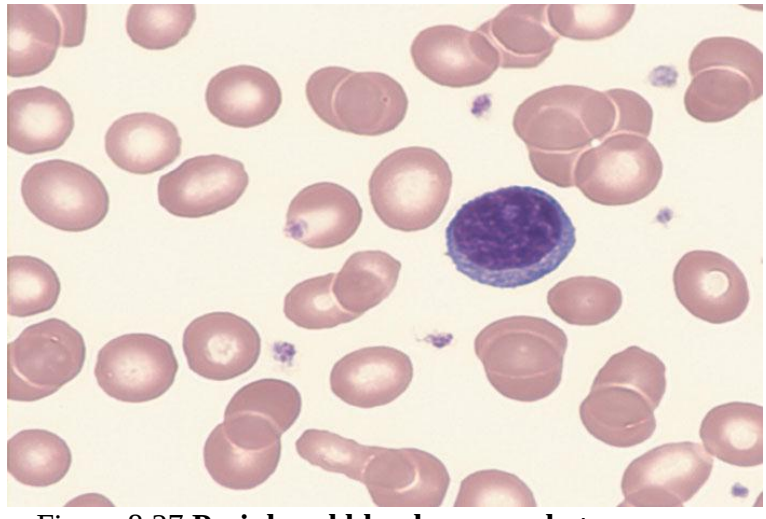


Figure 8.27 **Peripheral blood smear—heterozygous.**

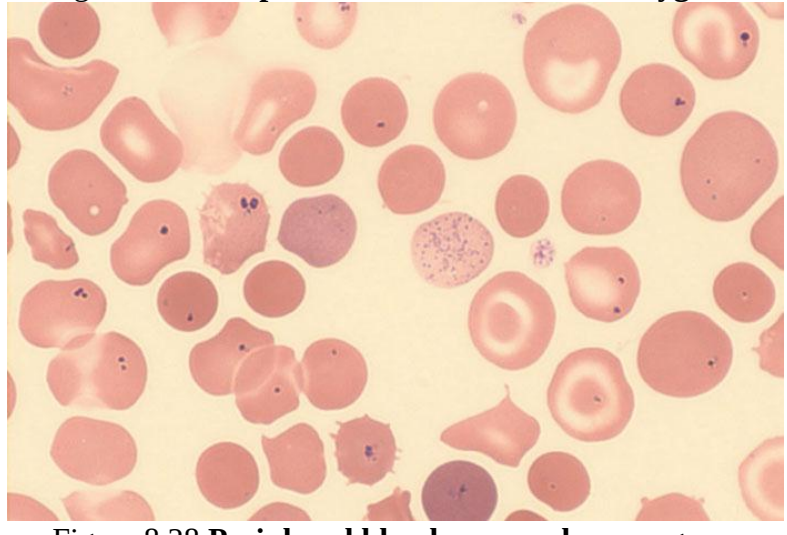


Figure 8.28 **Peripheral blood smear—homozygous.**

Pathology

1. The non–a hemoglobin chain is a λ-b–globin hybrid in which the N-terminal
2. end of the λ chain is fused to the C-terminal end of a b chain
3. Believed to arise during meiosis from aberrant recombination of misaligned λ and b chains on separate chromosomes
4. Two hybrid chains combine with two a chains to form hemoglobin Lepore
5. Hemoglobin Lepore is stable and has normal functional properties, except a slight increase in oxygen
6. The abnormal chains are ineffectively synthesized, leading to an excess of a chains that precipitate, leading to cell membrane damage in inflexibility— hemolytic anemia is the result [(1)]

Clinical Features

1. Common in middle and eastern Europe

Homozygotes

2. Described as a condition that resembles thalassemia intermedia
3. Variable anemia depending on racial group
4. Symptoms develop with the first 5 years of life
5. Hepatosplenomegaly is significant
6. Skeletal abnormalities may exist also with growth retardation

Heterozygotes

7. Mild anemia and the condition resembles thalassemia minor
8. May be asymptomatic
9. Slight splenomegaly [(1)]

Laboratory Features

Homozygotes

1. Hemoglobin level is usually 4.0–11.0 g/dL
2. Microcytic, hypochromic anemia

3. Anisocytosis poikilocytosis, codocytes, basophilic stippling, Pappenheimer bodies (Figure 8.28)

Heterozygotes

1. Hemoglobin level is slightly decreased
2. Microcytic, hypochromic anemia (Figure 8.27)

Bone Marrow

Erythroid marrow expands and produces abnormal cells
Ineffective erythropoiesis contributes to the anemia as the abnormal cells are destroyed

Hemoglobin Electrophoresis (Cellulose Acetate, pH 8.4)

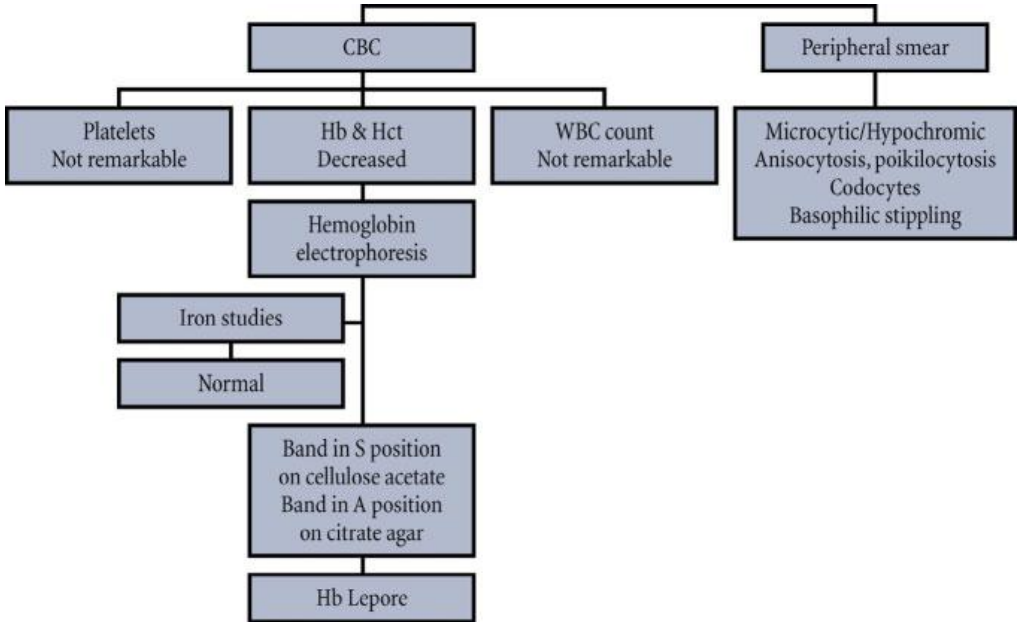
Homozygotes

1. 0% hemoglobin A
2. 0% hemoglobin A2
3. 75% hemoglobin F
4. 25% hemoglobin Lepore (hemoglobin Lepore migrates like hemoglobin S)

Heterozygotes

1. 75–85% hemoglobin A
2. About 2% hemoglobin A2
3. 1–6% hemoglobin F
4. 7–15% hemoglobin Lepore [(1)]

Diagnostic Scheme



Hereditary persistence fetal hemoglobin

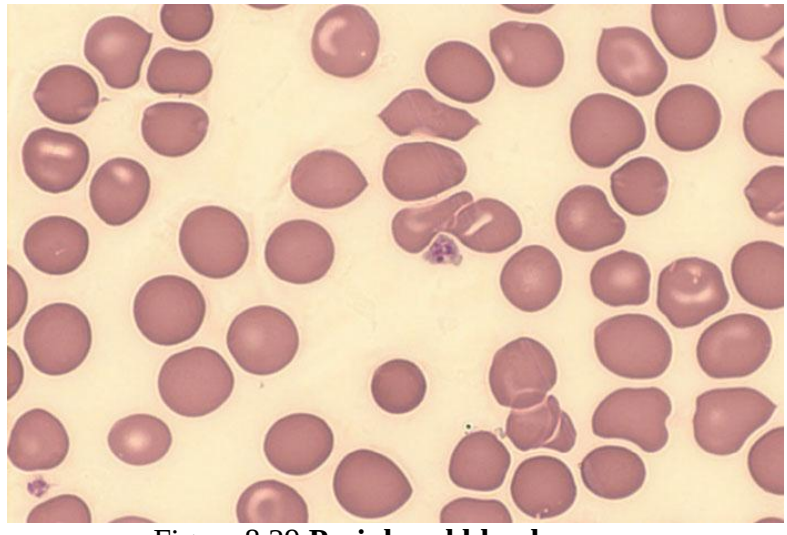


Figure 8.29 **Peripheral blood smear.**

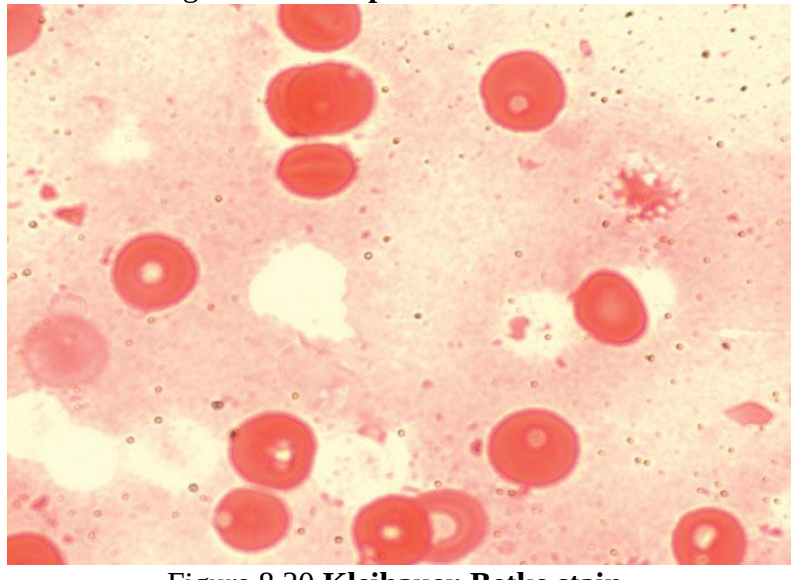


Figure 8.30 **Kleihauer-Betke stain.**

Pathology

1. Deletion/inactivation of λ and b genes
2. Absence of λ and b chain synthesis is compensated for by increased g chain production into adult life, causing the increased levels of hemoglobin F
3. Two types exist:
4. Pancellular (Black, Greek)
5. Heterocellular (Swiss)
6. Hemoglobin F has normal to slightly higher oxygen affinity; thus, patients are usually asymptomatic
7. Slightly increased oxygen affinity will lead to increased erythropoiesis [(1)]

Clinical Features

1. Usually no or minimal anemia
2. In the homozygous state, there are no findings suggestive of thalassemia (abnormal growth, splenomegaly) [(1)]

Laboratory Features

White Blood Cells

Not remarkable

Platelets

Not remarkable

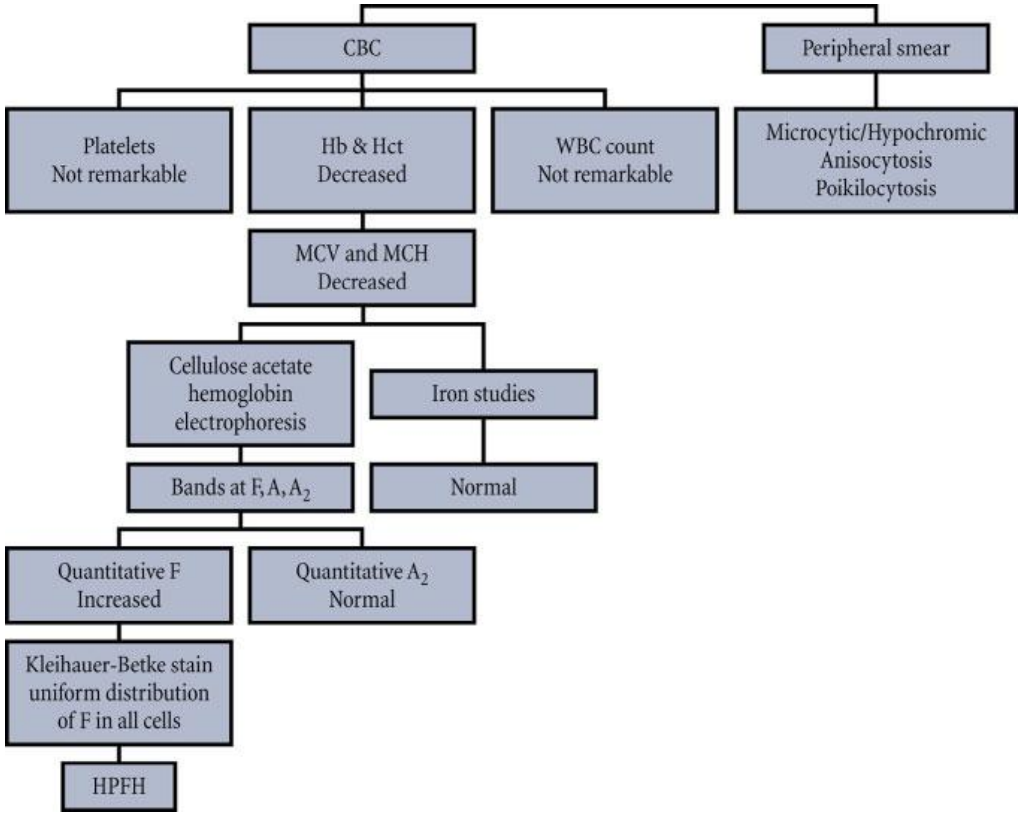
Red Blood Cells

1. Microcytic/hypochromic anemia
2. Mild erythrocytosis
3. Mean corpuscular volume decreased
4. Anisocytosis
5. Poikilocytosis
6. Target cells present (Figure 8.29)

Hemoglobin Electrophoresis

1. Homozygotes
2. 100% hemoglobin F
3. Heterozygotes
4. 10–30% hemoglobin F
5. 1–2% hemoglobin A2 [1]

Diagnostic Scheme



Sickle-cell (Hemoglobin S) disease

Sickle-cell disease results from a single glutamic acid to valine substitution at position 6 of the beta globin polypeptide chain. It is inherited as an autosomal recessive trait. Homozygotes only produce abnormal beta chains that make haemoglobin S (HbS, termed SS), and this results in the clinical syndrome of sickle-cell disease. Heterozygotes produce a mixture of normal and abnormal beta chains that make normal HbA and HbS (termed AS), and this results in the clinically asymptomatic sickle-cell trait. [3]

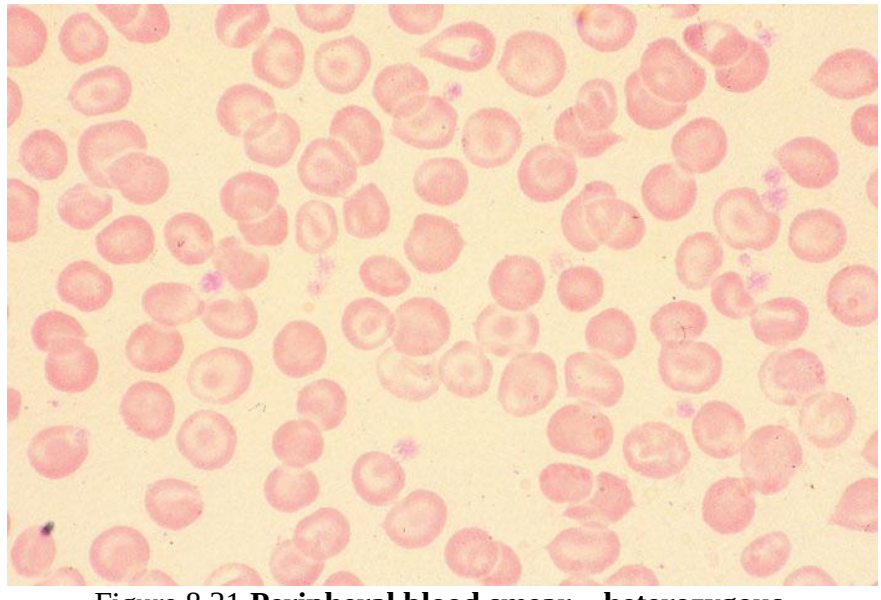


Figure 8.31 **Peripheral blood smear—heterozygous.**

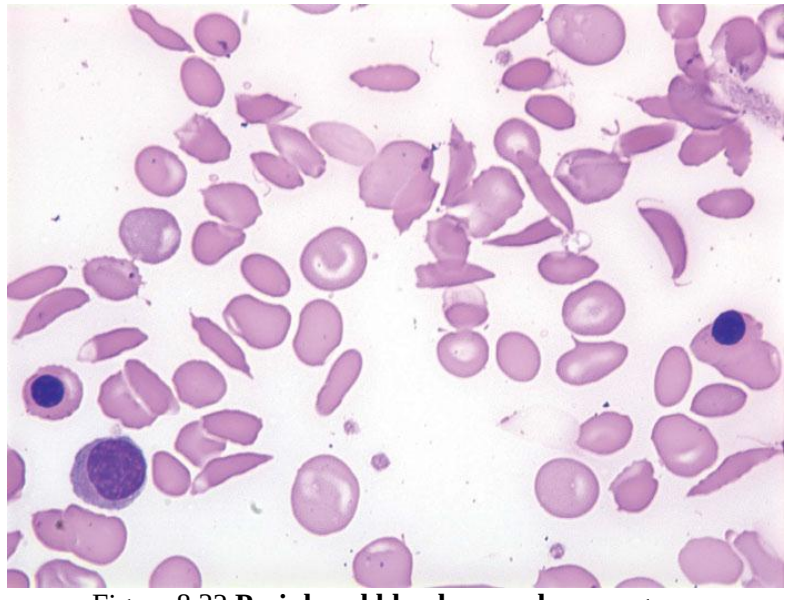


Figure 8.32 **Peripheral blood smear homozygous.**

Epidemiology

The heterozygote frequency is over 20% in tropical Africa (see Fig. 24.24). In black American populations, sickle-cell trait has a frequency of 8%. Individuals with sickle-cell trait are relatively resistant to the lethal effects of *falciparum* malaria in early childhood; the high prevalence in equatorial Africa can be explained by the selective survival advantage it confers in areas where *falciparum* malaria is endemic. However, homozygous patients with sickle-cell anaemia do not have correspondingly greater resistance to *falciparum* malaria. [(3)]

Pathology

1. A single nucleotide base change in the codon responsible for the synthesis of the sixth amino acid in the b-globulin chain, resulting in the substitution of valine for glutamic acid
2. The deoxyhemoglobin polymerizes within the red blood cell

3. Extravascular hemolysis of sickled cells takes place, causing a chronic hemolytic anemia [(1)]

Pathogenesis

When haemoglobin S is deoxygenated, the molecules of haemoglobin polymerise to form pseudocrystalline structures known as 'tactoids'. These distort the red cell membrane and produce characteristic sickle-shaped cells (Fig. 2). The polymerisation is reversible when reoxygenation occurs. The distortion of the red cell membrane, however, may become permanent and the red cell 'irreversibly sickled'. The greater the concentration of sickle-cell haemoglobin in the individual cell, the more easily tactoids are formed, but this process may be enhanced or retarded by the presence of other haemoglobins. Thus, the abnormal haemoglobin C variant participates in the polymerisation more readily than haemoglobin A, whereas haemoglobin F strongly inhibits polymerisation. [(3)]

Clinical features

Sickling is precipitated by hypoxia, acidosis, dehydration and infection. Irreversibly sickled cells have a shortened survival and plug vessels in the microcirculation. This results in a number of acute syndromes, termed 'crises', and chronic organ damage, as shown in Figure 8.36. [(3)]

Vaso-occlusive crisis

Plugging of small vessels in the bone produces acute severe bone pain. This affects areas of active marrow: the hands and feet in children (so-called dactylitis) or the femora, humeri, ribs, pelvis and vertebrae in adults. Patients usually have a systemic response with tachycardia, sweating and a fever. This is the most common crisis. [(3)]

Sickle chest syndrome

This may follow on from a vaso-occlusive crisis and is the most common cause of death in adult sickle disease. Bone marrow infarction results in fat emboli to the lungs which cause further sickling and infarction, leading to ventilatory failure if not treated. (3)

Sequestration crisis

Thrombosis of the venous outflow from an organ causes loss of function and acute painful enlargement. In children the spleen is the most common site. Massive splenic enlargement may result in severe anaemia, circulatory collapse and death. Recurrent sickling in the spleen in childhood results in infarction and adults may have no functional spleen. In adults the liver may undergo sequestration with severe pain due to capsular stretching. (3)

Aplastic crisis

Infection of adult sicklers with human erythrovirus 19 results in a severe but self-limiting red cell aplasia. This produces a very low haemoglobin which may cause heart failure. Unlike in all other sickle crises, the reticulocyte count is low. (3)

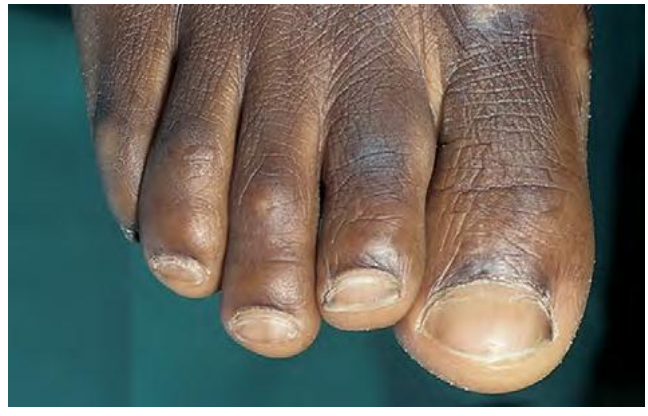


Figure 8.33 **Sickle cell anemia.** A and B, The toes of the patient, show irregularities in length. (7)

Table 8.2 **Clinical features Sickle-cell (Hemoglobin S) disease**
Homozygous
1. A disease state that occurs in approximately 0.3–1.3% of American Blacks 2. Person with this disorder may present with: • Fever • Effusion of joints • Bone deformities • Loss of renal function • Priapism • Enlarged liver • Ocular manifestations • Leg ulcers • Frequent infections • Decreased spleen function • Complications during pregnancy • Cerebrovascular accidents 3. Acute episodes may be manifest
Heterozygous
4. The individual possesses one normal beta gene and one S gene 5. In American Blacks, the frequency is approximately 8% 6. No clinical symptoms are associated with the trait, but episodes of hematuria may occur (1)

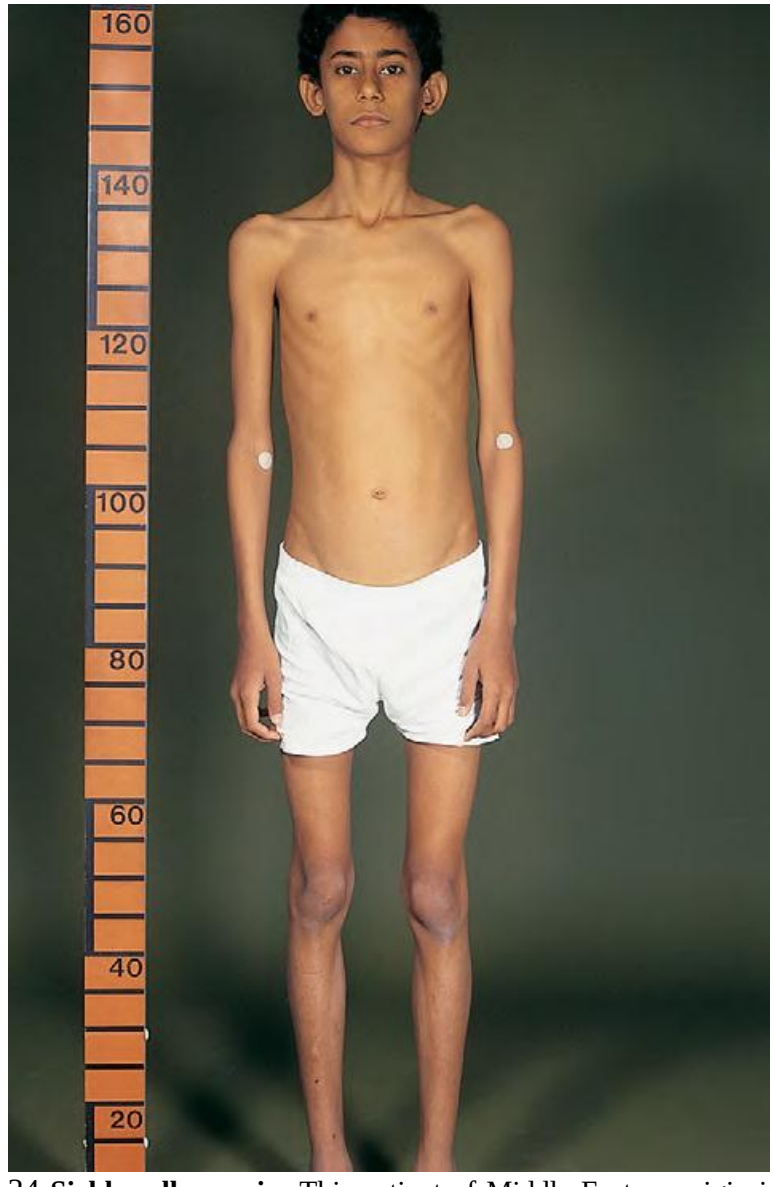


Figure 8.34 **Sickle cell anemia:** This patient of Middle Eastern origin is tall with long thin limbs, a large arm span, and narrow pectoral and pelvic girdles. Sexual development is normal. [7]

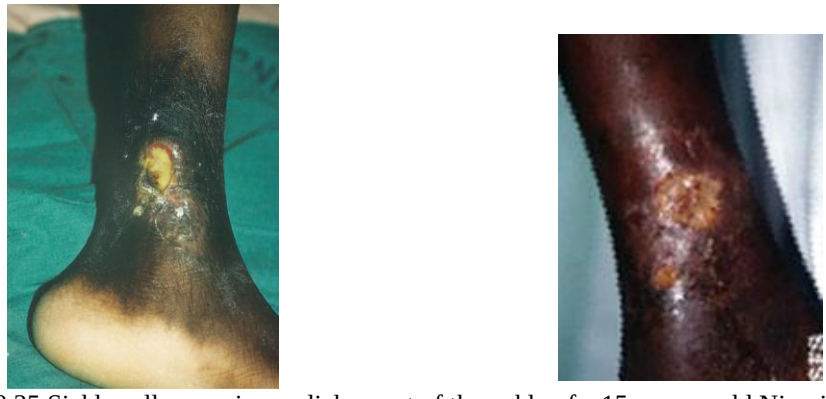


Figure 8.35 Sickle cell anaemia: medial aspect of the ankle of a 15 - year - old Nigerian boy showing necrosis and ulceration. (7)

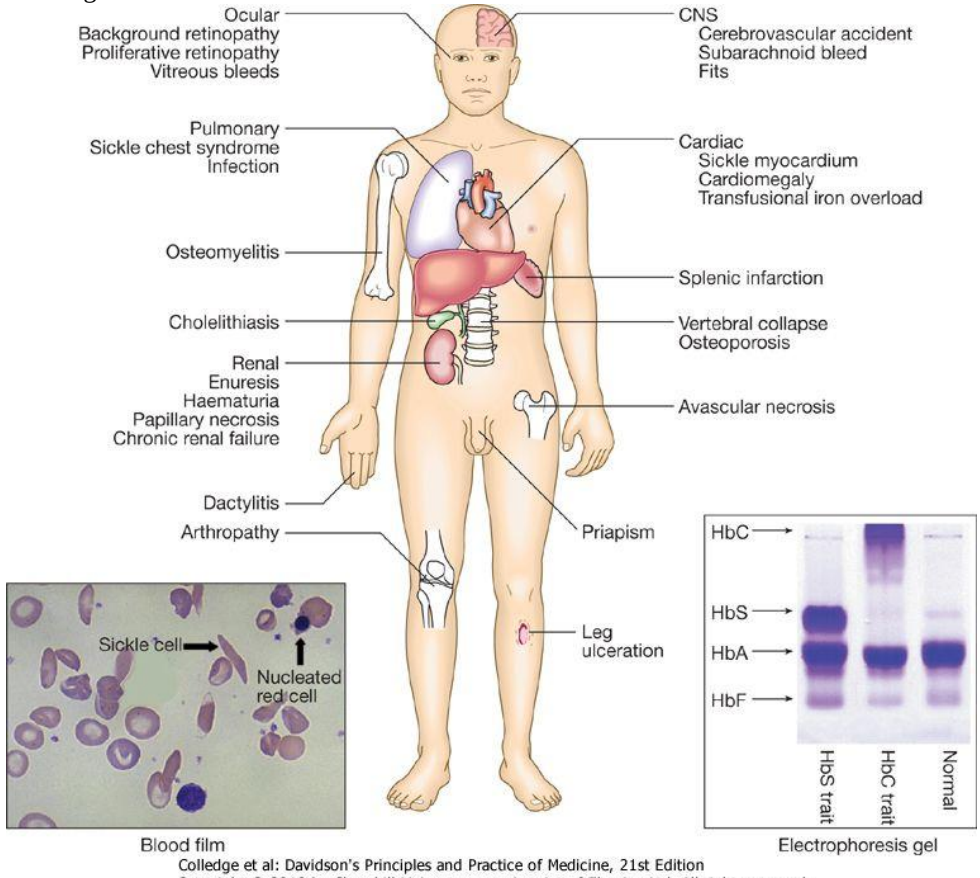


Figure 8.36 **Clinical manifestations of sickle-cell disease.**

Laboratory Features

Homozygous

White Blood Cells

Usually increased during a crisis, may be up to 25 × 109/L

Platelets

Normal to increased

Red Blood Cells

1. Normocytic/normochromic anemia
2. Hemoglobin level 6.5–10.0 g/dL
3. Reticulocyte count is increased (10–20%)
4. Red blood cell distribution width is increased
5. Smear shows polychromasia, codocytes, Howell-Jolly bodies, nucleated red blood cells, and drepanocytes (Fig. 8.31, 8.32)

Bone Marrow

Erythroid hyperplasia caused by chronic hemolysis

Hemoglobin Electrophoresis

1. 0% hemoglobin A
2. 80–99% hemoglobin S
3. Slightly increased hemoglobin A2
4. 1–20% hemoglobin F

Heterozygous

White Blood Cells

Not remarkable

Platelets

Not remarkable

Red Blood Cells

1. Codocytes
2. Hemoglobin and hematocrit levels normal

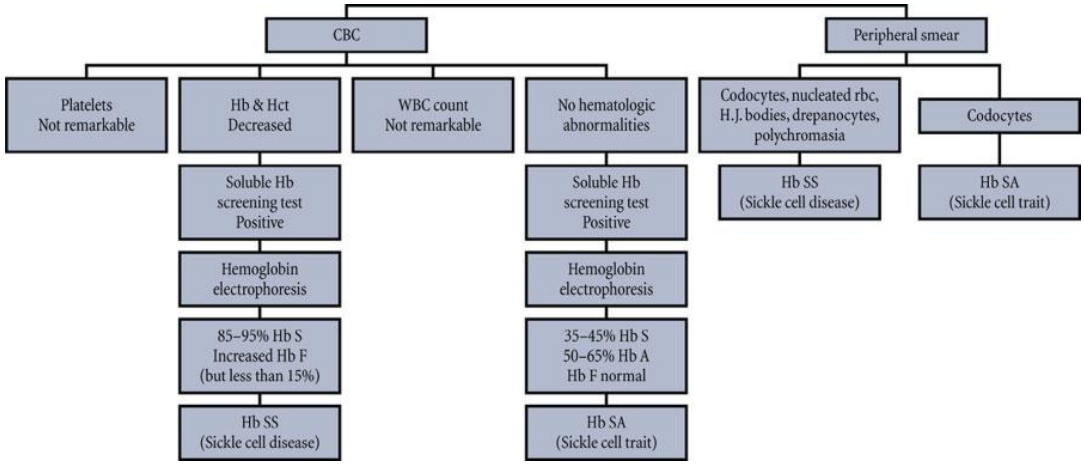
Bone Marrow

Not remarkable

Hemoglobin Electrophoresis

1. 50–65% hemoglobin A
2. 35–45% hemoglobin S
3. Normal to slightly increased hemoglobin A2
4. Normal hemoglobin F [1]

Diagnostic Scheme



Management

All patients with sickle-cell disease should receive prophylaxis with daily folic acid, and penicillin V to protect against pneumococcal infection which may be lethal in the presence of hyposplenism. These patients should be vaccinated against pneumococcus and, where vaccine is available, *Haemophilus influenzae* B and hepatitis B. [3]

Vaso-occlusive crises are managed by aggressive rehydration, oxygen therapy, adequate analgesia (which often requires opiates) and antibiotics. Transfusion should be with fully genotyped blood wherever possible. Simple top-up transfusion may be used in a sequestration or aplastic crisis. A regular transfusion programme to suppress HbS production and maintain the HbS level below 30% may be indicated in patients with recurrent severe

complications such as cerebrovascular accidents in children or chest syndromes in adults. Exchange transfusion, in which a patient is simultaneously venesected and transfused to replace HbS with HbA, may be used in life-threatening crises or to prepare patients for surgery. [3]

A high HbF level inhibits polymerisation of HbS and reduces sickling. Patients with sickle-cell disease and high HbF levels have a mild clinical course with few crises. Some agents are able to increase synthesis of HbF and this has been used to reduce the frequency of severe crises. The oral cytotoxic agent hydroxycarbamide has been shown to have clinical benefit with acceptable side-effects in children and adults who have recurrent severe crises.

Relatively few allogeneic stem-cell transplants from HLA-matched siblings have been performed but this procedure appears to be potentially curative (Table 8.3). [3]

Table 8.3 **Management of acute painful crisis in opioid naïve adults with sickle cell disease.** Higher doses may be required for patients who have previously received opioids.

Morphine/diamorphine

1. 0.1 mg/kg i.v./s.c. every 20 min until pain controlled, then
2. 0.05–0.1 mg/kg i.v./s.c. (or oral morphine) every 2–4 hours
3. Patient controlled analgesia (PCA) when pain controlled

Patient controlled analgesia (PCA) (example for adults > 50 kg)

Diamorphine

1. Continuous infusion: 0–10 mg/h
2. PCA bolus dose: 2–10 mg
3. Dose duration: 1 min
4. Lockout time: 20–30 min

Adjuvant oral analgesia

1. Paracetamol 1 g 6 hourly
2. +/− Ibuprofen* 400 mg 8 hourly
3. Or diclofenac* 50 mg 8 hourly

Laxatives (all patients)

For example:

1. Lactulose 10 ml × 2 daily
2. Senna 2–4 tablets daily
3. Sodium docusate 100 mg × 2 daily
4. Macrogol 1 sachet daily
5. Lubiprostone

Other adjuvants

Anti-pruritics

Hydroxyzine 25 mg × 2 as required

Antiemetics

1. Prochlorperazine 5–10 mg × 3 as required
2. Cyclizine 50 mg × 3 as required

Anxiolytic

Haloperidol 1–3 mg oral/i.m. × 2 as required

* Caution advised with NSAIDs in renal impairment.

Adapted from Rees DC, Olujohungbe AD, Parker NE et al. Guidelines for the management of the acute painful crisis in sickle cell disease.
British Journal of Haematology 2003; 120(5): 744–752.

i Box 8.1 Management of acute painful crisis in opioid naïve adults with sickle cell disease. Higher doses may be required for patients who have previously received opioid. [8]

Prognosis

In Africa few children with sickle-cell anaemia survive to adult life without medical attention. Even with standard medical care, approximately 15% die by the age of 20 years and 50% by the age of 40 years. [3]

Hemoglobin S/b Thalassemia

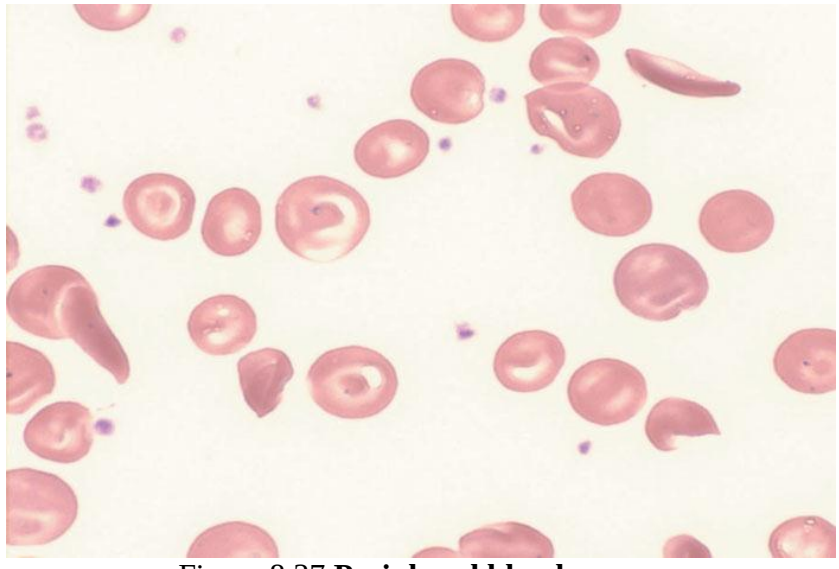


Figure 8.37 **Peripheral blood smear.**

Pathology

b thalassemia gene reduces the rate of synthesis of betaA chain, resulting in a predominance of beta S [(1)]

Clinical Features

1. S/b0—severity comparable to that seen in sickle cell anemia
2. S/b+—milder clinical course comparable to SC disease
3. Splenomegaly [(1)]

Laboratory Features

White Blood Cells

Not remarkable

Platelets

Not remarkable

Red Blood Cells

1. Microcytic/hypochromic anemia
2. Decreased mean corpuscular volume (Figure 8.37)

Hemoglobin Electrophoresis

1. S/b0—mostly hemoglobin S, increased A2, variable F and no A
2. S/b+—hemoglobin S about 11%, A2 about 6% [(1)]

Hemoglobin S/C disease

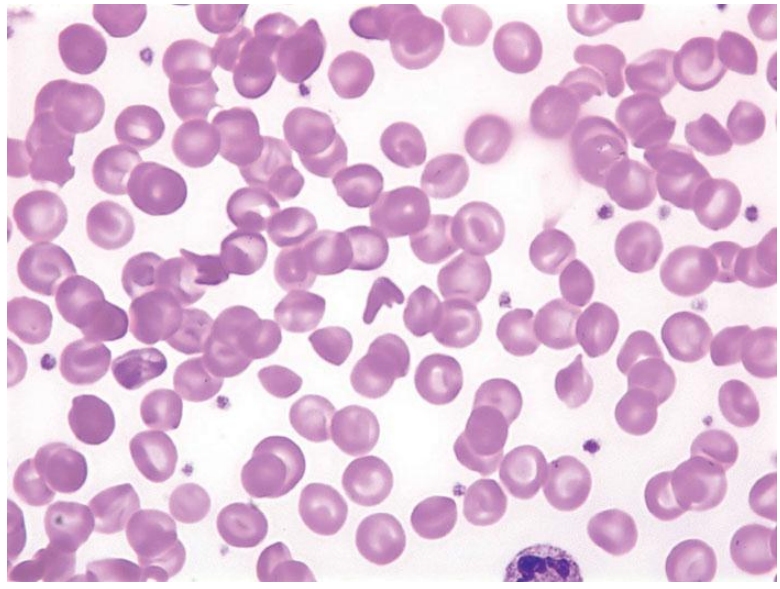


Figure 8.38 **Peripheral blood smear.**

Pathology

1. Both b chains are abnormal
2. Less frequent and less severe than sickle cell anemia
3. More severe than sickle trait of C trait [(1)]

Clinical Features

1. Splenomegaly
2. Proliferative retinopathy

3. Aseptic necrosis of long bones
4. Muscle, bone, and joint pain
5. Hematuria
6. Acute pulmonary disease
7. Splenic infarction
8. Vaso-occlusive crisis during pregnancy, surgery, or medical emergency [1]

Laboratory Features

White Blood Cells

Not remarkable

Platelets

Not remarkable

Red Blood Cells

1. Knizocytes, stomatocytes, and target cells present
2. Increased mean corpuscular hemoglobin
3. Microcytic anemia
4. Sickling not prominent
5. SC crystals (Figure 8.28)

Bone Marrow

Normoblastic hyperplasia

Hemoglobin Electrophoresis

1. Equal amounts of hemoglobin S and hemoglobin C
2. 1–2% hemoglobin F
3. Trace of A2 [1]

Unstable hemoglobins

Amino acid substitutions that reduce solubility or increase susceptibility to oxidation produce unstable hemoglobins that precipitate, forming inclusion bodies injurious to the RBC membrane. Representative mutations are those that interfere with contact points between the *a* and *b* subunits [e.g., Hb Philly ($b^{35Tyr\rightarrow Phe}$)], alter the helical segments [e.g., Hb Genova ($b^{28Leu\rightarrow Pro}$)], or disrupt interactions of the hydrophobic pockets of the globin subunits with heme [e.g., Hb Koln ($b^{98Val\rightarrow Met}$)]. The inclusions, called *Heinz bodies*, are clinically detectable by staining with supravital dyes such as crystal violet. (10)

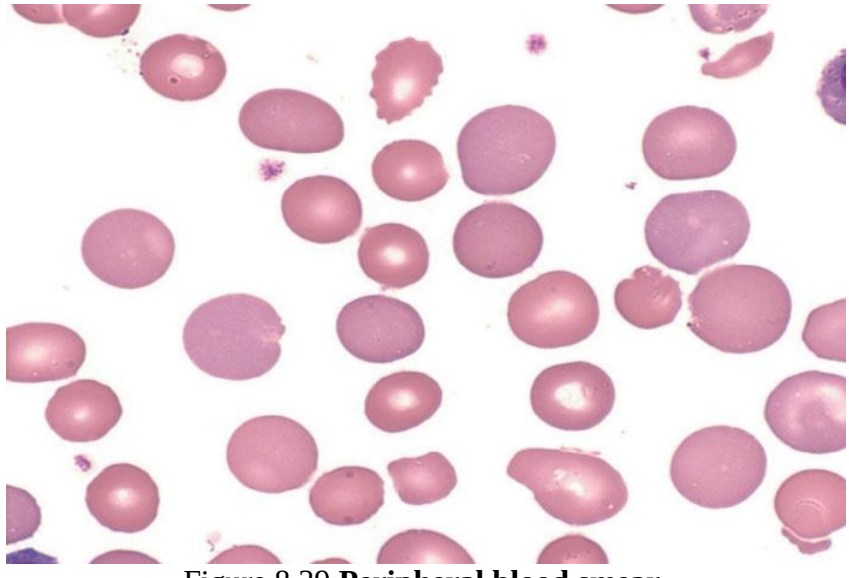


Figure 8.39 **Peripheral blood smear.**

Pathology

1. Amino acid substitutions in critical internal portions of the globin chains

2. Abnormal hemoglobin precipitates as Heinz bodies, which attach to the inner surface of the membrane and can cause cell rigidity, membrane damage, and, thus, erythrocyte hemolysis
3. Homozygous state is incompatible with life
4. Oxygen stability may be increased or decreased depending on where the amino acid substitution is located
5. Hemoglobin with high oxygen affinity is usually accompanied by erythrocytosis
6. Oxygen dissociation curve is shifted to the left
7. Decreased amount of oxygen released to the tissues and increased
8. erythropoietin levels
9. Hemoglobin with decreased oxygen affinity may be asymptomatic
10. Oxygen dissociation curve is shifted to the right
11. Increased amount of oxygen delivered to the tissues [(1)]

Clinical Features

1. Hemoglobin denaturation and hemolysis may occur spontaneously
2. Symptoms may occur only after drug administration, infection, or other changes to the normal environment
3. Jaundice and splenomegaly occur because of increased red blood cell hemolysis
4. Excretion of dark urine
5. Cyanosis may result from the formation of sulfhemoglobin
6. Methemoglobin [(1)]

Laboratory Features

White Blood Cells

Not remarkable

Platelets

Not remarkable

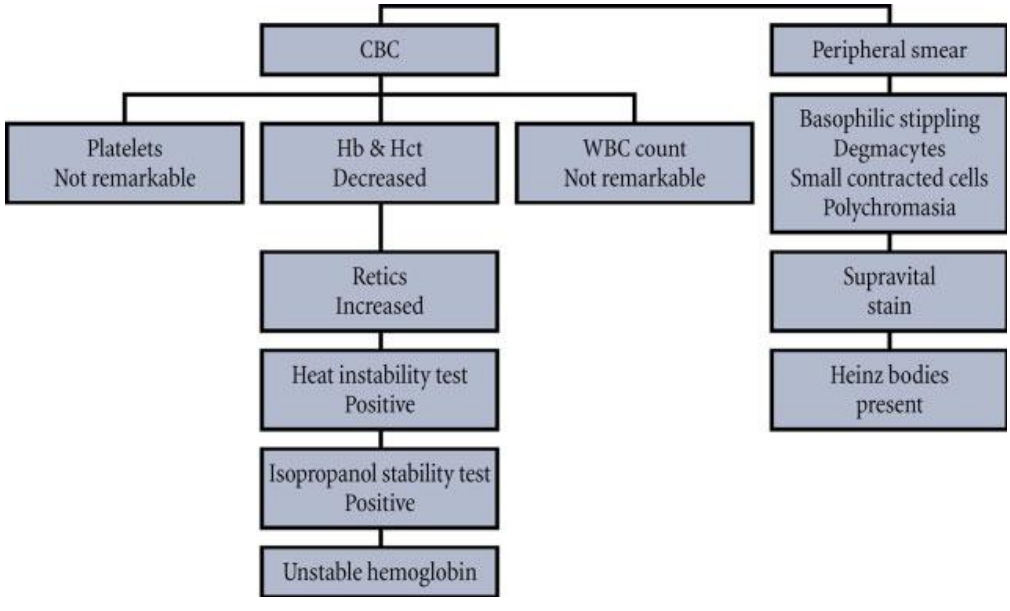
Red Blood Cells

1. Normochromic/normocytic anemia
2. Slight decrease in mean corpuscular hemoglobin and mean corpuscular
3. hemoglobin concentration
4. Reticulocyte count is increased
5. Basophilic stippling, bite cells, and small contracted cells may be present (Figure 8.29)
6. Osmotic fragility abnormal after 24-hour incubation
7. Heat instability test result is positive
8. Isopropanol stability test result is positive

Hemoglobin electrophoresis is abnormal in about 45% of cases—A2 and F are sometimes increased

Heinz bodies are seen on brilliant cresyl blue stain [(1)]

Diagnostic Scheme



Appendices

Normal Values

- *Lab Reference Values*
- *International Systems of units*
- *Pregnancy and Medical Therapeutics*
- *Reference*

Appendices

Barnes-Jewish Hospital Laboratory Reference Values

Reference values for the more commonly used laboratory tests are listed in table 9.1. These values are given in the units currently used at Barnes-Jewish Hospital and in Systeme International (SI) units, which are used in many areas of the world. Individual reference values can be population and method dependent (Table 9.1). [4]

Table 9.1 **Lab Reference Values** [4]

Test	Current units	Factor[a]	SI units
Common serum chemistries			
Albumin	3.6â€“5. g/	10	36â€“50 g/L
Ammonia (plasma)	9â€“33 mcmol/L	1	9â€“33 mcmol/L
Bilirubin			
Total[b]	0.3â€“1.1 mg/dL	17.1	5.13â€“18.80 mcmol/L
Direct	0â€“0.3 mg/dL	17.1	0â€“5.1 mcmol/L
Blood gases (arterial)			
pH	7.35â€“7.45	1	7.35â€“7.45
PO_2	80â€“105 m Hg	0.133	10.6â€“14.0 kPa
PCO_2	35â€“45 mm Hg	0.133	4.7â€“6.0 kPa
Calcium			
Total	8.6â€“10.3 mg/dL	0.25	2.15â€“2.58 mmol/L
Ionized	4.5â€“5.1 mg/dL	0.25	1.13â€“1.28 mmol/L
CO_2 content (plasma)	22â€“32 mmol/L	1	22â€“32 mmol/L
Ceruloplasmin	18â€“46 mg/dL	0.063	1.5â€“2.9 mcmol/L
Chloride	97â€“110 mmol/L	1	97â€“110 mmol/L
Cholesterol[c]			
Desirable	<200 mg/dL	0.0259	<5.18 mmol/L
Borderline high	200â€“239 mg/dL	0.0259	5.18â€“6.19 mmol/L
High	â‰¥240 mg/dL	0.0259	6.22 mmol/L
HDL cholesterol[c]	>35 mg/dL	0.0259	>0.91 mmol/L
Copper (total)	75â€“145 mg/dL	0.157	11.8â€“22.8 mmol/L
Creatinine[b]			
Male, age 4â€“20 yr	0.2â€“1.2 mg/dL	88.4	18â€“106 mcmol/L

Female, age 4â€“20 yr	0.2â€“1.2 mg/dL	88.4	18â€–106 mcmol/L
Male, age 20â€–69 yr	0.7â€–1.5 mg/dL	88.4	62â€–133 mcmol/L
Female, age 20â€–69 yr	0.6â€–1.4 mg/dL	88.4	53â€–124 mcmol/L
Male, age â‰¥70 yr	0.7â€–1.7 mg/dL	88.4	62â€–150 mcmol/L
Female, age â‰¥70 yr	0.6â€–1.5 mg/dL	88.4	53â€–133 mcmol/L
Ferritin			
Male adult	20â€–323 ng/mL	2.25	45â€–727 pmol/L
Female adult	10â€–291 ng/mL	2.25	23â€–655 pmol/L
Folate			
Plasma	3.1â€–12.4 ng/mL	2.27	7.0â€–28.1 nmol/L
Red cell	186â€–645 ng/mL	2.27	422â€–1,464 nmol/L
Glucose, fasting (plasma)	65â€–109 mg/dL	0.055	3.58â€–6.00 mmol/L
Haptoglobin	30â€–220 mg/dL	0.01	0.3â€–2.2 g/L
Hemoglobin A1c (estimated)	4.0%â€–6.0%	0.01	0.04â€–0.06
Iron (total) (age >13 yr)			
Male	45â€–160 mcg/dL	0.179	8.1â€–31.3 mcmol/L
Female	30â€–160 mcg/dL	0.179	5.4â€–31.3 mcmol/L
Iron-binding capacity	220â€–420 mcg/dL	0.179	39.4â€–75.2 mcmol/L
Transferrin saturation	20%â€–50%	0.01	0.2â€–0.5
Lactate (plasma)	0.7â€–2.1 mmol/L	1	0.7â€–2.1 mmol/L
Magnesium	1.3â€–2.2 mEq/L	0.5	0.65â€–1.10 mmol/L
Osmolality	275â€–300 mOsm/kg	1	275â€–300 mmol/kg
Phosphate	2.5â€–4.5 mg/dL	0.323	0.8â€–11.45 mmol/L
Potassium (plasma)	3.3â€–4.9 mmol/L	1	3.3â€–4.9 mmol/L
Protein, total (plasma)	6.5â€–8.5 g/dL	10	65â€–85 g/L
Sodium	135â€–145 mmol/L	1	135â€–145 mmol/L
Triglycerides, fasting[c]	<250 mg/dL	0.0113	<2.8 mmol/L
Trop nin I			
Normal	0.1 ng/mL	100	60 ng/L
Indeterminant	0.1â€–1.4 ng/mL	100	70â€–140 ng/L
Abnormal	â‰¥1.5 ng/mL	100	â‰¥150 ng/L
Urea nitrogen	8â€–25 mg/dL	0.357	2.9â€–8.9 mmol/L
Uric acid[b]	3â€–8 mg/dL	59.5	179â€–476 mcmol/L
Vitamin B12	180â€–1,000	0. 38	133â€–738 pmol/L

	pg/mL		
Common serum enzymatic activities			
Aminotransferases			
Alanine (ALT, SGPT)	7â€“53 International Units/L	0.01667	0.12â€“0.88 mckat/L
Aspartate (AST, SGOT)	11â€“47 International Units/L	0.01667	0.18â€“0.78 mckat/L
Amylase	25â€“115 International Unit /L	0.01667	0.42â€“1.92 mckat/L
Creatine kinase			
Male	30â€“200 International Units/L	0.01667	0.50â€“3.33 mc/kat/L
Female	20â€“170 International Units/L	0.01667	0.33â€“2.83 mc/kat/L
MB fraction	0â€“7 International Units/L	0.01667	0â€“0.12 mc/k t/L
Gamma-glutamyl			
transpeptidase (GGT)			
Male	11â€“50 International Units/L	0.01667	0.18â€“0.83 mckat/L
Female	7â€“32 International Units/L	0.01667	0.12â€“0.53 mckat/L
Lactate dehydrogenase[b]	100â€“250 International Units/L	0.01667	1.67â€“4.17 mck t/L
Lipase	<100 International Units/L	0.01667	<1.67 mckat/L
5â€²-Nucleotidase	2â€“16 International Units/L	0.01667	0.0â€“30.27 mckat/L
Phosphatase, acid	0â€“0.7 International	16.67	0â€“11.6 nkat/L

	Units/L		
Phosphatase, alkaline[d]			
Age 10â€“15 yr	130â€“550 International Units/L	0.01667	2.17â€“9.17 mc/kat/L
Age 16â€–20 yr	70â€–260 International Units/L	0.01667	1.17â€–4.33 mc/kat/L
Age >20 yr	38â€–126 International Units/L	0.01667	0.13â€–2.10 mc/kat/L
Common serum hormone values[e]			
ACTH, fas ing (8 am, supine)	<60 pg/mL	0.22	<13.2 pmol/L
Aldosterone[f]	10â€–160 ng/L	2.77	28â€–443 mmol/L
Cortisol (plasma, morning)	6â€–30 mg/dL	0.027	0.16â€–0.81 mcmol/L
FSH			
Male	1â€–8 International Units/L	1	1â€–8 International Units/L
Female			
Follicul r	4â€–13 International Units/L	1	4â€–13 International Units/L
Luteal	2â€–13 International Units/L	1	2â€–13 International Units/L
Midcycle	5â€–22 International Units/L	1	5â€–22 International Units/L
Postmenopausal	20â€–138 International Units/L	1	20â€–138 International Units/L
Gastrin, fasting	0â€–130 pg/mL	1	0â€–130 ng/L
Growth hormone, fasting			
Male	<5 ng/mL	1	<5 mcg/L
Female	<10 ng/mL	1	<10 mcg/L
17-Hydroxyprogesterone			
Male adult	<200 ng/dL	0.03	<6.6 nmol/L

Female			
Follicular	<80 ng/dL	0.03	<2.4 nmol/L
Luteal	<235 ng/dL	0.03	<8.6 nmol/L
Postmenopausal	<51 ng/dL	0.03	<1.5 nmol/L
Insulin, fasting	315 microunits/L	7.18	144 pmol/L
LH			
Male	2â€“12 International Units/L	1	2â€“12 International Units/L
Female			
Follicular	1â€“1 International Units/L	1	1â€“18 International Units/L
Luteal	20 International Units/L	1	20 International Units/L
Midcycle	24â€“105 International Units/L	1	24â€“105 International Units/L
Postmenopausal	15â€“62 International Units/L	1	15â€“62 International Units/L
Parathyroid hormone	12â€“72 pg/mL	â€”	â€”
Progesterone			
Male	<0.5 ng/mL	3.18	<1.6 nmol/L
Female			
Follicular	0.1â€“1.5 ng/mL	3.18	0.32â€“4.80 nmol/L
Luteal	2.5â€“28.0 ng/mL	3.18	8â€“89 nmol/L
First trimester	9â€“47 ng/mL	3 18	29â€“149 nmol/L
Third trimester	55â€“255 ng/mL	3.18	175â€“811 nmol/L
Postmenopausal	<0.5 ng/mL	3.18	<1.6 nmol/L
Prolactin			
Male	1.6â€“18.8 ng/mL	1	1.6â€“18.8 mcg/L
Female	1.4â€“24.2 ng/mL	1	1.4â€“24.2 mcg/L
Renin activity (plasma)[g]	0.9â€“3.3 ng/mL hr	0.278	0.25â€“0.91 ng/(L sec)
Testosterone, total			
Male	270â€“1,070 ng/dL	0.0346	9.3â€“37.0 nmol/L
Female	6â€“86 ng/dL	0.0346	0.2â€“13.0 nmol/L
Testosterone, free			

Male	9â€“30 ng/dL	0.0346	0.3â€“11.0 pmol/L
Female	0.3â€“1.9 ng/dL	0.0 46	0.001â€“30.26 pmol/L
Thyroxine, total (T4)	4.5â€“12.0 mcg/dL	12.9	58â€“155 nmol/L
Thyroxine, free	0.7â€“1.8 ng/dL	12.9	10.3â€“34.8 pmol/L
uptake[h]	30%â€“46%	0.01	0.3â€“0.46
Triiodothyronine (T3)	45â€“132 ng/dL	0.0154	0.91â€“2.70 nmol/L
T4 index[i]	1.5â€“4.5	1	1.5â€“4.5
TSH	0.35â€“6.20 microunits/mL	1	0.35â€“6.20 microunits/L
Vitamin D, 1,25-dihydroxy	15â€“60 pg/mL	2.4	36â€“144 pmol/L
Vitamin D, 25-hydroxy	10â€“55 ng/mL	2.49	25â€“137 nmol/L
Common urinary chemistries			
Delta-aminolevulinic acid	1.5â€“7.5 mg/d	7.6	11.4â€“53.2 mcmol/d
Amylase	0.04â€“0.30 International Units/min	16.67	0.6â€“75.00 nkat/min
	60â€“450 Units/24 hr		
Calcium	50â€“250 mg/d	0.250	1.25â€“6.25 mmol/d
Catecholamines	<540 mcg/d	â€”	â€”
Dopamine	65â€“400 mcg/d	â€”	â€”
Epi ephrine	<20 mcg/d	5.5	<110 nmol/d
Norepinephrine	15â€“80 mcg/d	5.9	88.5â€“472.0 nmol/d
Copper	15â€“60 mcg/d	0.0157	0.24â€“0.95 mcmol/d
Cortisol, free	9â€“53 mcg/d	2.76	25â€“146 nmol/d
Creatinine			
Male	0.8â€“1.8 g/d	8.84	7.1â€–15.9 mmol/d
Female	0.6â€–1.5 g/d	8.84	5.3â€–13.3 mmol/d
5-Hydroxyindoleacetic acid	<6 mg/d	5.23	<47 mcmol/d
Metanephrine	<1.3 mg/d	5.46	<7.1 mcmol/d
Oxalate			
Male	7â€“44 mg/d	11.4	80â€–502 mcmol/d
Female	4â€–31 mg/d	11.4	46â€–353 mcmol/d
Porphyrins			
Coproporphyrin			
Male	0â€–96 mcg/d	1.53	0â€–110 nmol/d

Female	0â€“60 mcg/d	1.54	0â€“92 nmol/d
Uroporphyrin			
Male	0â€“46 mcg/d	1.2	0â€“32 nmol/d
Female	0â€“22 mcg/d	1.2	0â€“26 nmol/d
Protein	0â€“150 mg/d	0.001	0â€“0.150 g/d
Vanillylmandelic acid (VMA)	<8 mg/d	5.05	<40 mcmol/d
Common hematologic values			
Coagulation			
Bleeding time[j]	2.5â€“9.5 min	60	150â€–570 sec
Fibrin degradation products	<8 mcg/mL	â€”	â€”
Fibrinogen[k]	150â€–400 mg/dL	0.01	1.5â€–4.0 g/L
Partial thromboplastin time (activated)	24â€–34 sec	1	24â€–34 sec
Prothrombin time[l]	10.5â€–14.5 sec	1	10.5â€–14.5 sec
INR	0.78â€–1.22	â€”	â€”
Thrombin time	11.3â€–18.5 sec	1	11.3â€–18.5 sec
CBC			
Hematocrit			
Male	40.7%â€–50.3%	0.01	0.407â€–0.503
Female	36.1%â€–44.3%	0.01	0.361â€–0.443
Hemoglobin			
Male	13.8â€–17.2 g/dL	0.620[m]	8.56â€–10.70 mmol/L
Female	12.1â€–15.1 g/dL	0.620	7.50â€–9.36 mmol/L
Erythrocyte count			
Male	4.5â€–5.7 —[f] 10^6/microliters	1	4.55.7 10 —[f]^{12}/L
Female	3.9â€–5.0 —[f] 10^6/microliters	1	3.9â€–5.0 10 —[f]^{12}/L
Mean corpuscular hemoglobin	26.7â€–33.7 pg/cell	0.062	1.66â€–2.09 fmol/cell
Mean corpuscular hemoglobin concentration	32.7â€–35.5 g/dL	0.620	20.3â€–22.0 mmol/L
Mean corpuscular volume	80.0â€–97.6 mcm^3	1	80.0â€–97.6 fL
Red cell distribution width	11.8%â€–14.6%	0.01	0.118â€–0.146
Leukocyte profile			

Total	3.8â€“9.8 —Á 10^3/microliters	1	3.8â€“9.8 10 —Í^9/L
Lymphocytes	1.2â€“3.3 —Á 10^3/microliters	1	1.2â€“3.3 10 —Í^9/L
Mononuclear cells	0.2â€“0.7 —Á 10^3/microliters	1	0.2â€“0.7 10 —Í^9/L
Granulocytes	1.8â€“6.6 —Á 10^3/microliters	1	1.8â€“6.6 10 —Í^9/L
Platelet count	140â€“440 —Á 10^3/microliters	1	140â€“440 10 —Í^9/L
Erythrocyte sedimentation rate			
Male, <50 yr	0â€“15 sec		
Male, >50 yr	0â€“20 sec		
Female, <50 yr	0â€“20 sec		
Female, >50 yr	0â€“30 sec		
Reticulocyte count			
Adults	0.5%â€“1.5%	0.01	0.005â€“0.015
Children	2.5%â€“6.5%	0.01	0.025â€“0.065
Immunology testing			
Complement (total hemolytic)[n]	118â€“226 units/mL		
C3	75â€“165 mg/dL	0.01	0.85â€“1.85 g/L
C4	12â€“42 mg/dL	0.01	0.12â€“0.54 g/L
Immunoglobulin			
IgA	70â€“370 mg/dL	0.01	0.70â€“3.70 g/L
IgM	30â€“210 mg/dL	0.01	0.30â€“2.10 g/L
IgG	700â€“1450 mg/dL	0.01	7.00â€“14.50 g/L
Therapeutic agents			
Amitriptyline (+ nortriptyline)	150â€“250 mcg/L		
Carbamazepine	4â€“12 mg/L	4.23	17â€“51 mcmol/L
Clonazepam	10â€“50 mcg/mL	3.17	32â€“159 nmol/L
Cyclosporine (whole blood)	183â€“335 ng/mL		Exact range depends on the type of transplant
Digoxin	0.8â€“2.0 mcg/L	1.28	1.0â€“2.6 nmol/L
Disopyramide	2â€“5 mg/L	2.95	6â€“15 mcmol/L
Ethosuximide	40â€“75 mg/L	7.08	283â€“531 mcmol/L
Imipramine	150â€“300 mcg/L	3.57	536â€“1071 nmol/L
Desipramine	100â€“300 mcg/L	3.75	375â€“1,125 nmol/L

Lithium	0.6â€“1.3 mmol/L	1	0.6â€“1.3 mmol/L
Nortriptyline	50â€“150 mcg/L	3.8	190â€“665 nmol/L
Phenobarbital	10â€–40 mg/L	4.3	43â€–172 mcmol/L
Phenytoin (diphenylhydantoin)	10â€–20 mg/L	3.96	40â€–79 mcmol/L
Primidone			
Primidone	5â€–15 mg/L	4.58	23â€–69 mcmol/L
Phenobarbital	1â€–5 mcg/L	4.3	6â€–9 mcmol/L
Procainamide	4â€–10 mg/L	4.23	17â€–42 mcmol/L
Procainamide +			
N-acetylprocainamide	6â€–20 mg/L		
Quinidine	2â€–5 mg/L	3.08	6.2â€–15.4 mcmol/L
Salicylate[o]	20â€–290 mg/L	0.0072	0.14â€–2.10 mmol/L
Theophylline	10â€–20 mg/L	5.5	55â€–110 mcmol/L
Valproic acid	50â€–100 mg/L	6.93	346â€–693 mcmol/L
Antimicrobials			
Amikacin			
Trough	1â€–8 mg/L	1.71	1.7â€–13.7 mcmol/L
Peak	20â€–30 mg/L	1.71	34â€–51 mcmol/L
5-Fluorocytosine			
Trough	20â€–60 mg/L		
Peak	50â€–100 mg/L		
Gentamicin			
Trough	0.5â€–2.0 mg/L	2.09	1.0â€–4.2 mcmol/L
Peak	6â€–10 mg/L	2.09	12.5â€–20.9 mcmol/L
Ketoconazole			
Trough	1 mg/L	â€”	â€”
Peak	1â€–4 mg/L	â€”	â€”
Sulfamethoxazole			
Trough	75â€–120 mg/L	â€”	â€”
Peak	100â€–150 mg/L	â€”	â€”
Tobramycin			
Trough	0.5â€–2.0 mg/L	2.14	1.1â€–4.3 mcmol/L
Peak	6â€–10 mg/L	2.14	12.8â€–21.4 mcmol/L
Trimethoprim			
Trough	2â€–8 mg/L	â€”	â€”
Peak	5â€–15 mg/L	â€”	â€”
Vancomycin			
Trough	5â€–15 mg/L	0.69	3.5â€–10.4 mcmol/L

Peak	20â€“40 mg/L		13.8â€“27.0 mcmol/L

ACTH, adrenocorticotropic hormone; fL, femtoliter; fmol, femtomole; FSH, follicle-stimulating hormone; HDL, high-density lipoprotein; INR, international normalized ratio; katal, mole/sec; kPa, kilopascal; LH, luteinizing hormone; ¯µkat, microkatal; nkat, nanokatal; pmol, picomole; TSH, thyroid-stimulating hormone.

[a]A more complete list of multiplication factors for converting conventional units to SI units can be found in *Ann Intern Med* 1967;106:114, and in *The SI for the Health Professions.* Geneva: World Health Organization, 1977.

[b]Variation occurs with age and gender. This range includes both genders and persons older than 5 yr.

[c]National Institutes of Health Congress Development Panel on Triglycerides, HDL, and Coronary Artery Diseases, *JAMA* 1993;269:505.

[d]Higher values (up to 350 ¯µU/mL) can be normal in persons younger than 20 yr.

[e]Because most hormones are measured by immunologic techniques and because hormones may vary in molecular weight (e.g., gastrin), most are expressed as mass/L. The reference ranges are method dependent.

[f]Supine, normal unit diet; in the upright position, the reference range is 40â€“310 ng/L.

[g]High-sodium diet, supplemented with sodium, 3 g/d.

[h]Replaces T_3 resin uptake.

[i]T_4 ¯· (T uptake).

[j]Template modified after Ivy.

[k]Determined by the Clauss method.

[l]Normal ranges for prothrombin times vary according to the reagent used. Therefore, we report an INR with all prothrombin times ordered.

[m]This factor assumes a unit molecular weight of 16,000; assuming a unit molecular weight of 64,500, we have a multiplication factor of 0.156.

[n]CH_{50} is the reciprocal of dilution of sera required to lyse 50% of sheep erythrocytes.

[o]Therapeutic range for treatment of rheumatoid arthritis [(4)]

International Systems of units

Notes on International Systems of units (SI Units)

Système International (SI) units are a specific subset of the metre-kilogram-second system of units and were agreed upon as the everyday currency for commercial and scientific work in 1960, following a series of international conferences organised by the International Bureau of Weights and Measures. SI units have been adopted widely in clinical laboratories, but non-SI units are still used in many countries. For that reason, values in both units are given for common measurements throughout this textbook and commonly used non-SI units are shown in this appendix. However, the SI unit system is recommended (Table 9.2, 9.3) [3]

Table 9.2 Examples of basic SI units [3]

Length	metre (m)
Mass	kilogram (kg)
Amount of substance	mole (mol)
Energy	joule (J)
Pressure	pascal (Pa)
Volume	The basic SI unit of volume is the cubic metre (1000 litres). For convenience, however, the litre (l) is used as the unit of volume in laboratory work.

Table 9.3 Examples of decimal multiples and submultiples of SI units

Factor	*Name*	*Prefix*
10^{6}	mega-	M
10^{3}	kilo-	K
10^{-1}	deci-	D
10^{-2}	centi-	C
10^{-3}	milli-	M
10^{-6}	Micro-	M
10^{-9}	nano-	N
10^{-12}	pico-	P
10^{-15}	Femto-	F

Exceptions to the use of SI units

By convention, blood pressure is excluded from the SI unit system and is measured in mmHg (millimetres of mercury) rather than pascals.

Mass concentrations (e.g. g/l, µg/l) are used in preference to molar concentrations for all protein measurements, and for substances which do not have a sufficiently well-defined composition.

Some enzymes and hormones are measured by 'bioassay', in which the activity in the sample is compared with the activity (rather than the mass) of a standard sample which is provided from a central source. For these assays, results are given in standardised 'units', or 'international units', which depend upon the activity in the standard sample and may not be readily converted to mass units. (3)

Pregnancy and Medical Therapeutics

Using medications during pregnancy or lactation always creates discussion between the patient and the physician. There is a continuous balance among treating a maternal condition for the safety of the mother, treating a maternal condition for the safety of the fetus, and potential drug toxicity to the fetus. [4]

It is important to remember that the risk to the embryo or fetus changes throughout gestation, such that many of the more teratogenic medications can be relatively safe during certain parts of the pregnancy or during lactation.

Many factors play a role in the possible teratogenic nature of a medication, such as the genetic susceptibility of the fetus, maternal ability to absorb and metabolize medications, other environmental factors, the developmental stage of the embryo or fetus, dose and duration of exposure, activity of metabolites, and drugâ€“drug interactions.

Placental transport is very important and occurs more readily with medications that are of low molecular weight, lipid soluble, nonpolar, and nonprotein bound.Gestational timing of medications is very important.

There is thought to be an â€œall-or-noneâ€ phenomenon for an embryo during the first 2 weeks after conception. It is during this time that exposure to a teratogen is believed to either cause enough damage to the embryo that death or a spontaneous miscarriage occurs, or no damage or sufficient repair occurs such that there are no lasting effects. This is a common period of fetal exposure, given that many women may not yet know they are pregnant.

The first trimester is the very important time of organogenesis, during which both adequate control of maternal disease states (e.g., diabetes), and limiting

exposure to teratogens (e.g., radiation exposure and many medications) are equally important and may require consultation with an obstetrician or maternal fetal medicine specialist.

The remainder of pregnancy is a period of cell growth and differentiation that can be inhibited by certain medications in varying doses and duration of exposure.

Finally, lactation represents a time during which certain medications may be transported to the fetus through breast milk. The cellular mechanisms through which this occurs are different than during placental transport, and certain medications (e.g., warfarin) that can be teratogenic or cause disastrous complications when passage occurs in utero can be very safe during lactation, given transportation in only an inactive form. (4)

Risk Factor Categories

Category A: Controlled studies in women fail to demonstrate a risk to the fetus in the first trimester, there is no evidence of a risk in later trimesters, and the possibility of fetal harm appears remote. Very few drugs have sufficient safety profiles, and therefore the table excludes this category. The only medications classified as category A are each of the vitamins when used in Recommended Dietary Allowance (RDA)-recommended doses, levothyroxine, the antiemetic doxylamine, and the electrolytes potassium citrate, potassium chloride, and potassium gluconate. (4)

Category B: Either animal studies have not demonstrated a fetal risk but there are no controlled studies in pregnant women, or animal studies have shown an adverse effect that was not confirmed in controlled studies in women in the first trimester, and there is not evidence of a risk in the later trimesters. (4)

Category C: Either studies in animals have revealed adverse effects on the fetus (teratogenic or embryocidal or other) and there are no controlled studies in women, or studies in women and animals are not available. These drugs should only be given if the potential benefit justifies the potential risk to the fetus. (4)

Category D: There is positive evidence of human fetal risk, but the benefits from use in pregnant women may be acceptable despite the risk, as if the drug is needed in a [4]

life-threatening situation or for serious diseases for which safer drugs are ineffective or cannot be used. [4]

Category X: Studies in animals or humans have demonstrated fetal abnormalities or there is evidence of fetal risk based on human experience, and the risk of the use of the drug in pregnant women clearly outweighs any possible benefit. These drugs are contraindicated in women who are or may become pregnant (Table 9.4). [4]

Table 9.4 **Pregnancy and Medical Therapeutics** [4]

Class of medication	Category B	Category C	Category D	Category X
Analgesics	Acetaminophen Diclofenac* Hydromorphone* Ibuprofen* Indomethacin* Meperidine* Methadone* Naproxen* Oxycodone* Oxymorphone*	Triptans (antimigraine) Aspirin* Butalbital Butorphanol* Celecoxib* Codeine* Fentanyl* Hydrocodone* Ketorolac* Morphine* Propoxyphene* Sufentanil* Tramadol	*	Dihydroergotamine Ergotamine
Anesthetics	Halothane Isoflurane Ketamine Lidocaine (local) Propofol Ropivacaine (local)	Nitrous oxide Sevoflurane		
Antibiotics	Azithromycin Aztreonam Cephalosporins Clavulanate Clindamycin Ethambutol Meropenem Metronidazole Penicillins Polymyxin B	Gentamycin Bacitracin Chloramphenicol Chloroquine Clarithromycin Dapsone Imipenem-cilastatin Isoniazid Linezolid	Quinine Streptomycin Tetracyclines	

	Sulbactam Tazobactam Vancomycin	Pyrazinamide Quinolones** Rifampin Spectinomycin Sulfonamides Trimethoprim		
Anticonvulsants	Magnesium sulfate	Ethosuximide Gabapentin Lamotrigine Levetiracetam Oxcarbazepine Topiramate Zonisamide	Carbamazepine Clonazepam Diazepam Phenobarbital Phenytoin Primidone Valproic acid	
Antidepressants/ antipsychotics/ anxiolytics	Buproprion Buspirone Zolpidem	Aripiprazole Chlorpromazine Clozapine Haloperidol Olanzapine Quetiapine Risperidone SSRIs[a] TCAs Thioridazine Ziprasidone	Alprazolam Chlordiazepoxide Clonazepam Diazepam Lithium Lorazepam Midazolam Oxazepam	Other benzodiazepines
Antifungals	Amphotericin B Clotrimazole	Caspofungin Fluconazole Griseofulvin Ketoconazole Miconazole Nystatin Terconazole		
Antihistamines	Cetirizine Chlorpheniramine Diphenhydramine Loratadine Meclizine	Fexofenadine Hydroxyzine Promethazine		
Antilipemics	Cholestyramine Colestipol Niacin[b]	Clofibrate Gemfibrozil		Statins
Antiretrovirals[c]	Atazanavir Didanosine Emtricitabine Ritonavir Saquinavir Tenofovir	Efavirenz Indinavir Lamivudine Lopinavir Nevirapine Zidovudine		
Antivirals	Acyclovir Famciclovir Valacyclovir	Amantadine Foscarnet Ganciclovir Oseltamivir Rimantadine		

Bisphosphonates		Alendronate Ibandronate Risedronate	Pamidronate	
Cardiovascular drugs	Methyldopa Hydrochlorothiazide[d]	Acetazolamide Adenosine Calcium-channel blockers Clonidine Digoxin Esmolol Flecainide Hydralazine Isosorbides Labetalol[d] Metoprolol[d] Minoxidil Nitroglycerin (B/C) Nitroprusside Prazosin Propranolol[d] Terazosin	ACE Inhibitors Amiodarone ARBs Atenolol	
Dermatology	Azelaic acid Clindamycin Erythromycin	Benzoyl peroxide Tretinoin (topical)	Doxycycline	Isotretinoin
Gastrointestinal	Cimetidine Famotidine Lactulose Lansoprazole Loperamide Meclizine Mesalamine Metoclopramide Ondansetron Opium tincture Orlistat Pantoprazole Ranitidine Sucralfate Ursodiol	Docusate Droperidol Kaolin Mineral oil Omeprazole Prochlorperazine Promethazine Senna Simethicone		Misoprostol
Hematologic	Argatroban Clopidogrel Dalteparin Dypyridamole Enoxaparin Lepirudin	Alteplase Epoetin alfa Filgrastim Heparin Pentoxifylline Streptokinase	Aminocaproic acid	Warfarin
Hormones	Acarbose Desmopressin Insulin Metformin	Adrenal hormones[e] Calcitonin Glipizide Glyburide	Methimazole Propylthiouracil Tamoxifen	Danazol Estrogens Iodide^{131} Leuprolide

	Somatostatin Troglitazone Vasopressin Micronized progesterone	Melatonin Repaglinide Rosiglitazone Tolbutamide	Hydroxyprogesterone[f]	Mifepristone Testosterone
Respiratory	Acetylcysteine Budesonide Cromolyn sodium Ipratropium Montelukast Zafirlukast	Albuterol Corticosteroids (inhaled) Dextromethorphan Guaifenesin Salmeterol Theophylline		
Urologic	Oxybutynin Phenazopyridine	Tolterodine Trospium		

ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blockers; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant.
*All of these can be considered category D if used in large doses or for prolonged duration.
**Although classified as category C by the manufacturer, use with caution, given concern for floroquinolone-induced fetal cartilage damage. This is somewhat controversial, but most obstetricians avoid their use in pregnancy.
[a]Recent data suggest that there may be concern for increased risk of persistent pulmonary hypertension with exposure in the latter half of pregnancy or neonatal withdrawl syndromeâ€"although usually self-limitedâ€"with exposure in the third trimester. Fluoxitine is the most studied and has been followed the longest, and setraline still has a good safety profile. Much of this is still hotly debated in the literature.
[b]Considered category C if used in doses for lipid treatment or above RDA doses.
[c]Efavirenz is generally not recommended in the first trimester. Consider consultation with a specialist who has experience in HIV and pregnancy because there are specific recommendations for treating HIV in pregnancy that differ from nonpregnant treatment regimens.
[d]There is concern for use of beta-blockers in the second and third trimesters given reports of intrauterine growth restriction and reduced placental weights, although labetalol has the most safety data of the class. Diuretics should not be used to treat gestational hypertension.
[e]Adrenal hormones (e.g., cortisol, dexamethasone, hydrocortisone, and prednisone) are generally regarded as category C, although some argue that one should try to avoid use during the first trimester, given some concern for teratogenic data in animals and human epidemiologic studies.
[f]Avoid in the 1st trimester. The American college of Obstetrics & Gynecology (ACOG) supports its use in pregnancy if history of spontaneous preterm delivery. (4)

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