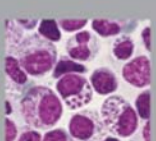

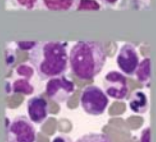

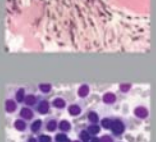

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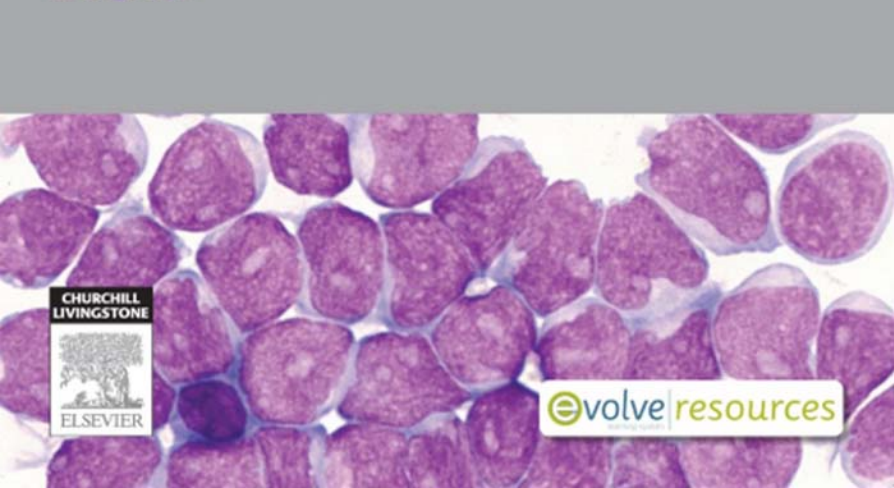

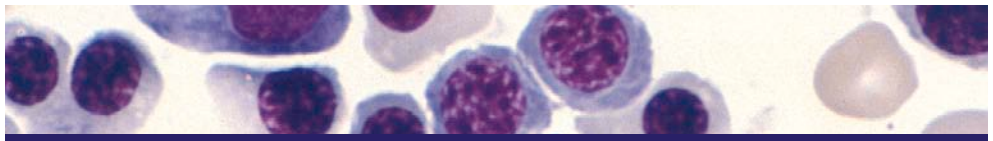

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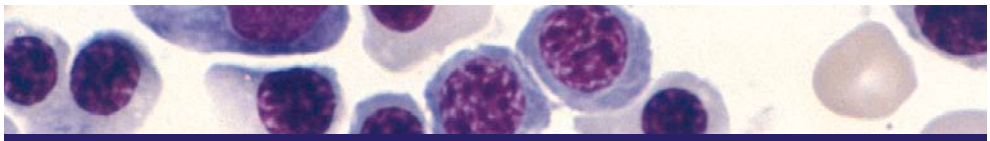

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CONTENTS

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PREFACE

The third edition of *Microscopic Haematology: A Practical Guide for the Laboratory,* maintains the standard and picture quality achieved in the second edition. The third edition includes descriptions of neoplasms according to the fourth edition of the *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Additional red cell disorders and white cell neoplasms, including non-Hodgkin lymphoma have been included in this edition. An additional ninety-two images have also been included.

I am indebted to a number of people for their assistance in compiling this book. I would like to thank Professor Robert Lindeman (Director of the Department of Haematology at the Prince of Wales Hospital, Sydney) for allowing me to access all the blood films and bone marrow slides in our laboratory. I am especially indebted to Michael Oakey and Virginia Bentink for their invaluable help and experience in producing a CD-ROM of all 450 images. Thank you to Pauline Dalzell for her expert assistance in updating the cytogenetics of haemopoietic and lymphoid neoplasms. Above all, I wish to thank Narelle Woodland (Senior Lecturer and Coordinator of Haematology at the University of Technology, Sydney) for her advice and continued support during my writing of this third edition.

Gillian Rozenberg

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ABBREVIATIONS

ABL1	Abelson murine leukaemia viral oncogene homolog1
aCML	atypical chronic myeloid leukaemia
add	addition
ADP	adenosine diphosphate
AIDS	acquired immunodeficiency syndrome
AIHA	autoimmune haemolytic anaemia
ALL	acute lymphoblastic leukaemia
AMEGA	amegakaryocytic
AML	acute myeloid leukaemia
AP-AAP	alkaline phosphatase-anti-alkaline phosphatase
APL	acute promyelocytic leukaemia
ATLL	adult T-cell leukaemia/lymphoma
ATPase	adenosine triphosphatase
ATRA	all-trans retinoic acid
AUL	acute undifferentiated leukaemia
BCL2	B-cell CLL/lymphoma 2
BCL6	B-cell CLL/lymphoma 6
BCL10	B-cell CLL/lymphoma 10
BCR	breakpoint cluster region
BL	Burkitt lymphoma
BM	bone marrow
B-PLL	B-cell prolymphocytic leukaemia
BSS	Bernard-Soulier syndrome
CCND1	cyclin D1
cCD	cytoplasmic cluster of differentiation
CD	cluster of differentiation
CDA	congenital dyserythropoietic anaemia
CEL	chronic eosinophilic leukaemia
CHL	classical Hodgkin lymphoma
CLL/SLL	chronic lymphocytic leukaemia/small lymphocytic lymphoma
CMML	chronic myelomonocytic leukaemia
CM	cutaneous mastocytosis
CML	chronic myelogenous leukaemia
CML-AP	chronic myelogenous leukaemia-accelerated phase
CML-BP	chronic myelogenous leukaemia-blast phase
CML-CP	chronic myelogenous leukaemia-chronic phase
CMV	cytomegalovirus
CNL	chronic neutrophilic leukaemia
CNS	central nervous system
CSF	cerebrospinal fluid
CTCL	cutaneous T-cell lymphoma
cyt-μ	cytoplasmic
DAT	direct antiglobulin test
DBA	Diamond-Blackfan anaemia
DC	dyskeratosis congenita

DEB	diepoxybutane
del	deletion
der	derivative
DIC	disseminated intravascular coagulation
DLBCL	diffuse large B-cell lymphoma
DNA	deoxyribonucleic acid
EBV	Epstein-Barr virus
EDTA	ethylenediamine tetraacetic acid
ESR	erythrocyte sedimentation rate
ET	essential thrombocythaemia
ETV6	ETS variant gene
EWS	Ewing sarcoma
FA	Fanconi anaemia
FGFR1	fibroblast growth factor receptor 1
FISH	fluorescence in situ hybridisation
FLI1	interleukin 1 family, member 7 (zeta)
FLT3	FMS-related tyrosine kinase 3
G-CSF	granulocyte colony-stimulating factor
GP	glycoprotein
GPS	gray platelet syndrome
G-6-PD	glucose-6-phosphate dehydrogenase
HbCS	haemoglobin Constant Spring
HbF	fetal haemoglobin
HbH	haemoglobin H
HCL	hairy cell leukaemia
H&E	haematoxylin and eosin
HE	hereditary elliptocytosis
HELLP	haemolysis, elevated liver enzymes and low platelet count
HEMPAS	hereditary erythroblastic multinuclearity with a positive acidified serum test
HES	hypereosinophilic syndrome
HIV	human immunodeficiency virus
HL	Hodgkin lymphoma
HPP	hereditary pyropoikilocytosis
HS	hereditary spherocytosis
HTLV-1	human T-cell leukaemia virus (human T-lymphotrophic virus) type 1
HUS	haemolytic uraemic syndrome
i	isochromosome
IGH	IgG heavy chain Locus
IGK	immunoglobulin kappa
IGL	immunoglobulin lambda
IL3	interleukin 3
IM	infectious mononucleosis
inv	inversion
ISSD	infantile sialic acid storage disease
ITP	idiopathic thrombocytopenic purpura
JAK2	Janus Kinase 2
JMML	juvenile myelomonocytic leukaemia
KIT	V-KIT Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
LCH	Langerhans' cell histiocytosis
LDHL	lymphocyte-depleted classical Hodgkin lymphoma

LGL	large granular lymphocyte
LPL	lymphoplasmacytic lymphoma
LRCHL	lymphocyte-rich classical Hodgkin lymphoma
MALT	mucosa-associated lymphoid tissue
MALT1	mucosa-associated lymphoid tissue lymphoma translocation gene 1
MCCHL	mixed cellularity classical Hodgkin lymphoma
MCH	mean cell haemoglobin
MCHC	mean cell haemoglobin concentration
MCL	mast cell leukaemia
MCV	mean cell volume
MDS	myelodysplastic syndrome
MDS/MPD,U	myelodysplastic/myeloproliferative neoplasm, unclassifiable
MDS-U	myelodysplastic syndrome, unclassifiable
MHA	May-Hegglin anomaly
MLL	mixed lineage leukaemia gene
MPAL	mixed phenotype acute leukaemia
MPN,U	myeloproliferative neoplasm, unclassifiable
MPO	myeloperoxidase
MPV	mean platelet volume
MYC	V-MYC avian myelocytomatosis viral oncogene homolog
NaF	sodium fluoride
NAP	neutrophil alkaline phosphatase
N/C ratio	nuclear cytoplasmic ratio
NEC	necrotising enterocolitis
NHL	non-Hodgkin lymphoma
NK	natural killer
NLPHL	nodular lymphocyte predominant Hodgkin lymphoma
NOS	not otherwise specified
NPM1	nucleophosmin/nucleoplasmin family member 1
NRBCs	nucleated red blood cells
NSCHL	nodular sclerosis classical Hodgkin lymphoma
PB	peripheral blood
PAS	periodic acid-Schiff
PBX1	pre-B-cell leukaemia transcription factor 1
PCH	paroxysmal cold haemoglobinuria
PDGFRA	platelet-derived growth factor receptor, alpha
PDGFRB	platelet-derived growth factor receptor, beta
PDW	platelet distribution width
Ph	Philadelphia chromosome
PK	pyruvate kinase
PLL	prolymphocytic leukaemia
PMF	primary myelofibrosis
PNH	paroxysmal nocturnal haemoglobinuria
PV	polycythaemia vera
RA	refractory anaemia
RAEB	refractory anaemia with excess blasts
RAEB-F	refractory anaemia with excess blasts with fibrosis
RARA	retinoic acid receptor alpha gene
RBC	red blood cell
RARS	refractory anaemia with ring sideroblasts
RCC	refractory cytopenia of childhood

RCMD	refractory cytopenia with multilineage dysplasia
RCMD-RS	refractory anaemia with multilineage dysplasia and ring sideroblasts
RCUD	refractory cytopenia with unilineage dysplasia
RDW	red cell distribution width
RN	refractory neutropenia
RNA	ribonucleic acid
RT	refractory thrombocytopenia
RUNX1	runt-related transcription factor 1
SBB	Sudan black B
SDS	Shwachman-Diamond syndrome
SIg	surface immunoglobulin
SM	systemic mastocytosis
SMZL	splenic marginal zone lymphoma
t	translocation
TAM	transient abnormal myelopoiesis
t-AML	therapy-related acute myeloid leukaemia
TAR	thrombocytopenia with absent radii
TCR	T-cell receptor
TdT	terminal deoxynucleotidyl transferase
TEC	transient erythroblastopenia of childhood
T-LGL	T-cell large granular lymphocytic leukaemia
t-MDS	therapy-related myelodysplastic syndrome
t-MDS/MPN	therapy-related myelodysplastic syndrome/myeloproliferative neoplasm
TP53	Tumour protein p53
T-PLL	T-cell prolymphocytic leukaemia
TTP	thrombotic thrombocytopenic purpura
WAS	Wiskott-Aldrich syndrome
WBC	white blood cell
WCC	white cell count
WHO	World Health Organization
ZBTB16	zinc finger-and BTB domain-containing protein 16

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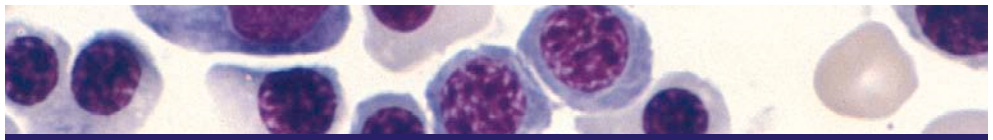
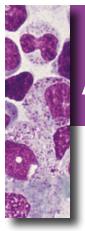


PART A

ERYTHROCYTES



A1 Erythropoiesis

NORMOBLASTIC ERYTHROPOIESIS

Erythropoiesis is divided into a number of stages. The earliest recognisable red cell precursor in the bone marrow is known as the proerythroblast; this gives rise to the basophilic erythroblast, the polychromatic erythroblast, the orthochromatic erythroblast, the polychromatic red cell (reticulocyte) and the mature red cell.

Normal erythropoiesis is characterised by the following progressive changes:

(a) Reduction in cell size

(b) Maturing of the cytoplasm: as the cytoplasm gradually acquires haemoglobin it changes from a basophilic to an eosinophilic colour. This change is accompanied by a gradual loss of RNA

(c) Maturing of the nucleus: the chromatin strands gradually become condensed and pyknotic; nucleoli are lost and the nucleus is finally extruded at the orthochromatic stage while the cell is still within the bone marrow. The resulting polychromatic red cell or reticulocyte still contains some RNA, which, after a period of 1–2 days, completely disappears and a fully haemoglobinised mature red cell or erythrocyte results.

These cell characteristics are seen in fixed preparations stained with a Romanowsky stain.

Proerythroblast

The proerythroblast varies from 12 to 20 μm in diameter and has a large nucleus that occupies most of the cell. The chromatin strands are fine, giving an even reticular appearance. Nucleoli are present. The cytoplasm is intensely basophilic—much more so than is seen in blast cells of the white cell series. Refer to Fig A1-1.

Basophilic erythroblast

The basophilic erythroblast varies from 10 to 16 μm in diameter. The nucleus is still relatively large and the chromatin strands are thick, giving a coarse appearance; there are no nucleoli present. The cytoplasm is still very basophilic. Refer to Fig A1-2.

Polychromatic erythroblast

The polychromatic erythroblast varies from 8 to 14 μm in diameter. The nucleus is smaller and the chromatin strands more dense, tending to form clumps giving a characteristic cartwheel-shaped appearance. The cytoplasm is no longer basophilic but polychromatic or mauve coloured as it has begun to acquire haemoglobin. Refer to Fig A1-3.

Orthochromatic erythroblast

The orthochromatic erythroblast varies from 8 to 10 μm in diameter. The nucleus is small, with a coarse, pyknotic chromatin pattern. The cytoplasm is pale pink with a polychromatic hue signifying that it has acquired more haemoglobin. As the cell matures, the nucleus becomes smaller and is finally extruded whilst still within the bone marrow. Refer to Fig A1-4.

Polychromatic red cell

The polychromatic red cell is a young erythrocyte that is slightly larger than the mature red cell. It is polychromatic in colour since it still contains some RNA remnants, which can be demonstrated by the use of a supravital stain such as new methylene blue or brilliant cresyl blue, in which case the cell is termed a reticulocyte. Once this cell has lost all its RNA, it develops into a mature fully haemoglobinised red cell or erythrocyte. Refer to Fig A1-5.

Mature red cell

The mature red cell (erythrocyte) is a biconcave disc approximately 7 μm in diameter with an area of central pallor occupying less than one-third of its diameter. Red cells exhibit an eosinophilic reaction when stained with any of the Romanowsky stains. The average life span of a red cell is 120 days. Refer to Fig A1-6.

MEGALOBLASTIC ERYTHROPOIESIS

Megaloblasts are abnormal erythroblasts produced in the bone marrow of patients deficient in vitamin B_{12} and/or folic acid. Vitamin B_{12} and folic acid are vital for DNA synthesis and thus for the normal maturation and growth of red cells.

Megaloblastic changes occur in all stages of red cell maturation. Megaloblastic erythropoiesis is classified according to the normoblastic series: promegaloblast, basophilic megaloblast, polychromatic megaloblast, orthochromatic megaloblast and mature macrocyte.

Megaloblasts differ from normoblastic erythroblasts in the following respects:

(a) They are larger at every stage of their development.
(b) Nuclear maturation is abnormal, since vitamin B_{12} and folic acid are vital for DNA synthesis. Deficiency or absence of either leads to abnormal maturation of the nucleus and asynchronous development; the nucleus lags behind the cytoplasm at every stage in the maturation process. This is most evident in the polychromatic megaloblast, where the cytoplasm is polychromatic and the chromatin strands of the nucleus are still very fine and open—unlike the polychromatic erythroblast, where they are dense and form clumps.
(c) Mitoses are common and sometimes abnormal in appearance.

Refer to Figs A1-7 to A1-12.

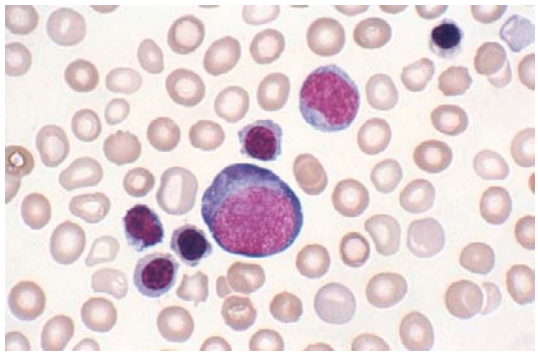


Figure A1-1
Proerythroblast and polychromatic erythroblasts in the peripheral blood of a newborn infant with haemolytic disease of the newborn. (x 1000)

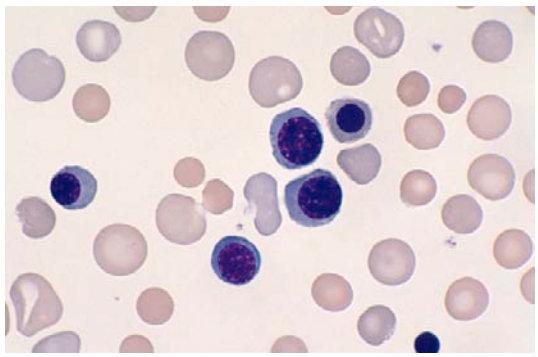


Figure A1-2
Basophilic and polychromatic erythroblasts in the peripheral blood of a newborn infant with haemolytic disease of the newborn. (x 1000)

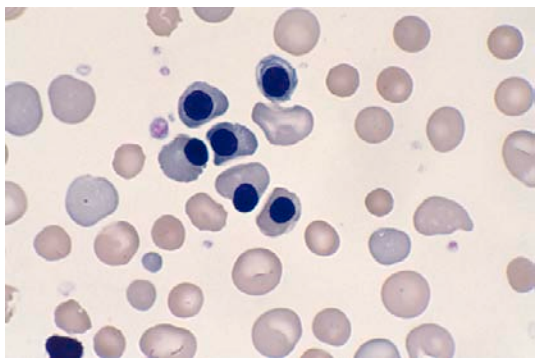


Figure A1-3
Polychromatic erythroblasts in the peripheral blood of a newborn infant with haemolytic disease of the newborn. (x 1000)

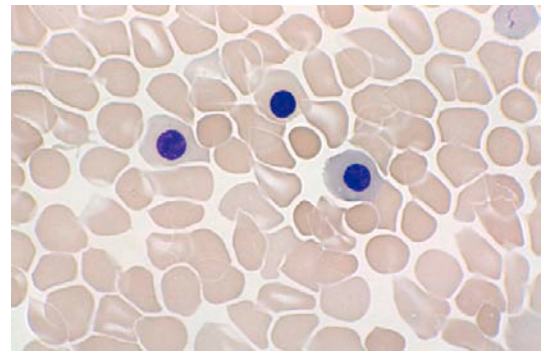


Figure A1-4
Polychromatic and orthochromatic erythroblasts in the peripheral blood of a newborn infant with haemolytic disease of the newborn. (x 1000)

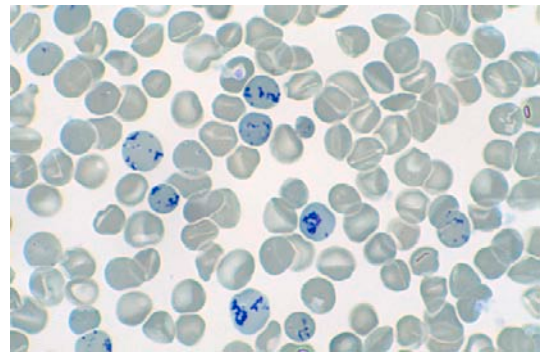


Figure A1-5
Reticulocytes in the peripheral blood stained with new methylene blue stain. (x 1000)

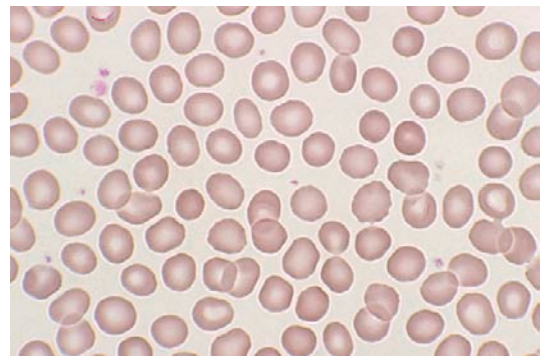


Figure A1-6
Mature red cells in the peripheral blood. (x 1000)

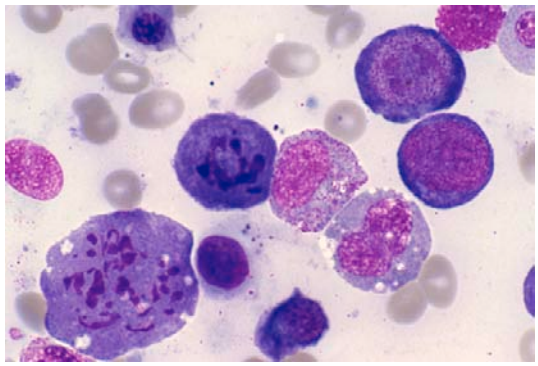


Figure A1-7
Abnormal mitoses in the bone marrow in megaloblastic anaemia. (x 1000)

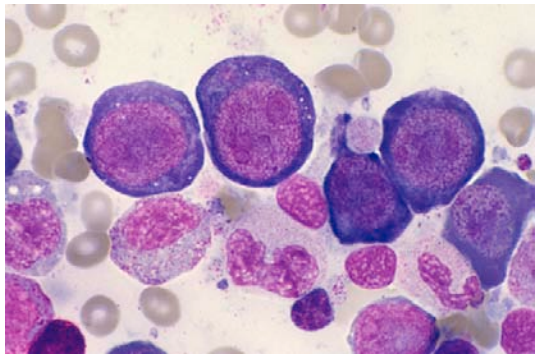


Figure A1-8
Promegaloblast, basophilic megaloblasts and myeloid precursors in a bone marrow aspirate from a patient with megaloblastic anaemia. (x 1000)

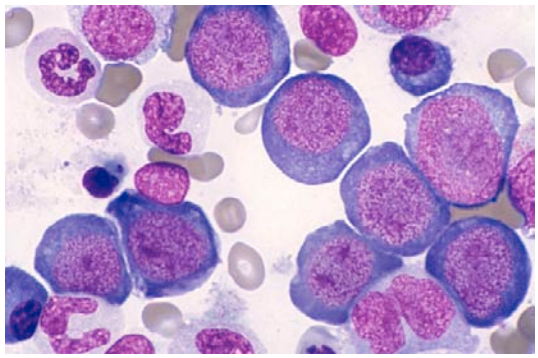


Figure A1-9
Basophilic megaloblasts in the bone marrow in megaloblastic anaemia. (x 1000)

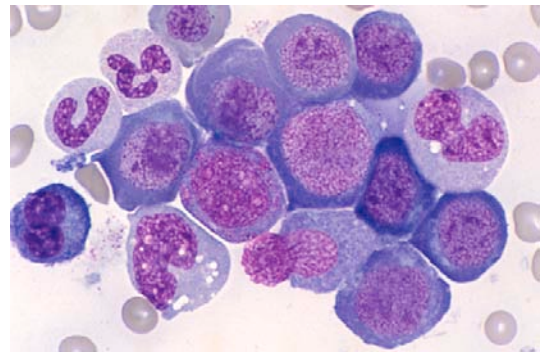


Figure A1-10
Basophilic and polychromatic megaloblasts in the bone marrow in megaloblastic anaemia. (x 1000)

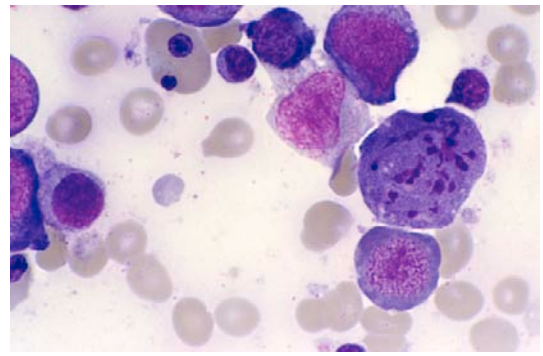


Figure A1-11
Polychromatic and orthochromatic megaloblast in the bone marrow in megaloblastic anaemia. (x 1000)

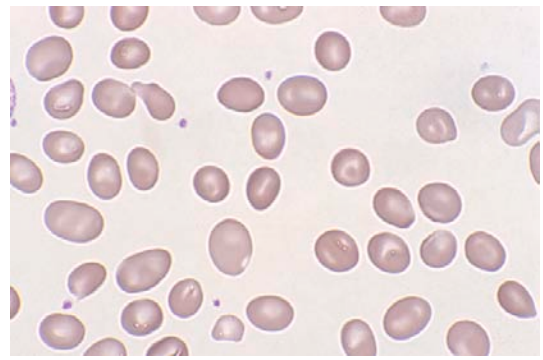
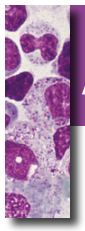


Figure A1-12
Megaloblastic mature red cells in the peripheral blood in megaloblastic anaemia. (x 1000)



A2 Deficiency anaemias

IRON DEFICIENCY ANAEMIA

Iron deficiency anaemia occurs when the iron content of the body is less than normal. It is characterised by decreased or absent iron stores, low serum iron concentration, high transferrin with low saturation, low haemoglobin concentration, low haematocrit and low red cell number. The red cells in iron deficiency anaemia are microcytic and hypochromic. Specific red cell parameters such as the mean cell volume (MCV) and mean cell haemoglobin (MCH) are reduced, while the red cell distribution width (RDW) is increased.

A major cause of iron deficiency anaemia is blood loss. It may also result from an inadequate diet and rarely from malabsorption. Pregnancy and growth are associated with greater requirements for iron; thus the risk of development of iron deficiency is high at these times. Microcytic hypochromic red cells are characterised by an MCV less than 80 fL and an MCH less than 27 pg. Red cell size may be assessed by comparing the red cell with a small lymphocyte.

The classical features found on the blood film in iron deficiency include anisocytosis, microcytes, hypochromasia, elliptocytes, and pencil cells and fragmented cells. Thrombocytosis is often present. When iron-deficiency anaemia is treated, a dimorphic blood film will result, that is, one in which there are two distinct populations of red cells: microcytic and hypochromic as well as normocytic and normochromic.

Severe cases of iron deficiency anaemia may also be detected in the bone marrow by the presence of smaller than normal erythroblasts with ragged and incompletely haemoglobinised cytoplasm. Iron stores may be assessed from the bone marrow by performing a Perl's Prussian blue stain. Haemosiderin, which is present in the marrow fragments, will stain a turquoise colour in the presence of iron; decreased or absent haemosiderin is characteristic of iron deficiency. Refer to Figs A2-1 to A2-4.

MEGALOBLASTIC ANAEMIA

Megaloblastic anaemia is due to a lack of vitamin B_{12} and/or folic acid. Vitamin B_{12} deficiency is usually due to malabsorption. One form of malabsorption is pernicious anaemia, an autoimmune disease in which there is a lack of intrinsic factor production by the gastric parietal cells. Less commonly, vitamin B_{12} deficiency results from dietary insufficiency. Folic acid deficiency results from an inadequate diet, particularly of leafy green vegetables and fruit, the additional requirements of pregnancy and, less frequently, from impaired absorption.

Classical features of megaloblastic anaemia are seen in both the peripheral blood and bone marrow. Nuclear cytoplasmic asynchrony is a characteristic feature leading to macrocytic red cells with an MCV ranging from 100 to 150 fL. The red cells are oval in shape and may contain basophilic stippling and Howell-Jolly bodies. Teardrop poikilocytes are often present. The neutrophils are hypersegmented and giant metamyelocytes can be seen in the bone marrow. Megaloblastic anaemia due to inadequate diet often coexists with a microcytic hypochromic anaemia due to the presence of iron deficiency. In such cases, hypochromic microcytes will also be present and the blood picture is described as a 'mixed' deficiency. Refer to Figs A2-5 to A2-9.

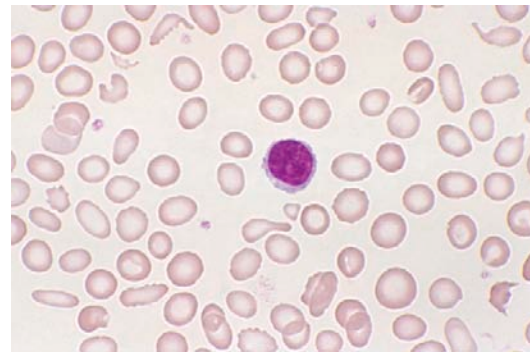


Figure A2-1
Iron deficiency anaemia: peripheral blood film showing hypochromic microcytes, elliptocytes and fragmented cells. (x 1000)

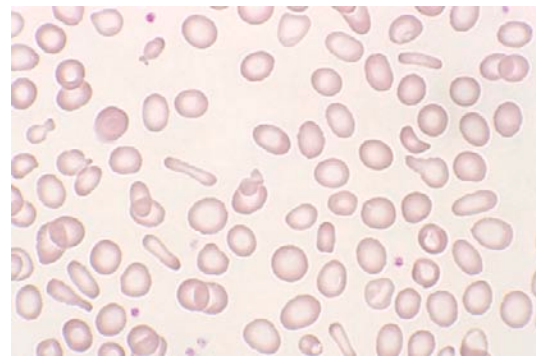


Figure A2-2
Iron deficiency anaemia: peripheral blood film showing hypochromic microcytes, elliptocytes, pencil cells and fragmented cells. (x 1000)

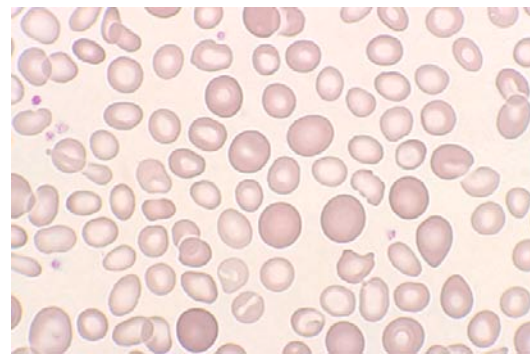


Figure A2-3
Response to iron therapy in a child: dimorphic blood picture showing two populations of red cells: hypochromic microcytic and normochromic normocytic. (x 1000)

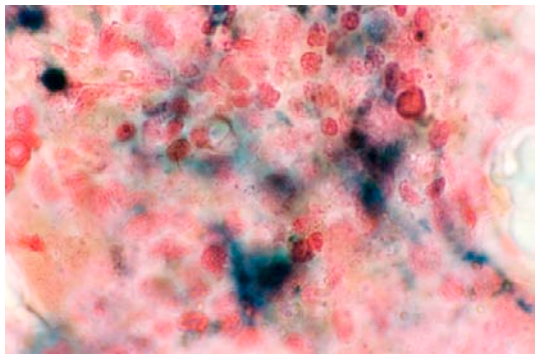


Figure A2-4
Perl's Prussian blue stain showing haemosiderin in a fragment of bone marrow. (x 1000)

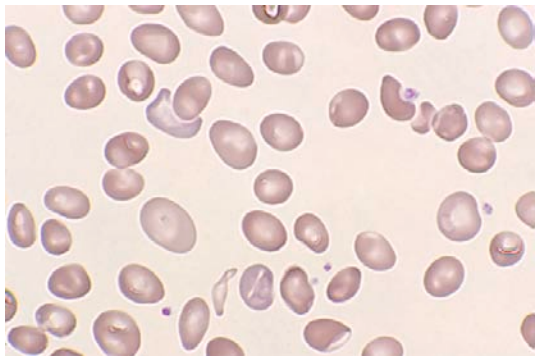


Figure A2-5
Megaloblastic anaemia: peripheral blood showing many oval macrocytes. (x 1000)

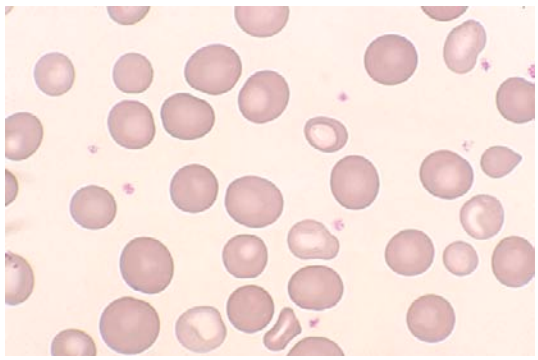


Figure A2-6
Round macrocytes in the peripheral blood in alcoholic liver disease with low serum and red cell folate levels. (x 1000)

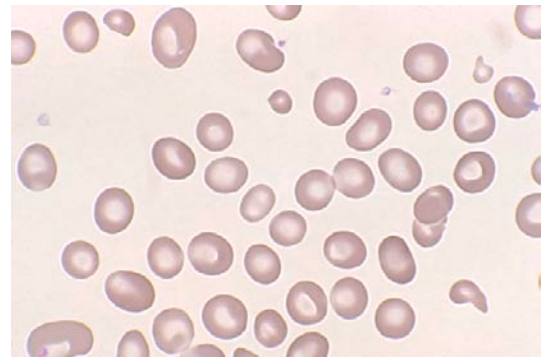


Figure A2-7
'Mixed deficiency' in the peripheral blood film from a 4-month-old child with vitamin B_{12}, folic acid and iron deficiency. This child was being breastfed by a vegan mother. (x 1000)

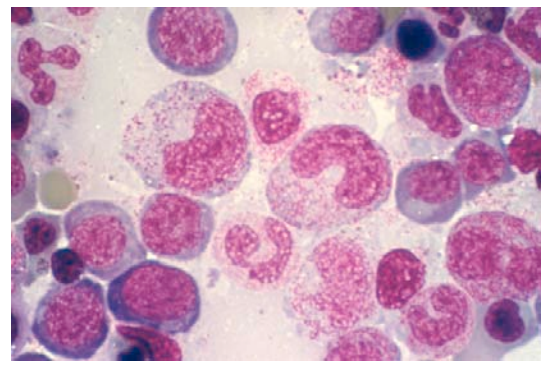


Figure A2-8
Megaloblastic anaemia: bone marrow showing two giant metamyelocytes. (x 1000)

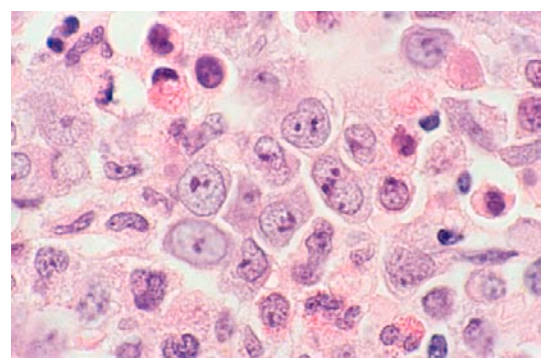
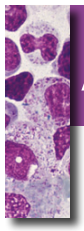


Figure A2-9
Megaloblastic anaemia: bone marrow trephine infiltrated with megaloblasts. (H&E) (x 1000)



A3 Haemolytic anaemias

AUTOIMMUNE HAEMOLYTIC ANAEMIA

Autoimmune haemolytic anaemia (AIHA) is due to antibodies produced by the body's immune system against its own red cells. These antibodies are either warm or cold and in some instances may have a wide thermal amplitude extending from warm to cold.

Warm-antibody AIHA is the most common type; the antibodies produced are of the IgG class, which have maximal activity at 37°C. Cold-antibody AIHA results from the production of antibodies of the IgM class, which act at temperatures below 37°C.

Examination of the blood film from a case of AIHA reveals the presence of spherocytes, polychromasia, and nucleated red cells. In cold AIHA, auto-agglutination will also be present.

The diagnosis of AIHA is established by performing a direct antiglobulin test (DAT) on the patient's red cells. A positive result indicates the presence of antibody or complement on the red cell surface, thus confirming the diagnosis of AIHA. The DAT can also be used to differentiate AIHA from hereditary spherocytosis (HS): both disorders have a similar blood picture but HS is characterised by a negative DAT. Refer to Figs A3-1 to A3-3.

PAROXYSMAL COLD HAEMOGLOBINURIA (PCH)

Paroxysmal cold haemoglobinuria is an autoimmune haemolytic anaemia described by Julius Donath and Karl Landsteiner in 1904. It occurs in children under 5 years of age. The blood picture resembles that of an AIHA with spherocytes, reticulocytes and nucleated red cells. It is positive for the Donath-Landsteiner antibody which is a polyclonal IgG that binds to various red cell antigens such as I, i, P and p on the red cell surface. The P antigen is its primary target. The polyclonal IgG anti-P autoantibody binds to red blood cell surface antigens in the cold. When the blood returns to the warmer central circulation, the red cells are lysed with complement, giving rise to intravascular haemolysis. The anaemia is DAT (C3d) positive. The blood film sometimes shows monocytic and granulocytic erythrophagocytosis. Refer to Figs A3-4 to A3-6.

NON-IMMUNE HAEMOLYTIC ANAEMIA

Clostridial sepsis

Septicaemias induced by *Clostridium welchii* and *C. perfringens* give rise to severe rapidly progressive intravascular haemolytic anaemia characterised by the presence of microspherocytes. It is thought that these bacteria produce a toxin containing a proteolytic agent capable of destroying spectrin. This toxin is responsible for red cell membrane destruction often involving the entire red cell mass. Refer to Figs A3-7 and A3-8.

Paroxysmal nocturnal haemoglobinuria (PNH)

PNH is a haemopoietic stem cell disorder present in red cells, white cells and platelets. This red cell abnormality predisposes the red cells to intravascular complement-mediated lysis. Lack of red cell membrane proteins, in particular the 'decay accelerating factor'

(DAF CD55), the 'membrane inhibitor of reactive lysis' protein (MIRL CD59) and the 'homologous restriction factor' (HRF), leads to severe clinical haemolysis. These proteins negatively regulate the haemolytic action of complement on red cells. Patients with PNH present with a severe anaemia with Hb levels ranging from less than 5.0 g/L to normal. They may also present with pancytopenia as well as aplastic anaemia. There is a macrocytosis due to the presence of increased reticulocytes and in some cases a microcytic hypochromic anaemia due to iron deficiency. Refer to Fig A3-9.

Haemolytic anaemia due to lead poisoning

The ingestion of lead interferes with haem synthesis. It does so by inhibiting several of the enzymes directly involved with haem synthesis. Pyrimidine 5′-nucleotidase is one such enzyme. In its absence, pyrimidine nucleotides accumulate in the red cells, preventing iron from being incorporated into haem at a normal rate. This leads to a shortened red cell life span resulting in a mild haemolytic anaemia. The blood film shows characteristic fine to coarse basophilic stippling in the red cells as seen with any of the Romanowsky stains. The anticoagulant ethylenediamine tetraacetic acid (EDTA) can mask lead-induced stippling if blood films are not made fresh and fixed immediately.

Refer to Fig A3-10.

MICROANGIOPATHIC HAEMOLYTIC ANAEMIA

The term 'microangiopathic' means small vessel disease; hence microangiopathic haemolytic anaemia results from physical damage to red cells as they pass through very small orifices or damaged and sclerosed vessels.

The blood film shows increased numbers of red cell fragments that have characteristically sharp projections. These fragments are referred to as schistocytes, red cells produced by a microangiopathic process. They are fractured or ripped as they pass across strands of fibrin in damaged vessels or as they pass across a damaged or prosthetic heart valve. Thrombocytopenia is a classical finding in some types of microangiopathic haemolytic anaemia.

A variety of disorders are associated with a microangiopathic blood picture, namely haemolytic uraemic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), human immunodeficiency virus (HIV) infection, disseminated intravascular coagulation (DIC), valvular heart disease, HELLP or preeclampsia of pregnancy, necrotising enterocolitis (NEC), malignancy and acute renal failure. Microangiopathic haemolytic anaemia may also result from the use of the immunosuppressive agent cyclosporin. Refer to Figs A3-11 to A3-20.

Haemolytic uraemic syndrome (HUS)

HUS occurs most commonly in infancy and early childhood and is initiated by infection with *Escherichia coli* strain 0157. This bacterium produces a verocytotoxin that is attracted to the vascular endothelium, especially the endothelium lining the glomeruli of the kidney. This toxin induces severe glomerulonephritis that in turn leads to a microangiopathic blood picture.

The blood film of HUS shows schistocytes and a marked thrombocytopenia. Refer to Fig A3-11.

Thrombotic thrombocytopenic purpura (TTP)

TTP is a microangiopathic haemolytic anaemia seen mostly in adults. It is characterised by a pentad of clinical features, namely fever, thrombocytopenia, anaemia, neurological symptoms and renal disease; schistocytes are seen on the blood film.

TTP has been reported in patients with the acquired immunodeficiency syndrome (AIDS)-related complex. Refer to Fig A3-12.

Disseminated intravascular coagulation (DIC)

DIC occurs when small blood vessels become blocked by platelet and fibrin thrombi, thus altering the patency of the vessel and inducing intravascular haemolysis. The blood film shows schistocytes and thrombocytopenia. Refer to Fig A3-14.

Valvular heart disease

Microangiopathic haemolytic anaemia occurs in valvular heart disease and also in some patients who have had prosthetic heart valves inserted. The high shear forces produced by the abnormal blood flow seen in such disorders produce a blood film characterised by the presence of schistocytes, classical of a microangiopathic process. The platelet count is invariably normal. Refer to Fig A3-15.

Malignancy

A microangiopathic haemolytic anaemia may be associated with metastatic carcinoma, especially mucin-secreting adenocarcinoma of the breast and stomach. Metastases occurring in the microvascular system, especially the lung, give rise to a microangiopathic blood picture with thrombocytopenia. Refer to Fig A3-16.

Cyclosporin

The immunosuppressive agent cyclosporin is nephrotoxic and hence may give rise to a microangiopathic blood picture with thrombocytopenia. Refer to Fig A3-18.

HELLP syndrome

The HELLP syndrome (haemolysis, elevated liver enzymes and low platelet count) is a multisystem syndrome occurring in severe preeclampsia and eclampsia. It affects both primiparous and multiparous women in the third trimester of pregnancy. HELLP is characterised by a microangiopathic haemolytic anaemia, hepatic dysfunction, renal failure and in severe cases, DIC.

Delivery of the fetus is the initial treatment; however, the disease remains active after delivery and appears to achieve peak intensity during the 24–48 hour post delivery period. Refer to Fig A3-20.

OXIDANT-DRUG-INDUCED HAEMOLYTIC ANAEMIA

The use of oxidant drugs can be easily recognised from the blood film, provided that the patient has not had a splenectomy. Two frequently used oxidant drugs, dapsone and sulfasalazine (Salazopyrin), may give rise to a Heinz-body-positive haemolytic anaemia resulting in a blood picture characterised by the presence of bite and blister cells. Heinz bodies are precipitates of denatured haemoglobin and are the manifestation of the oxidative challenge that the red cell has suffered. They are rapidly removed or pitted out by the spleen, giving rise to bite cells. Should the red cell membrane of the bite cell rejoin, a blister cell will result. The red cells of premature and term neonates are more susceptible to oxidants. Prolonged exposure to naphthalene may give rise to a haemolytic anaemia in infants despite normal levels of the enzyme glucose-6-phosphate dehydrogenase (G-6-PD). Refer to Figs A3-21 to A3-24.

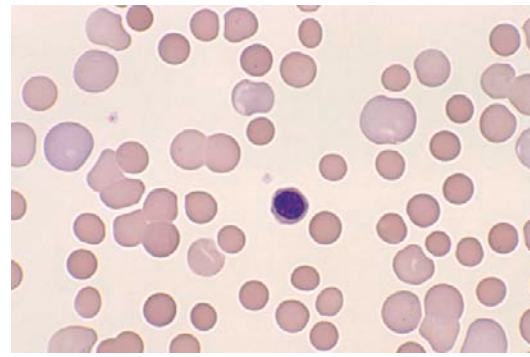


Figure A3-1
Autoimmune haemolytic anaemia (warm antibody): peripheral blood film showing spherocytes, reticulocytes (polychromasia) and a nucleated red cell. (x 1000)

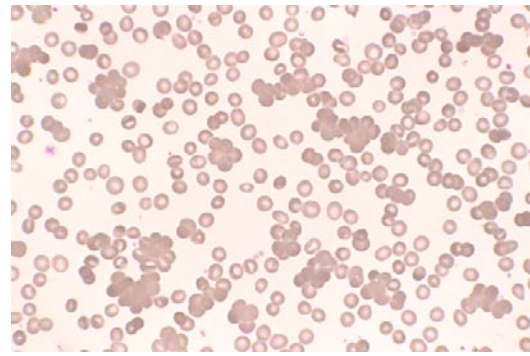


Figure A3-2
Autoimmune haemolytic anaemia (cold antibody): peripheral blood film showing marked auto-agglutination. Blood samples with cold agglutination will have a falsely raised mean cell volume (MCV) unless the sample is prewarmed to 37°C prior to processing through the blood cell analyser. (x 400)

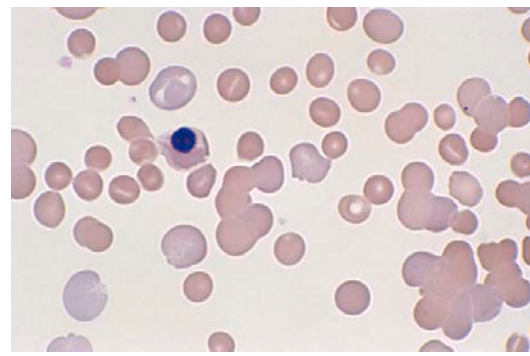


Figure A3-3
Peripheral blood film showing the presence of a wide-thermal-amplitude auto-antibody giving rise to features of both a warm and cold autoimmune haemolytic anaemia. (x 1000)

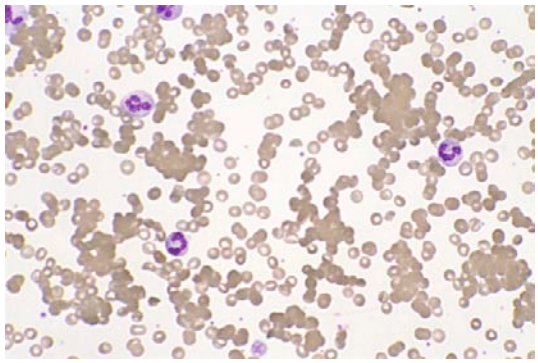


Figure A3-4
Paroxysmal cold haemoglobinuria: Donath Landsteiner antibody positive haemolytic anaemia. Peripheral blood film showing auto-agglutination, spherocytes and reticulocytes. (x 400)

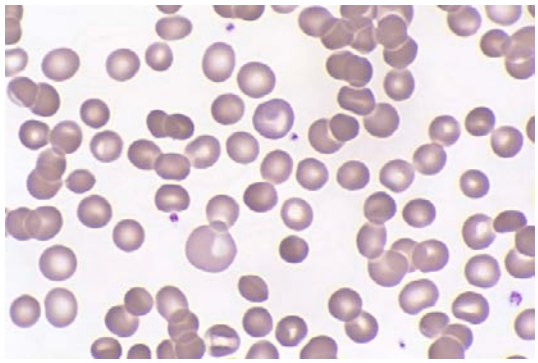


Figure A3-5
Paroxysmal cold haemoglobinuria: Donath Landsteiner antibody positive haemolytic anaemia. Peripheral blood film showing auto-agglutination, spherocytes and reticulocytes. (x 1000)

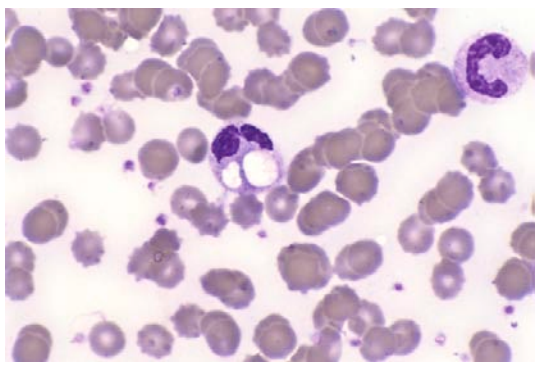


Figure A3-6
Paroxysmal cold haemoglobinuria: Donath Landsteiner antibody positive haemolytic anaemia. Peripheral blood film showing granulocytic erythrophagocytosis. (x 1000)

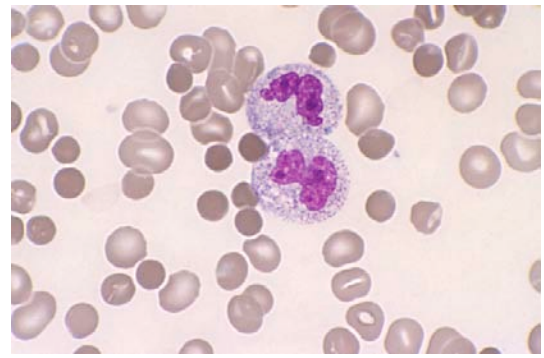


Figure A3-7
Clostridial sepsis: peripheral blood film from a case of *C. Perfringens* septicaemia showing toxic granulation in the neutrophils and microspherocytes. (x 1000)

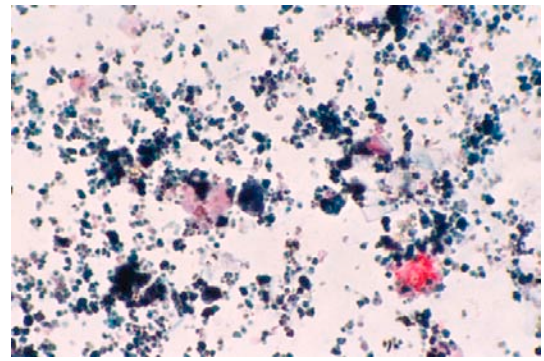


Figure A3-8
Clostridial sepsis: Perl's Prussian blue stain on the urine deposit from the case in Figure A3-7 showing a strongly positive urinary haemosiderin indicative of intravascular haemolysis. (x 1000)

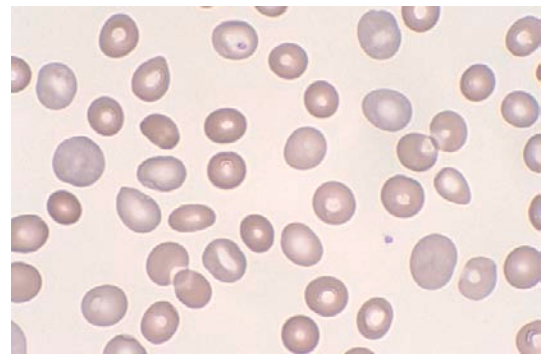


Figure A3-9
Paroxysmal nocturnal haemoglobinuria: peripheral blood film showing a macrocytic anaemia together with increased polychromasia or reticulocytes. (x 1000)

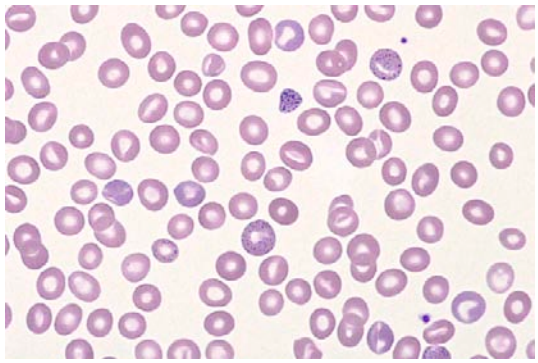


Figure A3-10
Lead poisoning: peripheral blood film showing coarse basophilic stippling in the red cells of a patient with a lead level of 5.95 µmol/l. (x 1000)

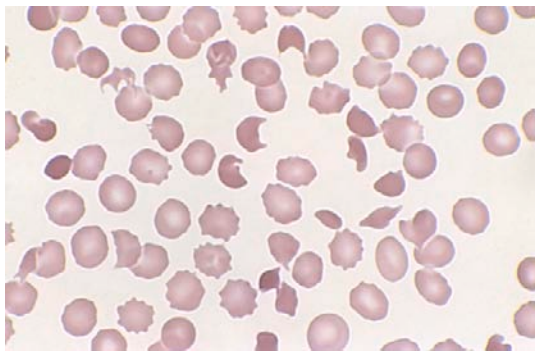


Figure A3-11
Haemolytic uraemic syndrome: peripheral blood film showing schistocytes and thrombocytopenia. (x 1000)

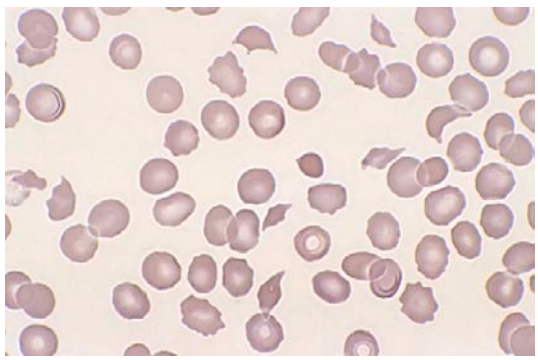


Figure A3-12
Thrombotic thrombocytopenic purpura: peripheral blood film showing schistocytes and thrombocytopenia. (x 1000)

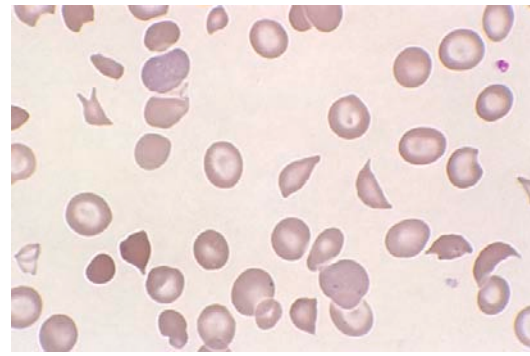


Figure A3-13
HIV infection: peripheral blood film showing schistocytes and thrombocytopenia. (x 1000)

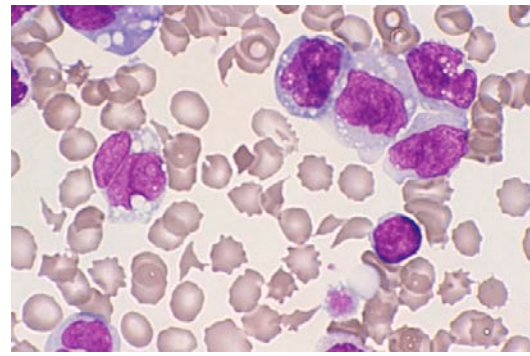


Figure A3-14
Disseminated intravascular coagulation: peripheral blood film from a patient with chronic myelomonocytic leukaemia (CMML) showing a marked number of schistocytes and thrombocytopenia. (x 1000)

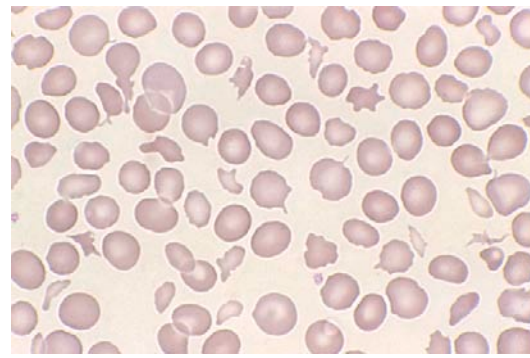


Figure A3-15
Valvular heart disease: peripheral blood film showing the presence of schistocytes. The platelet count is usually normal in valvular heart disease. (x 1000)

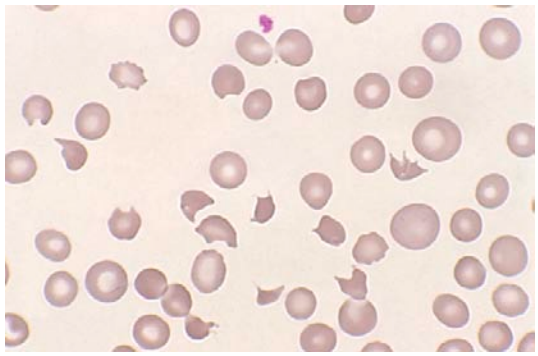


Figure A3-16
Mucin-secreting cancer of the stomach: peripheral blood film showing schistocytes and thrombocytopenia. (x 1000)

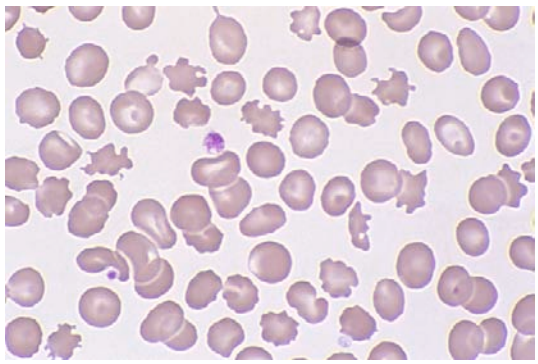


Figure A3-17
Acute renal failure: peripheral blood film showing increased numbers of burr cells. (x 1000)

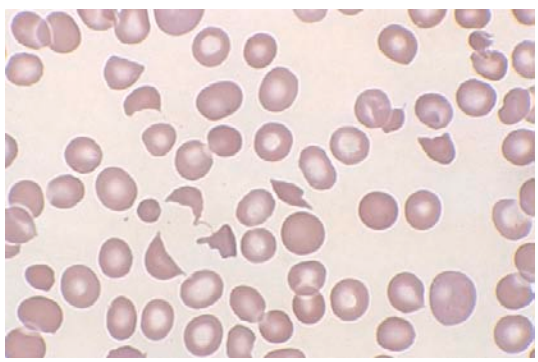


Figure A3-18
Cyclosporin-induced microangiopathic haemolytic anaemia following a peripheral blood stem cell transplant showing the presence of schistocytes and thrombocytopenia. (x 1000)

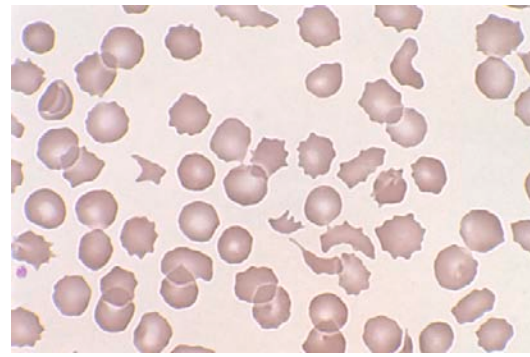


Figure A3-19
Necrotising enterocolitis: peripheral blood film in a premature neonate showing schistocytes and thrombocytopenia. (x 1000)

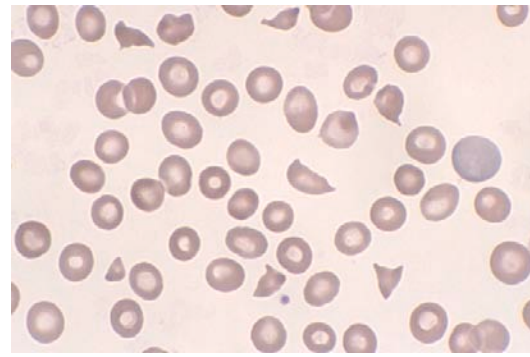


Figure A3-20
HELLP syndrome: peripheral blood film showing the presence of schistocytes in a primiparous woman in the third trimester of pregnancy. (x 1000)

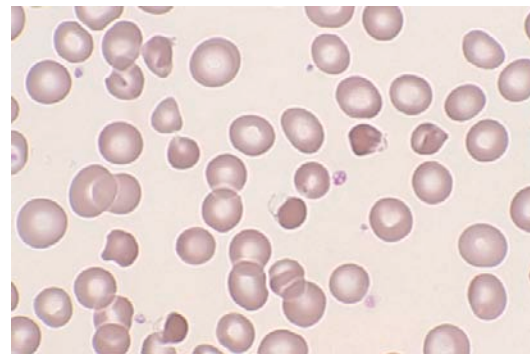


Figure A3-21
Oxidant drug (dapsone)-induced haemolytic anaemia: peripheral blood film showing the presence of bite and blister cells. (x 1000)

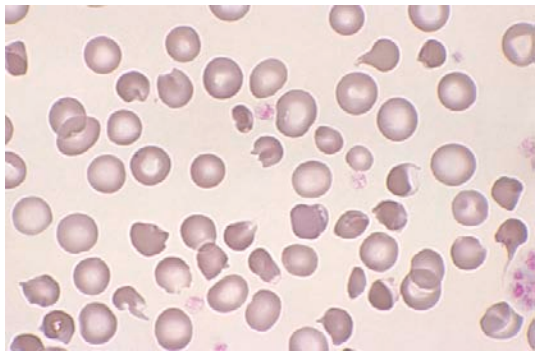


Figure A3-22
Naphthalene-induced haemolytic anaemia: peripheral blood film from a 19-day-old neonate showing bite and blister cells with a normal level of G-6-PD. (x 1000)

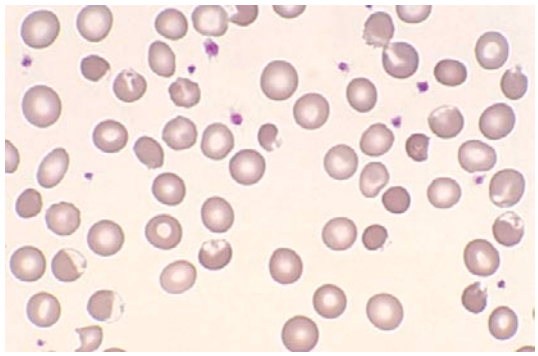


Figure A3-23
G-6-PD deficiency: peripheral blood film showing the presence of bite and blister cells following ingestion of fava beans (favism). (x 1000)

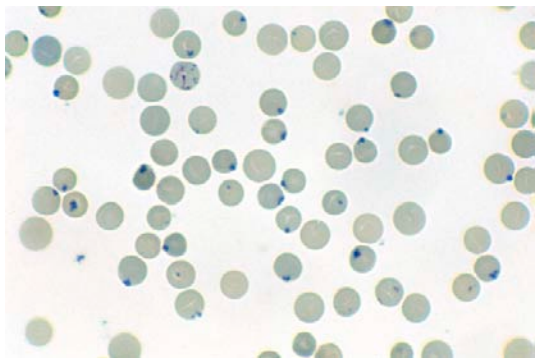
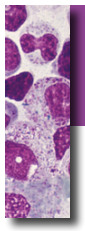


Figure A3-24
Heinz bodies in the peripheral blood film of a splenectomised patient who is being treated with the oxidant drug dapsone. New methylene blue stain. (x 1000)



A4 Haemoglobin disorders

The thalassaemias are hereditary anaemias that occur as a result of a mutation that affects the synthesis of normal haemoglobin. Normal haemoglobin consists of two pairs of dissimilar polypeptide chains, α-like and non-α(β, γ or δ). Each chain encloses an iron-containing porphyrin known as haem. The normal haemoglobins are:

- Haemoglobin A, consisting of two α- and two β-globin chains
- Haemoglobin A_2, consisting of two α- and two δ-globin chains
- Haemoglobin F, consisting of two α- and two γ-globin chains.

α-Thalassaemia is characterised by a reduction or total lack of α-globin chains and β-thalassaemia by a reduction or total lack of β-globin chains. A microcytic hypochromic anaemia is associated with both α- and β-thalassaemia.

THE α-THALASSAEMIAS

α-Thalassaemia commonly occurs in populations from South-East Asia, the Mediterranean, Africa and China. It can arise when any number of the four α-globin genes are either reduced or absent and hence can be divided into four groups.

Number of genes affected	Condition
1	Silent carrier α-thalassaemia trait
2	α-Thalassaemia trait
3	Haemoglobin H (HbH) disease
4	Hydrops fetalis

Silent carrier α-thalassaemia trait

Silent carrier α-thalassaemia trait is characterised by minimal or no haematological changes, and the haemoglobin level and the mean cell volume (MCV) are low normal. There is no obvious microcytosis seen on the blood film. The diagnosis of silent carrier α-thalassaemia trait is made by family studies and/or gene analysis.

α-Thalassaemia trait

This trait is characterised by a microcytic hypochromic blood film; the average MCV is 68 fL and the average mean cell haemoglobin (MCH) is 22 pg. HbH inclusion bodies (β_4) are present after the blood is incubated at 37°C for 2 hours with a supravital stain such as brilliant cresyl blue. These inclusion bodies represent precipitates of HbH and give the cell a golfball-like appearance. Haemoglobin electrophoresis is normal in α-thalassaemia trait; thus it is vital to detect the occasional HbH cell whose presence enables the diagnosis of α-thalassaemia trait to be made. Refer to Fig A4-1.

Haemoglobin H disease

HbH disease is characterised by a markedly microcytic hypochromic blood film with increased numbers of target cells and red cell fragments. The average MCV is 57 fL and the average MCH is 21 pg. HbH inclusions are present in the great majority of red cells. Refer to Figs A4-2 and A4-3.

Hydrops fetalis

Infants with hydrops fetalis are delivered stillborn at 30–40 weeks. The hydrops is due to a failure to produce α-globin chains. If a blood film can be obtained from the stillborn, it will show a characteristic population of large hypochromic macrocytes, marked polychromasia, basophilic stippling and increased numbers of nucleated red cells. Refer to Fig A4-4.

Haemoglobin constant spring

Haemoglobin H disease can be associated with a haemoglobin known as Haemoglobin Constant Spring (HbCS). HbCS is an alpha chain variant rather than a deletion. The alpha chain is elongated by 31 additional amino acid residues at the C-terminal end making it very unstable. The presence of HbCS causes the red cells to break down faster than usual giving rise to a severe anaemia.

The red cells of HbCS are large and different from those seen in any of the other forms of thalassaemia. They are markedly overhydrated relative to those of the deletional forms of alpha thalassaemia. Coarse basophilic stippling is a characteristic feature of HbCS. Refer to Fig A4-5.

THE β-THALASSAEMIAS

β-Thalassaemia commonly occurs in populations of Mediterranean and African origin, as well as in the Middle East, India, Pakistan, China and South-East Asia.

The β-thalassaemias include four syndromes: silent carrier β-thalassaemia trait, β-thalassaemia trait, β-thalassaemia intermedia and β-thalassaemia major.

Silent carrier β-thalassaemia trait

Silent carrier β-thalassaemia trait is characterised by minimal or no haematological changes. The haemoglobin level and the MCV are low normal and there is no obvious microcytosis seen on the blood film. Characteristically, silent carriers of β-thalassaemia have normal levels of HbA_2. Diagnosis of silent carrier β-thalassaemia trait is made by family studies and/or gene analysis.

β-Thalassaemia trait

This trait is characterised by a microcytic hypochromic red cell picture together with target cells, elliptocytes, and basophilic stippling. The average MCV is 63 fL and the average MCH is 20 pg.

In β-thalassaemia trait, the HbA2 level is increased above 3.5% and may be as high as 8.0%, while the HbF level is elevated in approximately 50% of patients, ranging from less than 1% to 5%. Refer to Fig A4-6.

β-Thalassaemia intermedia

This is a more severe form of β-thalassaemia trait but less severe than β-thalassaemia major. At the most severe end of the scale patients are transfusion dependent while at the less severe end they are transfusion independent. The red cell changes are more severe than those found in β-thalassaemia trait, with increased numbers of red cell poikilocytes. Teardrop poikilocytes are a prominent feature. Refer to Fig A4-7.

β-Thalassaemia major

As the neonate has substantial HbF at birth, anaemia in these patients usually develops during the first few months of life and becomes progressively worse in time. These infants will be transfusion dependent by the end of the first year of life; a later onset of the condition would suggest a case of thalassaemia intermedia.

β-Thalassaemia major is characterised by Hb levels as low as 30 g/L and variable amounts of HbF according to the transfusion status at the time of measurement. The acid elution or Kleihauer test shows that the HbF is evenly distributed among the red cells. The blood film shows marked red cell poikilocytosis, microcytosis and hypochromasia, target cells, basophilic stippling, Pappenheimer bodies (siderotic granules) and a reticulocytosis with increased numbers of nucleated red cells. As a result of frequent transfusions, the blood picture is often dimorphic, and consequently the MCV and MCH are difficult to define. Refer to Fig A4-8.

ABNORMAL HAEMOGLOBINS

Haemoglobins C, E and S (HbC, HbE and HbS) are abnormal haemoglobins characterised by an amino acid substitution in the β-globin chain.

Haemoglobin C

HbC ($\alpha_2\beta_2^{6Glu \rightarrow Lys}$) is an abnormal haemoglobin produced by the replacement of glutamic acid with lysine at the sixth position on the β chain. It is found in West Africans, particularly from Ghana and the Upper Volta.

HBC TRAIT

Individuals with HbC trait are clinically normal. Target cells are present on an otherwise normal blood film.

HBCC DISEASE

HbCC disease is associated with a haemolytic anaemia; the haemoglobin level ranges from 80 to 120 g/L. The blood film shows marked numbers of target cells, red cell fragments and microspherocytes. Upon careful examination, the red cells will be seen to contain intraerythrocytic crystals that dissolve readily when oxygen is released to the tissues. The MCV and MCH are slightly reduced as a result of the marked number of target cells that result from potassium efflux from the red cells, shrinking their contents with dehydration, leading to an increased ratio of surface area to volume. Refer to Fig A4-10.

IN VITRO TEST FOR DETECTION OF HBC

An in vitro test to demonstrate the presence of HbC crystals may be performed by adding 3% NaCl to the red cells and examining a wet preparation under a coverslip after 4 hours or longer. Hypertonic dehydration of the red cells produces tetrahedral crystals in up to 75% of the cells. Refer to Fig A4-11.

HAEMOGLOBIN E

HbE ($\alpha_2\beta_2^{26Glu \rightarrow Lys}$) is an abnormal haemoglobin produced by the replacement of glutamic acid with lysine at position 26 on the β chain. It is found in South-East Asians.

HBE TRAIT

Individuals with HbE trait are asymptomatic, with haemoglobin levels of 120 g/L average MCV of 74 fL and an average MCH of 25 pg. Occasional target cells may be present. Refer to Fig A4-12.

HBEE DISEASE

HbEE disease is also asymptomatic; the haemoglobin level is rarely less than 100 g/L. The red cell indices are distinctly abnormal, with an average MCV of 58 fL and an average MCH of 20 pg. The red cells are markedly microcytic and hypochromic, with a marked number of target cells present. Refer to Fig A4-13.

Haemoglobin S

HbS ($\alpha_2\beta_2^{6Glu \rightarrow Val}$) is an abnormal haemoglobin produced by the replacement of glutamic acid with valine at the sixth position on the β chain, and is found in the African as well as in the American black population.

HBS TRAIT

Individuals with HbS trait are clinically asymptomatic, with normal haemoglobin levels and a normal blood picture.

HBSS DISEASE

HbSS disease is characterised by a mild to moderate normochromic anaemia. The blood picture shows a reticulocytosis with a varying number of sickle cells. Sickle cells are biconcave discs that, upon deoxygenation, change shape to become sickle-shaped. Sickling is associated with the formation of liquid crystals or tactoids of HbS that run parallel to the long axis of the cell and cause the characteristic sickle shape. When the cells containing HbS enter the fine capillaries of the body, they become deoxygenated, change shape and thus block off those capillaries. This process gives rise to small infarcts throughout the body, especially in the spleen. As a result of this process, blood films of patients with sickle cell disease demonstrate features of autosplenectomy, namely Howell-Jolly bodies, Pappenheimer bodies, target cells and nucleated red cells. Refer to Fig A4-17.

IN VITRO SICKLING TEST FOR DETECTION OF HBS

The reducing agent sodium dithionite induces red cells containing HbS to sickle. A mixture of red cells and sodium dithionite placed on a glass slide and sealed with a coverslip will reveal the presence of sickle-shaped cells within 1–12 hours. Refer to Fig A4-18.

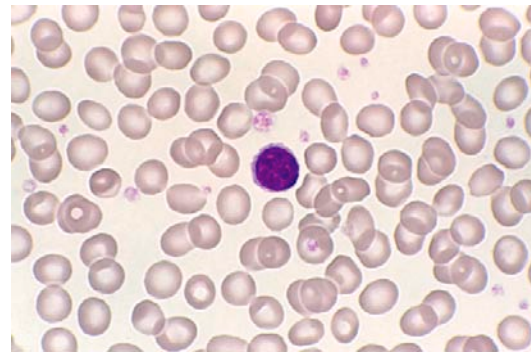


Figure A4-1
α-Thalassaemia trait: peripheral blood film showing a homogeneous population of microcytic hypochromic red cells. (x 1000)

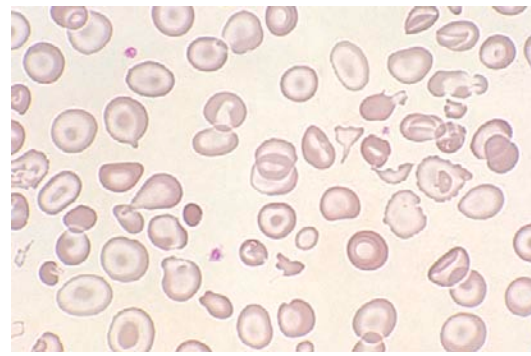


Figure A4-2
HbH disease: peripheral blood film showing microcytes, hypochromasia, target cells and fragmented cells. Cells resembling 'bite' cells may be present. These cells are a feature of an unstable Hb and are not indicative of an oxidant drug. (x 1000)

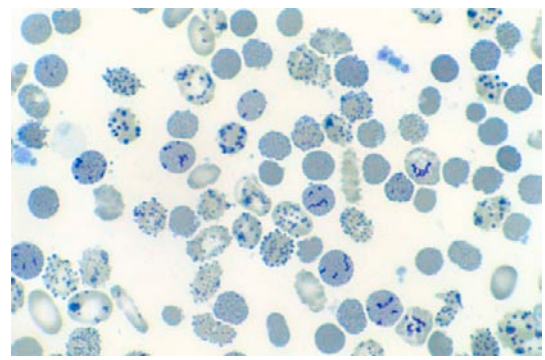


Figure A4-3
HbH disease: cresyl blue stain of peripheral blood film showing HbH inclusion bodies (β4) that are precipitates of HbH as a result of redox action of the cresyl blue dye. (x 1000)

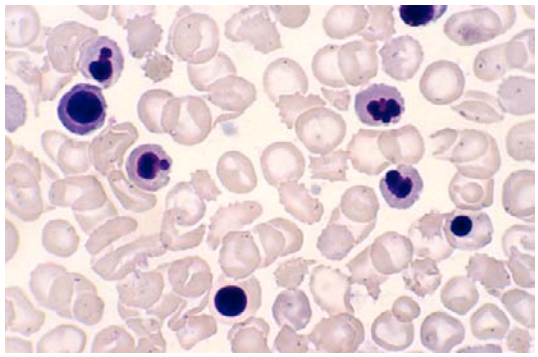


Figure A4-4
Hydrops fetalis: peripheral blood film from a stillborn infant showing hypochromic macrocytes, polychromasia, basophilic stippling and increased numbers of dysplastic nucleated red cells. (x 1000)

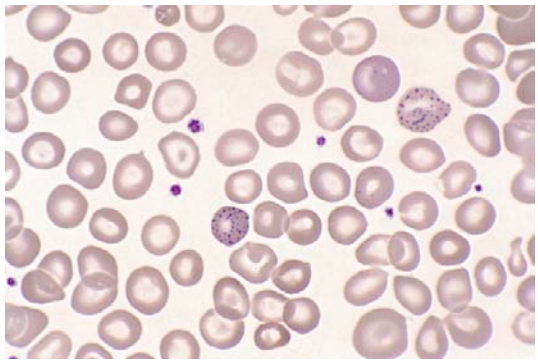


Figure A4-5
HbHCS: peripheral blood film showing microcytes, hypochromasia and coarse basophilic stippling. (x 1000)

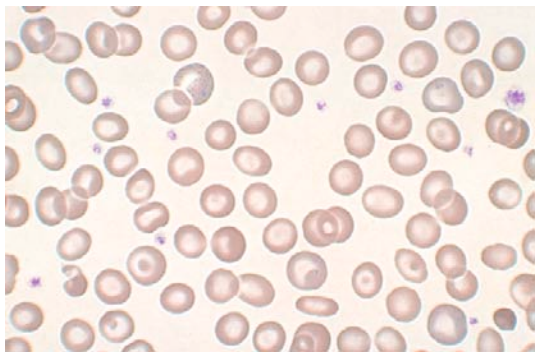


Figure A4-6
β-Thalassaemia trait: peripheral blood film showing microcytes, hypochromasia, target cells and basophilic stippling (insoluble aggregates of free α chains). (x 1000)

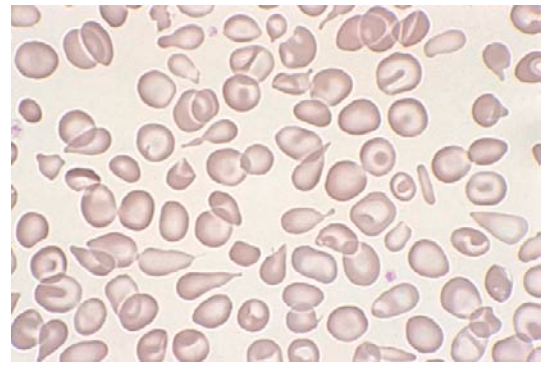


Figure A4-7
β-Thalassaemia intermedia: peripheral blood film showing microcytes, hypochromasia and increased red cell changes including teardrops. (x 1000)

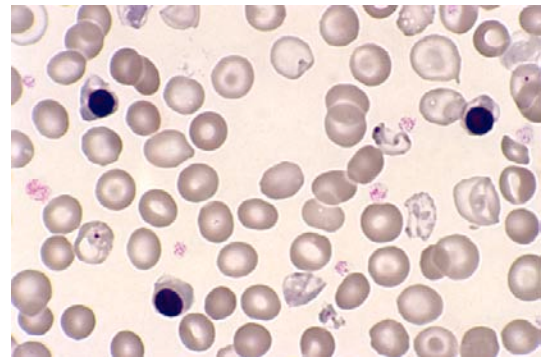


Figure A4-8
β-Thalassaemia major: peripheral blood film from a patient following splenectomy showing marked red cell changes, microcytes, hypochromasia, target cells, fragments, Howell-Jolly bodies, Pappenheimer bodies, reticulocytes and nucleated red blood cells. (x 1000)

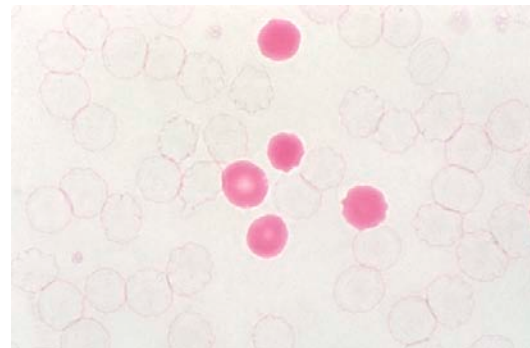


Figure A4-9
Kleihauer (acid-elution test) demonstrating the presence of HbF in fetal red cells. The cells containing adult Hb appear as ghost cells. (x 1000)

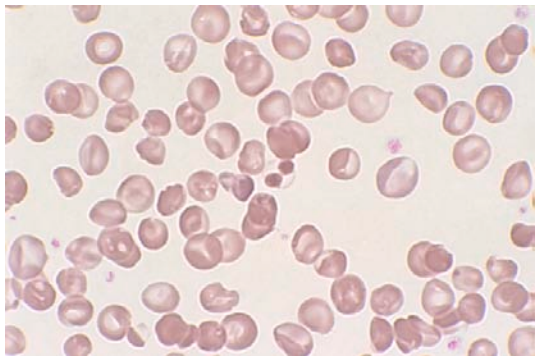


Figure A4-10
HbCC disease: peripheral blood film showing occasional microcytes and target cells. Note the intraerythrocytic crystals within some of the red cells. (x 1000)

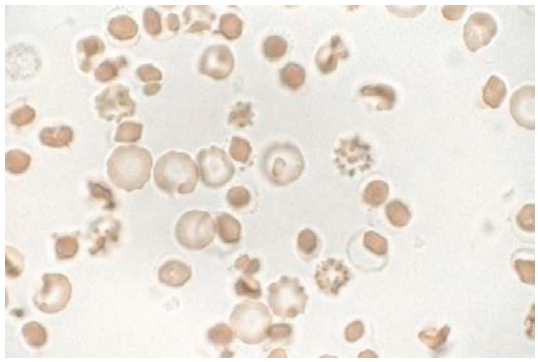


Figure A4-11
HbCC disease: in vitro demonstration of tetrahedral crystals of HbCC in peripheral blood. (x 1000)

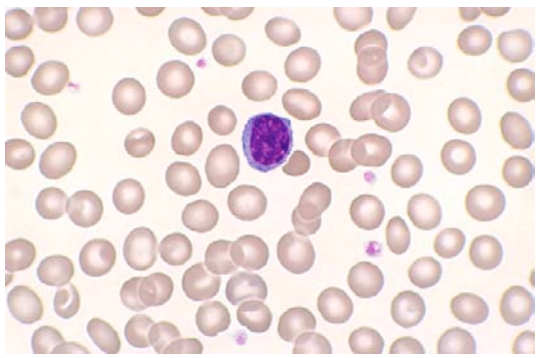


Figure A4-12
HbE trait: peripheral blood film showing a homogeneous population of microcytic hypochromic red cells. (x 1000)

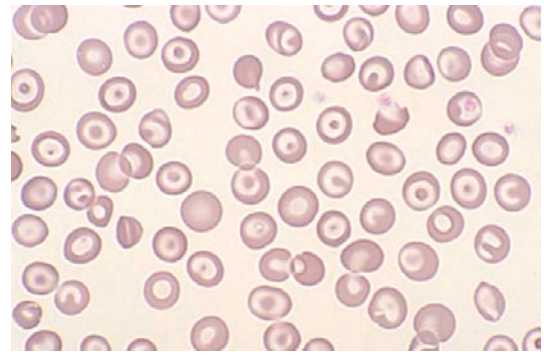


Figure A4-13
HbEE disease: peripheral blood film showing microcytic hypochromic red cells with a marked number of target cells. (x 1000)

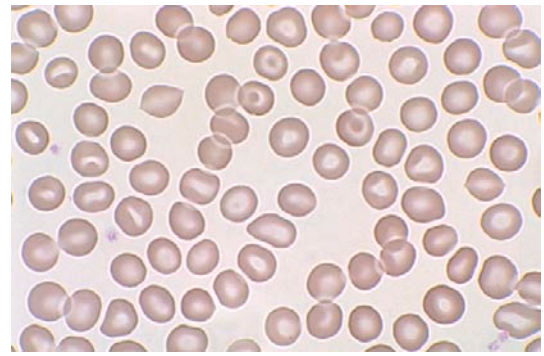


Figure A4-14
HbE/α-thalassaemia trait: peripheral blood film showing microcytic hypochromic red cells with an occasional target cell. (x 1000)

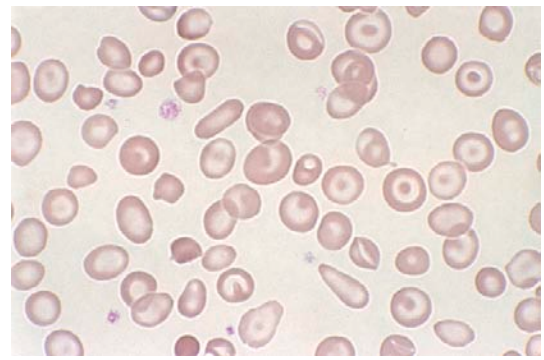


Figure A4-15
HbE/β-thalassaemia trait: peripheral blood film showing microcytic hypochromic red cells with elliptocytes and target cells. (x 1000)

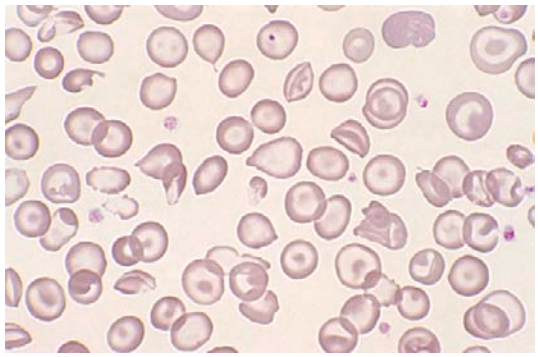


Figure A4-16
HbE/HbH disease: peripheral blood film showing marked red cell changes, microcytic hypochromic red cells, target cells and fragments. (x 1000)

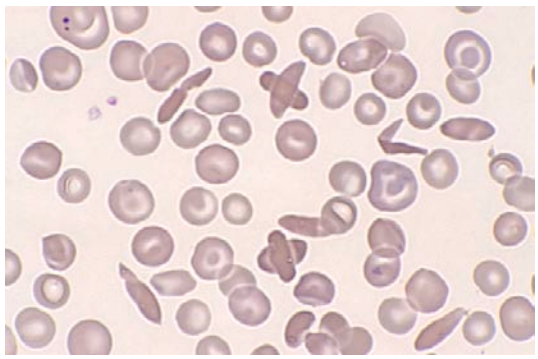


Figure A4-17
HbSS disease: peripheral blood film showing sickle cells, an occasional Howell-Jolly body and reticulocyte. (x 1000)

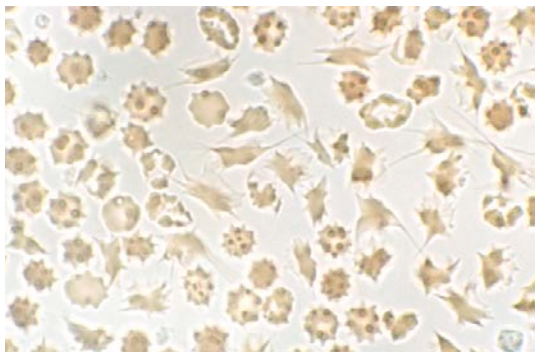


Figure A4-18
HbS: in vitro sodium dithionite preparation showing sickle cells in the peripheral blood. (x 1000)

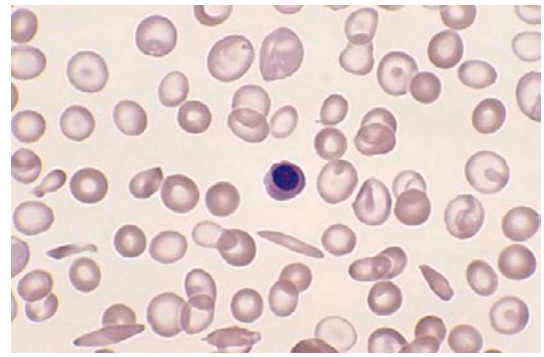
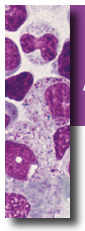


Figure A4-19
HbS/β-thalassaemia trait: peripheral blood film showing microcytic hypochromic cells with target and sickle cells as well as an occasional nucleated red cell. (x 1000)



A5 Red cell membrane disorders

HEREDITARY SPHEROCYTOSIS (HS)

HS is characterised by red cells that lack an area of central pallor, have a smaller diameter than normal and are intensely haemoglobinised. These cells are known as spherocytes. The presence of spherocytes results in a raised mean cell haemoglobin concentration (MCHC) of about 380 g/L. The mean cell haemoglobin (MCH) and mean cell volume (MCV) are within the normal range.

Spherocytes result from an intracorpuscular red cell membrane defect. Deficiency of spectrin, ankyrin and band 3 protein leads to uncoupling of the skeletal lipid bilayer resulting in membrane loss in the form of microvesicles. This loss of surface area leads to the formation of spherocytes.

HS is associated with a raised reticulocyte count and erythroid hyperplasia of the marrow. Spherocytes haemolyse more readily in hypotonic salt solutions, resulting in a tail on the osmotic fragility curve. Refer to Figs A5-1 and A5-2.

BURNS

Third-degree burns induce changes on the blood film that can be seen almost immediately after the event. Direct action of heat at 49°C denatures spectrin in the red cell membrane, giving rise to membrane budding, fragmentation, microcytes and microspherocytes. The presence of microcytes and microspherocytes falsely elevates the platelet number—hence the need for a manual count. Refer to Figs A5-3 and A5-4.

LIVER DISEASE

Obstructive liver disease is characterised by the presence of target cells and round macrocytes. Target cells have a characteristic distribution of haemoglobin in the cell centre as well as around the periphery. Their ratio of surface area to volume is greater than normal since the red cell membrane is expanded by the accumulation of lecithin and cholesterol from free exchange with plasma lipids. In obstructive jaundice and hepatitis with biliary obstruction, there is an increase in free cholesterol and lecithin in the plasma due to the bile salts that inhibit the activity of the enzyme lecithin-cholesterol acyl transferase, which normally esterifies cholesterol. Refer to Fig A5-6.

SPUR CELL ANAEMIA

Spur cell anaemia is seen in hepatocellular disease rather than obstructive liver disease; alcoholic liver disease is a classic example. Spur cells are produced in two stages. First, excess cholesterol produced by the patient's diseased liver increases the surface area of the red cell, resulting in a red cell with a scalloped or undulating periphery. In the second stage, these scalloped cells are converted to spur cells by a process of splenic conditioning. Over a period of a few days, the membrane lipids as well as the

increased surface area are lost and the cell becomes rigid and assumes the appearance of a spur cell.

The life span of a spur cell is shortened owing to splenic sequestration; thus patients with spur cell anaemia have a moderately severe haemolytic anaemia. Spur cell anaemia is usually seen in fulminant hepatocellular liver disease. Refer to Fig A5-7.

HEREDITARY ELLIPTOCYTOSIS (HE)

The elliptocyte (ovalocyte) is an oval biconcave disc that varies in shape from being slightly oval to a cylindrical elongated cell. The elliptocytes of HE demonstrate both quantitative and qualitative abnormalities in two major proteins comprising the membrane skeleton, namely spectrin and protein 4.1. There are various types of HE; the silent carrier, mild HE, HE with infantile poikilocytosis and chronic haemolytic HE. Mild HE is the type that is commonly seen in the laboratory. These patients usually have normal haemoglobin levels, but a mild compensated anaemia may be present. While approximately 5% of elliptocytes are seen on normal blood films, between 30% and 100% are seen on the blood film of mild HE. Refer to Fig A5-8.

SOUTH-EAST ASIAN OVALOCYTOSIS

This disorder is characterised by the presence of oval red cells, many of which contain one or two transverse bars that give the cells the appearance of double stomatocytes. This abnormality results from increased ankyrin binding and decreased protein 3 mobility, leading to the production of rigid red cells. This rigidity acts as a protective mechanism against all strains of malaria, including *Plasmodium falciparum*.

South-East Asian ovalocytosis is seen in up to 30% of people of Melanesian stock in Malaysia and Melanesia, particularly in the lowland tribes where malaria is endemic. Refer to Fig A5-9.

HEREDITARY STOMATOCYTOSIS (HYDROCYTOSIS)

The Na^+/K^+ ATPase pump is greatly increased in hereditary stomatocytosis. The influx of Na^+ into red cells exceeds the loss of K^+ exiting from red cells. This leads to an increase in monovalent cation content causing the movement of water into the red cells. Hence the red cells swell and are transformed from discocytes to bowl forms. These bowl forms are known as stomatocytes and have an increased MCV.

The defect in this disorder is due to the deficiency of a membrane protein known as protein 7.2b or stomatin. The function of this protein is to regulate membrane Na^+ permeability. Stomatocytes require increased energy to protect them against osmotic rupture. They are also vulnerable to splenic sequestration. Patients with hereditary stomatocytosis have haemolytic anaemia. They are jaundiced with splenomegaly and often develop pigment gallstones later in life. Splenectomy may diminish the rate of haemolysis in these patients.

Inheritance of stomatocytosis (hydrocytosis) is autosomal-dominant. Refer to Fig A5-10.

HEREDITARY XEROCYTOSIS

Hereditary xerocytosis is a rare autosomal-dominant haemolytic anaemia. The red cells are dehydrated as there is a loss of potassium exiting the cell compared with the amount of sodium entering the cell. Thus the intracellular cation content and water content is reduced. The enzyme involved in the passage of anions across the red cell membrane, glyceralderhyde-3-phosphate dehydrogenase, is increased.

The blood picture is that of a severe haemolytic anaemia. The MCV may be slightly increased due to the presence of increased reticulocytes. Target cells are prominent and there may be some 'puddling' of haemoglobin towards the periphery of some of the cells. Refer to A5-11.

ABETALIPOPROTEINAEMIA

Abetalipoproteinaemia is a rare autosomal-recessive disorder characterised by the presence of acanthocytic red cells on the peripheral blood film. The primary defect is due to a mutation and lack of activity in the microsomal triglyceride transfer protein needed to bind lipids to the β-apolipoprotein in plasma. The plasma triglycerides are almost absent and the plasma cholesterol is markedly decreased. There is an increase in sphingomyelin in the outer half of the red cell membrane bilayer, increasing the surface layer of the cell. This β-apolipoprotein defect leads to the production of acanthocytic red cells, about 50–90% of the red cells being acanthocytes. The sphingomyelin accumulates with cell ageing; hence the nucleated precursor red cells and the reticulocytes are not affected.

Abetalipoproteinaemia is characterised clinically by ataxic neurologic disease, retinitis pigmentosa (often leading to blindness) and fat malabsorption. The neurologic abnormalities present between 5 and 10 years of age and continue until death in the second or third decade. Despite the marked acanthocytosis seen in these patients, anaemia and haemolysis are not seen. The haemoglobin levels are normal. Refer to Fig A5-12.

HEREDITARY PYROPOIKILOCYTOSIS (HPP)

HPP is an extremely rare disorder presenting in infancy and characterised by the presence of extreme poikilocytosis with red cell budding, triangular fragments, spherocytes and elliptocytes. The MCV is significantly reduced owing to the presence of a large number of fragments.

Whereas normal red cells fragment at 49°C, the red cells in HPP fragment at 45–46°C. Prolonged heating at 37°C will also induce fragmentation; thus these patients suffer from a severe microangiopathic anaemia that is partially corrected by splenectomy. Refer to Fig A5-13.

VITAMIN E DEFICIENCY

Vitamin E (α-tocopherol) is a fat-soluble vitamin that appears to serve as an antioxidant in humans. Nutritional deficiency of vitamin E is extremely rare as α-tocopherol occurs in many food products and the daily requirement is only 5–7 mg. Vitamin E deficiency in humans is virtually limited to the neonatal period and to pathologic states associated with chronic fat malabsorption. Low birthweight infants are born with low serum and tissue concentrations of vitamin E. When these infants are fed a diet unusually rich in polyunsaturated fatty acids and inadequate vitamin E, a haemolytic anaemia will develop by 4–6 weeks of age. The anaemia is associated with morphologic alterations of the red cell membrane; a red cell similar to a spur cell is produced. A haemolytic anaemia due to increased splenic sequestration follows. Treatment with vitamin E produces a prompt reversal of this process. Modifications of infant formulas have all but eliminated vitamin E deficiency in the preterm infant. Refer to Fig A5-14.

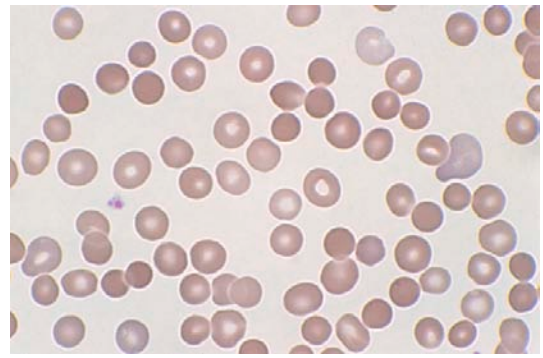


Figure A5-1
Hereditary spherocytosis: peripheral blood film showing spherocytes and increased numbers of reticulocytes. (x 1000)

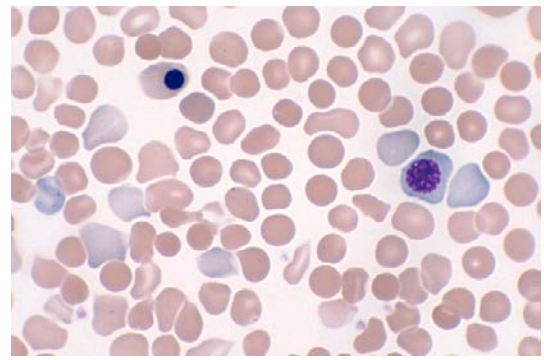


Figure A5-2
Hereditary spherocytosis in a 1-day-old neonate showing marked numbers of spherocytes, polychromasia and nucleated red cells. (x 1000)

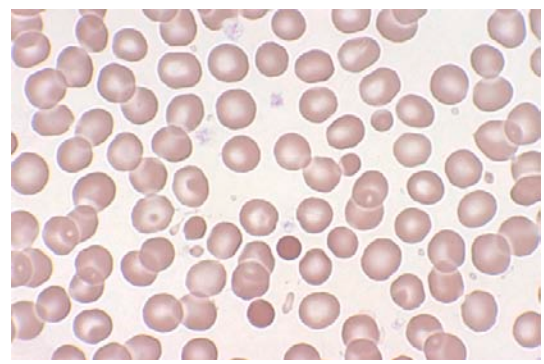


Figure A5-3
Burns: peripheral blood film showing spherocytes and microspherocytes as well as red cell budding and microcytes. (x 1000)

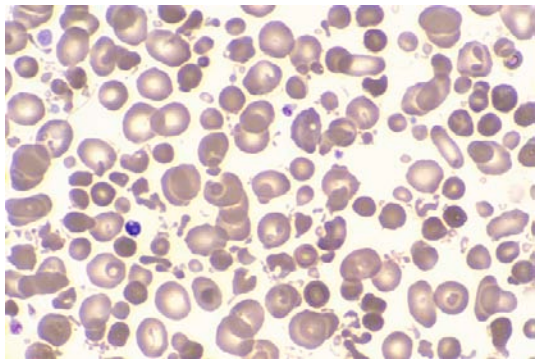


Figure A5-4
Burns: peripheral blood film showing marked numbers of spherocytes, microspherocytes, microcytes and marked red cell budding. (x 1000)

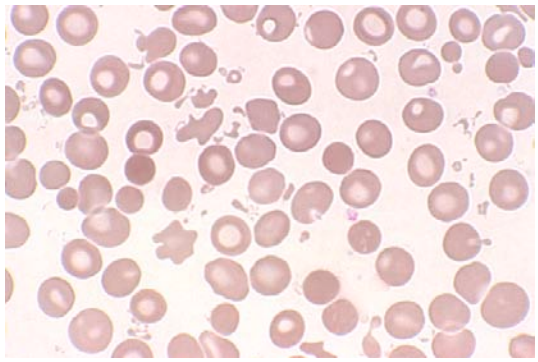


Figure A5-5
Blood film from a specimen left in a courier van on a very hot day. Note the increased number of red cell fragments; the platelet count was falsely elevated due to the red cell fragmentation. (x 1000)

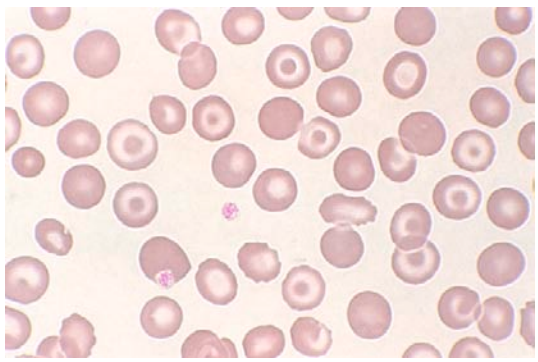


Figure A5-6
Liver disease: peripheral blood film showing the presence of target cells and round macrocytes. (x 1000)

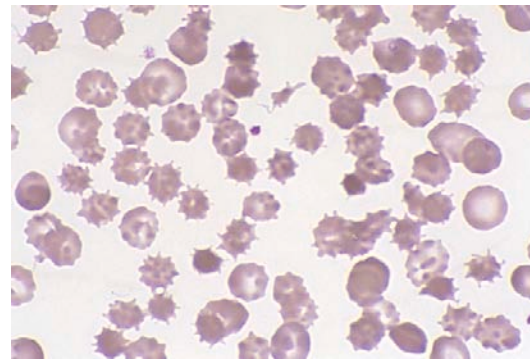


Figure A5-7
Spur cell anaemia: spur cells in the peripheral blood in fulminant liver disease secondary to alcohol. (x 1000)

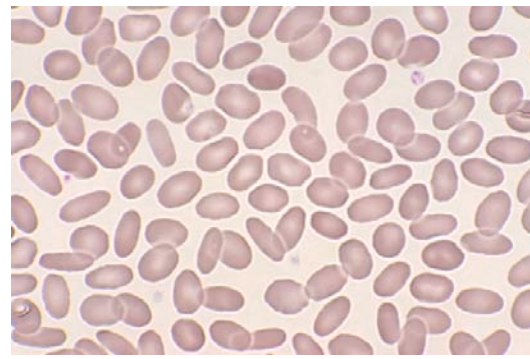


Figure A5-8
Hereditary elliptocytosis: peripheral blood film showing characteristic oval and elongated elliptocytes with rounded ends. (x 1000)

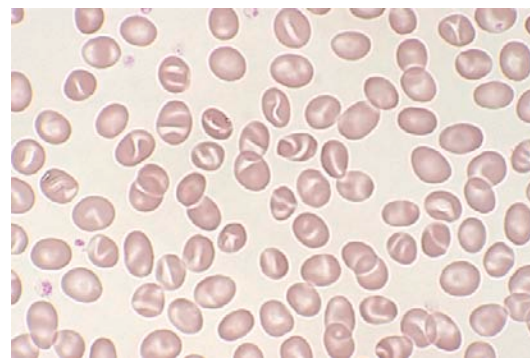


Figure A5-9
South-East Asian ovalocytosis: peripheral blood film showing oval-shaped stomatocytes, some with two transverse slits. (x 1000)

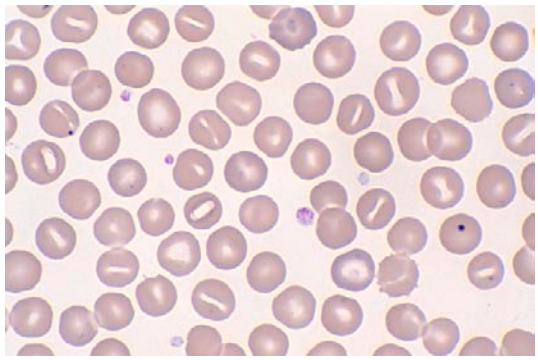


Figure A5-10
Hereditary stomatocytosis (hydrocytosis): macrocytic red cells with a single slit instead of the characteristic area of central pallor. This is a post-splenectomy picture with an MCV of 130.6 fL. (x 1000)

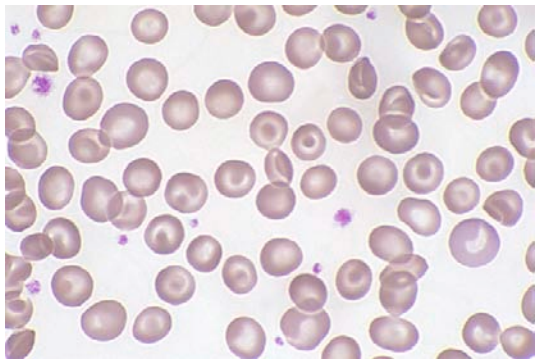


Figure A5-11
Hereditary xerocytosis: peripheral blood film showing target cells as well as 'puddling' of haemoglobin towards the periphery of some red cells. (x 1000)

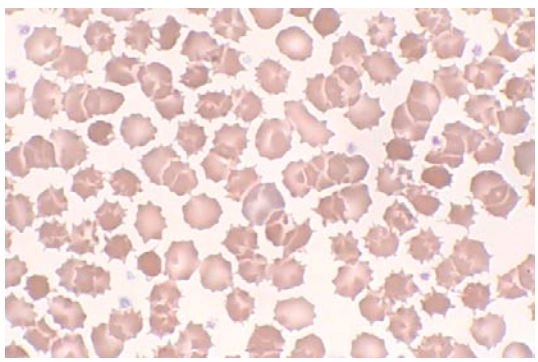


Figure A5-12
Abetalipoproteinaemia: peripheral blood film showing marked numbers of acanthocytes. (x 1000)

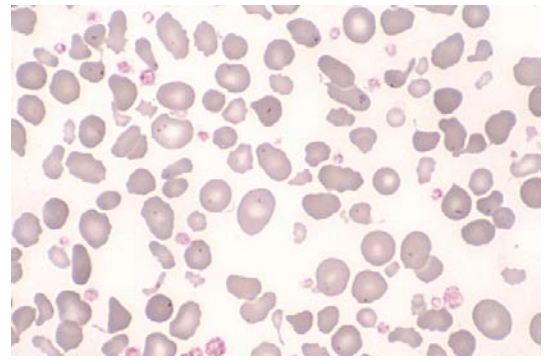


Figure A5-13
Hereditary pyropoikilocytosis: peripheral blood film showing the presence of spherocytes, extreme poikilocytosis with fragments, triangulocytes and bizarre red cells shapes. (x 1000)

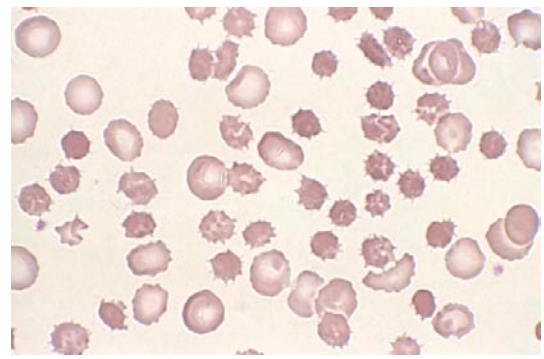
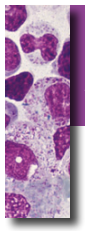


Figure A5-14
Vitamin E deficiency: peripheral blood film showing red cells similar in appearance to those of spur cell anaemia. Vitamin E is an antioxidant. (x 1000)



A6 Miscellaneous red cell abnormalities

SPLENECTOMY

Post-splenectomy blood films show the following features: target cells, acanthocytes, spherocytes, nucleated red cells, Howell-Jolly bodies, Pappenheimer bodies and Heinz bodies. This presence of intracellular remnants within the red cells attests to the culling or pitting function of the spleen. The spleen is also responsible for the surface remodelling of red cells, that is, it removes surplus membrane from red cells such as target cells that have an increased ratio of surface area to volume—hence the appearance of target cells in the first weeks following splenectomy. Refer to Figs A6-1 to A6-4.

LIPAEMIC BLOOD (GHOST CELLS)

Hyperlipidaemia may be detected on the blood film by the presence of ghost-like red cells. It is thought that excess lipid coats the red cell membrane, preventing complete methanol fixation and producing a ghost cell or a cell with an indistinct membrane. Refer to Fig A6-5.

CRYOPROTEIN

The prefix 'cryo' designates a property of precipitating in the cold and redissolving when warmed. Cryoproteins are either immunoglobulins (IgM, IgG or IgA) (cryoglobulins in plasma or serum) or cryofibrinogen (in plasma only). They occur in disorders such as plasma cell myeloma, Waldenström macroglobulinaemia, rheumatoid arthritis, Sjögren's syndrome, renal disease and hepatic disease. Cryoproteins may interfere with some laboratory tests such as the erythrocyte sedimentation rate (ESR) and the white and red cell count. Blood samples from patients with a cryoprotein should be prewarmed to 37°C prior to processing through the blood cell analyser.

Cryofibrinogen may be recognised on the blood film by the presence of aggregates of amorphous material occurring throughout the film and especially towards the tail. Refer to Fig A6-6.

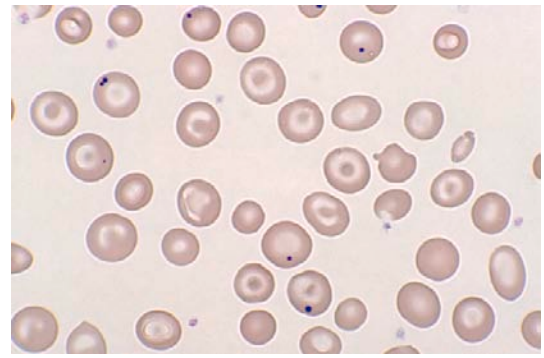


Figure A6-1
Splenectomy: peripheral blood film showing target cells and Howell-Jolly bodies (nuclear remnants). (x 1000)

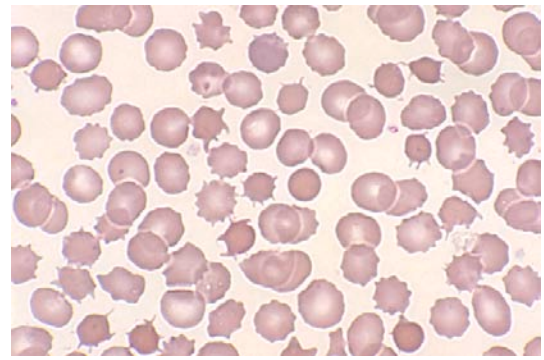


Figure A6-2
Splenectomy: peripheral blood film showing increased numbers of acanthocytes (hyperchromic red cells with an irregular number of fine spines). (x 1000)

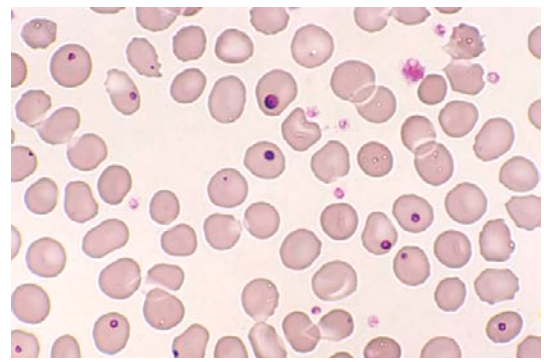


Figure A6-3
Splenectomy: peripheral blood film showing increased numbers of Howell-Jolly bodies. (x 1000)

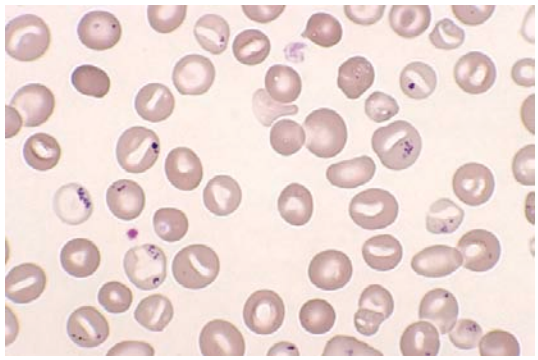


Figure A6-4
Splenectomy: peripheral blood film showing many Pappenheimer bodies. (x 1000)

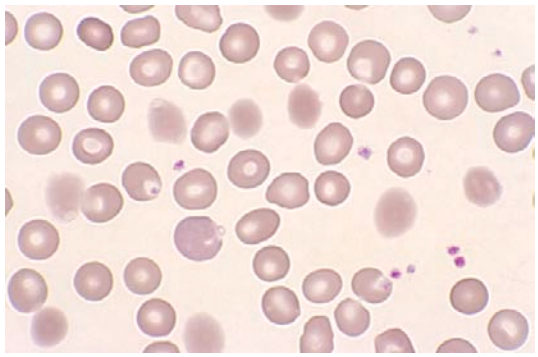


Figure A6-5
Hyperlipidaemia: peripheral blood film showing the presence of ghost cells with an indistinct cell membrane. (x 1000)

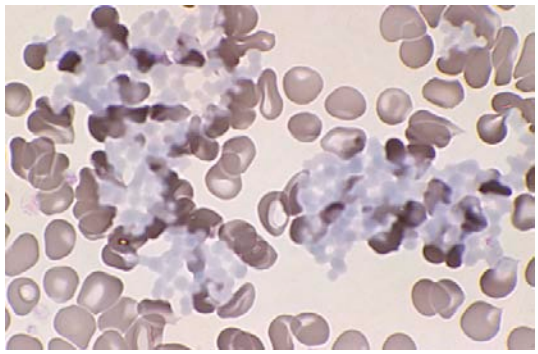
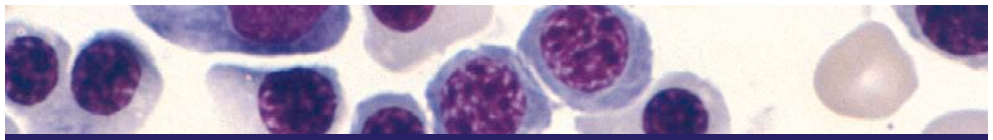
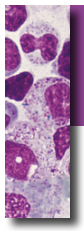


Figure A6-6
Cryoprotein: peripheral blood film showing amorphous aggregates of cryofibrinogen in a patient with plasma cell myeloma. (x 1000)



PART B

LEUCOCYTES AND PLATELETS



B1 Myeloid cells

MYELOID MATURATION

The earliest recognisable cell in the myeloid series is the myeloblast, which gives rise sequentially to the promyelocyte, myelocyte, metamyelocyte, band form and finally the mature neutrophil (polymorphonuclear granulocyte), eosinophil or basophil.

Myeloblast

The myeloblast varies from 15 to 20 µm in diameter and has a large round nucleus that occupies about 80% of the cell. The chromatin is arranged in fine strands, giving an evenly stained reticular appearance with approximately one to three nucleoli. The cytoplasm is very basophilic and agranular. Refer to Fig B1-1.

Promyelocyte

The promyelocyte is the next stage in the maturation process. It is approximately the same size as the myeloblast, and the nucleus still contains nucleoli but the chromatin strands are coarser and hence stain less evenly. The cytoplasm is still basophilic but now contains azurophilic or primary granules. Refer to Fig B1-2.

Myelocyte

The myelocyte may be up to 25 µm in diameter. The nucleus is round and the chromatin strands are thicker and stain more deeply than in the promyelocyte. There are no nucleoli present. The nuclear to cytoplasmic (N/C) ratio is decreased. Primary granules may be seen in the early myelocyte while the late or more developed myelocyte contains specific or secondary granules. The composition of these granules determines whether the myelocyte will develop into a neutrophil, eosinophil or basophil. Refer to Figs B1-3 and B1-4.

Metamyelocyte

The metamyelocyte, which is between 10 and 18 µm in diameter, is characterised by an indented nucleus resembling the shape of a broad bean. The chromatin strands are thicker and stain more deeply than in the myelocyte, and the cytoplasm contains many fine specific granules. Refer to Fig B1-5.

Band form

The band form, 10 to 15 µm in diameter, is smaller than the metamyelocyte. It has a deeply indented U-shaped nucleus composed of coarsely clumped chromatin. The cytoplasm is pink and contains many specific granules. Refer to Fig B1-6.

Neutrophil

The neutrophil is the most mature stage in the myeloid series (together with the eosinophil and basophil). The neutrophil is approximately 12 to 14 µm in diameter. The nucleus is lobulated, having three or four lobes connected by thin strands of chromatin.

The cytoplasm is pink and contains specific granules. A morphologic difference between neutrophils of males and females is that female neutrophils have nuclear appendages shaped like a drumstick. These appendages are attached to one lobe of the nucleus by a thin strand of chromatin. Refer to Fig B1-7.

Eosinophil

The eosinophil develops through the same stages as the neutrophil, the specific eosinophilic granules first appearing at the myelocyte stage. Apart from the difference in granules, the eosinophilic myelocyte, metamyelocyte and band form have the same structural characteristics as their neutrophilic counterparts. The mature eosinophil is a round cell 12 to 17 µm in diameter and is a phagocytic cell (although less so than the neutrophil). It is characterised by the presence of large round granules with an affinity for acid dyes such as eosin. The nucleus has two or three lobes. Eosinophils are attracted to sites of antigen-antibody interaction in tissues, especially in cases of foreign protein sensitisation such as allergic disorders and parasitic infections. Refer to Fig B1-8.

Basophil

The basophil develops through the same stages as the neutrophil and the eosinophil. Similarly, the specific granules appear at the myelocyte stage. The basophil is a round cell approximately 10 to 14 µm in diameter and its nucleus is bilobed. The granules are large and bluish-black in colour and are present in the cytoplasm as well as overlying and partly obscuring the nucleus. The granules contain heparin and histamine. Their phagocytic power is weak; they degranulate and release histamine at sites of inflammation or when allergens react with IgE. Heparin is probably not released from basophils. Refer to Fig B1-9.

ABNORMAL MYELOID CELLS

Hypersegmented neutrophil

A hypersegmented neutrophil is one in which the nucleus has six or more lobes. An increased number of hypersegmented neutrophils are found in megaloblastic anaemia and following antimetabolite cytotoxic therapy. Refer to Fig B1-13.

Pelger-Huët anomaly

This is a congenital anomaly. In the heterozygous form, the neutrophils have one or two nuclear lobes. The chromatin pattern is dense and pyknotic, and often the lobes are joined by a fine strand of chromatin giving the appearance of spectacles. In the homozygous form, the neutrophils contain only single, round nuclei with a dense chromatin pattern. Refer to Fig B1-15.

Toxic granulation

The presence of blue-black granules within the cytoplasm of neutrophils is known as toxic granulation. These granules are azurophilic granules that have become activated owing to the presence of a bacterial infection or a toxic state. Refer to Fig B1-16.

Toxic vacuolation

Toxic vacuolation is commonly seen in the cytoplasm of neutrophils containing toxic granules. These vacuoles, known as phagocytic vacuoles, contain phagocytosed bacteria. Vacuolation may also be induced in neutrophils that have been stored in EDTA for more than 24 hours. Refer to Fig B1-17 and B1-18.

Döhle bodies

Döhle bodies are blue-staining bodies present in the cytoplasm of toxic neutrophils. They consist of endoplasmic reticulum. Refer to Fig B1-19.

Leukaemoid reaction

A leukaemoid reaction is one in which there is proliferation in the myeloid line resulting in a blood picture resembling leukaemia. There is a marked increase in the number of neutrophils together with an increase in the numbers of myeloblasts, promyelocytes, myelocytes, metamyelocytes and band forms. Leukaemoid reactions may be associated with bacterial infections, cytokine therapy such as granulocyte colony-stimulating factor (G-CSF) or severe illness such as malignancy. Toxic granulation is often a feature of a leukaemoid reaction. Refer to Figs B1-20 and B1-21.

Agranular neutrophils

Agranular neutrophils may be seen in bacterial infections after the neutrophil has undergone the process of degranulation. They are also a characteristic feature of the myelodysplastic syndrome. Refer to Figs B1-22 and B1-23.

May-Hegglin anomaly (MHA)

This is a congenital disorder characterised by the presence of basophilic inclusions of RNA within the cytoplasm of neutrophils (see Part C). These inclusions resemble Döhle bodies. Thrombocytopenia with giant platelets is seen in approximately one-third of patients with May-Hegglin anomaly. Refer to Figs B1-24 and C7-6.

Alder granulation

This is coarse granulation seen in neutrophils. It resembles toxic granulation but differs in colour in that it stains a reddish brown with Romanowsky stains. Alder granulation is also present in eosinophils, basophils and monocytes. It is important to distinguish Alder granulation from toxic granulation. Refer to Fig B1-25.

Chédiak-Higashi anomaly

This is a congenital anomaly characterised by giant granules in the cytoplasm of neutrophils and lymphocytes. The granules result from the fusion of primary and secondary granules to form giant granules. These granules are not attracted to the phagocytic vacuole during bacterial infections; hence bactericidal activity is impaired. Refer to Fig B1-26.

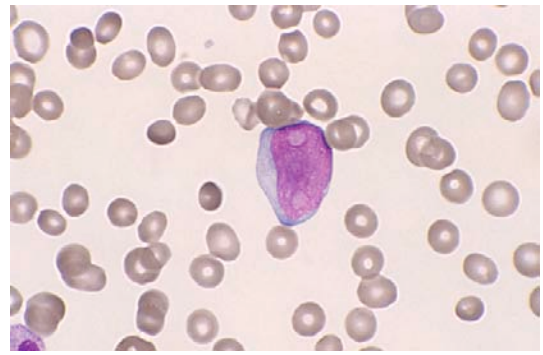


Figure B1-1
Myeloblast in peripheral blood.
(x 1000)

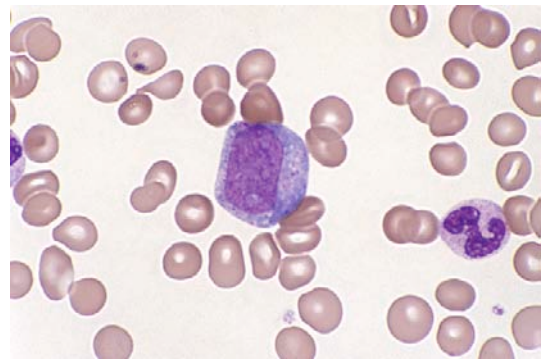


Figure B1-2
Promyelocyte in peripheral blood.
(x 1000)

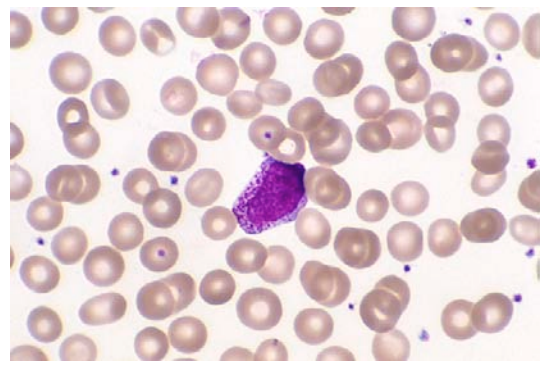


Figure B1-3
Myelocyte (early) in peripheral blood.
(x 1000)

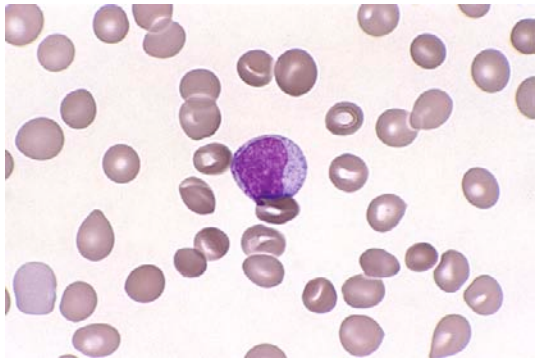


Figure B1-4
Myelocyte (late) in peripheral blood.
(x 1000)

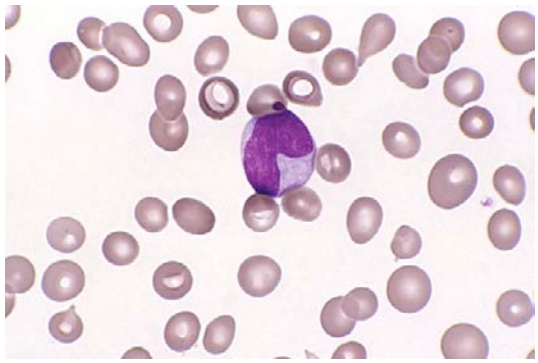


Figure B1-5
Metamyelocyte in peripheral blood.
(x 1000)

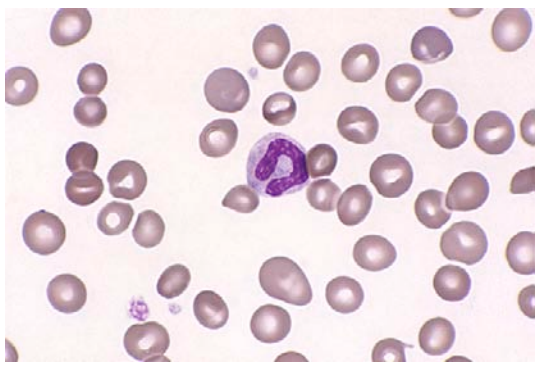


Figure B1-6
Band form in peripheral blood.
(x 1000)

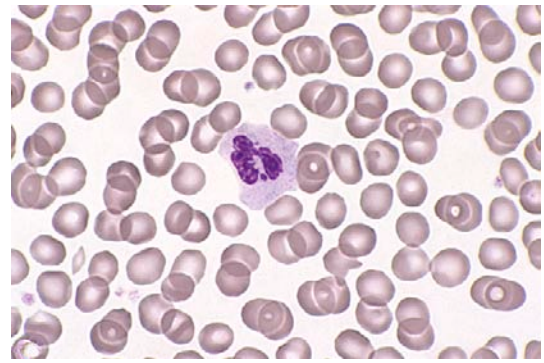


Figure B1-7
Neutrophil showing female appendage (drumstick) in peripheral blood. (x 1000)

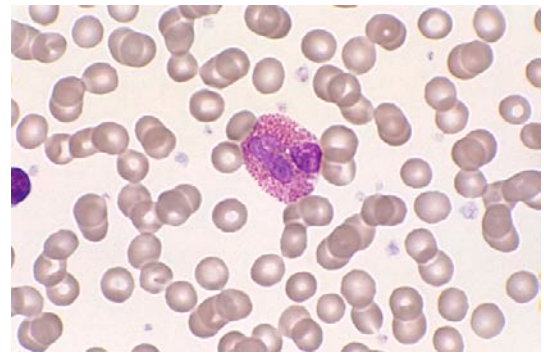


Figure B1-8
Eosinophil in peripheral blood. (x 1000)

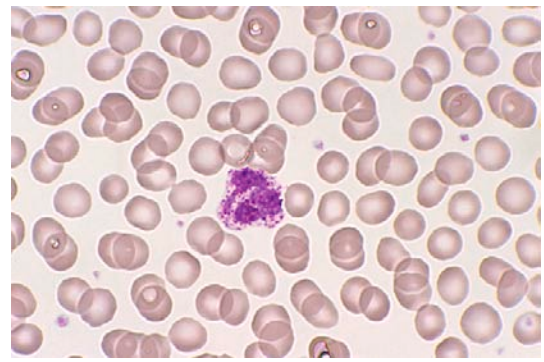


Figure B1-9
Basophil in peripheral blood. (x 1000)

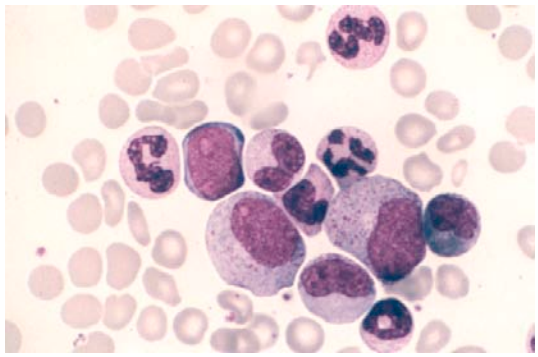


Figure B1-10
Myeloblast, promyelocyte, myelocyte, metamyelocytes and neutrophils in peripheral blood. (x 1000)

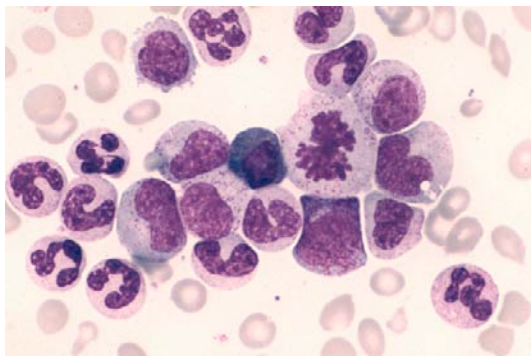


Figure B1-11
Mitosis in anaphase present in the bone marrow in chronic myelogenous leukaemia, *BCR-ABL1* positive. (x 1000)

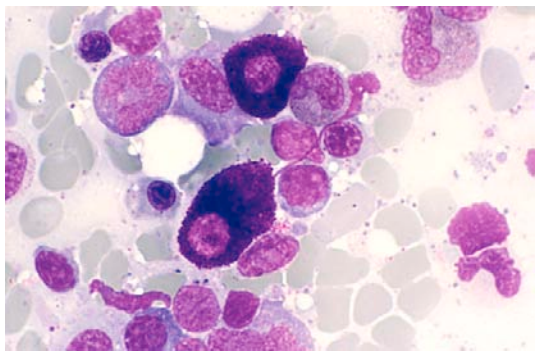


Figure B1-12
Mast cells in the bone marrow showing cytoplasm packed with bluish-black granules. (x 1000)

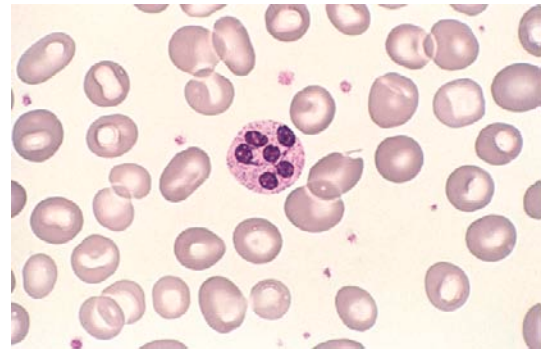


Figure B1-13
Hypersegmented neutrophil: peripheral blood film showing a neutrophil nucleus with six lobes. (x 1000)

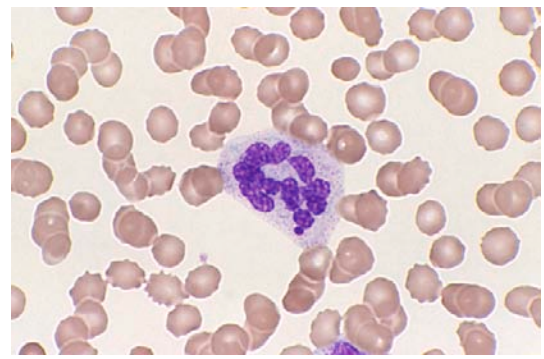


Figure B1-14
Macropolycyte: occasionally seen in the peripheral blood film and of unknown significance. (x 1000)

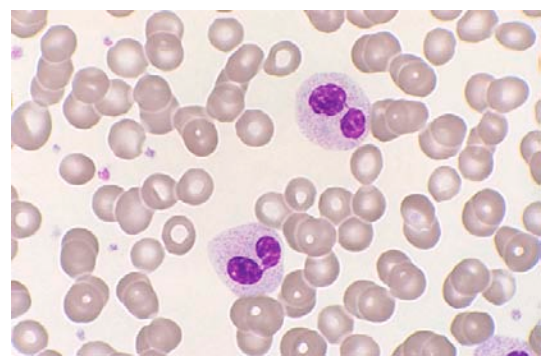


Figure B1-15
Pelger-Huët anomaly: peripheral blood film showing classical 'spectacle form' nuclei. (x 1000)

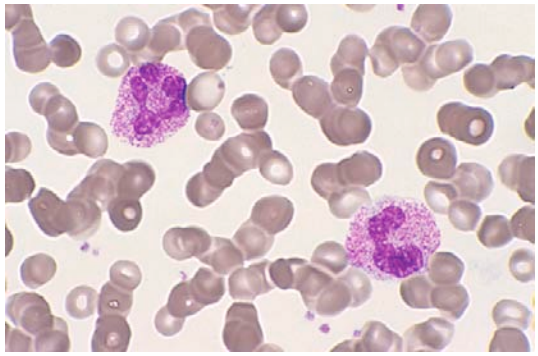


Figure B1-16
Toxic granulation in neutrophils in peripheral blood. (x 1000)

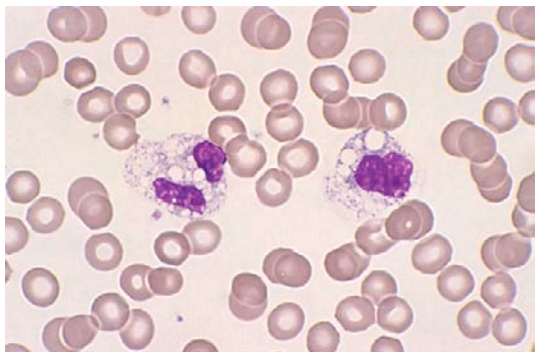


Figure B1-17
Toxic vacuolation: peripheral blood film showing toxic vacuolation in the neutrophils resulting from a bacterial infection. (x 1000)

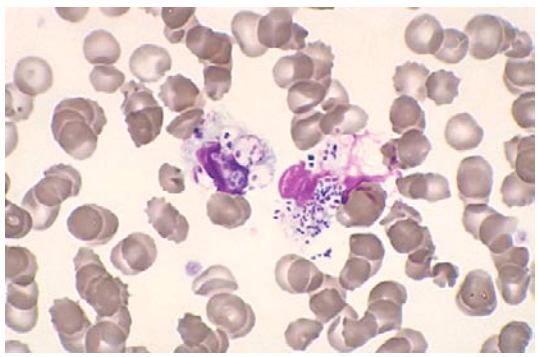


Figure B1-18
Toxic vacuolation: neutrophils showing phagocytosed bacteria in a case of severe septicaemia. (x 1000)

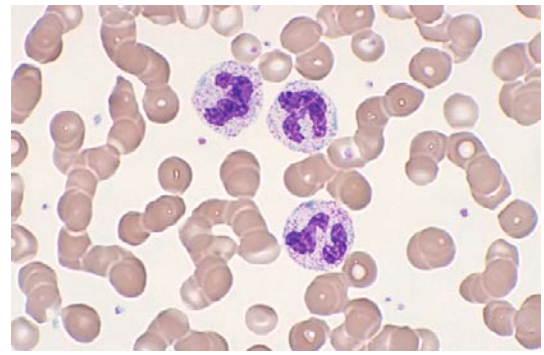


Figure B1-19
Döhle bodies in the cytoplasm of toxic neutrophils in peripheral blood. (x 1000)

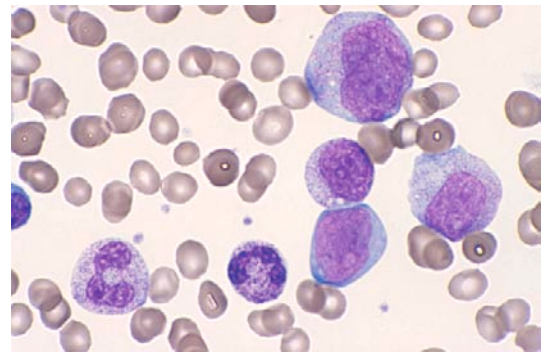


Figure B1-20
Leukaemoid reaction: peripheral blood film showing a response to cytokine (G-CSF) therapy. (x 1000)

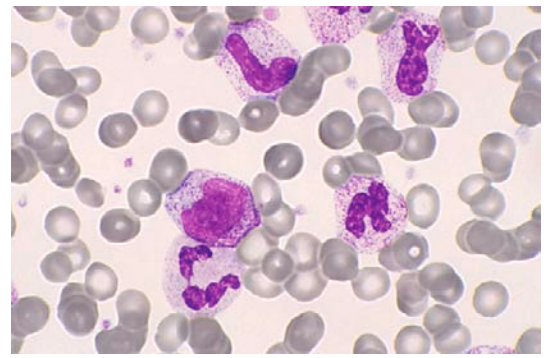


Figure B1-21
Leukaemoid reaction in peripheral blood in a case of carcinoma of the lung. (x 1000)

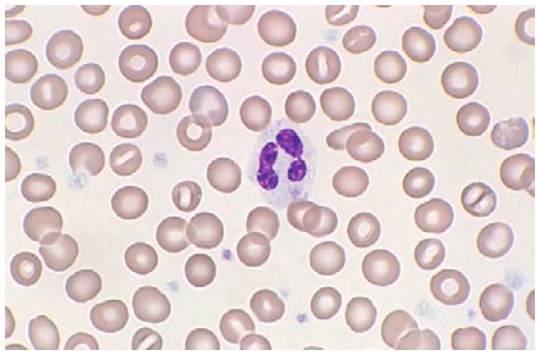


Figure B1-22
Peripheral blood film showing a hypogranular neutrophil that has degranulated during septicaemia. (x 1000)

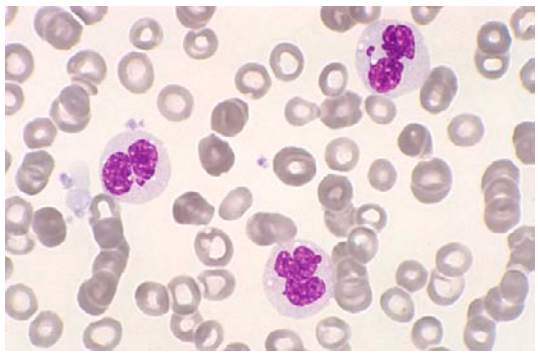


Figure B1-23
Peripheral blood film showing agranular neutrophils in a case of refractory anaemia. (x 1000)

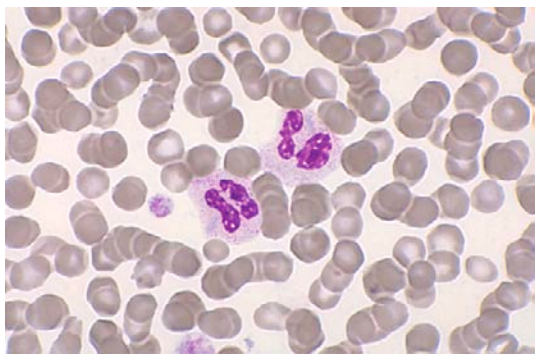


Figure B1-24
May-Hegglin anomaly: peripheral blood film showing inclusions of RNA within the neutrophil cytoplasm as well as thrombocytopenia and large platelets. (x 1000)

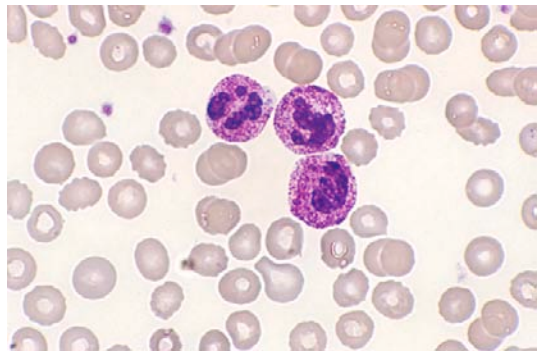


Figure B1-25
Alder granulation: peripheral blood film showing neutrophils whose cytoplasm is packed with coarse reddish-brown granules. (x 1000)

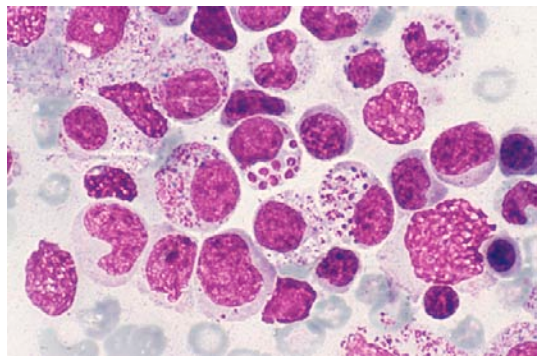


Figure B1-26
Chédiak-Higashi anomaly: characteristic giant granules within the cytoplasm of bone marrow myeloid precursors. (x 1000) (Courtesy of Dr Boyd Webster)

MYELOPROLIFERATIVE NEOPLASMS: THE WORLD HEALTH ORGANIZATION CLASSIFICATION

Chronic myelogenous leukaemia, *BCR-ABL1* positive

Chronic myelogenous leukaemia (CML) is a myeloproliferative neoplasm originating from the bone marrow. It is consistently associated with *BCR-ABL1* fusion due to t(9;22)(q34;q11.2) resulting in the formation of the Philadelphia (Ph) chromosome. CML occurs more frequently in males, affecting both adolescents and adults with the majority of cases seen in the 25–60 year age group.

There are three phases of CML: the initial phase or chronic phase (CP) followed by the accelerated phase (AP), and the blast phase (BP).

CHRONIC PHASE (CP)

The peripheral blood shows a leucocytosis with a total white cell count in the range of 12–1000 × 10^9/L and a median count of ~100 × 10^9/L. Approximately 50% of the cells are mature neutrophils while the less mature cells are present in diminishing frequency with <2% blasts. An absolute basophilia is invariably present and an eosinophilia is common. The platelet count ranges from normal to 1000 × 10^9/L.

The bone marrow shows a similar picture. The blast cell count is <5%.

More than 10% blasts indicate disease progression. Refer to Fig B1-27 to B1-29.

ACCELERATED PHASE (AP)

Diagnosis of the accelerated phase is made if any of the following are present.

An increase in the white cell count, >10 × 10^9/L or an increase in the platelet count, >1000 × 10^9/L, despite therapy; thrombocytopenia, <100 × 10^9/L unrelated to therapy; increasing splenomegaly despite therapy; increasing basophilia, >20% basophils and 10–19% myeloblasts in the peripheral blood or bone marrow. Refer to Fig B1-30.

BLAST PHASE (BP)

Diagnosis of the blast phase is made when the blast count is equal or greater than 20% in either the peripheral blood or bone marrow. In approximately 70% of cases the blast lineage is myeloid whereas in approximately 20–30% of cases the blast cell lineage is lymphoid. Refer to Fig B1-31.

CYTOGENETICS

Chronic phase (CML-CP)

- 100% of cases exhibit *BCR-ABL1* fusion, 95% due to t(9;22)(q34;q11.2) resulting in formation of the Philadelphia chromosome (Ph). The remaining cases have either variant translocations or cryptic *BCR-ABL1* fusion.

Transformation to (CML-AP) or (CML-BP)

- t(9;22)(q34;q11.2) plus one or more of the following: i(17q), +8, +19 and an additional Ph chromosome in 80% of transformed CMLs.

CYTOCHEMISTRY/IMMUNOPHENOTYPE

- Neutrophil alkaline phosphatase is decreased in CML.

Immunophenotyping is only appropriate if there is an increase in the number of blast cells. When in accelerated phase, the blasts may express the following:

- $CD13^+$, $CD33^+$ (myeloid markers)
- $CD11c^+$, $CD14^+$, $CD116^+$ (monocytoid markers)
- $CD41^+$, $CD61^+$ (megakaryocytic markers)
- TdT^+, $HLA\text{-}DR^+$, $CD10^+$, $CD19^+$, $CD34^+$ (lymphoid markers)

Chronic neutrophilic leukaemia (CNL)

CNL is a rare disorder occurring in the very old, usually over 80 years of age. It has also been reported in adolescents. The peripheral blood has an absolute neutrophil count of ≥25 × 10^9/L. There may be an increase in the number of band forms but very few promyelocytes, myelocytes and metamyelocytes. Myeloblasts are almost never seen. The neutrophils often show coarse granulation but may also appear normal.

The bone marrow is hypercellular and may show an increase in myeloid precursors. Myeloblasts and promyelocytes are not increased. Monocytes are < 1 × 10^9/L. CNL is diagnosed by exclusion from all other myeloproliferative neoplasms. Refer to Fig B1-32.

CYTOGENETICS

- The *BCR-ABL1* fusion gene and Ph chromosome are absent in CNL.
- Cytogenetic studies are normal in about 90% of cases.
- The changes that may occur in 10% of cases include +8, +9, +21, del(20q), del(11q) and del(12p).

CYTOCHEMISTRY/IMMUNOPHENOTYPE

- Neutrophil alkaline phosphatase is increased in CNL.
- As the number of blast cells is not increased, immunophenotyping is not indicated.

Polycythaemia vera (PV)

Polycythaemia vera is a disorder characterised by an increase in red cell mass. PV occurs in middle-aged to older males and less commonly females, with the majority of cases occurring in the 50–60 year age group. Two-thirds of cases have a relative and absolute granulocytosis in the range of 12.0–25.0 × 10^9/L and approximately 50% of cases have a thrombocytosis in the range 450–800 × 10^9/L. Refer to Figs B1-33 to B1-35.

There are three phases of PV:

1 A pre-polycythaemic phase
2 An overt polycythaemic phase
3 A 'spent phase' or post-polycythaemic myelofibrosis phase.

PRE-POLYCYTHAEMIC PHASE AND OVERT POLYCYTHAEMIA

The characteristic features of these phases are the increase in erythropoiesis, granulopoiesis and megakaryopoiesis in both the PB and BM. The red cell morphology is normocytic and normochromic. In some cases iron deficiency is present. This is due to therapeutic venesection or chronic gastrointestinal bleeding in which case the red cells are microcytic and hypochromic. Occasional immature granulocytes may be present. Megakaryocytes are increased, especially in cases where there is a thrombocytosis. As the overt polycythaemic phase progresses, erythropoiesis decreases and the red cell mass normalises.

'SPENT PHASE' AND POST-POLYCYTHAEMIC MYELOFIBROSIS

The 'spent phase' is characterised by a decrease in erythropoiesis and granulopoiesis. The blood film is leucoerythroblastic with teardrop poikilocytes. There is a gradual increase in the number of immature granulocytes. Less than 10% myeloblasts may be present in either the peripheral blood or bone marrow. The bone marrow becomes increasingly fibrotic while the spleen increases in size secondary to extra-medullary haematopoiesis.

CYTOGENETICS

More than 95% of patients carry the gain-of-function mutation of the Janus 2 kinase gene, *JAK2* V617F; however, these mutations are not specific for PV. A functionally similar mutation in exon 12 of *JAK2* may also be present.

At diagnosis, 10–20% of cases present with one or a combination of the following abnormalities: del(20q), +8, +9, alterations of 9p, gains of 1q and del(13q).

IMMUNOPHENOTYPE

No abnormal phenotypic features have been described in PV.

Primary myelofibrosis (PMF)

PMF is a clonal myeloproliferative neoplasm characterised by an increase in granulocytes and abnormal megakaryocytes in the bone marrow.

PMF predominantly occurs between 60 and 70 years of age, affecting both sexes almost equally. A normocytic normochromic anaemia is common. Some patients may present with a microcytic hypochromic anaemia possibly due to secondary gastrointestinal bleeding.

There are two stages of PMF: the prefibrotic early stage and the fibrotic stage.

PREFIBROTIC EARLY STAGE

The BM is hypercellular with an increase in granulocytes together with a left shift. Myeloblasts are not increased. The megakaryocytes are abnormal in size and form clusters. Refer to Figs B1-36 and B1-37.

FIBROTIC STAGE

In this stage the BM may vary from being hypercellular to normocellular or hypocellular being partially replaced by fibrous connective tissue. Extramedullary haematopoiesis occurs in the spleen and sometimes in the liver. There is a left shift in the granulocyte lineage with <10% myeloblasts. The presence of ≥20% myeloblasts is indicative of acute transformation. Atypical megakaryocytes occurring in clusters are a feature of the fibrotic stage.

The splenomegaly may be such that a splenectomy is required. In such cases a reduction or absence of teardrop poikilocytes in the peripheral blood will be noted, suggesting that the teardrop formation takes place mainly in the spleen.

Bone marrow aspiration invariably results in a dry tap and the diagnosis is established on trephine biopsy. Refer to Figs B1-38 and B1-39.

It should be noted that a similar leucoerythroblastic blood picture with teardrop poikilocytes may be produced by malignant bone marrow infiltration. Refer to Figs B1-40 to B1-42.

CYTOGENETICS

- Approximately 50% of patients carry the *JAK2* V617F mutation gene.
- There is no *BCR-ABL1* fusion gene or Ph chromosome.
- Up to 30% of patients with PMF have cytogenetic abnormalities: del(13q), del(20q), der(6)t(1;6), partial trisomy 1q, +8 and/or +9, –7/del(7q), –5/del(5q).

IMMUNOPHENOTYPE

- No abnormal phenotypic features have been described in PMF.

Essential thrombocythaemia (ET)

ET is a chronic myeloproliferative neoplasm involving the megakaryocytic lineage. It is characterised by a sustained platelet count of ≥450 × 10^9/L in the PB and increased numbers of large, mature megakaryocytes in the BM. As there is no gene or biological marker associated with ET, other causes of thrombocytosis such as infection, haemorrhage and reactive thrombocytosis must be excluded prior to making a diagnosis of ET.

ET occurs predominantly in the 50–60 year age group but is sometimes seen in younger patients aged between 20 and 40 years. Both males and females are affected equally by this neoplasm.

The platelets in the PB show anisocytosis ranging from small to large and sometimes giant size. Hypogranular and agranular forms may be present. The white cell count and differential is usually normal. At least 50% of patients have a microcytic hypochromic blood picture as a result of impaired platelet function leading to chronic blood loss from the gastrointestinal tract.

The bone marrow is hypercellular with an increase in the number of megakaryocytes. The megakaryocytes are increased in size with hyperlobulated nuclei, often forming clusters within the marrow. Refer to Figs B1-43 and B1-44.

CYTOGENETICS

- No specific cytogenetic abnormality has been identified in ET.
- There is no *BCR-ABL1* fusion gene or Ph chromosome.
- Approximately 40–50% of cases carry the *JAK2* V617F mutation.
- 5% to 10% of cases may have a cytogenetic abnormality, of which +8, abnormalities of chromosome 9, del(7q), del(17p) and del(20q) are the most frequent.

IMMUNOPHENOTYPE

- No abnormal phenotypic features have been described in ET.

Chronic eosinophilic leukaemia (CEL) and the hypereosinophilic syndrome (HES)

CEL is a rare myeloproliferative neoplasm characterised by a proliferation of eosinophils in the peripheral blood and bone marrow. The absolute eosinophil count in the peripheral blood is $\geq 1.5 \times 10^9$/L while the percentage of blasts in the peripheral blood and bone marrow is less than 20%. Demonstration of a clonal population of eosinophils is necessary to make a diagnosis of CEL. If this is not possible and there is no increase in blast cells in the bone marrow, a diagnosis of HES is made. HES is also characterised by an absolute eosinophil count of $\geq 1.5 \times 10^9$/L persisting for at least six months. HES is associated with organ involvement. The eosinophils in both CEL and HES are morphologically abnormal. They may be increased in size and lack granules, resulting in clear blue areas in the cytoplasm that are devoid of granules. The eosinophils are often vacuolated and show both hyposegmented and hypersegmented nuclei. Refer to Figs B1-45 to B1-47.

CYTOGENETICS

- There is no *BCR-ABL1* fusion gene or Ph chromosome present in CEL.
- If present, a diagnosis of CML rather than CEL should be made.
- No specific cytogenetic changes characterise CEL; however, +8, –7, i(17q), del(20q) and –Y have been reported. Some cases may show *PDGFRA* (4q12) or *PDGFRB* (5q33) rearrangements, including a cryptic fusion of *FIP1L1-PDGFRA* resulting from a 4q12 deletion. This rearrangement is only detectable by FISH analysis and is not specific for CEL.
- The *JAK2* mutation may be present in some cases.

IMMUNOPHENOTYPE

- No abnormal phenotypic features have been described in CEL or HES.

Mastocytosis

Mastocytosis is a clonal neoplastic proliferation of mast cells either in the dermis, known as cutaneous mastocytosis (CM) or in internal organs, known as systemic mastocytosis (SM). Mastocytosis occurs at any age.

CUTANEOUS MASTOCYTOSIS (CM)

CM commonly occurs in very young children, sometimes presenting at birth. It presents either as a solitary mastocytoma or as urticaria pigmentosa. Only in rare cases does it involve the bone marrow and progress to mast cell leukaemia. A generalised itching and a heparin-like coagulopathy result from the production of histamine by the mast cell granules. Fatal haemorrhage may result from the heparin-like coagulopathy. CM usually regresses spontaneously during early childhood. Refer to Fig C3-7.

SYSTEMIC MASTOCYTOSIS (SM)

SM is usually confined to adults, presenting after the second decade of life. The bone marrow is almost always involved as are other organs such as the spleen, lymph nodes, liver and gastrointestinal tract. Skin lesions occur in more than 50% of patients. Refer to Fig B1-48 and B1-49.

Mast cell leukaemia (MCL)

In mast cell leukaemia the number of mast cells equals or exceeds 20% of all the nucleated cells within the bone marrow. The marrow shows a diffuse infiltrate of mast cells that are often atypical in appearance with hypogranular cytoplasm and irregularly shaped nuclei. Nucleoli are often prominent. Should the number of mast cells be less than 10% then a diagnosis of 'aleukaemic' MCL is made.

CYTOGENETICS

- *JAK2* V617F may sometimes be present.
- Mastocytosis may be associated with somatic activating point mutations within *KIT*. In the majority of cases, codon 816 mutations in the tyrosine kinase domain occur.

IMMUNOPHENOTYPE

- $CD9^+$, $CD33^+$, $CD45^+$, $CD68^+$, $CD117^+$, $CD2^+/CD25^+$

Myeloproliferative neoplasm, unclassifiable (MPN, U)

Myeloproliferative neoplasm, unclassifiable encompasses those cases with clinical, laboratory and morphological characteristics of a myeloproliferative neoplasm but which do not fit into a specific category. MPN, U is divided into three groups.

The first group includes those cases that present with early features of a myeloproliferative neoplasm such as a peripheral blood thrombocytosis and a variable degree of neutrophilia but which are not severe enough to be labelled polycythaemia vera (PV), primary myelofibrosis (PMF) or essential thrombocythaemia (ET). The second group includes cases that present with an advanced chronic myeloproliferative neoplasm. The bone marrow shows dense fibrosis and osteosclerosis as well as an increase in blast cells, masking the underlying neoplasm while the third group includes cases with a myeloproliferative neoplasm but in whom a coexisting neoplasm or inflammatory process masks the diagnostic features of the particular neoplasm. Refer to Figs B1-50 and B1-51.

CYTOGENETICS

- No specific cytogenetic abnormality has been identified in MPN, U.
- There is no *BCR-ABL1* fusion gene, Ph chromosome or rearrangements of *PDGFRA*, *PDGFRB*, *FGFR1* in MPN, U.

IMMUNOPHENOTYPE

- No abnormal phenotypic features have been described in MPN, U.

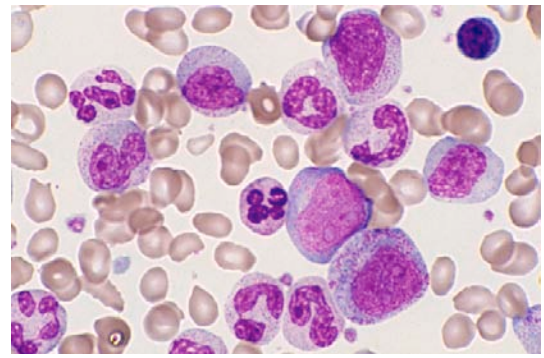


Figure B1-27
Chronic myelogenous leukaemia, *BCR-ABL1* positive-chronic phase: peripheral blood film showing myeloblast, promyelocyte, myelocytes, band forms and neutrophils. (x 1000)

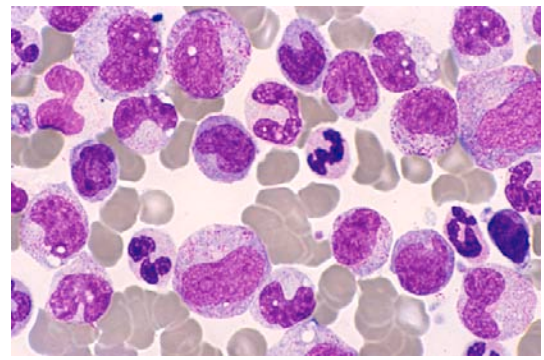


Figure B1-28
Chronic myelogenous leukaemia, *BCR-ABL1* positive-chronic phase: bone marrow showing increased myelopoiesis. (x 1000)

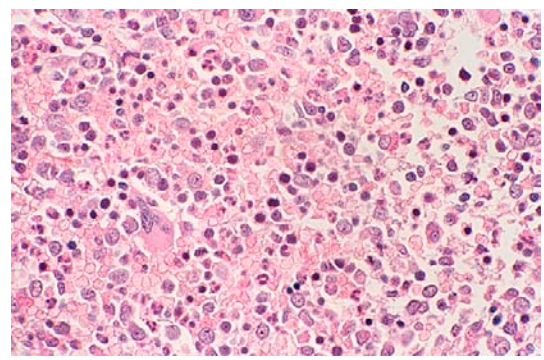


Figure B1-29
Chronic myelogenous leukaemia, *BCR-ABL1* positive-chronic phase: bone marrow trephine showing a hypercellular marrow with increased myelopoiesis. (H&E) (x 400)

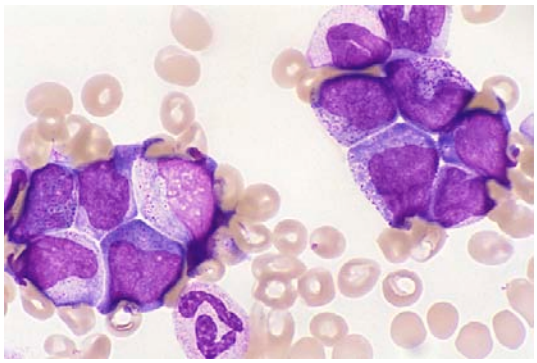


Figure B1-30
Chronic myelogenous leukaemia, *BCR-ABL1* positive-accelerated phase: peripheral blood film showing an increase in myeloblasts, promyelocyte, myelocytes and metamyelocytes. (x 1000)

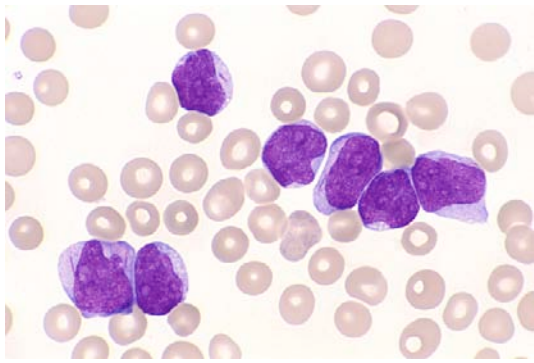


Figure B1-31
Chronic myelogenous leukaemia, *BCR-ABL1* positive-blast phase: peripheral blood showing myeloblasts, many of which contain Auer rods. (x 1000)

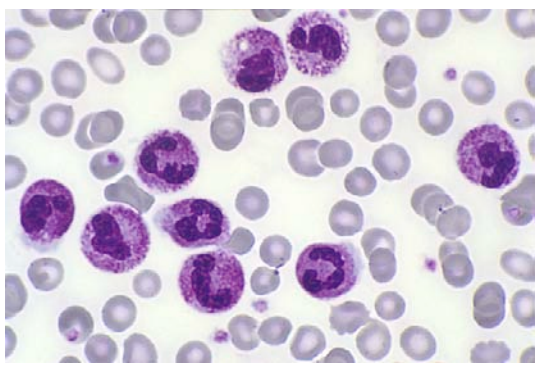


Figure B1-32
Chronic neutrophilic leukaemia: peripheral blood film showing an increase in mature neutrophils with coarsely granulated cytoplasm. (x 1000)

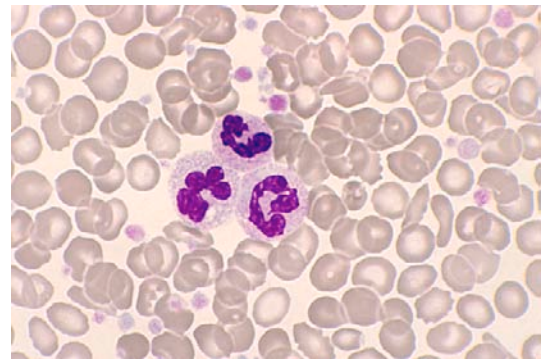


Figure B1-33
Polycythaemia vera: peripheral blood film showing increased numbers of red cells, granulocytes and platelets, including many large abnormal platelets. (x 1000)

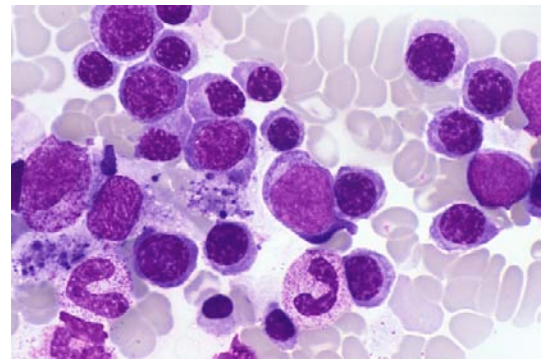


Figure B1-34
Polycythaemia vera: bone marrow showing an increase in myeloid precursors and nucleated red cells. (x 1000)

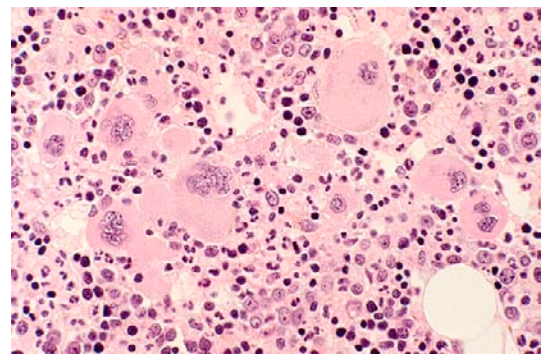


Figure B1-35
Polycythaemia vera: bone marrow trephine showing increased granulopoiesis and megakaryopoiesis. (H&E) (x 400)

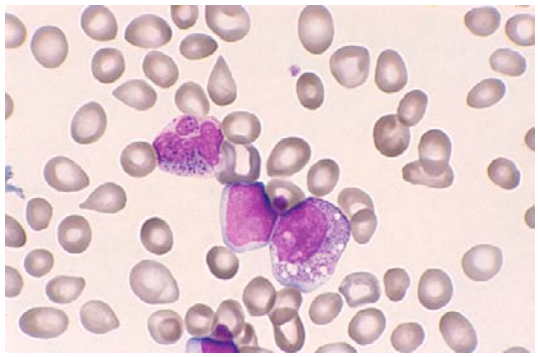


Figure B1-36
Primary myelofibrosis: peripheral blood film showing a myeloblast and myelocyte with teardrop poikilocytes. (x 1000)

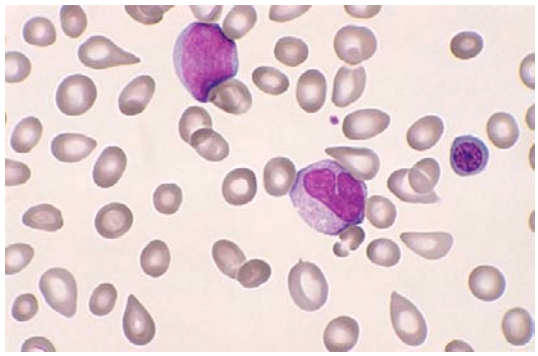


Figure B1-37
Primary myelofibrosis: peripheral blood film showing a myeloblast, metamyelocyte and nucleated red cell with teardrop poikilocytes. (x 1000)

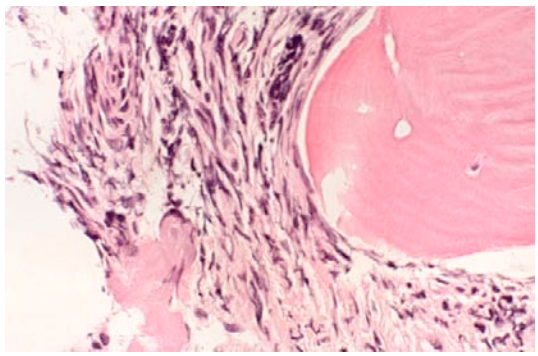


Figure B1-38
Primary myelofibrosis: bone marrow trephine demonstrating increased fibrosis. (H&E) (x 400)

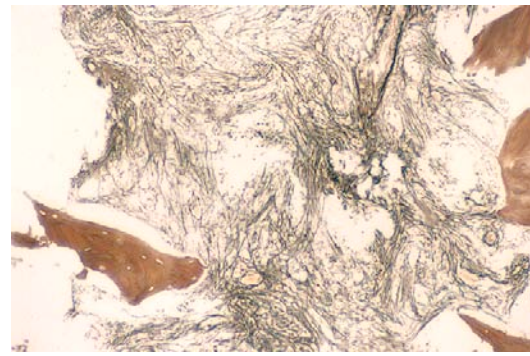


Figure B1-39
Primary myelofibrosis: reticulin stain demonstrating increased fibrosis in a bone marrow trephine. (x 200)

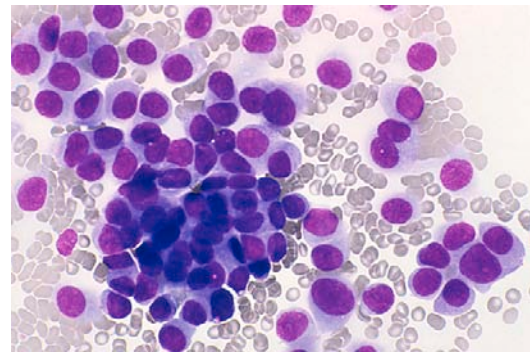


Figure B1-40
Malignant, non-haemopoietic cells infiltrating the bone marrow. (x 400)

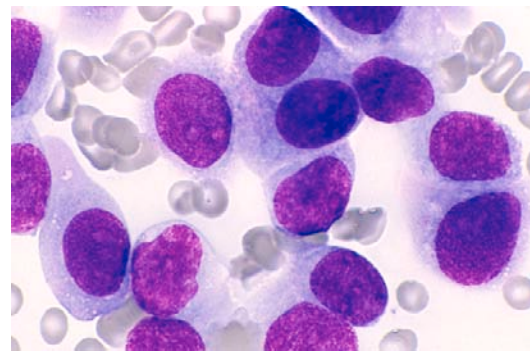


Figure B1-41
Malignant non-haemopoietic cells infiltrating the bone marrow. These primitive cells have nuclei with a fine chromatin pattern, nucleoli and basophilic cytoplasm. (x 1000)

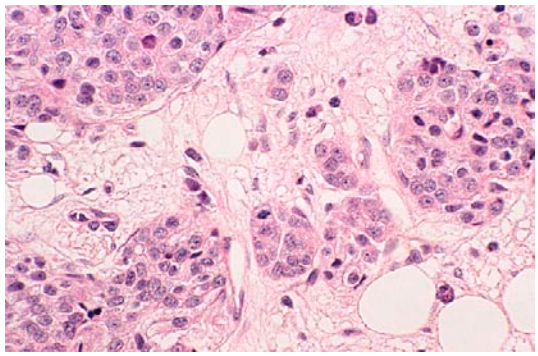


Figure B1-42
Malignant cell infiltration seen in the bone marrow trephine. (H&E) (x 400)

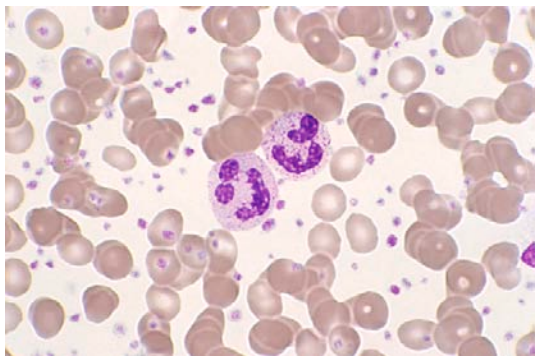


Figure B1-43
Essential thrombocythaemia: peripheral blood film showing marked thrombocytosis with a platelet count of 2129 x 10^9/L. (x 1000)

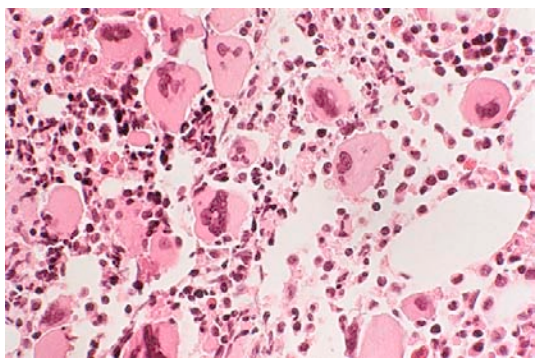


Figure B1-44
Essential thrombocythaemia: bone marrow trephine showing an increase in megakaryocytes. (H&E) (x 400)

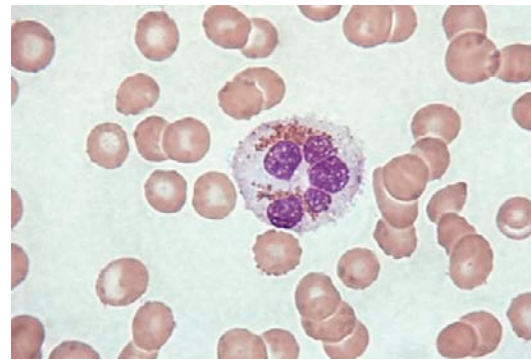


Figure B1-45
Hypereosinophilic syndrome: peripheral blood showing a hypogranular eosinophil. (x 1000)

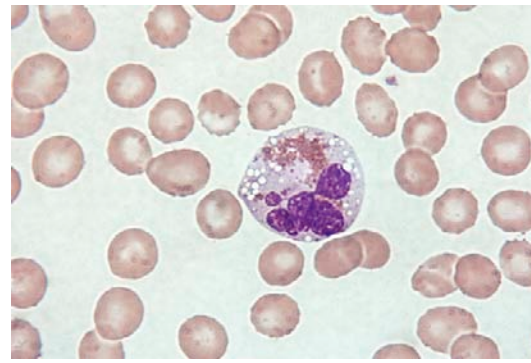


Figure B1-46
Hypereosinophilic syndrome: peripheral blood showing a hypersegmented, hypogranular and vacuolated eosinophil. (X 1000)

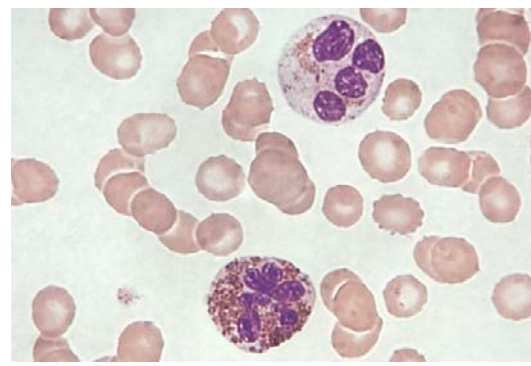


Figure B1-47
Hypereosinophilic syndrome: peripheral blood showing abnormal eosinophils. (x 1000)

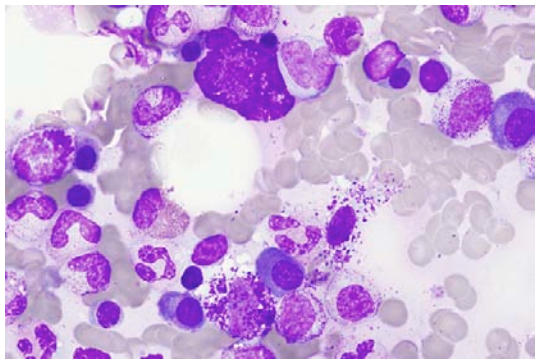


Figure B1-48
Systemic mastocytosis: mast cells infiltrating the bone marrow. (x 1000) (Courtesy of Westmead Hospital)

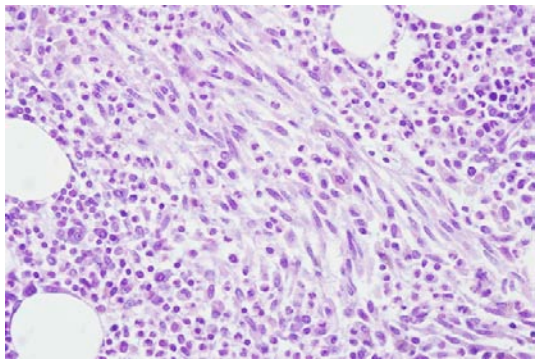


Figure B1-49
Systemic mastocytosis: bone marrow trephine showing a lesion of mast cells with a spindle appearance. (x 400) (Courtesy of Westmead Hospital)

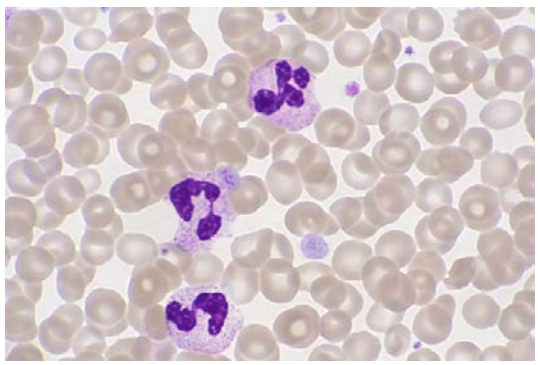


Figure B1-50
Myeloproliferative neoplasm, unclassifiable (group 1): peripheral blood showing a thrombocytosis with large platelets and neutrophilia. (x 1000)

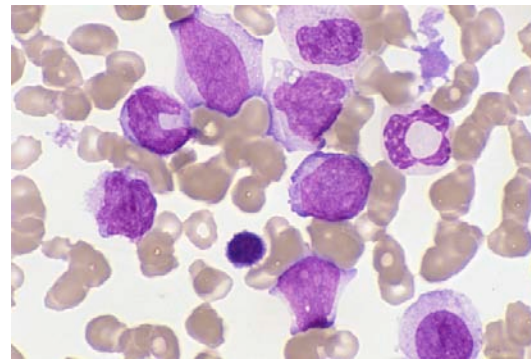


Figure B1-51
Myeloproliferative neoplasm, unclassifiable (group 2): peripheral blood showing a leucoerythroblastic picture with blast cells and myeloid precursors. (x 1000)

MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS

Chronic myelomonocytic leukaemia (CMML)

CMML is a clonal haematopoietic neoplasm with both myeloproliferative and myelodysplastic characteristics. It occurs predominantly in the over-50-years age group. CMML is characterised by a persistent monocytosis of >1 × 10^9/L and is usually in the range of 2–5 × 10^9/L. The *BCR-ABL1* fusion gene and Ph chromosome are absent. Dysplasia is present in one or more of the myeloid lineages. There are less than 20% blasts (promonocytes are counted as blasts) in both the peripheral blood and bone marrow. The monocytes of CMML are often morphologically abnormal.

CMML is divided into two subgroups, CMML-1 and CMML-2 according to the number of blasts in the peripheral blood and the bone marrow. CMML-1 is characterised by <5% blasts (including promonocytes) in the peripheral blood and <10% blasts in the bone marrow. CMML-2 is characterised by 5–19% blasts (including promonocytes) in the peripheral blood and 10-19% blasts in the bone marrow. Refer to Figs B1-52 to B1-54.

CYTOGENETICS

- Cytogenetic abnormalities occur in 20–40% of cases of CMML, most frequently +8, –7/del(7q), i(17q) and structural abnormalities of chromosome 12q

IMMUNOPHENOTYPE

- $CD13^+$, $CD14^{+/-}$, $CD33^+$, $CD64^{+/-}$, $CD68^{+/-}$

Atypical chronic myeloid leukaemia, *BCR-ABL1* negative (aCML)

Atypical chronic myeloid leukaemia (aCML) is a neoplasm with myeloproliferative and myelodysplastic characteristics. It occurs in older males and less commonly in females; the majority of cases occurring in the 70–80 year age group; however, the neoplasm has also been reported in teenagers.

The total white cell count is ≥13 × 10^9/L. It may be higher, in the range of 35–95 × 10^9/L or as high as 300 × 10^9/L. The number of blasts present in the peripheral blood is usually <5%. The number of myeloid precursors ranges from 10–20%. A mild basophilia may be present, usually less than 2%. Monocytes are not increased, usually less than 10%. The number of blasts in the bone marrow is less than 20%. Dysgranulopoiesis such as abnormally segmented nuclei with pelgeroid features and hypogranular cytoplasm is a characteristic feature of aCML. Thrombocytopenia is common. Dysplasia in the erythroid and megakaryocytic lineages is variable.

CYTOGENETICS

- There is no *BCR-ABL1* fusion gene, Ph chromosome or rearrangements of *PDGFRA*, *PDGFRB*.
- The *JAK2* V617F mutation may occur in some cases.
- Abnormalities occur in 80% of cases of aCML: +8, del(20q), abnormalities of chromosomes 12, 13, 14, 17, 19 and rarely i(17q).

CYTOCHEMISTRY/IMMUNOPHENOTYPE

- The neutrophil alkaline phosphatase varies from low to normal to elevated.
- No specific immunophenotypic abnormality has been identified in aCML.

Juvenile myelomonocytic leukaemia (JMML)

Juvenile myelomonocytic leukaemia (JMML) is a clonal neoplasm characterised by an increase in granulocytes and monocytes. It occurs mainly in children from 1 month to 14 years of age with the majority of cases occurring in children less than 3 years. The

neoplasm is more common in males than females. It is sometimes preceded by an eczematous rash that when biopsied shows a leukaemic infiltrate. Ten per cent of cases are associated with the clinical disorder neurofibromatosis type 1.

JMML is characterised by an elevated white cell count that is usually $<100 \times 10^9$/L with a left shift in the myeloid line. There is a peripheral blood monocytosis of $>1 \times 10^9$/L with <20% blasts (plus promonocytes) in the peripheral blood and bone marrow.

Dysplasia in JMML is mild, occurring more often in the granulocytic lineage than in the erythroid or megakaryocytic lineage. Haemoglobin F is increased for age. It ranges from 38–70% of total haemoglobin with a uniform distribution throughout the red cells. HbA_2 is normal or decreased. Refer to Figs B1-55 to B1-58.

CYTOGENETICS

- The *BCR-ABL1* fusion gene and Ph chromosome are absent.
- Monosomy 7 occurs in approximately 25% of cases but is not specific for JMML.

IMMUNOPHENOTYPE

- No specific immunophenotype is seen in JMML.

Myelodysplastic/myeloproliferative neoplasm, unclassifiable (MDS/MPD, U)

Myelodysplastic/myeloproliferative neoplasm, unclassifiable (MDS/MPD, U) is a neoplasm characterised by the presence of both myelodysplastic and myeloproliferative features yet does not fulfill the criteria for CMML, aCML or JMML. Anaemia, often with a macrocytosis and dimorphic red cells together with a thrombocytosis of $\geq 450 \times 10^9$/L and a leucocytosis of $\geq 13 \times 10^9$/L characterise this neoplasm. There are <20% blasts in both the peripheral blood and bone marrow. More than 10% blasts is indicative of transformation to a more aggressive stage. The bone marrow is hypercellular with dysplastic features in at least one or more cell lineage. The peripheral blood and bone marrow findings support a diagnosis of a myelodysplastic syndrome as well as a myeloproliferative neoplasm.

When making a diagnosis of MDS/MPD, U it is important to ascertain whether the dysplastic features present are drug induced. If this is indeed so, the disorder would not be classified in the MDS/MDP-U category. Only when there is no preceding history of a myeloproliferative, myelodysplastic process can the classification MDS/MPD, U be used.

CYTOGENETICS

- No specific cytogenetic abnormality has been identified in MDS/MPD, U.
- There is no *BCR-ABL1* fusion gene, Ph chromosome, rearrangements of *PDGFRA*, *PDGFRB* and *FGFR1*, isolated del(5q) or t(3;3)/inv(3).

IMMUNOPHENOTYPE

- No abnormal phenotypic features have been described in MDS/MPD, U.

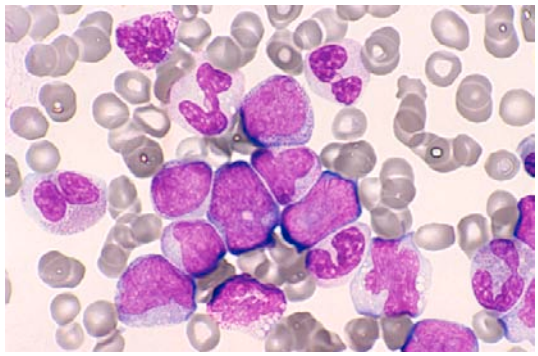


Figure B1-52
Chronic myelomonocytic leukaemia (group 1): peripheral blood film showing increased numbers of myeloid and monocytoid cells. (x 1000)

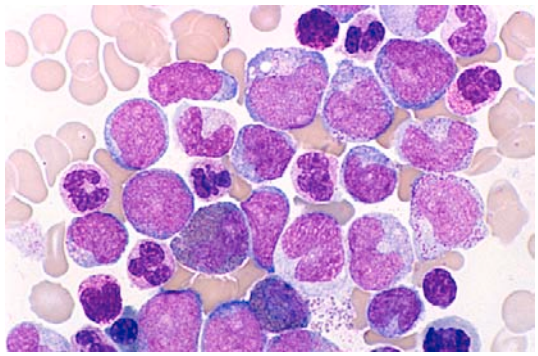


Figure B1-53
Chronic myelomonocytic leukaemia (group 1): bone marrow showing increased numbers of myeloid and monocytoid cells. (x 1000)

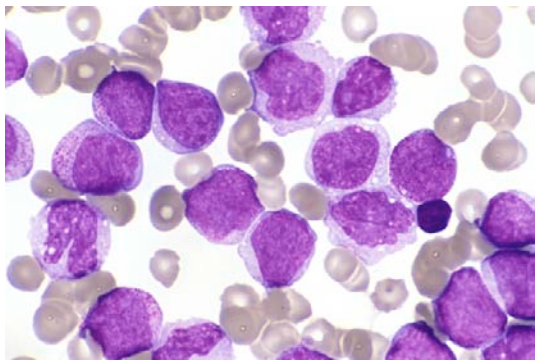


Figure B1-54
Chronic myelomonocytic leukaemia (group 2): bone marrow showing an increase in blast cells together with increased numbers of myeloid and monocytoid cells. (x 1000)

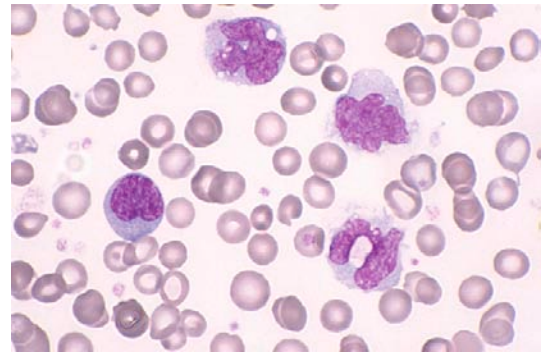


Figure B1-55
Juvenile myelomonocytic leukaemia: peripheral blood film showing a monocytosis and a metamyelocyte. (x 1000)

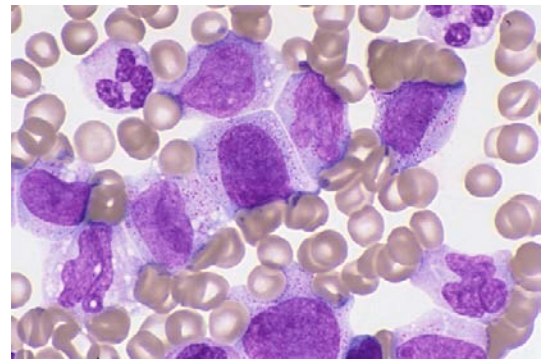


Figure B1-56
Juvenile myelomonocytic leukaemia: peripheral blood film showing a monocytosis and an increase in myeloid precursors. (x 1000)

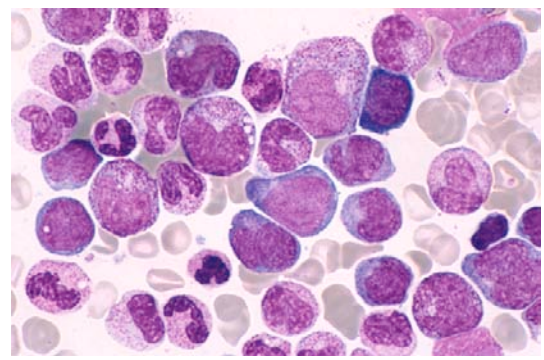


Figure B1-57
Juvenile myelomonocytic leukaemia: bone marrow showing an increase in myelopoiesis. (x 1000)

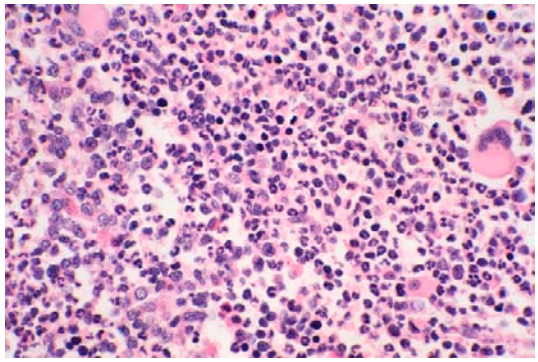


Figure B1-58
Juvenile myelomonocytic leukaemia: bone marrow trephine showing a hypercellular marrow with increased myelopoiesis. (x 400)

MYELODYSPLASTIC SYNDROMES/NEOPLASMS

The term 'myelodysplastic' describes a state of abnormal bone marrow proliferation and differentiation, usually afflicting the elderly and in approximately 85% of cases culminating in acute myeloblastic leukaemia. Pre-leukaemia and smoldering leukaemia were the terms used previously to describe this group of neoplasms. Myelodysplastic syndrome (MDS) results from the neoplastic transformation of a single pluripotent stem cell leading to abnormalities in one or more of the erythroid, myeloid or megakaryocytic cell lines being detected in both the peripheral blood and bone marrow.

Morphological abnormalities

RED CELLS

One or more of the following features may be present:

(a) A mean red cell volume (MCV) ranging from low to high but a red cell distribution width (RDW) that is always high, indicating that there is a marked degree of anisocytosis or dimorphism
(b) Red cell poikilocytosis including elliptocytes and teardrop poikilocytes
(c) Nucleated red cells and Howell-Jolly bodies indicating abnormal red cell maturation
(d) Basophilic stippling
(e) Pappenheimer bodies and ring sideroblasts reflecting defective erythroid maturation and impaired utilisation of iron
(f) Nuclear budding, multinuclearity and internuclear and intercytoplasmic bridging
(g) Megaloblastoid changes.

WHITE CELLS

One or more of the following features may be present:

(a) Neutropenia in 50% of cases
(b) Monocytosis
(c) Hypogranularity and/or agranularity of the myeloid line
(d) Pseudo Pelger-Huët anomaly or pelgeroid changes in the myeloid line
(e) Myeloid precursors including myeloblasts.

PLATELETS

One or more of the following features may be present:

(a) Mild to moderate thrombocytopenia in about 25% of patients
(b) Platelet anisocytosis with giant forms (raised mean platelet volume (MPV) and platelet distribution width (PDW))
(c) Micromegakaryocytes with small hypolobulated nuclei as well as hypersegmented megakaryocytes in the bone marrow.

WHO classification

The WHO classification divides the syndromes into seven groups according to the number of myeloblasts and ring sideroblasts in the peripheral blood and bone marrow (Table B1-1).

Refractory cytopenia with unilineage dysplasia (RCUD)

Refractory cytopenia with unilineage dysplasia RCUD is a myelodysplastic syndrome associated with unicytopenia or bicytopenia. The cytopenias are defined as having a haemoglobin <100 g/L, an absolute neutrophil count $<1.8 \times 10^9$/L and platelet count $<100 \times 10^9$/L. RCUD includes three groups namely refractory anaemia (RA), refractory neutropenia (RN) and refractory thrombocytopenia (RT).

Table B1-1 The WHO classification of myelodysplastic syndromes/neoplasms

Subtype	Peripheral blood	Bone marrow
1. Refractory cytopenias with unilineage dysplasia (RCUD) Refractory anaemia (RA) Refractory neutropenia (RN) Refractory thrombocytopenia (RT)	Unicytopenia or bicytopenia No or rare blasts (<1%)	Unilineage dysplasia: ≥10% of the cells in one myeloid lineage <5% blasts <15% of erythroid precursors are ring sideroblasts
2. Refractory anaemia with ring sideroblasts (RARS)	Anaemia No blasts	≥15% of erythroid precursors are ring sideroblasts Erythroid dysplasia only <5% blasts
3. Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenia(s) No or rare blasts (<1%) No Auer rods <1 × 10^9/L monocytes	Dysplasia in ≥10% of the cells in ≥2 myeloid lineages (neutrophil and/or erythroid precursors and/or megakaryocytes) <5% blasts in marrow No Auer rods ±15% ring sideroblasts
4. Refractory anaemia with excess blasts-1 (RAEB-1)	Cytopenia(s) <5% blasts No Auer rods <1 × 10^9/L monocytes	Unilineage or multilineage dysplasia 5–9% blasts No Auer rods
5. Refractory anaemia with excess blasts-2 (RAEB-2)	Cytopenia(s) 5–19% blasts Auer rods ± <1 × 10^9/L monocytes	Unilineage or multilineage dysplasia 10–19% blasts Auer rods ±
6. Myelodysplastic syndrome unclassified (MDS-U)	Cytopenia(s) ≤ 1% blasts	Unequivocal dysplasia in <10% of cells in one or more myeloid cell lines when accompanied by cytogenetic abnormality considered as presumptive evidence for a diagnosis of MDS <5% blasts
7. MDS associated with isolated del(5q)	Anaemia Usually normal or increased platelet count No or rare blasts (<1%)	Normal to increased megakaryocytes with hypolobulated nuclei <5% blasts Isolated del(5q) cytogenetic abnormality No Auer rods

Reproduced from Swerdlow SH, Campo E, Harris NL et al (eds): WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, Volume 2, 4th edn. Lyon, IARC, 2008.

Refractory anaemia (RA)

RA affects mainly the red cell series. The red cells show a degree of anisocytosis with a raised RDW. Macrocytes and microcytes appear together on the blood film—usually in an elderly patient whose initial presentation is anaemia. The neutrophils and platelets are usually normal in number and morphology. The red cell precursors in the bone marrow show dysplastic changes in ≥10% of the erythroid precursors. These changes include nuclear budding, multinuclearity, internuclear and intercytoplasmic bridging as well as megaloblastoid changes. Blast cells are rarely seen. There are <1% blasts in the peripheral blood and <5% blasts in the bone marrow. There are also <15% ring sideroblasts present in the bone marrow. Refer to Figs B1-59 and B1-60.

Refractory neutropenia (RN)

RN is characterised by ≥10% dysplastic neutrophils in the peripheral blood and bone marrow. The characteristic changes are nuclear hypolobulation and hypogranulation. Refer to Figs B1-61 and B1-62.

Refractory thrombocytopenia (RT)

RT is characterised by ≥10% dysplastic megakaryocytes in at least 30 megakaryocytes. The characteristic changes are hypolobulated and hyperlobulated megakaryocytes as well as micromegakaryocytes. The megakaryocytes may be increased or decreased in number. The peripheral blood platelets demonstrate both anisocytosis and variation in granularity. Refer to Fig B1-63.

CYTOGENETICS

- No specific cytogenetic abnormality has been identified in RA.
- Cytogenetic abnormalities may be present in up to 50% of cases. When present, they are del(20), +8, –5/del(5q), –7/del(7q).

IMMUNOPHENOTYPE

- Immunophenotyping may be indicated in RA as aberrant immunophenotypic features of erythroid precursors can be found by flow cytometry.
- Immunophenotyping is not indicated in RN or RT.

Refractory anaemia with ring sideroblasts (RARS)

Refractory anaemia with ring sideroblasts (RARS) is a myelodysplastic syndrome affecting the red cell series only. It is characterised by a dimorphic population of red cells with a high RDW. The red cells on the peripheral blood film may contain basophilic stippling as well as numerous Pappenheimer bodies. The Perl's Prussian blue stain on the bone marrow shows the presence of ≥15% sideroblasts. A sideroblast is a red cell precursor whose nucleus is surrounded by a minimum of 5 siderotic granules. These granules cover at least one-third of the nucleus. The red cells in the marrow demonstrate dysplastic changes including nuclear lobulation and megaloblastoid features.

There are no blasts in the peripheral blood and <5% blasts in the bone marrow. There is no evidence of dysplasia in the myeloid line. The platelets may be large with a raised mean platelet volume (MPV) and platelet distribution width (PDW). Refer to Figs B1-64 to B1-66.

CYTOGENETICS

- No specific cytogenetic abnormality has been identified in RARS.
- However, cytogenetic abnormalities may be present in 5–10% of cases often involving a single chromosome.

IMMUNOPHENOTYPE

- Immunophenotyping may be indicated in RARS.

Refractory cytopenia with multilineage dysplasia (RCMD)

Refractory cytopenia with multilineage dysplasia (RCMD) is a myelodysplastic syndrome characterised by unicytopenia, bicytopenia or pancytopenia as well as dysplastic changes occurring in two or three cell lines. The red cells in the peripheral blood are characteristically dimorphic while the neutrophils are hypogranular and/or agranular with pseudo Pelger-Huët or pelgeroid features. There are <1% blasts in the peripheral blood and <5% blasts in the bone marrow. The peripheral blood also shows $<1 \times 10^9$/L monocytes.

Red cell precursors in the bone marrow show nuclear budding, multinuclearity and megaloblastoid changes. The megakaryocytes may also show dysplastic features such as

micromegakaryocytes with small hypolobulated nuclei as well as hypersegmented megakaryocytes. Approximately 15% ring sideroblasts may be present in the bone marrow in RCMD. Refer to Figs B1-67 to B1-69.

CYTOGENETICS

- No specific cytogenetic abnormality has been identified in RCMD.
- Cytogenetic abnormalities may include –5/del(5q), –7/del(7q), +8 and del(20q).
- Complex karyotypic abnormalities may be present in up to 50% of cases.

IMMUNOPHENOTYPE

- Immunophenotyping may be indicated in RCMD.

Refractory anaemia with excess blasts (RAEB)

Refractory anaemia with excess blasts (RAEB) is a myelodysplastic syndrome characterised by unilineage or multilineage dysplasia. The red cells in the peripheral blood are characteristically dimorphic while the neutrophils are hypogranular and/or agranular with pseudo Pelger-Huët or pelgeroid forms.

Red cell precursors in the bone marrow show abnormally lobulated and budding nuclei, multinucleated forms with megaloblastoid features as well as internuclear bridging. The megakaryocytes range from micromegakaryocytes with small hypolobulated nuclei to hypersegmented megakaryocytes with separated nuclei.

RAEB is divided into two subgroups according to the number of blasts in the peripheral blood and the bone marrow. RAEB-1 is defined as having <5% blasts in the peripheral blood and 5–9% blasts in the bone marrow. The peripheral blood also shows $<1 \times 10^9$/L monocytes. RAEB-2 is defined as having 5–19% blasts in the peripheral blood and 10–19% blasts in the bone marrow. The peripheral blood also shows $<1 \times 10^9$/L monocytes.

The presence of Auer rods in the blasts qualifies as a case of RAEB-2 irrespective of the number of blasts present. Refer to Figs B1-70 to B1-72

RAEB with fibrosis (RAEB-F)

Approximately 15% of patients with MDS have increased fibrosis in the bone marrow and have been categorised as RAEB-F. Morphologically this group may overlap with acute panmyelosis with myelofibrosis, a subcategory of acute myeloid leukaemia and related precursor neoplasms.

CYTOGENETICS

- No specific cytogenetic abnormality has been identified in RAEB. Cytogenetic abnormalities may be present in 30–50% of cases. When present, they are –5/del(5q), –7/del(7q), +8 and del(20q). Complex karyotypic abnormalities may be present.

IMMUNOPHENOTYPE

- $CD34^+$, HLA-DR^+, $CD38^+$, $CD117^+$, $CD13^{+/-}$, $CD33^{+/-}$
- In RAEB-F CD61 and CD42b will be positive.

Myelodysplastic syndrome, unclassifiable (MDS-U)

Myelodysplastic syndrome, unclassifiable (MDS-U) is a subtype of MDS which cannot be classified under any of the MDS syndromes. The diagnosis of MDS-U can be made when any of the following morphological findings are present:

(a) Patients with refractory cytopenia with unilineage dysplasia (RCUD) or with refractory cytopenia with multilineage dysplasia (RCMD) but with only 1% blasts in the peripheral blood qualify for MDS-U

(b) Patients with refractory cytopenia with unilineage dysplasia (RCUD) associated with pancytopenia. RCUD only allows for a single cytopenia or bi-cytopenia
(c) Patients with cytopenias and <1% blasts in the peripheral blood and <5% blasts in the bone marrow and who have dysplasia in <10% of cells in one or more of the myeloid lineages are also categorised in the MDS-U category.

Patients with MDS-U are usually older; however, the syndrome may also affect the younger patient. It may also result from exposure to chemotherapy agents and radiation therapy.

CYTOGENETICS

- No specific cytogenetic abnormality has been identified in MDS-U.

IMMUNOPHENOTYPE

- Immunophenotyping is not relevant in MDS-U.

Myelodysplastic syndrome with isolated del(5q)

Myelodysplastic syndrome with del(5q) occurs mainly in women with a median age of 67 years. It affects mainly the red cell precursors and the megakaryocytes. The patients usually present with severe refractory anaemia. The dysplastic changes in the red cells are variable. The platelet count is either normal or increased while the megakaryocytes in the bone marrow are hypolobulated and either normal or increased in number.

There may be a slight leucopenia but the number of blasts in the peripheral blood and bone marrow is not increased. The percentage of blast cells in the peripheral blood is <1% while the number in the bone marrow is <5%. Auer rods are absent.

MDS del(5q) is associated with an isolated del(5q) cytogenetic abnormality. Should there be any other cytogenetic abnormality the particular case should not be included in this category. Refer to Figs B1-75 and B1-76.

CYTOGENETICS

- The sole genetic abnormality is an interstitial deletion of the long arm of chromosome 5. If other karyotypic abnormalities are present the case should be re-categorised. The size of the deletion and the breakpoints involved may vary; however, bands 5q31 and 5q33 are invariably deleted. It has been reported that a small number of cases with MDS del(5q) have a *JAK2* V617F mutation.

IMMUNOPHENOTYPE

- Immunophenotyping is not relevant in MDS del(5q).

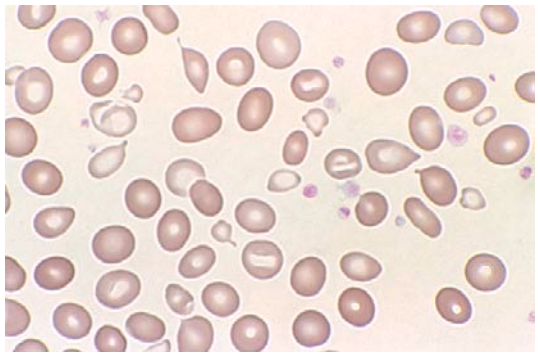


Figure B1-59
Refractory cytopenia with unilineage dysplasia. Refractory anaemia: peripheral blood showing marked red cell anisocytosis, hypochromic microcytes and normochromic macrocytes (dimorphism) as well as large platelets. (x 1000)

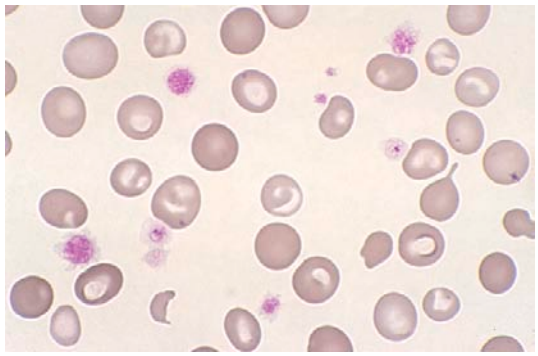


Figure B1-60
Refractory cytopenia with unilineage dysplasia. Refractory anaemia: peripheral blood showing a dimorphic red cell picture with giant platelets. (x 1000)

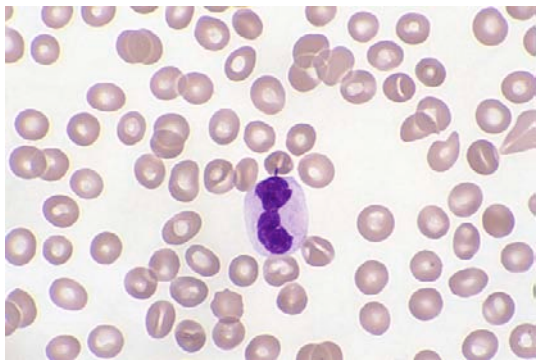


Figure B1-61
Refractory cytopenia with unilineage dysplasia. Refractory neutropenia: peripheral blood showing an agranular pelgeroid neutrophil. (x 1000)

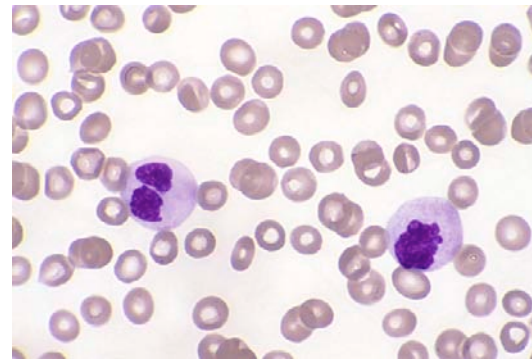


Figure B1-62
Refractory cytopenia with unilineage dysplasia. Refractory neutropenia: peripheral blood film showing hypogranular pelgeroid neutrophils. (x 1000)

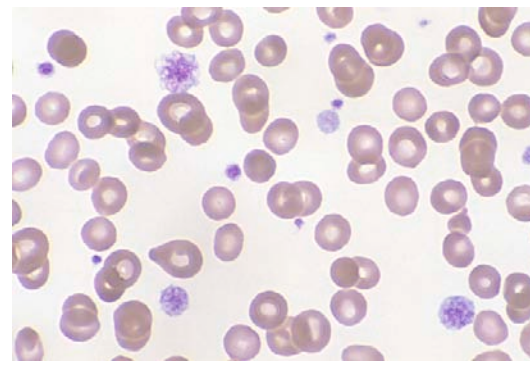


Figure B1-63
Refractory cytopenia with unilineage dysplasia. Refractory thrombocytopenia: peripheral blood film showing variation in both platelet size and granularity. (x 1000)

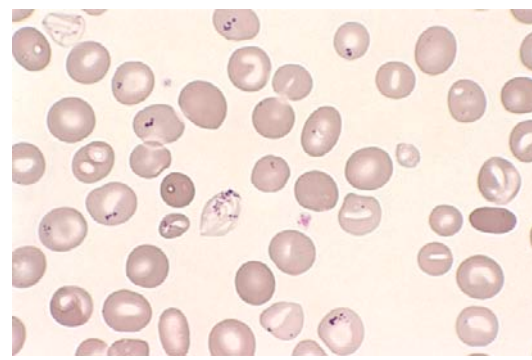


Figure B1-64
Refractory anaemia with ring sideroblasts: peripheral blood film showing a marked number of Pappenheimer bodies in a dimorphic population of red cells. (x 1000)

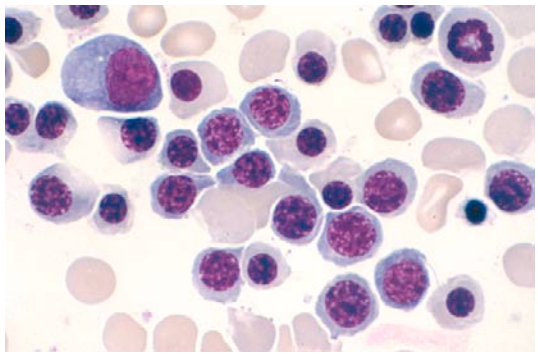


Figure B1-65
Refractory anaemia with ring sideroblasts: bone marrow showing a group of dyserythropoietic erythroblasts. (x 1000)

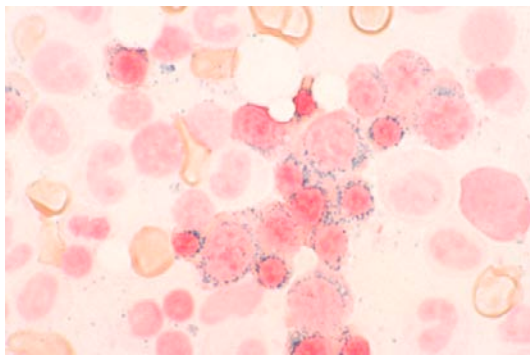


Figure B1-66
Refractory anaemia with ring sideroblasts: iron stain (Perl's Prussian blue) on bone marrow showing ring sideroblasts. (x 1000)

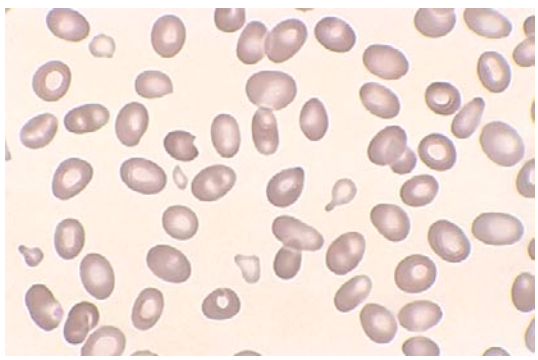


Figure B1-67
Refractory cytopenia with multilineage dysplasia: peripheral blood film showing a dimorphic population of red cells. (x 1000)

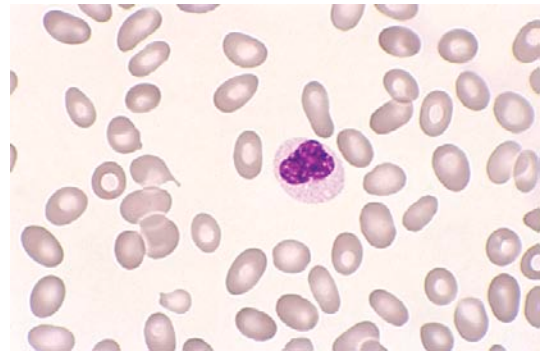


Figure B1-68
Refractory cytopenia with multilineage dysplasia: peripheral blood film showing red cell anisocytosis and a hypogranular pelgeroid neutrophil. (x 1000)

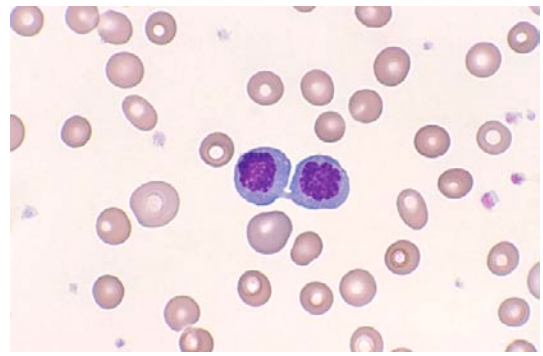


Figure B1-69
Refractory cytopenia with multilineage dysplasia: bone marrow showing intercytoplasmic bridging between two red cell precursors. (x 1000)

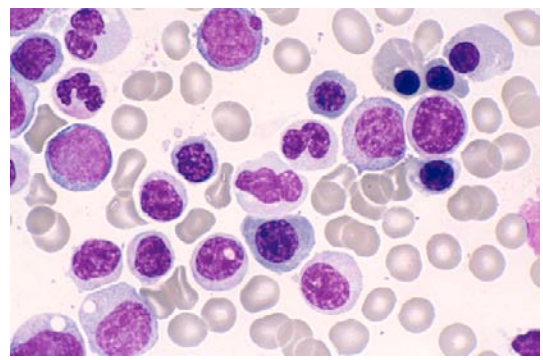


Figure B1-70
Refractory anaemia with excess blasts: bone marrow showing myeloblasts, pelgeroid neutrophils and dysplastic red cell precursors. (x 1000)

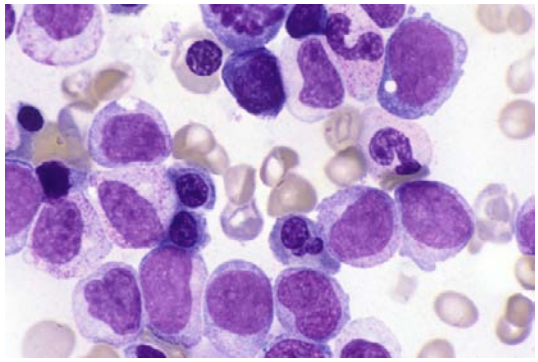


Figure B1-71
Refractory anaemia with excess blasts: bone marrow showing pelgeroid myelocytes and band form neutrophils as well as dysplastic erythropoiesis with nuclear budding. (x 1000)

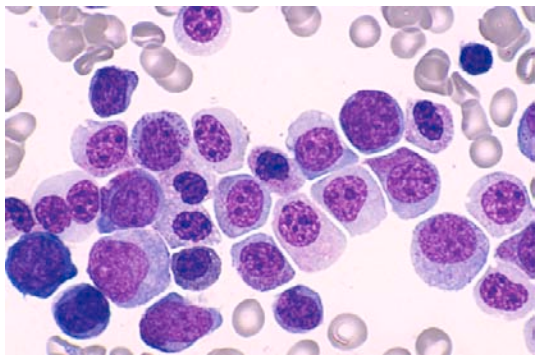


Figure B1-72
Refractory anaemia with excess blasts: hypercellular marrow showing increased numbers of dysplastic myeloid cells. (x 1000)

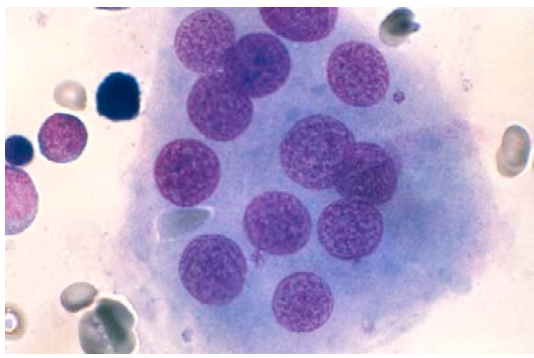


Figure B1-73
Dyshaemopoiesis: methotrexate toxicity showing a hypersegmented megakaryocyte in the bone marrow of a patient exposed to the antimetabolite methotrexate. Antimetabolites induce prominent dysplastic changes resembling those seen in primary myelodysplastic syndromes. (x 1000)

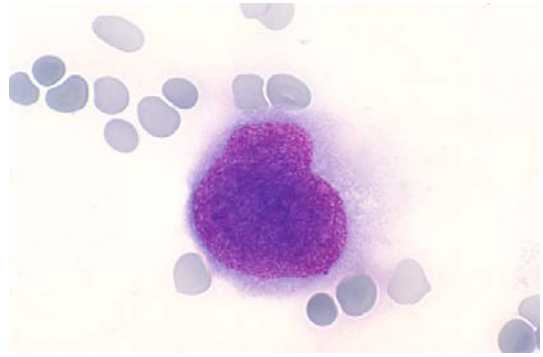


Figure B1-74
Dyshaemopoiesis: methotrexate toxicity showing a hypolobulated megakaryocyte in the bone marrow of a patient exposed to the antimetabolite methotrexate. (x 1000)

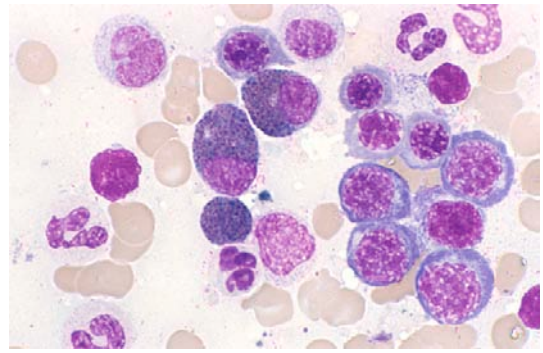


Figure B1-75
Myelodysplastic syndrome with isolated del(5q): bone marrow showing dysplastic erythroid precursors. (x 1000)

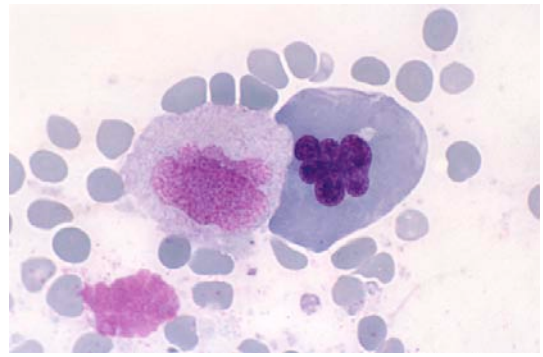


Figure B1-76
Myelodysplastic syndrome with isolated del(5q): bone marrow showing multinuclearity in a red cell precursor. (x 1000)

MYELOID-SPECIFIC STAINS

Neutrophil alkaline phosphatase (NAP)

NAP may be demonstrated in the cytoplasm of neutrophils and to a lesser extent in band forms. The enzyme is present in the specific granules as well as in tubular structures in the neutrophil cytoplasm.

A high NAP score is seen in neutrophilia of infection, leukaemoid reactions, late pregnancy, PV and some cases of myelofibrosis. NAP activity may be used to differentiate myeloproliferative disorders from CML (the NAP score is raised in myeloproliferative disorders but low in CML). Refer to Fig B1-77.

Myeloperoxidase (MPO)

Myeloperoxidase (MPO) is a lysosomal enzyme found in the azurophilic granules of the myeloid and monocyte lineages. Developing and mature granulocytes give positive MPO reactions. Leukaemic myeloblasts of the WHO classification acute myeloid leukaemia, NOS—namely AML without maturation and AML with maturation—are positive while those with features suggestive of AML with minimal differentiation are negative. Eosinophils, basophils, promonocytes and mature monocytes all give positive MPO reactions, while monoblasts, lymphocytes and lymphoblasts give negative reactions. Auer rods in leukaemic myeloblasts are also MPO positive.

The main value of the MPO stain is in differentiating between the myeloblasts of acute myeloid leukaemia, not otherwise specified (NOS) and the lymphoblasts of the precursor B- and T-cell neoplasms. Immature cells that show MPO activity can be confidently referred to as myeloblasts while blasts that are MPO negative could be any other type of blast cell. Refer to Fig B1-78.

Sudan black B (SBB)

SBB stains the lipid membrane surrounding the azurophilic granules of the myeloid and monocyte lineages; hence it closely parallels the MPO reaction. Similarly, lymphocytes and lymphoblasts fail to react. Positivity in the myeloid lineage increases with maturation. Discrete scattered positivity is associated with the monocyte lineage, the mature monocyte demonstrating a sparsely but evenly distributed positivity. The SBB stain also allows differentiation between acute myeloblastic and acute lymphoblastic leukaemia. Refer to Fig B1-79.

Esterases

Nine esterase isoenzymes are present in leucocytes. ‘Specific esterases’ are found in granulocytes and can be demonstrated by the substrate naphthol AS-D chloroacetate while ‘non-specific’ esterases are found in monocytes, lymphocytes, megakaryocytes and platelets and can be demonstrated by the substrate α-naphthyl acetate.

Naphthol AS-D chloroacetate esterase positivity is found in mature and immature granulocytes and in general parallels the MPO and SBB reactions. Little or no enzyme activity is demonstrated in lymphocytes and monocytes.

α-naphthyl acetate esterase positivity is present in lymphocytes and monocytes. The α-naphthyl acetate reaction should always be performed with and without sodium fluoride (NaF) since monocyte esterases are sensitive to NaF, giving a negative reaction, while lymphocyte esterases resist NaF, giving a positive reaction.

The positivity of normal lymphocytes is variable: B cells are negative, T cells have a dot-like perinuclear positivity while null lymphocytes have scattered granules. These patterns may be reflected in pathologic states except in the B cells of hairy cell leukaemia (HCL), which demonstrate crescentic positivity.

The naphthol AS-D chloroacetate esterase and the α-naphthyl acetate esterase reactions have been widely used in combination in a single method called the dual or combined esterase reaction. The end result is the demonstration, in the same preparation, of both types of esterase. This procedure simplifies the cytochemical characterisation of leukaemic cells. Refer to Figs B1-80 to B1-82.

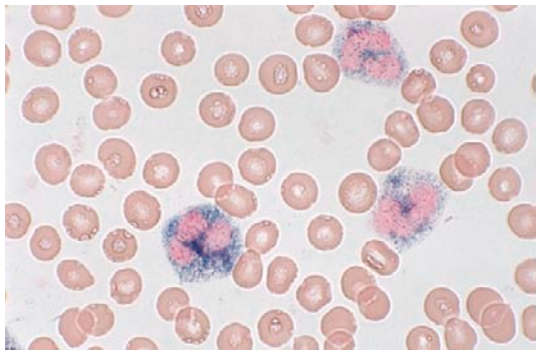


Figure B1-77
Neutrophil alkaline phosphatase (NAP): peripheral blood film in a case of late pregnancy showing strong positivity in the neutrophils. The secondary granules stain blue-black, resulting in a high NAP score. (x 1000)

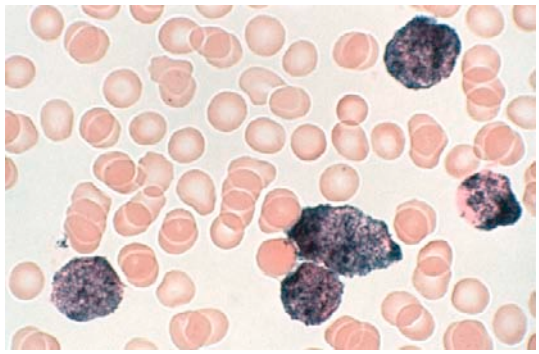


Figure B1-78
Myeloperoxidase (MPO): myeloblasts seen in the bone marrow of a patient with promyelocytic leukaemia. The primary granules stain a turquoise colour. (x 1000)

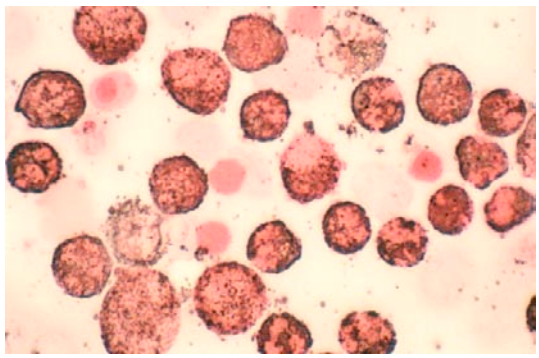


Figure B1-79
Sudan black B (SBB): myeloblasts seen in the bone marrow of a patient with acute myelogenous leukaemia with maturation. The primary granules are staining black. (x 1000)

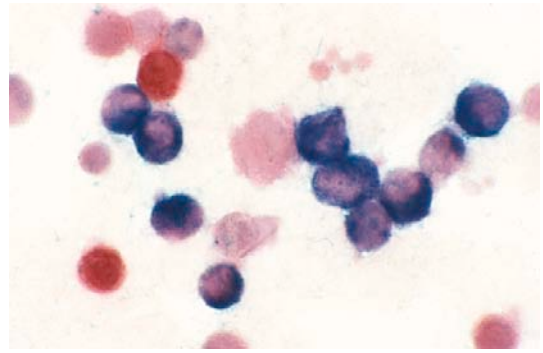


Figure B1-80
Dual esterase: a case of acute myelomonocytic leukaemia showing two populations of cells within the bone marrow: myeloblasts containing bluish-black granules that are naphthol AS-D chloroacetate-positive and monoblasts containing reddish-brown granules that are α-naphthyl acetate-positive. (x 1000)

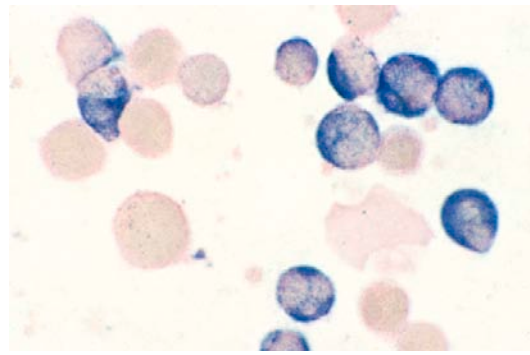


Figure B1-81
Dual esterase: NaF inhibition of the bone marrow in the case shown in B1-80. The monoblast esterases are sensitive to NaF and do not stain in its presence. (x 1000)

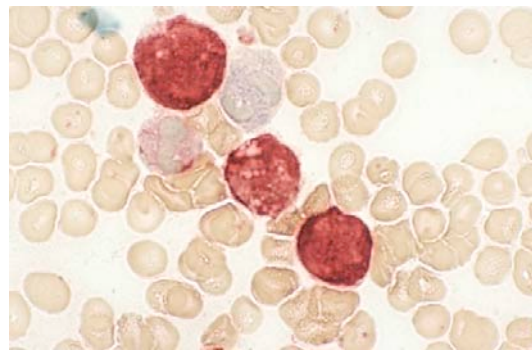
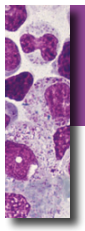


Figure B1-82
Dual esterase: monoblasts staining in the bone marrow in a case of acute monoblastic leukaemia. (x 1000)



B2 Monocytes and macrophages

MONOCYTIC MATURATION

Monocytes are produced in the bone marrow. They migrate to the peripheral blood and then to organs of the reticuloendothelial system. Monocytes differentiate into macrophages or histiocytes. They then migrate to the tissues, where they act as phagocytes. In the tissues, they clear the body of microbial organisms and of aged damaged cells by a process of pinocytosis and phagocytosis.

The maturation of monocytes is divided into three stages: the monoblast, promonocyte and mature monocyte.

Monoblast

The monoblast varies in diameter from 14 to 18 µm. It has a round to oval nucleus occupying about 80% of the cell, and one or two nucleoli are present. The cytoplasm is basophilic and lacks granules. Refer to Figs B2-1 and B2-3.

Promonocyte

The promonocyte varies in diameter from 14 to 18 µm. The nucleus is indented and often contains a nucleolus. The cytoplasm is blue-grey and may contain a few fine azurophilic granules. Refer to Figs B2-2 and B2-3.

Monocyte

The monocyte varies in diameter from 12 to 18 µm. The nucleus is folded and lobulated, with two or more lobes. The cytoplasm is grey-blue with numerous fine azurophilic granules. Vacuolation is a characteristic feature of the mature monocyte.

Disorders of the monocyte/macrophage system involve monocytic proliferation in the blood and bone marrow as well as congenital and acquired macrophage disorders. Monocyte proliferative disorders are closely associated with the myeloid line of cells involving leukaemic states, for example, myelodysplastic syndrome, juvenile myelomonocytic leukaemia and myelomonocytic leukaemia. A small number of cases of chronic monocytic leukaemia have also been described. Refer to Figs B2-3 to B2-5.

MONOCYTE AND MACROPHAGE DISORDERS

Gaucher disease

Gaucher disease is a lipid storage disease characterised by the presence of macrophages throughout the bone marrow and organs of the reticuloendothelial system. These macrophages, known as Gaucher cells, measure between 20 and 100 µm in diameter, and have a striated cytoplasm resembling onion skin that is due to the accumulation of glucocerebroside stored within their cytoplasm. The fundamental biochemical defect is due to a severe reduction of the enzyme glucocerebroside β-glucosidase, which catalyses the hydrolytic splitting off of glucose in normal tissues, including peripheral leucocytes.

Gaucher disease is recessively inherited and is found in all populations but has a higher frequency among Ashkenazi Jews. The principal features of the disease include a massive splenomegaly and, less frequently, hepatomegaly. Anaemia and thrombocytopenia are usually present, leading to the need for a splenectomy in such patients.

Laboratory diagnosis depends upon the presence of Gaucher cells in the bone marrow and the demonstration of a deficiency in lysosomal β-glucosidase activity measured in leucocytes. Refer to Fig B2-6.

Niemann-Pick disease

Niemann-Pick disease is characterised by a deficiency of the enzyme sphingomyelinase leading to a build-up of sphingomyelin, cholesterol and other cell membrane lipids, which accumulate within foam cells throughout the reticuloendothelial system.

Foam cells range in size from 20 to 100 μm in diameter and their cytoplasm is packed with small droplets of lipid—hence the description 'foam cells'. The presence of these cells in the bone marrow provides the simplest means for diagnosis.

Sea-blue histiocytes are also present in the bone marrow of Niemann-Pick disease. The cytoplasm of these histiocytes is filled with large granules containing ceroid that stain greenish-blue with Romanowsky stains.

Niemann-Pick disease is recessively inherited and is also found in high frequency among Ashkenazi Jews.

Anaemia may be present in some patients but the majority of cases have a normal haemoglobin level. On examination of the blood film, the majority of lymphocytes are noted to contain cytoplasmic vacuolation. These vacuoles represent lipid-filled lysosomes. Refer to Figs B2-7 to B2-9.

Reactive haemophagocytic syndrome

This is characterised by a proliferation of macrophages or histiocytes within organs of the reticuloendothelial system. The syndrome may be associated with a systemic viral infection such as Epstein-Barr virus (EBV), cytomegalovirus (CMV), herpes simplex or varicella. Sometimes the syndrome may be associated with a bacterial, fungal or protozoal infection. It may also occur in malignant histiocytosis.

The clinical signs of the syndrome include fever, lethargy and myalgia. Young children often have an associated splenomegaly or hepatosplenomegaly.

Patients present with severe anaemia, leucopenia and thrombocytopenia. Bone marrow examination reveals an increase in macrophages, many of which have phagocytosed erythrocytes, leucocytes and platelets. Refer to Figs B2-10 to B2-13.

Langerhans cell histiocytosis (LCH)

Langerhans cell histiocytosis (LCH) (histiocytosis X) affects mainly children in the first 4 years of life but may occur in patients 20 years and under. It is categorised according to the level of dissemination. It can be localised, infiltrating the skin, skull, ribs and long bones. It can also be disseminated, involving the lymph nodes, liver, spleen and bone marrow. In disseminated LCH the bone marrow contains lipid-laden macrophages and eosinophilic granulomas. Localised disease in bone is associated with a peripheral blood eosinophilia. Refer to Figs B2-16 to B2-18.

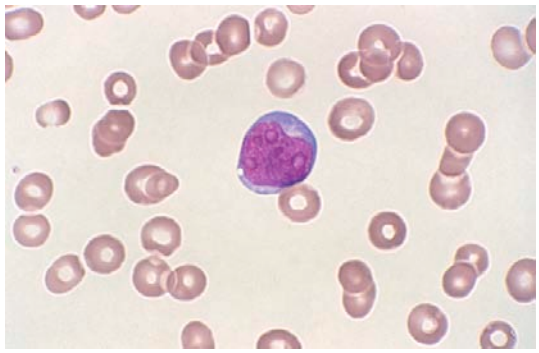


Figure B2-1
Monoblast in peripheral blood.
(x 1000)

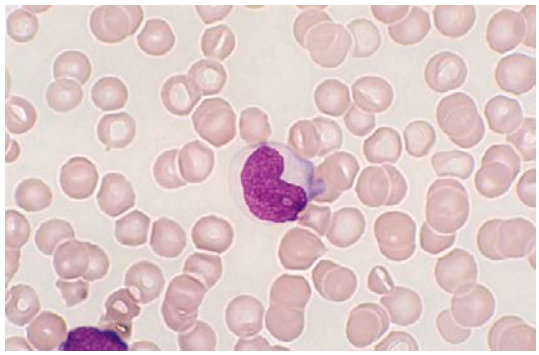


Figure B2-2
Promonocyte in peripheral blood.
(x 1000)

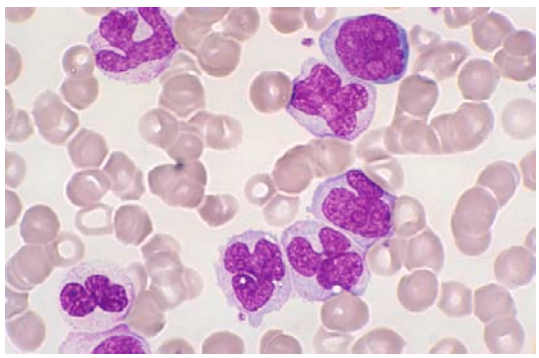


Figure B2-3
Monoblast, promonocytes and monocytes in peripheral blood.
(x 1000)

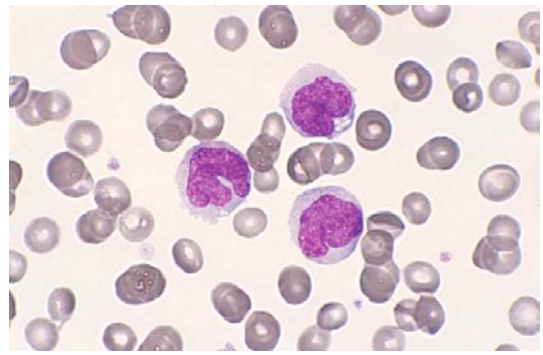


Figure B2-4
Monocytes in peripheral blood demonstrating folded, lobulated nuclei. (x 1000)

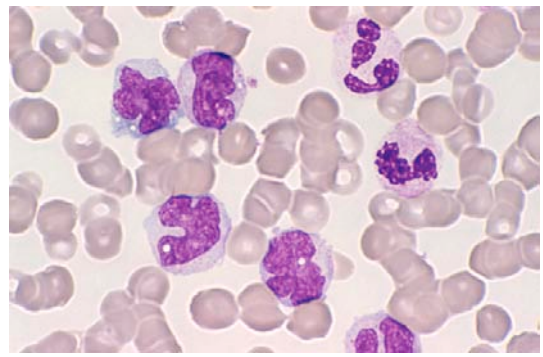


Figure B2-5
Monocytes and neutrophils in peripheral blood. (x 1000)

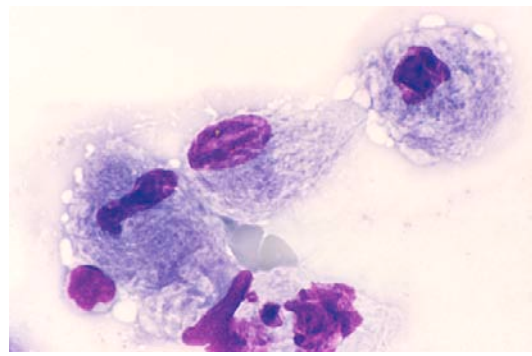


Figure B2-6
Gaucher disease: bone marrow showing Gaucher macrophages with a striated pattern within the cytoplasm that resembles the skin of an onion. (x 1000)

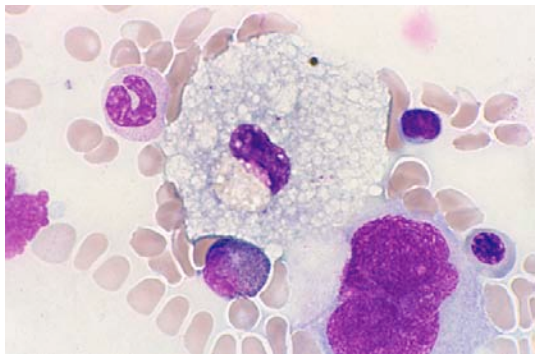


Figure B2-7
Niemann-Pick disease: bone marrow showing a foamy macrophage whose cytoplasm is filled with droplets of lipid. (x 1000)

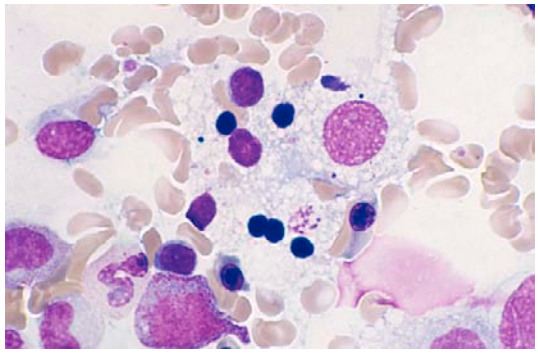


Figure B2-8
Niemann-Pick disease: bone marrow showing a foamy macrophage that has phagocytosed several lymphocyte nuclei. (x 1000)

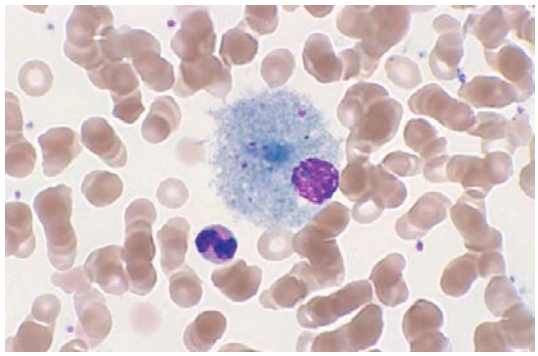


Figure B2-9
Niemann-Pick disease: bone marrow showing a sea-blue histiocyte whose cytoplasm is filled with large blue granules containing ceroid. (x 1000)

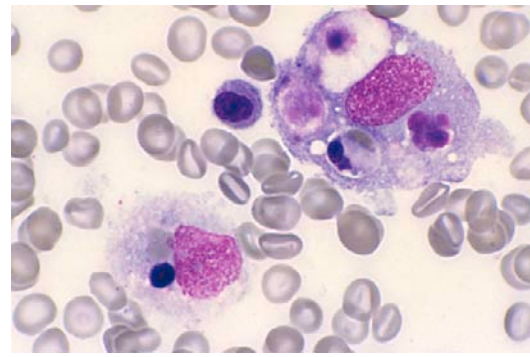


Figure B2-10
Reactive haemophagocytic syndrome: macrophages present in the bone marrow of a 6-year-old child that have phagocytosed red cells and white cell nuclei. (x 1000)

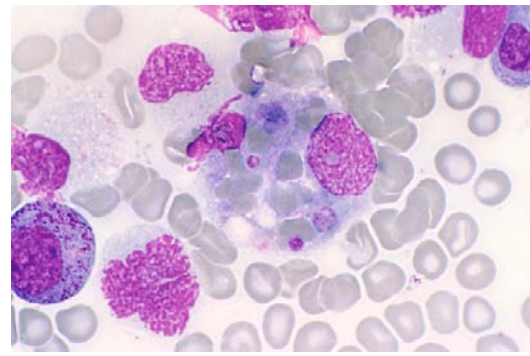


Figure B2-11
Reactive haemophagocytic syndrome: bone marrow from the case shown in B2-10 showing a macrophage that has phagocytosed erythrocytes. (x 1000)

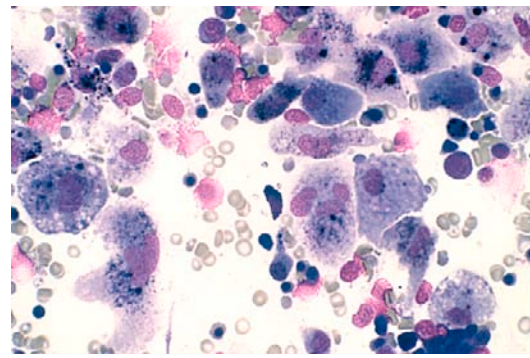


Figure B2-12
Reactive haemophagocytic syndrome: macrophage infiltration of the marrow in an 18-month-old child. (x 1000)

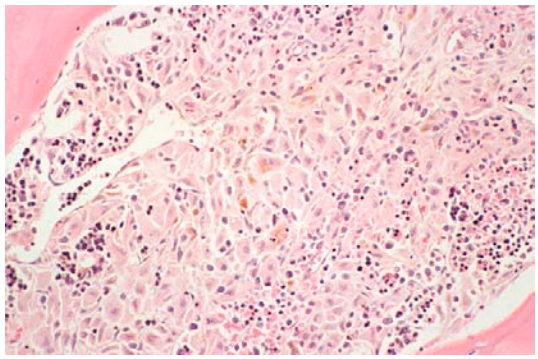


Figure B2-13
Reactive haemophagocytic syndrome: bone marrow trephine from the case shown in B2-12. (x 200)

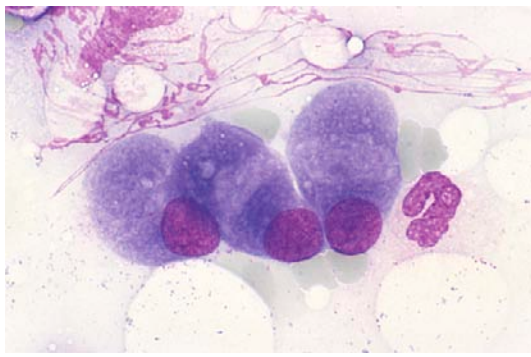


Figure B2-14
Osteoblasts: bone marrow showing large macrophages approximately 30 μm in diameter with eccentrically placed nuclei and light blue cytoplasm. (x 1000)

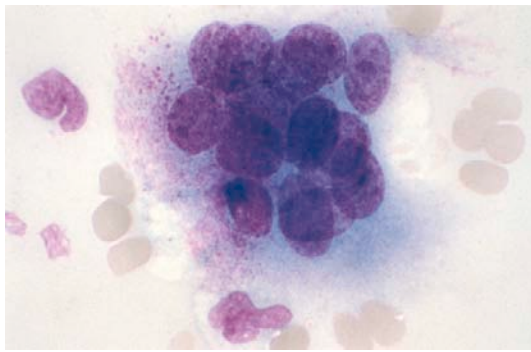


Figure B2-15
Osteoclast: bone marrow showing a macrophage approximately 100 μm in diameter containing multiple nuclei with fine chromatin pattern and nucleoli. The cytoplasm is basophilic. Osteoblasts and osteoclasts are seen in the bone marrow of patients with osteoblastic reactions to tumours as well as in adults with Paget's disease. (x 1000)

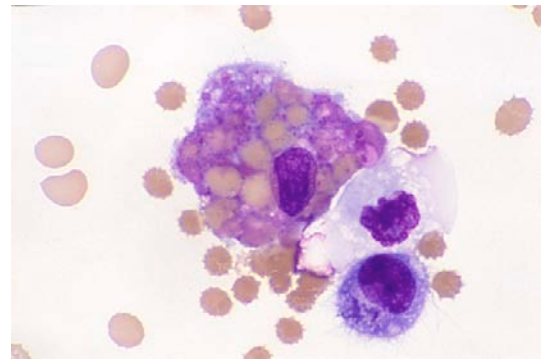


Figure B2-16
Langerhans cell histiocytosis (histiocytosis X): fine needle biopsy of the parotid gland of a 3-year-old child showing histiocytic erythrophagocytosis. (x 1000)

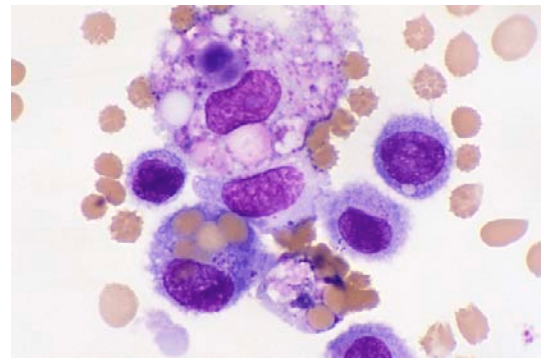


Figure B2-17
Langerhans cell histiocytosis (histiocytosis X): fine needle biopsy of the parotid gland of a 3-year-old child showing a histiocyte which has phagocytosed red cells, white cell nuclei and platelets. (x 1000)

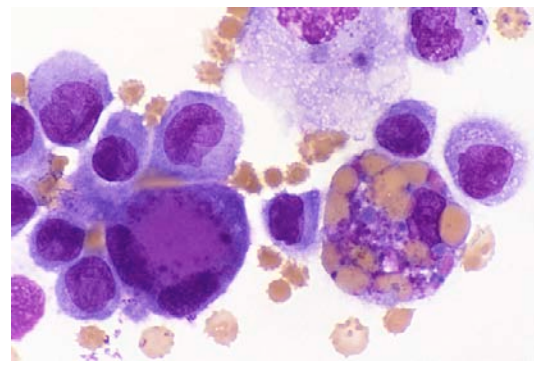


Figure B2-18
Langerhans cell histiocytosis (histiocytosis X): fine needle biopsy of the parotid gland of a 3-year-old child showing erythrophagocytosis. (x 1000)

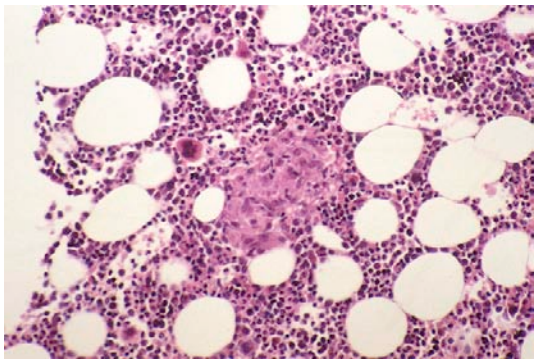
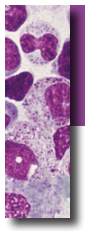


Figure B2-19
Bone marrow trephine from a child with sarcoidosis showing a large granuloma. (H&E) (x 400)



B3 Platelets

MEGAKARYOCYTIC MATURATION

Megakaryocytic maturation occurs within the bone marrow by a process known as endomitosis. The nucleus replicates in multiples of two without cytoplasmic division, only enlargement of volume. The cytoplasm becomes less basophilic as the megakaryocyte matures, acquiring azurophilic granules. Finally, platelets are produced by fragmentation of the megakaryocyte cytoplasm. Each megakaryocyte produces some 4000 platelets, which are approximately 1 to 2 µm in diameter.

Megakaryocytic maturation is divided into three stages: the megakaryoblast, promegakaryocyte and megakaryocyte.

Megakaryoblast

The megakaryoblast is a large cell about 20 to 30 µm in diameter. It has a single oval or kidney-shaped nucleus, several nucleoli and basophilic non-granular cytoplasm. Refer to Fig B3-1.

Promegakaryocyte

The promegakaryocyte is larger than the megakaryoblast and has 2 to 4 nuclei with several nucleoli. The cytoplasm is basophilic and contains fine azurophilic granules. Refer to Fig B3-2.

Megakaryocyte

The megakaryocyte is a large cell measuring 30 to 90 µm in diameter containing 8, 16 or 32 nuclei. The cytoplasm is less basophilic and shows diffuse azurophilic granulation. Refer to Figs B3-3 to B3-5.

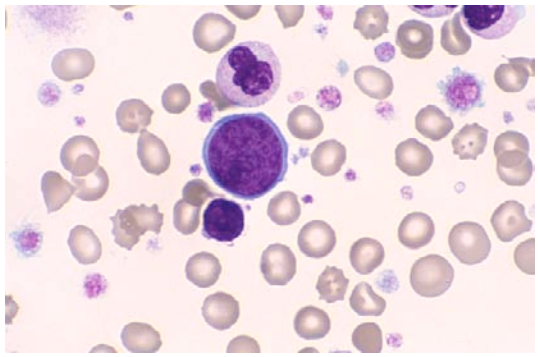


Figure B3-1
Megakaryoblast: blood film from a patient with chronic myelogenous leukaemia, *BCR-ABL1* positive who has undergone splenectomy showing a circulating megakaryoblast in the peripheral blood. (x 1000)

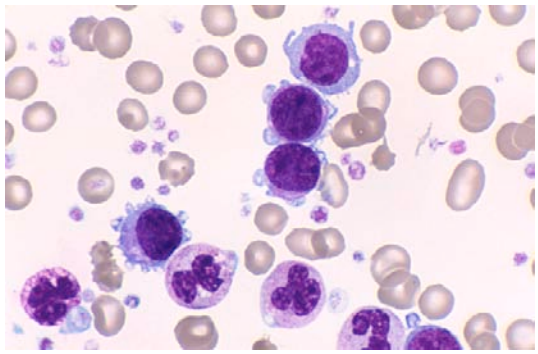


Figure B3-2
Promegakaryocytes: peripheral blood film from the case shown in B3-1 showing circulating promegakaryocytes. (x 1000)

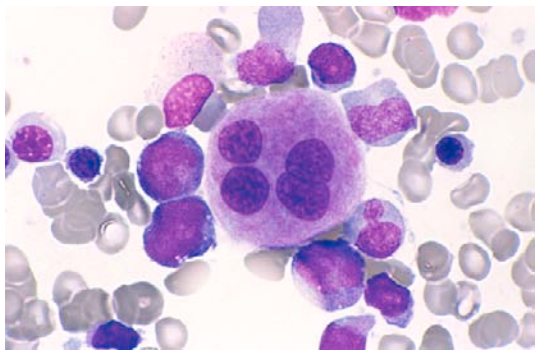


Figure B3-3
Megakaryocyte: bone marrow showing a mature megakaryocyte. (x 1000)

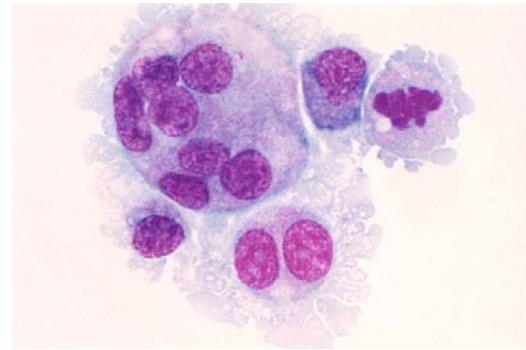


Figure B3-4
Megakaryocytes cultured from cord blood. (x 1000)

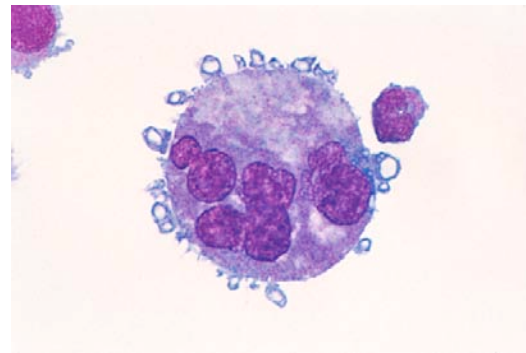


Figure B3-5
Megakaryocyte cultured from cord blood actively producing platelets from mature cytoplasm. (x 1000)

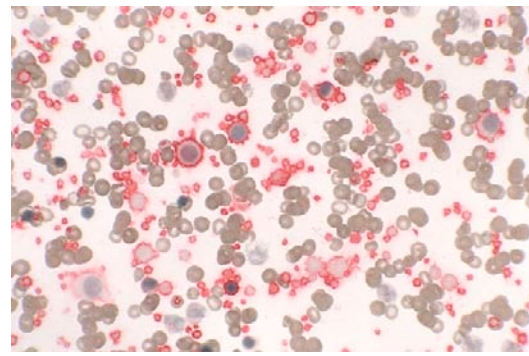


Figure B3-6
Promegakaryocytes and platelets from the case shown in B3-1 reacting with the antibody to CD61 (platelet glycoprotein 111a). (x 400)

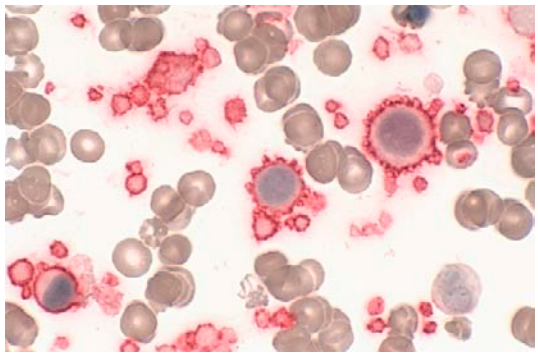


Figure B3-7
Promegakaryocytes and platelets from the case shown in B3-1 reacting with the antibody to CD61. (x 1000)

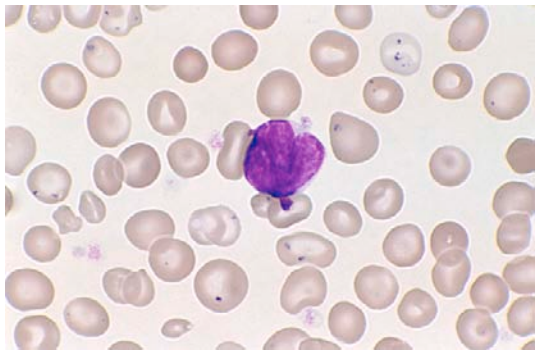


Figure B3-8
Megakaryocytic fragment: these are often present in the peripheral blood from patients with chronic myelogenous leukaemia, *BCR-ABL1* positive and other myeloproliferative neoplasms. (x 1000)

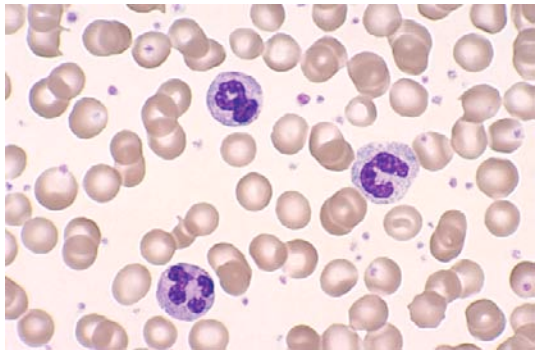


Figure B3-9
Thrombocytosis: peripheral blood film showing reactive thrombocytosis in a child with juvenile rheumatoid arthritis. (x 1000)

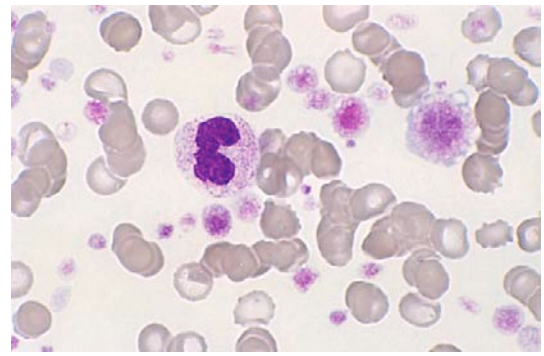


Figure B3-10
Macrothrombocytes: peripheral blood film showing large to giant platelets in a case of primary myelofibrosis following splenectomy. (x 1000)

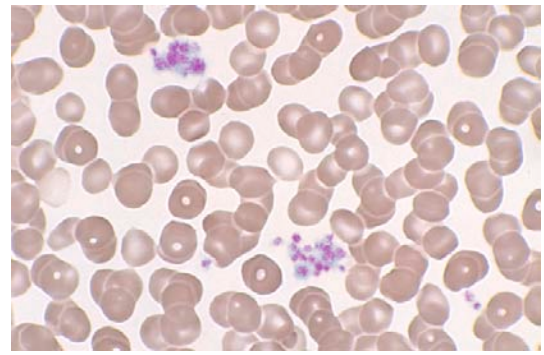


Figure B3-11
Platelet aggregates: platelet aggregates in EDTA samples of peripheral blood usually result from difficulty in collection or less frequently from anti-platelet anti-EDTA antibodies. The presence of platelet aggregates result in an erroneously low platelet count. (x 1000)

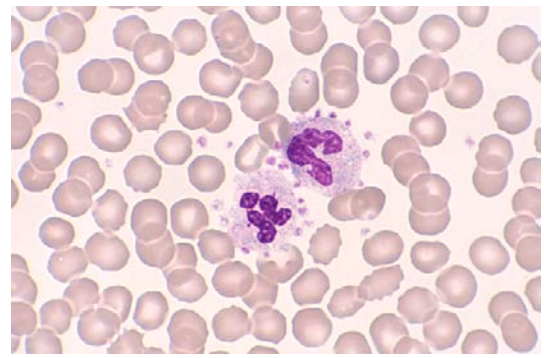
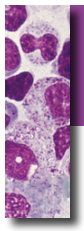


Figure B3-12
Platelet satellitism: this is a rarely seen phenomenon in which the platelets adhere to neutrophils. It occurs only in EDTA blood again resulting in an erroneously low platelet count. Its pathogenesis and significance are unknown. (x 1000)



B4 Lymphocytes

LYMPHOCYTIC MATURATION

Lymphocytes develop mainly in the lymphoid tissues of the body, namely the lymph nodes, the spleen and the lymphatic follicles within the bone marrow. Lymphocytic maturation is divided into three stages: the lymphoblast, prolymphocyte and lymphocyte (large and small). Maturation proceeds along two pathways: one in the thymus producing T lymphocytes (CD4 and CD8 cells) and the other in lymph nodes producing B lymphocytes.

Lymphoblast

The lymphoblast varies from 10 to 20 µm in diameter. It has a round nucleus that occupies about 80% of the cell. The chromatin is fine and contains one or two nucleoli. The cytoplasm is basophilic and agranular. Refer to Fig B4-1.

Prolymphocyte

The prolymphocyte varies in diameter from 10 to 18 µm. The nucleus is round, the chromatin denser and the cytoplasm more abundant compared with the lymphoblast. One prominent nucleolus is present within the nucleus. Refer to Fig B4-2.

Lymphocyte (small)

The small lymphocyte varies in diameter from 9 to 12 µm. The nucleus is round, with coarse clumped chromatin. The cytoplasm is scanty, sky blue in colour and agranular. It may be a T or B lymphocyte; differentiation between the two can only be achieved by examining immune surface markers. Refer to Fig B4-3.

Lymphocyte (large)

The large lymphocyte varies in diameter from 12 to 16 µm. The nucleus is round, with coarse clumped chromatin. The cytoplasm is abundant and sky blue in colour, and often contains a few fine azurophilic granules. Large granular lymphocytes are usually $CD8^+$ T lymphocytes. Refer to Fig B4-4.

REACTIVE LYMPHOCYTOSIS

Reactive lymphocytosis occurs in a number of viral illnesses: infectious mononucleosis, cytomegalovirus infection, varicella infection and viral hepatitis. It occurs in the bacterial infection *Bordetella pertussis* and is also seen in what is described as 'non-specific' acute infectious lymphocytosis. Such reactions must be clearly distinguished from lymphatic leukaemias or any other lymphoproliferative neoplasm.

Infectious mononucleosis (IM)

IM is an infection caused by the Epstein-Barr virus (EBV) and occurs in teenagers and young adults. The clinical presentation includes lassitude, fever, pharyngitis, lymphadenopathy and hepatosplenomegaly. The blood film shows a proliferation of virally

infected B lymphocytes with about 20% or more reactive lymphocytes that are activated T lymphocytes. These reactive lymphocytes have round or irregularly shaped nuclei, with abundant flowing cytoplasm that characteristically has a dark-staining periphery. Thrombocytopenia is a common feature in EBV infection. IM is heterophile antibody-positive, thus distinguishing it from the other virally induced lymphoproliferative disorders, which are all heterophile antibody-negative.

Some patients also present with a haemolytic anaemia resulting from the production of antibody by the B lymphocytes against the 'I' antigen on the red cells. In such cases, a degree of auto-agglutination of the red cells is seen on the blood film. Refer to Figs B4-5 to B4-7. See also Chapter C3.

Cytomegalovirus (CMV) infection

CMV infection usually occurs in patients between the ages of 20 and 50 years. The virus infects the neutrophils, which spread the infection to the macrophages. This produces a T-cell response leading to lymphocytosis and the presence of circulating reactive T lymphocytes in the peripheral blood. The clinical features of CMV are similar to those of IM with two exceptions: patients with CMV rarely present with pharyngitis or lymphadenopathy.

The reactive lymphocytes of CMV are morphologically indistinguishable from those of an EBV infection; the main difference between the two illnesses is that CMV infection is heterophile antibody-negative.

Thrombocytopenia is a common feature of CMV infection. Refer to Fig B4-8.

Varicella infection

Varicella infection (chickenpox) is commonly seen in young children but may also occur in adulthood. The classical features are thrombocytopenia and the presence in the peripheral blood of reactive T lymphocytes closely resembling those induced by EBV and CMV infections. Refer to Fig B4-9.

Viral hepatitis

Both hepatitis A and B viruses can produce a mononucleosis-like syndrome characterised by reactive lymphocytes on the blood film. Refer to Fig B4-10.

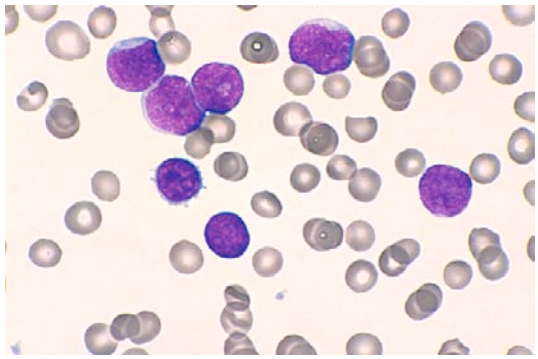


Figure B4-1
Lymphoblasts in peripheral blood. (x 1000)

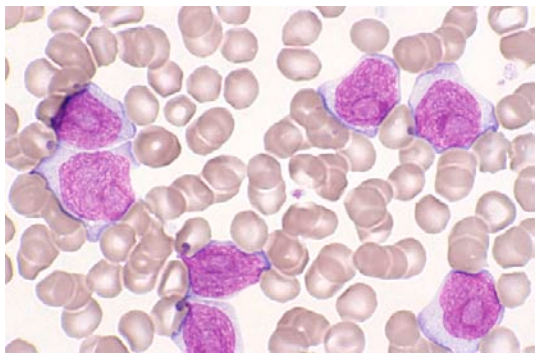


Figure B4-2
Prolymphocytes in peripheral blood. (x 1000)

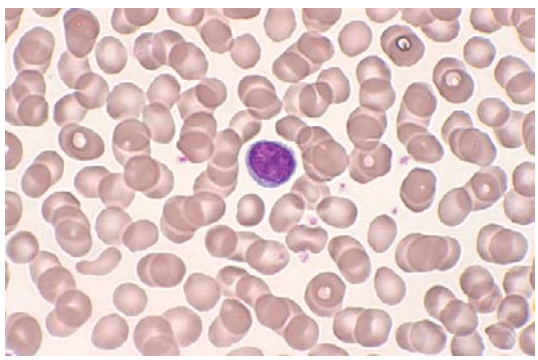


Figure B4-3
Small lymphocyte in peripheral blood. (x 1000)

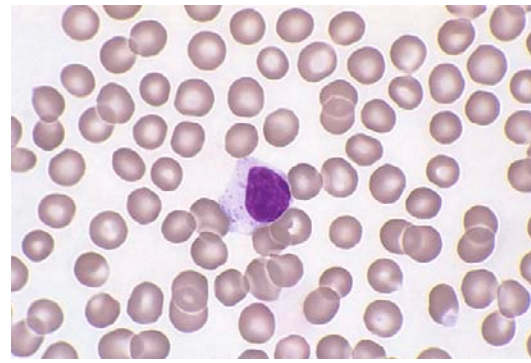


Figure B4-4
Large lymphocyte in peripheral blood. (x 1000)

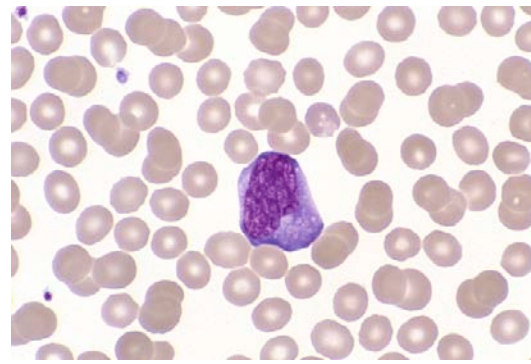


Figure B4-5
Infectious mononucleosis: peripheral blood film showing a reactive lymphocyte with an irregularly shaped nucleus and basophilic, flowing cytoplasm. (x 1000)

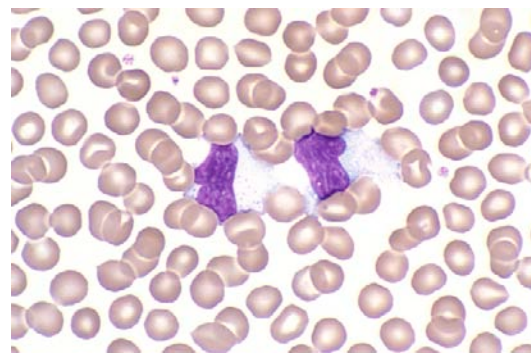


Figure B4-6
Infectious mononucleosis: peripheral blood showing reactive lymphocytes with basophilic, flowing cytoplasm. (x 1000)

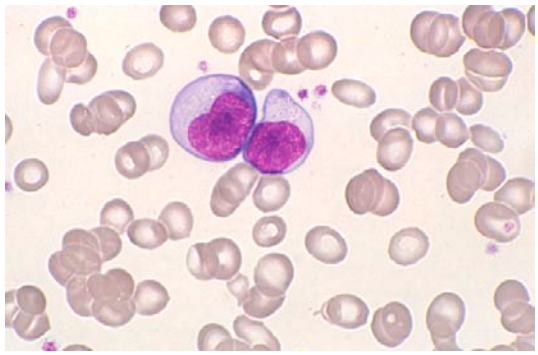


Figure B4-7
Infectious mononucleosis: haemolytic anaemia with infectious mononucleosis showing auto-agglutination of the red cells on the peripheral blood film. (x 1000)

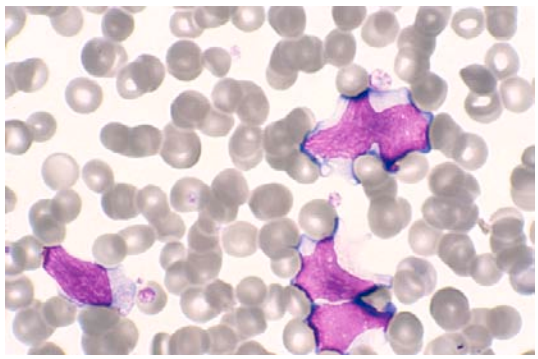


Figure B4-8
Cytomegalovirus infection: peripheral blood film showing reactive T lymphocytes morphologically indistinguishable from those of an Epstein-Barr virus infection showing irregularly shaped nuclei and flowing basophilic cytoplasm with dark-staining periphery. (x 1000)

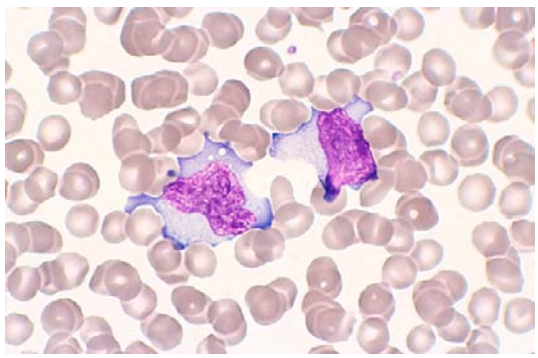


Figure B4-9
Varicella infection: peripheral blood film showing reactive T lymphocytes with irregularly shaped nuclei and basophilic flowing cytoplasm. (x 1000)

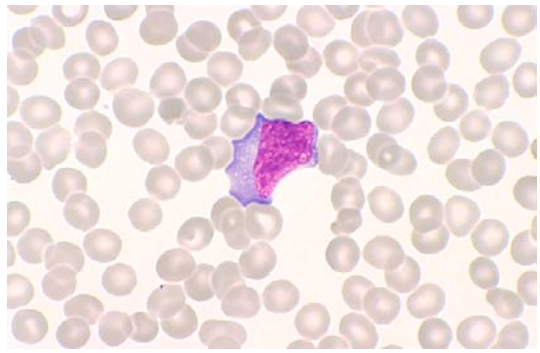


Figure B4-10
Viral hepatitis: peripheral blood film showing a reactive lymphocyte with similar characteristics to those found in other viral infections. (x 1000)

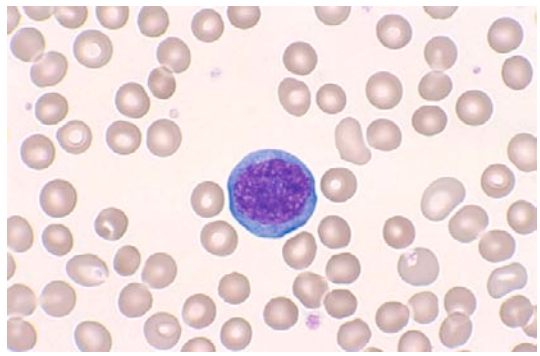


Figure B4-11
Türk cell: classically found in the peripheral blood film in dengue fever and non-specific viral infections. These cells have a large round eccentrically placed nucleus with intensely basophilic cytoplasm. (x 1000)

LYMPHOPROLIFERATIVE NEOPLASMS: THE WHO CLASSIFICATION

The World Health Organization (WHO) classification of tumours is based on cell morphology, immunophenotype and cytogenetics. A full description of the WHO classification is beyond the scope of this book; however, a broad outline of those neoplasms whose cells appear in both the peripheral blood and bone marrow are included. Cytogenetic findings and immunophenotyping are included where appropriate.

Precursor lymphoid neoplasms

- B lymphoblastic leukaemia/lymphoma, NOS
- T lymphoblastic leukaemia/lymphoma, NOS.

Mature lymphoid neoplasms

- Mature B-cell neoplasms
- Mature T-cell and NK-cell neoplasms.

PRECURSOR LYMPHOID NEOPLASMS

B lymphoblastic leukaemia/lymphoma, not otherwise specified (NOS)

B lymphoblastic leukaemia/lymphoma is a disease of precursor B lymphoblasts. B lymphoblastic leukaemia occurs mainly in young children with approximately 75% of cases occurring in children less than 6 years of age while B lymphoblastic lymphoma has a median age of 20 years. The lymphoblasts range from those with a high N:C ratio, fine-to-clumped chromatin pattern with inconspicuous nucleoli and scanty basophilic cytoplasm to those that are heterogeneous in cell size and have a nuclear chromatin pattern varying from finely dispersed to coarsely condensed. Nuclear clefting, indentation and folding are characteristic and gross irregularities in shape are common. Nucleoli are nearly always present, with variability in size and number. The amount of cytoplasm is variable and often abundant. The term 'B-lymphoblastic leukaemia' applies when there is involvement of the bone marrow and peripheral blood and the term 'B-lymphoblastic lymphoma' when there is significant nodal or extranodal involvement. The distinction between the two names is arbitrary. Refer to Figs B4-12 to B4-15.

CYTOGENETICS

B lymphoblastic leukaemia/lymphoma is usually classified depending on the specific cytogenetic abnormality present (see below). Other changes include del(6q), del(9p), del(12p) and amplification of *RUNX1* (usually detected by fluorescence in situ hybridisation (FISH) analysis).

B lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities

- *B lymphoblastic leukaemia/lymphoma with t(9;22)(q34;q11.2); BCR-ABL1*

Cytogenetically identical to the CML t(9;22) however the fusion protein differs. The CML t(9;22) generally produces p210 while the ALL t(9;22) generally produces p190. Other cytogenetic abnormalities may also be present.

- *B lymphoblastic leukaemia/lymphoma with t(v;11q23); MLL rearranged*

These cases have a rearrangement between the *MLL* locus and a large variety of translocation partners. The most common is t(4;11)(q21;q23) and others include t(11;19)(q23;p13), and t(6;11)(q27;q23).

Confirmation of *MLL* involvement by FISH analysis is recommended.

- *B lymphoblastic leukaemia/lymphoma with t(12;21)(p13;q22); ETV6-RUNX1*

This translocation results in *ETV6*(12p13) and *RUNX1*(21q22) fusion. It is not visible cytogenetically and is usually detected by FISH.

- *B lymphoblastic leukaemia/lymphoma with hyperdiploidy*

Usually exhibit a modal number between 50 and 65. Commonly gains of chromosomes 4, 6, 10, 14, 17, 18, 21 and X are seen. Gains usually appear as trisomies, however multiple copies of chromosomes 21 and X may be seen. Structural abnormalities may also be seen.

- *B lymphoblastic leukaemia/lymphoma with hypodiploidy*

Chromosome modal number can range from near haploid (23) to 45.

- *B lymphoblastic leukaemia/lymphoma with t(5;14)(q31;q32); IL3-IGH*

This translocation causes over expression of *IL3*.

- *B lymphoblastic leukaemia/lymphoma with t(1;19)(q23;P13.3); E2A-PBX1*

Often seen in an unbalanced form with just the der(19) t(1;19) present.

IMMUNOPHENOTYPE

Early-B ALL

- TdT^+, HLA-DR^+, SIg^-, cyt-μ^-, $CD10^-$, $CD19^+$, $CD22^+$, $CD34^+$

Common B ALL

- TdT^+, HLA-DR^+, SIg^-, cyt-μ^-, $CD10^+$, $CD19^+$, $CD22^-$, $CD34^+$

Pre-B ALL

- TdT^+, HLA-DR^+, SIg^-, cyt-μ^+, $CD10^+$, $CD19^+$, $CD22^+$, $CD34^-$

B-ALL

- TdT^-, HLA-DR^+, SIg^+, $CD10^-$, $CD19^+$, $CD20^+$, $CD22^+$, $CD34^-$

T lymphoblastic leukaemia/lymphoma, not otherwise specified (NOS)

T lymphoblastic leukaemia/lymphoma is a disease of precursor T lymphoblasts. T lymphoblastic leukaemia occurs more commonly in adolescents than in young children. It also occurs in adults of any age group. The lymphoblasts are small to medium in size, with a high N/C ratio, moderately condensed chromatin pattern and inconspicuous nucleoli. The nuclei are often folded and cleaved within scanty basophilic cytoplasm. The term 'T-lymphoblastic leukaemia' applies when there is involvement of the bone marrow and peripheral blood and the term 'T-lymphoblastic lymphoma' when there is significant nodal or extranodal involvement. The distinction between the two names is arbitrary.

Refer to Figs B4-16 to B4-18.

CYTOGENETICS

- Abnormal karyotype seen in 50–70% of cases, usually involving T-cell receptor (*TCR*) loci [alpha (*TRA*) and delta (*TRD*) at 14q11.2, beta (*TRB*) at 7q34, gamma (*TRG*) at 7p14] and a variety of partner chromosomes. Rearrangements include t(1;7)(p33;q34), t(7;9)(q34;q34), t(7;10)(q34;q24), t(7;11)(q34;p13), t(8;14)(q24;q11), t(10;14)(q24;q11), t(11;14)(p13;q11).

CYTOCHEMISTRY/IMMUNOPHENOTYPE

- T-cell lymphoblasts show focal positivity with the acid phosphatase stain on either the peripheral blood or the bone marrow film.
- TdT^+, HLA-DR^-, $CD1a^+$, $CD2^+$, $CD3^+$, $CD4^{+/-}$, $CD5^+$, $CD7^+$, $CD8^{+/-}$

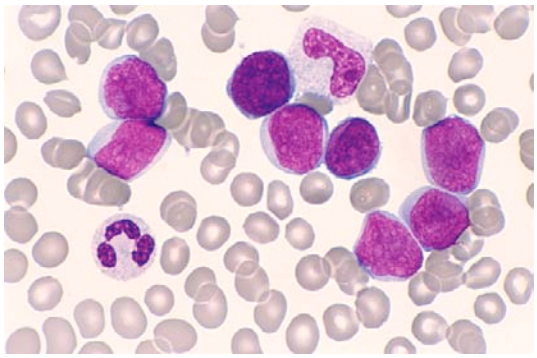


Figure B4-12
B-lymphoblastic leukaemia/lymphoma: peripheral blood film showing blast cells with a high N/C ratio, fine to clumped chromatin pattern and inconspicuous nucleoli. (x 1000)

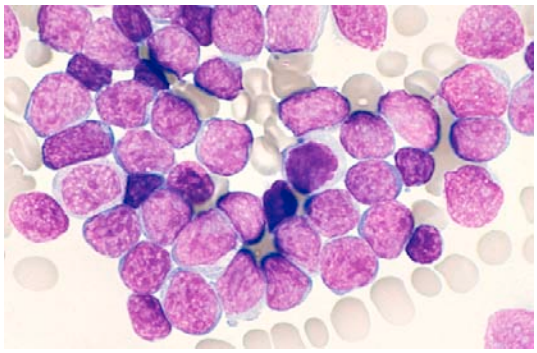


Figure B4-13
B-lymphoblastic leukaemia/lymphoma: bone marrow showing a homogeneous infiltrate of lymphoblasts. (x 1000)

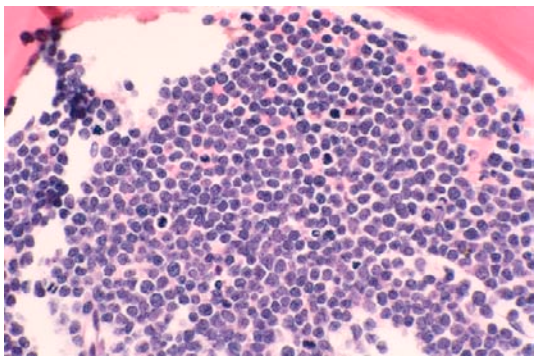


Figure B4-14
B-lymphoblastic leukaemia/lymphoma: bone marrow trephine showing complete replacement by lymphoblasts. (H&E) (x 400)

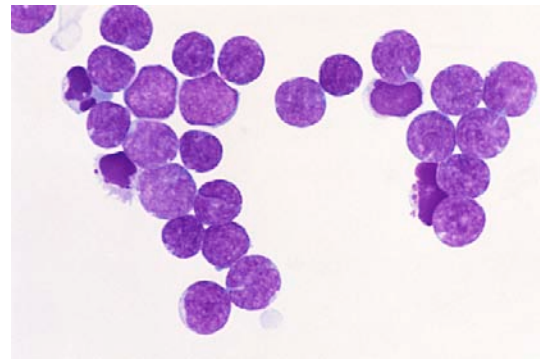


Figure B4-15
B-lymphoblastic leukaemia/lymphoma: central nervous system (CNS) relapse showing a heavy infiltrate of lymphoblasts. (x 1000)

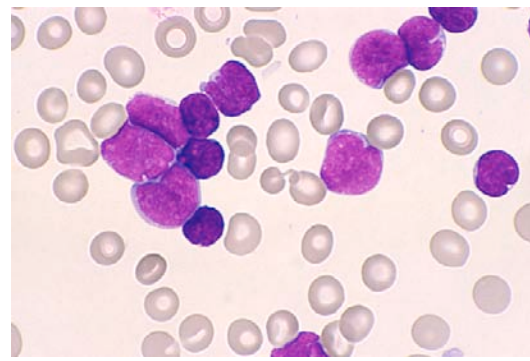


Figure B4-16
T-lymphoblastic leukaemia/lymphoma: peripheral blood showing blast cells with a high N/C ratio, fine to clumped chromatin pattern, cleaved nuclei and inconspicuous nucleoli. (x 1000)

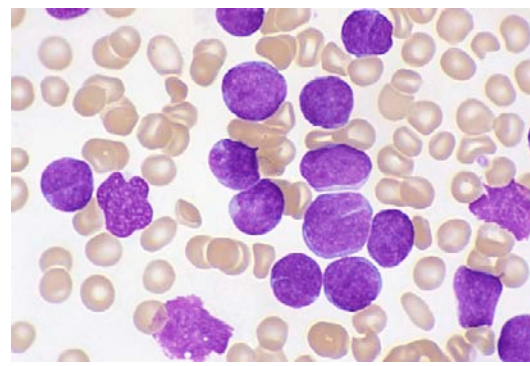


Figure B4-17
T-lymphoblastic leukaemia/lymphoma: peripheral blood showing cleaved lymphoblasts. (x 1000)

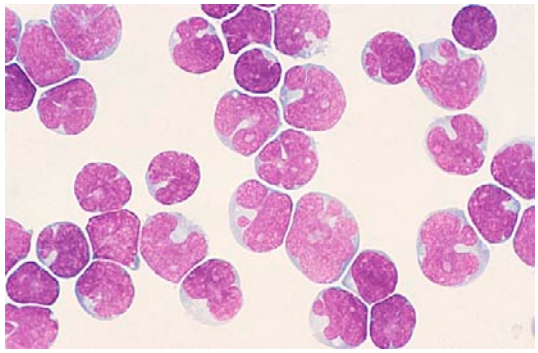


Figure B4-18
T-lymphoblastic leukaemia/lymphoma: central nervous system (CNS) relapse showing a heavy infiltrate of cleaved lymphoblasts. (x 1000)

MATURE B-CELL NEOPLASMS

Mature B-cell neoplasms result from malignant proliferation of lymphocytes in lymph nodes, spleen, tonsils, thymus, bone marrow and skin.

B-cell chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL)

B-cell CLL/SLL occurs in the older age group, usually over 65 years, affecting males more often than females. The neoplasm is asymptomatic in most patients; however, it is sometimes associated with an autoimmune haemolytic anaemia. CLL/SLL is diagnosed on a routine blood film. Diagnosis requires the presence of a clonal lymphocytosis persisting for 3 months or more and with bone marrow involvement. The lymph nodes, liver and spleen are often involved. The absolute lymphocyte count is $>10 \times 10^9$/L. The lymphocytes appear morphologically normal and there is usually a small percentage of 'smudge cells' present. These are fragile lymphocytes whose cytoplasm has been disrupted whilst spreading the blood film thus releasing a distorted nucleus or a 'smudge cell'. An occasional prolymphocyte, <2% may also be present. Increased numbers of prolymphocytes, more than 55%, would suggest B-cell prolymphocytic leukaemia. More than 90% of cases of CLL/SLL are of the B-cell lineage.

A normochromic normocytic anaemia is found in 15% of cases. The anaemia is the result of bone marrow replacement. Thrombocytopenia also occurs in about 15% of cases and again is related to bone marrow replacement and hypersplenism. Refer to Figs B4-19 to B4-23

CYTOGENETICS

- Routine cytogenetic analysis often results in a normal karyotype; however, FISH analysis identifies cytogenetic abnormalities in up to 80% of cases. Common abnormalities include del(13q), +12, del(11q) and del(17p).

IMMUNOPHENOTYPE

- SIg^+ (weak), $CD5^+$, $CD10^-$, $CD19^+$, $CD20^+$, $CD22^+$, $CD23^+$, $CD43^+$, $CD79a^+$

B-cell prolymphocytic leukaemia (B-PLL)

B-cell PLL occurs in the older age group, usually over 70 years, afflicting males more than females. Approximately 80% of cases of PLL are of B-cell lineage. The neoplasm is characterised by splenomegaly and a markedly elevated white cell count of $>100 \times 10^9$/L. The number of prolymphocytes must exceed 55% of the lymphoid cells in the peripheral blood. The prolymphocytes are larger than the lymphocytes of CLL. They have abundant basophilic cytoplasm, round nuclei with moderately condensed chromatin and a single large nucleolus. The bone marrow shows a nodular infiltrate of prolymphocytes. Refer to Fig B4-24.

CYTOGENETICS

- Karyotypes are often complex with abnormalities such as del(17p) (50%), del(13)(q14) (27%), del(11q) and, less commonly, +12.

IMMUNOPHENOTYPE

- SIg^+, $CD5^{-/+}$, $CD19^+$, $CD20^+$, $CD22^+$, $CD23^{-/+}$, $CD79a^+$

Splenic B-cell marginal zone lymphoma (SMZL)

Splenic marginal zone lymphoma (SMZL) is a chronic proliferation of B lymphocytes. The disease predominantly affects males between the ages of 60 and 70 years. Patients present with an enlarged spleen as well as, in some cases, lymphadenopathy and hepatomegaly.

The white cell count is usually raised, although it rarely exceeds 25 × 10^9/L. The lymphocytes in the peripheral blood have a characteristic morphology: they have round to oval nuclei and variable amounts of basophilic cytoplasm with thin, short villi, unevenly distributed and often concentrated towards one pole of the cell. Refer to Figs B4-25 and B4-26.

CYTOGENETICS

- Deletions and rearrangements of 7q (40%), +3q and t(11;14)(q13;q32) (rarely).

IMMUNOPHENOTYPE

- SIg$^+$, CD5$^-$, CD10$^-$, CD19$^+$, CD20$^+$, CD22$^+$, CD23$^-$, CD43$^-$, CD79a$^+$
- The absence of CD5 is useful in distinguishing SMZL from CLL/SLL

Hairy cell leukaemia (HCL)

Hairy cell leukaemia (HCL) is a B-cell lymphoproliferative neoplasm occurring in the 40–50 year age group, with the majority of cases presenting in males. There are two forms of HCL. The 'classical' form presents with pancytopenia, including monocytopenia while the 'variant' form presents with a mean white cell count of 35 × 10^9/L and does not have a monocytopenia. The lymphocytes of the classical form vary in size from 10 to 20 µm in diameter. They have an eccentrically placed nucleus which is round to oval in shape. The cytoplasm is basophilic with many hair-like projections around the entire circumference. The lymphocytes of the variant form are smaller with a diameter of 10 to 15 µm. The nucleus is generally centrally placed rather than eccentrically placed. In both forms there may be few hairy cells in the peripheral blood. The bone marrow shows a patchy or diffuse infiltrate and may result in a 'dry tap'. The trephine shows an infiltrate of lymphocyte nuclei surrounded by halos of pale-staining cytoplasm often described as having a 'fried-egg' appearance. This contrasts with the trephine of CLL/SLL which is heavily infiltrated with sheets of dense nuclei with very little cytoplasm. Refer to Figs B4-27 to B4-29.

CYTOCHEMISTRY

- The B-cells of HCL are tartrate-resistant acid phosphatase (TRAP) positive. The cytochemical stain 'acid phosphatase' is specific for T-cell neoplasms however the B-cells of HCL react strongly with this cytochemical stain.

CYTOGENETICS

Classical form

- There are no specific cytogenetic changes in HCL.
- Abnormalities of chromosomes 5 and 7 have been described.

Variant form

- No known specific cytogenetic changes. Karyotypes may be complex including abnormalities of 14q32 (*IGH*) or 8q24 (*MYC*) and deletions of 17p (*TP53*).

IMMUNOPHENOTYPE

Classical form

- CD19$^+$, CD20$^+$, CD22$^+$, CD103$^+$, CD11c$^+$, CD25$^+$

Variant form

- CD19$^+$, CD20$^+$, CD22$^+$, CD103$^{+/-}$, CD11c$^+$, CD25$^-$

Lymphoplasmacytic lymphoma (LPL)/Waldenström macroglobulinaemia

Lymphoplasmacytic lymphoma (LPL)/Waldenström macroglobulinaemia is a B-cell neoplasm occurring in males with a median age of 60 years. Patients present with lymphadenopathy, hepatosplenomegaly and fatigue. A normochromic normocytic anaemia is usually present, as well as a mild leucopenia and thrombocytopenia. A monoclonal IgM is

invariably present, causing hyperviscosity; cryoglobulins are also present in a small percentage of cases. The bone marrow contains a diffuse infiltrate of malignant lymphocytes as well as some with a plasmacytoid appearance. These cells are sometimes seen in the peripheral blood. The lymphocytes are small with a round, sometimes eccentrically placed nucleus and basophilic cytoplasm. Refer to Figs B4-30 to B4-32.

CYTOGENETICS

- No specific cytogenetic abnormality has been identified in LPL/Waldenström macroglobulinaemia.

IMMUNOPHENOTYPE

- SIg^+, $CD5^-$, $CD10^-$, $CD19^+$, $CD20^+$, $CD22^+$, $CD23^-$, $CD38^+$, $CD79a^+$, $CD103^-$
- Lack of CD5 positivity is useful in differentiating LPL/Waldenström macroglobulinaemia from CLL/SLL.

Hodgkin lymphoma (HL)

Hodgkin lymphoma (HL) has a bimodal age distribution with a peak in the 20 years age group and a gradual increase after 45 years. A lymphadenopathy, constitutional symptoms and a raised erythrocyte sedimentation rate characterise HL. Most patients have a normal blood count apart from increased rouleaux formation, although occasionally an eosinophilia is present.

The characteristic cell of HL is the Reed-Sternberg cell. It is a binucleated cell most commonly of B-cell lineage and, although classically found in the lymph node, may also be found in the bone marrow in a small percentage of newly diagnosed patients.

HL has been divided into two neoplasm categories according to the number of Reed-Sternberg cells present, the number of lymphocytes and the degree of fibrosis. The first category is nodular lymphocyte predominant Hodgkin lymphoma (NLPHL). The second, classical Hodgkin lymphoma (CHL) has been divided into four subgroups, according to the sites of involvement, clinical features, growth pattern, presence of fibrosis, composition of the cellular background and the number of Reed-Sternberg cells present. The frequency of Epstein-Barr virus (EBV) infection also varies between the subgroups. Refer to Figs B4-33 to B4-35.

The subgroups of CHL are as follows:

- Nodular sclerosis classical Hodgkin lymphoma (NSCHL)
- Mixed-cellularity classical Hodgkin lymphoma (MCCHL)
- Lymphocyte-rich classical Hodgkin lymphoma (LRCHL)
- Lymphocyte-depleted classical Hodgkin lymphoma (LDCHL)

CYTOGENETICS

- Numerous chromosomal anomalies occur in HL, none of which is specific for the disease.

IMMUNOPHENOTYPE

- *Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL):*
  $CD15^-$, $CD20^+$, $CD30^-$, $CD45^+$, $CD79a^+$, $CD75^+$
  EBV infection is consistently absent.
- *Classical Hodgkin lymphoma (CHL):*
  Nodular sclerosis classical Hodgkin lymphoma (NSCHL):
  $CD15^{+/-}$, $CD20^{-/+}$, $CD30^+$, $CD45^-$, $CD79a^{-/+}$, $CD75^-$
  EBV infection occurs in 10–40% of cases.
  Mixed cellularity classical Hodgkin lymphoma (MCCHL):
  $CD15^{+/-}$, $CD20^{-/+}$, $CD30^+$, $CD45^-$, $CD79a^{-/+}$, $CD75^-$
  EBV infection occurs in 75% of cases.

Lymphocyte-rich classical Hodgkin lymphoma (LRCHL):
CD15$^{+/-}$, CD20$^{-/+}$, CD30^{+}, CD45^{-}, CD79a$^{-/+}$, CD75^{-}
Lymphocyte-depleted classical Hodgkin lymphoma (LDHL):
CD15$^{+/-}$, CD20$^{-/+}$, CD30^{+}, CD45^{-}, CD79a$^{-/+}$, CD75^{-}
Most HIV-positive cases express EBV.

Non-Hodgkin lymphoma (NHL)

The non-Hodgkin lymphomas (NHL) are a miscellaneous group of disorders, both follicular and diffuse, named according to the site of involvement of the tumours, the appearance of the cells on histological examination, their immunophenotype and cytogenetic findings. Most are B-cell disorders. The malignant lymphocytes are found in the peripheral blood only occasionally. When present in the blood, lymphoma cells can be recognised as either large cells with a high N:C ratio, a non-cleaved nucleus and prominent nucleoli, or as smaller cells with more basophilic cytoplasm and a cleaved nucleus. It is important to distinguish these lymphoma cells from virally activated or reactive lymphocytes and from the blast cells of leukaemia.

Occasionally, less common forms of NHL can be recognised by the characteristic appearance of the malignant lymphocyte in the peripheral blood, namely splenic marginal zone lymphoma, cutaneous T-cell lymphoma and LPL/Waldenström macroglobulinaemia.

Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)

MALT lymphoma is a B-cell lymphoma occurring in adults with a median age of 61 years. It occurs most commonly in the gastrointestinal (GI) tract, especially in the stomach. It also occurs in the salivary gland, head and neck, lung, skin, thyroid and breast.

MALT lymphoma cells have a small- to medium-sized irregularly shaped nucleus with a dispersed chromatin pattern and inconspicuous nucleoli. They have abundant pale blue cytoplasm. Overall they have a monocytoid appearance. Approximately one-third of MALT lymphoma cells have a plasmacytoid appearance. Refer to Figs B4-36 to B4-38.

CYTOGENETICS

- t(11;18)(q21;q21)—results in *AP12*(11q21) and *MALT1*(18q21) fusion, usually occurs as the sole abnormality and mainly found in pulmonary and gastric tumours.
- t(1;14)(p22;q32)—results in juxtaposition of *BCL10*(1p22) and *IGH*(14q32). Detected in <2% of cases but may be more common in pulmonary tumours.
- t(14;18)(q32;q21)—results in juxtaposition of *MALT1*(18q21) and *IGH*(14q32) leading to deregulation of *MALT1* and is usually found in ocular adnexae/orbit and salivary gland lesions. This rearrangement is cytogenetically identical to the t(14;18) *IGH-BCL2* fusion and requires FISH for differentiation.
- Additional abnormalities include +3 and +18.

IMMUNOPHENOTYPE

- SIg^{+}, CD5^{-}, CD10^{-}, CD11c$^{+/-}$ (weak), CD19^{+}, CD20^{+}, CD79a^{+}, CD21^{+}, CD22^{+}, CD23^{-}, CD43$^{+/-}$, CD35^{+}

Follicular lymphoma

Follicular lymphoma is a B-cell lymphoma occurring in adults with a median age of 60 years. It occurs predominantly in the lymph nodes but also occurs in the spleen, peripheral blood and bone marrow. It can occur in extranodal sites such as the gastrointestinal tract, the eye, breast and testis.

There are two types of follicular lymphoma cells. Small to medium sized cells with cleaved nuclei, inconspicuous nucleoli and scanty pale blue cytoplasm and larger cells with round to oval nuclei, one to three nucleoli and a narrow border of pale blue cytoplasm. Follicular lymphoma cells are often three times the size of lymphocytes. Refer to Figs B4-39 and B4-40.

CYTOGENETICS

- The characteristic abnormality in follicular lymphoma is the t(14;18)(q32;q21) or its variants t(2;18)(p12;q21) and t(18;22)(q21;q11). These translocations result in juxtaposition of an immunoglobulin gene [*IGH* (14q32), *IGK* (2p12), *IGL* (22q11)] with the *BCL2* (18q21) locus resulting in deregulation of *BCL2*. Translocations of *BCL6* (3q27) have also been described involving the same immunoglobulin loci that is, t(3;14)(q27;q32), t(2;3)(p12;q27), t(3;22)(q27;q11). Additional abnormalities include loss of 1p, 6q, 10q and 17p, gains of chromosomes 1, 6p, 7, 8, 12q, X and 18q.

IMMUNOPHENOTYPE

- SIg^+, $CD5^-$, $CD10^+$, $CD19^+$, $CD20^+$, $CD79a^+$, $CD22^+$, $CD43^-$, $BCL2^+$, $BCL6^+$

Mantle cell lymphoma

Mantle cell lymphoma is a B-cell lymphoma occurring in adults with a median age of 60 years. It occurs predominantly in the lymph nodes but also occurs in the spleen and bone marrow. The peripheral blood may be involved. It can occur in extranodal sites such as the gastrointestinal tract.

Mantle cell lymphoma is classically a monomorphic lymphoid proliferation. The lymphoma cells are small to medium in size with irregularly shaped nuclei and inconspicuous nucleoli. They have a small rim of pale blue cytoplasm. Refer to Figs B4-41 to B4-43.

CYTOGENETICS

- Almost all cases exhibit t(11;14)(q13;q32) resulting in juxtaposition of *IGH* (14q32) and *CCND1* (11q13) leading to over expression of *CCND1*. Additional changes include –Y, -13, -9, -18, +3, +12, del(6q), del(1p), del(13q), del(10q), del(11q), del(9p) and del(17p).

IMMUNOPHENOTYPE

- SIg^+, $CD5^+$, $CD10^-$, $CD19^+$, $CD20^+$, $CD22^+$, $CD23^{-/+}$, $CD43^+$, cyclin $D1^+$, $BLC2^+$, $BCL6^-$

Diffuse large B-cell lymphoma, not otherwise specified (DLBCL)

Diffuse large B-cell lymphoma commonly occurs in the elderly with a median age of 70 years; however, it may occur in children and young adults. It usually presents de novo but may represent a transformation from a less aggressive lymphoma such as CLL/SLL, follicular lymphoma or mantle cell lymphoma. Forty per cent of cases of DLBCL present with nodal or extranodal disease. A small percentage of these cases also have bone marrow involvement while only a few cases have peripheral blood involvement. The extranodal sites involved include the gastrointestinal tract, mainly the stomach, bone, testis, spleen, salivary gland, thyroid, liver, kidney and adrenal gland.

The morphology of diffuse large B-cell lymphoma is quite variable as there are three variants of this neoplasm. The lymphoma cells vary from having oval to round nuclei with two to four nucleoli in some cases and a single, centrally placed nucleolus in others. The cytoplasm varies from scanty basophilic cytoplasm to abundant cytoplasm in others. Refer to Figs B4-44 to B4-47.

CYTOGENETICS

- No specific DLBCL cytogenetic abnormality; however, may show 'general lymphoma-associated' abnormalities including rearrangements of 3q27 (*BCL6* locus) (20–40%), t(14;18)(q32;q21) (20–30%), t(8;14)(q24;q32) or variant (10%). Karyotypes are often complex with multiple structural and numerical abnormalities.

IMMUNOPHENOTYPE

- $SIg^{+/-}$, $CD5^{-/+}$, $CD10^{-/+}$, $CD19^{+}$, $CD20^{+}$, $CD22^{+}$, $CD79a^{+}$
- Cases associated with the EBV may have a worse outcome compared with those who are EBV negative.

Burkitt cell lymphoma (BL)

Burkitt lymphoma is a B-cell lymphoma with a very short doubling time. It is a highly aggressive lymphoma, often occurring at extra-nodal sites. It is referred to as Burkitt cell leukaemia when the bone marrow is involved.

There are three clinical variants of Burkitt cell lymphoma:

ENDEMIC BURKITT CELL LYMPHOMA

This variant occurs in equatorial Africa and Papua New Guinea. It is the most common malignancy of childhood occurring in children between the age of 4 and 7 years.

SPORADIC BURKITT LYMPHOMA

This variant occurs throughout the world. Occurring mainly in children and young adults, it is associated with the Epstein-Barr virus (EBV).

IMMUNODEFICIENCY ASSOCIATED BURKITT LYMPHOMA

This variant is primarily associated with the human immunodeficiency virus (HIV).

The blast cells of BL are large and homogeneous. They have a dense but finely stippled nuclear chromatin pattern; and the nucleus is round with one or more prominent nucleoli. The cytoplasm is moderately abundant and intensely basophilic with prominent vacuolation. Whether the lymphoblasts are confined to the nodal or extranodal sites (Burkitt lymphoma) or whether they invade the bone marrow and peripheral blood (Burkitt cell leukaemia), the term used to describe the malignant state is arbitrary.

Burkitt cell lymphoma is commonly of the B-cell lineage but rare cases of T-cell lineage have been reported. Refer to Figs B4-48 to B4-52.

Cytogenetics

- The characteristic abnormality in BL is the t(8;22)(q24;q11) or its variants t(8;14)(q24;q32) and t(2;8)(p12;q24). These translocations result in juxtaposition of an immunoglobulin gene [*IGH* (14q32), *IGK* (2p12), *IGL* (22q11)] with the *MYC* (8q24) locus resulting in deregulation of the *MYC* oncogene. Additional abnormalities include gains of 1q, +7, +12 and 13q rearrangements.

Immunophenotype

- SIg^{+}, TdT^{-}, $HLA\text{-}DR^{-}$, $CD10^{+}$, $CD19^{+}$, $CD20^{+}$, $CD22^{+}$, $BCL6^{+}$, $CD38^{+}$, $CD77^{+}$, $CD43^{+}$ and $CD34^{-}$

MATURE T AND NK-CELL NEOPLASMS

T-cell prolymphocytic leukaemia (T-PLL)

Less than 20% of cases of PLL are T-PLL. This neoplasm occurs in adults with a median age of 65 years. It is characterised by hepatosplenomegaly and generalised lymphadenopathy. Skin infiltration occurs in approximately 20% of patients.

The white cell count in T-PLL is usually >100 × 10^9/L and may be >200 × 10^9/L in some cases. The lymphocytes are small to medium sized with non-granular basophilic cytoplasm. The nuclei vary in shape. They may be round, oval or markedly irregular in shape, sometimes cloverleaf shaped. They have a visible nucleolus that is much smaller in size compared with the B prolymphocyte of B-PLL.

T-cell prolymphocytes stain positively with α-naphthyl acetate esterase and acid phosphatase resulting in a dot-like positivity. Refer to Figs B4-53 and B4-54.

CYTOGENETICS

- The typical cytogenetic abnormality of T-PLL is inv(14)(q11q32) (80%) or t(14;14)(q11;q32) (10%). Additional changes include +8q [often as i(8q)], gain of 6q and loss of 6p, rearrangements of 22q, del(10p), del(18p) and del(11q).

IMMUNOPHENOTYPE

- TdT^-, $CD1a^-$, $CD2^+$, $CD3^+$(weak), $CD4^{+/-}$, $CD7^+$, $CD52^+$, $CD8^{-/+}$

T-cell large granular lymphocytic leukaemia (T-LGL)

T-LGL is a clonal proliferation of mature $CD3^+$, $CD8^+$ and T cell receptor (TCR) αβ-positive cytotoxic T lymphocytes. A persistent lymphocytosis of large granular lymphocytes for more than 6 months must be present in order to make a diagnosis of T-LGL. This neoplasm occurs in males and females with equal frequency. There is a wide age group for this neoplasm, usually between 45 and 75 years of age. The majority of cases of T-LGL have an indolent clinical course. They have a lymphocytosis, usually between 2 and 20 × 10^9/L large granular lymphocytes. They are severely neutropenic with neutrophil counts below 0.5 × 10^9/L. Severe anaemia due to pure red cell aplasia as well as a DAT-positive haemolytic anaemia can occur. Approximately 50% of cases have a splenomegaly; lymphadenopathy is rare and 30% of cases have an associated rheumatoid arthritis.

As not all cases of T-LGL have increased absolute lymphocyte counts, careful examination of the blood film is necessary to diagnose this neoplasm. The predominant cell on the blood film is the large granular lymphocyte with its abundant cytoplasm filled with fine-to-coarse azurophilic granules. Bone marrow involvement varies, but usually less than 50% of the cells in the bone marrow are large granular lymphocytes. Refer to Fig B4-55.

CYTOGENETICS

- There are no specific cytogenetic changes in T-LGL.

IMMUNOPHENOTYPE

- $CD3^+$, $CD4^-$, $CD5^-$, $CD7^-$, $CD8^+$, $CD16^+$, $CD57^+$, $TCR\alpha\beta^+$

Aggressive NK-cell leukaemia

NK-cell leukaemia occurs mostly in young to middle aged adults with a median age of 42 years. It is almost invariably associated with the Epstein-Barr virus. Patients present with fever, hepatosplenomegaly and sometimes lymphadenopathy. There is a leucocytosis together with anaemia, neutropenia and thrombocytopenia.

The leukaemic cells in the peripheral blood may vary in appearance from those resembling large granular lymphocytes to those with atypical nuclei with fine chromatin and distinct nucleoli. The cytoplasm is basophilic and contains fine to coarse azurophilic granules. The bone marrow is heavily infiltrated with the same leukaemic cells. NK-cell leukaemia is a rare form of leukaemia. It has an aggressive course resulting in death in 1–2 years. Some patients may even die within days or weeks of the initial presentation. Refer to Fig B4-56.

CYTOGENETICS

- Cytogenetic analysis is often normal. When a cytogenetic abnormality is present, del(6)(q21q25) and del(11q) are most frequently identified.

IMMUNOPHENOTYPE

- $CD2^+$, $CD3^-$, $CD56^+$, $CD57^-$

Adult T-cell leukaemia/lymphoma (ATLL)

Less than 5% of cases of CLL/SLL are ATLL. These patients have diffuse marrow infiltration as well as cutaneous tumour involvement. Central nervous system manifestations are also common. ATLL is caused by a retrovirus: human T-cell leukaemia virus type 1 (HTLV-1), also known as human T-lymphotrophic virus type 1.

The absolute lymphocyte count in ATLL is higher than that in CLL/SLL. The cells are morphologically indistinguishable from those of CLL/SLL. Some cases show nuclear convolutions, a characteristic feature of T cells rather than B cells. Refer to Fig B4-57.

CYTOGENETICS

- Cytogenetic abnormalities are seen in >90% of cases. Karyotypes are often complex involving rearrangements of chromosome 14 [inv(14)(q11q32), t(14;14)(q11;q32), or del/t(14)(q11)], gains of 7q and 3p and losses of 6q and 13q.

IMMUNOPHENOTYPE

- TdT^+, HLA-DR^+, $CD2^+$, $CD3^+$, $CD4^+$, $CD5^+$, $CD7^-$, $CD8^-$, $CD25^+$

Cutaneous T-cell lymphoma (CTCL—Mycosis fungoides and Sézary syndrome)

Within the T-cell lymphoma disorders, there are two malignant lymphomas, namely mycosis fungoides and Sézary syndrome. They both have a predisposition for the skin. The malignant cells characteristic of these two lymphomas are morphologically identical—hence their amalgamation under the classification cutaneous T-cell lymphoma.

Some cutaneous T-cell lymphomas are associated with the HTLV-1 retrovirus. The disease occurs in the 50-years- and -over age group and is more common in males than females. Patients present with skin lesions associated with severe itching. These lesions become plaques that in time develop into cutaneous tumours. Many patients also suffer from a generalised exfoliative erythroderma referred to as 'rouge homme'. Patients with cutaneous T-cell lymphoma have a T-cell lymphocytosis. These lymphocytes have a characteristic convoluted or cerebriform nuclear appearance and are called Sézary cells. Refer to Fig B4-58.

Mycosis fungoides:

Less than 1×10^9/L Sézary cells in peripheral blood
CD4/CD8 ratio of <10:1

Sézary syndrome:

More than 1×10^9/L Sézary cells in the peripheral blood
CD4/CD8 ratio of >10:1

CYTOGENETICS

- Cytogenetic abnormalities are seen in 40–70% of cases, often as complex karyotypes involving -10, -9 and +18. The chromosomes most frequently involved in structural alterations have been 1, 6, 8, 9, 10, 11 and 17.

IMMUNOPHENOTYPE

- *Mycosis fungoides*: $CD2^+$, $CD3^+$, $CD4^+$, $CD5^+$, $CD7^-$, CD 8^-
- *Sézary syndrome*: $CD2^+$, $CD3^+$, $CD4^+$, $CD5^+$, $CD7^{+/-}$, $CD8^-$

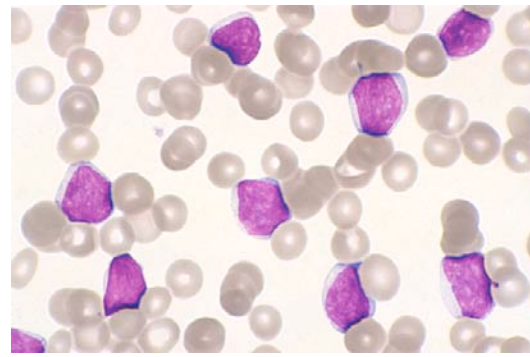


Figure B4-19
B-cell chronic lymphocytic leukaemia/small lymphocytic lymphoma: peripheral blood film showing a proliferation of morphologically normal B lymphocytes. (x 1000)

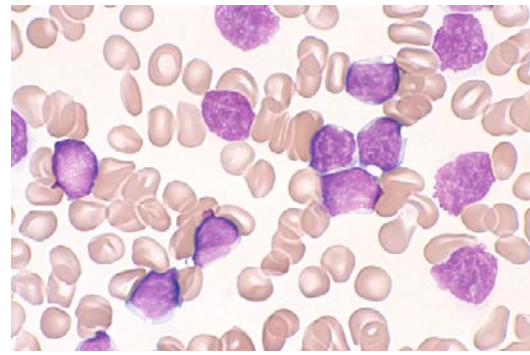


Figure B4-20
B-cell chronic lymphocytic leukaemia/small lymphocytic lymphoma: peripheral blood film showing a proliferation of morphologically normal B lymphocytes including smudge cells. (x 1000)

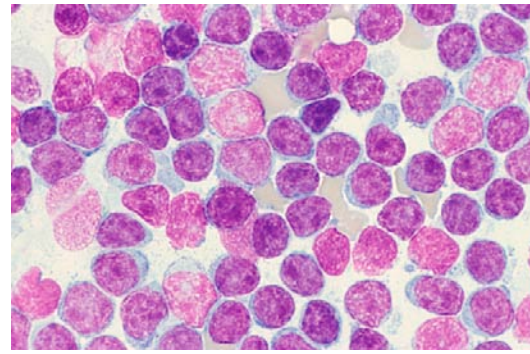


Figure B4-21
B-cell chronic lymphocytic leukaemia/small lymphocytic lymphoma: bone marrow showing a clonal population of B lymphocytes. (x 1000)

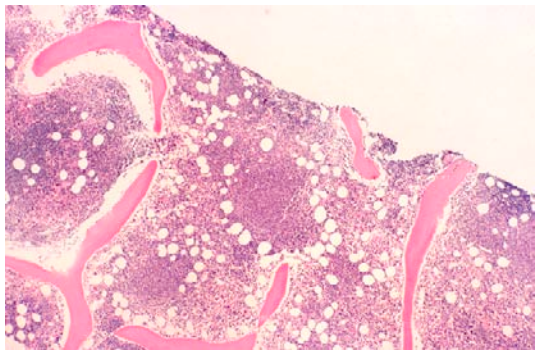


Figure B4-22
B-cell chronic lymphocytic leukaemia/small lymphocytic lymphoma: bone marrow trephine showing lymphocyte nodules. (H&E) (x 100)

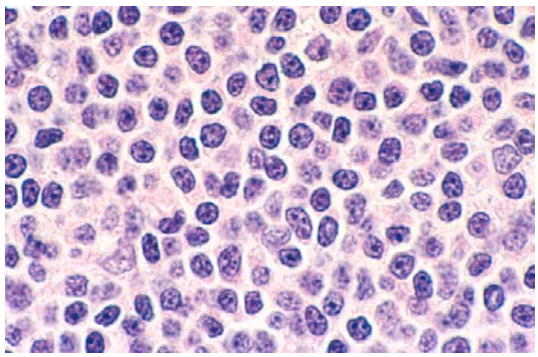


Figure B4-23
B-cell chronic lymphocytic leukaemia/small lymphocytic lymphoma: bone marrow trephine showing lymphocyte proliferation. (H&E) (x 1000)

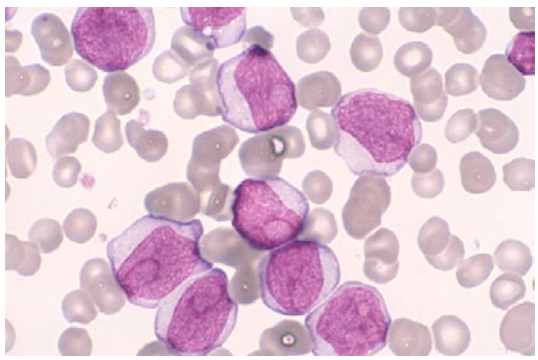


Figure B4-24
B-cell prolymphocytic leukaemia: peripheral blood film showing a clonal population of prolymphocytes with a characteristic single nucleolus in each cell. (x 1000)

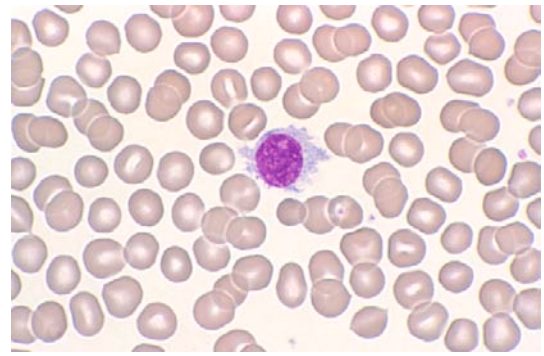


Figure B4-25
Splenic B-cell marginal zone lymphoma: peripheral blood film showing a splenic lymphoma cell with characteristic villi concentrated towards one pole of the cell. (x 1000)

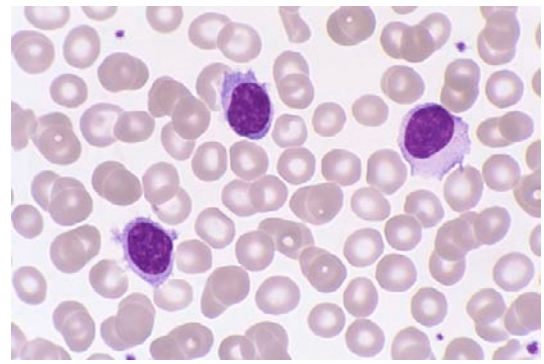


Figure B4-26
Splenic B-cell marginal zone lymphoma: peripheral blood film showing circulating splenic lymphoma cells. (x 1000)

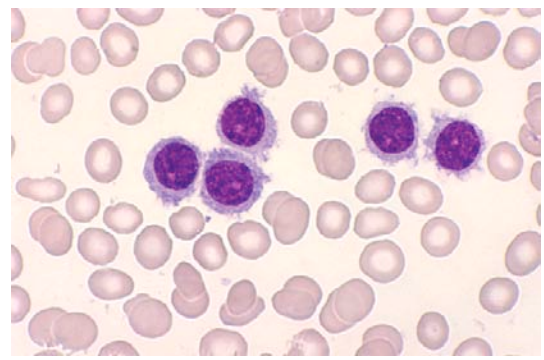


Figure B4-27
Hairy cell leukaemia (variant): peripheral blood film showing hairy lymphocytes with characteristic fine hairlike projections. (x 1000)

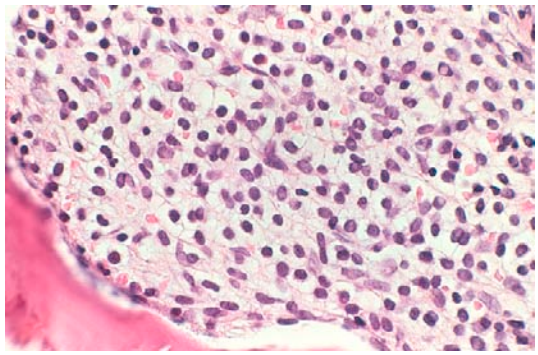


Figure B4-28
Hairy cell leukaemia: bone marrow trephine infiltrated by lymphocyte nuclei surrounded by halos of pale-staining cytoplasm. (H&E) (x 400)

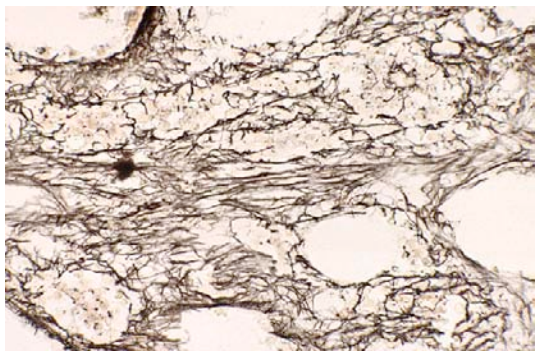


Figure B4-29
Hairy cell leukaemia: reticulin stain of bone marrow trephine showing reticulin fibres replacing haemopoietic tissue. (x 400)

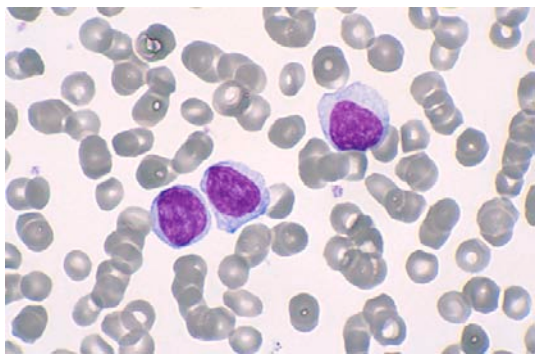


Figure B4-30
Lymphoplasmacytic lymphoma/ Waldenström macroglobulinaemia: peripheral blood film showing plasmacytoid lymphocytes (with features of both lymphocytes and plasma cells). (x 1000)

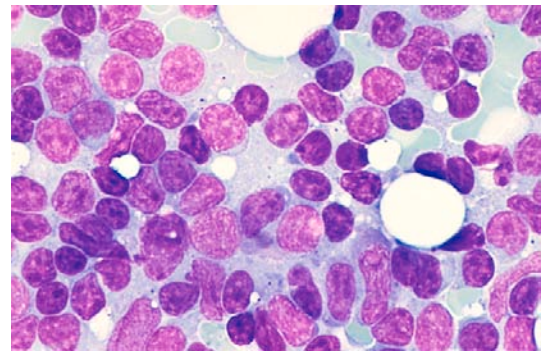


Figure B4-31
Lymphoplasmacytic lymphoma/ Waldenström macroglobulinaemia: bone marrow showing diffuse infiltrate of lymphocytes. (x 1000)

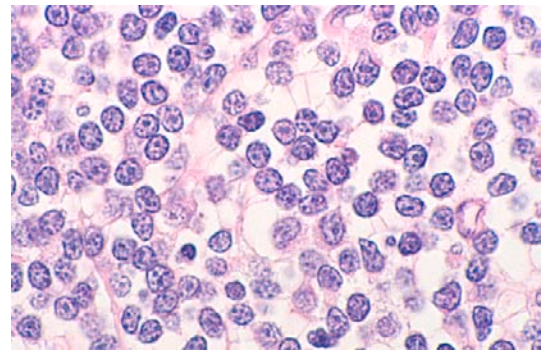


Figure B4-32
Lymphoplasmacytic lymphoma/ Waldenström macroglobulinaemia: bone marrow trephine showing an infiltrate of plasmacytoid lymphocytes. (H&E) (x 1000)

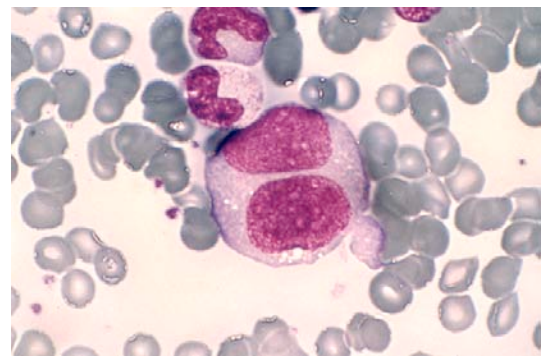


Figure B4-33
Hodgkin lymphoma: Reed-Sternberg cell in bone marrow. (x 1000)

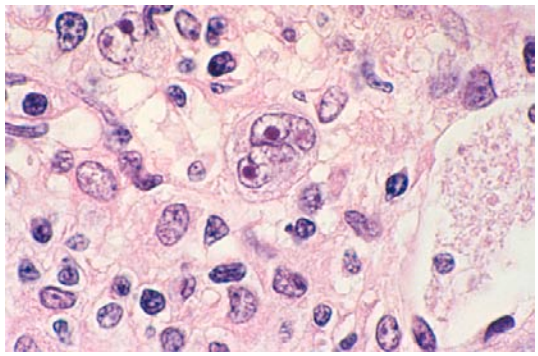


Figure B4-34
Hodgkin lymphoma: bone marrow trephine with a Reed-Sternberg cell in the centre of the field. (H&E) (x 1000)

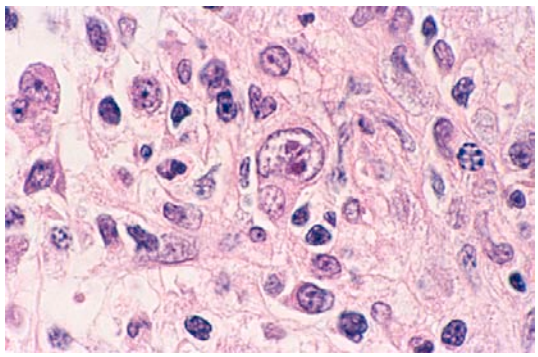


Figure B4-35
Hodgkin lymphoma: bone marrow trephine with a Reed-Sternberg cell in the centre of the field. (H&E) (x 1000)

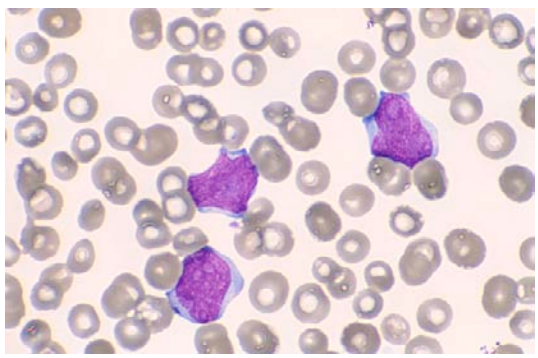


Figure B4-36
Non-Hodgkin lymphoma (MALT): peripheral blood film showing lymphoma cells with a high N/C ratio and inconspicuous nucleoli. (x 1000)

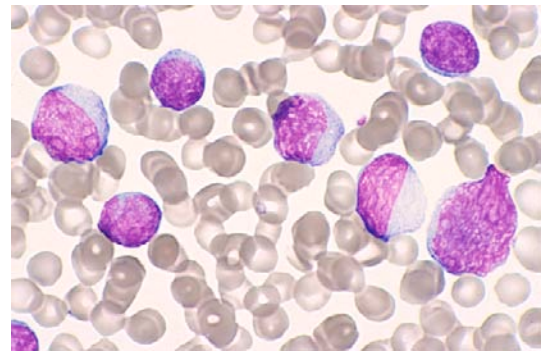


Figure B4-37
Non-Hodgkin lymphoma (MALT): peripheral blood film showing lymphoma cells with basophilic cytoplasm, dispersed chromatin pattern and inconspicuous nucleoli. (x 1000)

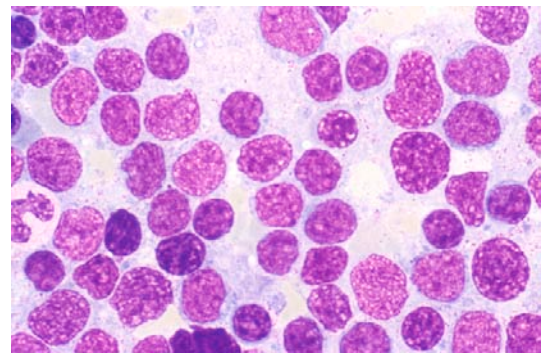


Figure B4-38
Non-Hodgkin lymphoma (MALT): bone marrow showing heavy infiltration with lymphoma cells. (x 1000)

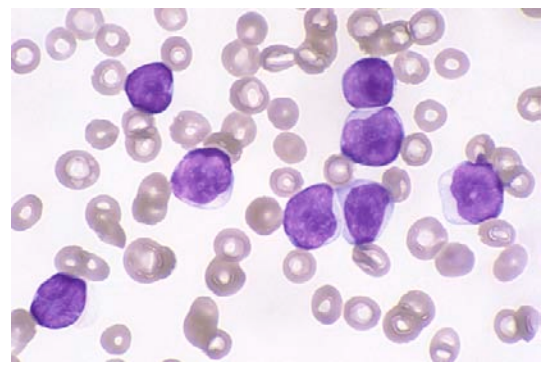


Figure B4-39
Non-Hodgkin lymphoma (follicular): peripheral blood showing lymphoma cells with a high N/C ratio, scanty basophilic cytoplasm and inconspicuous nucleoli. (x 1000)

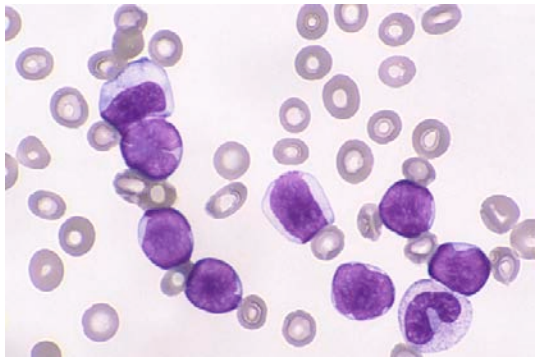


Figure B4-40
Non-Hodgkin lymphoma (follicular): peripheral blood showing lymphoma cells with a high N/C ratio, scanty basophilic cytoplasm and inconspicuous nucleoli. (x 1000)

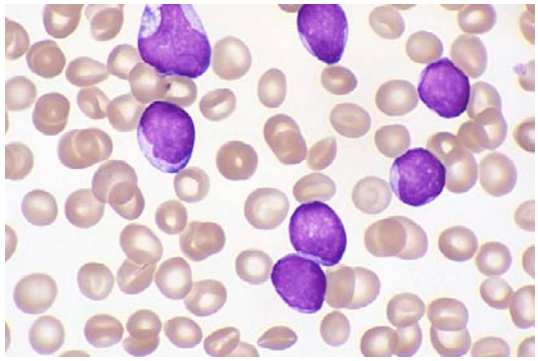


Figure B4-41
Non-Hodgkin lymphoma (mantle): peripheral blood showing lymphoma cells with a high N/C ratio, a small rim of cytoplasm and inconspicuous nucleoli. (x 1000)

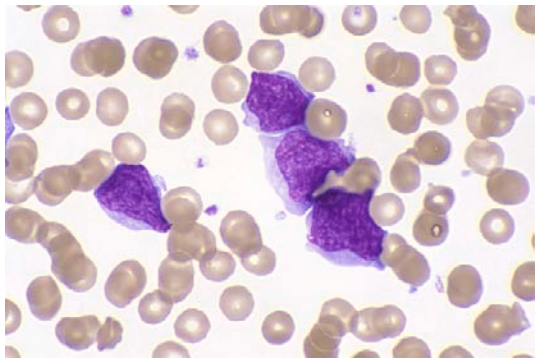


Figure B4-42
Non-Hodgkin lymphoma (mantle): peripheral blood showing lymphoma cells with irregularly shaped nuclei and inconspicuous nucleoli. (x 1000)

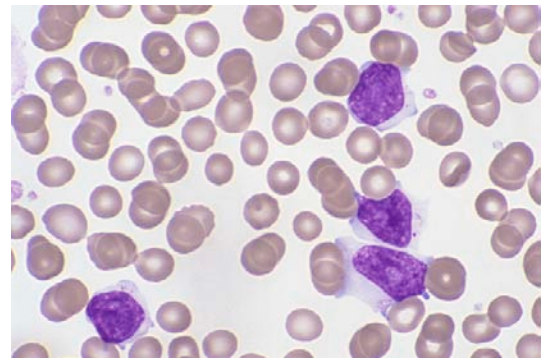


Figure B4-43
Non-Hodgkin lymphoma (mantle): peripheral blood showing medium-sized lymphoma cells with a high N/C ratio, basophilic cytoplasm and nucleoli. (x 1000)

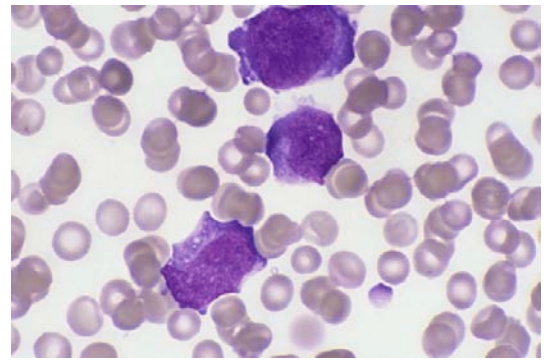


Figure B4-44
Diffuse large B-cell lymphoma: bone marrow with large lymphoma cells, irregularly shaped nuclei and very basophilic cytoplasm. (x 1000)

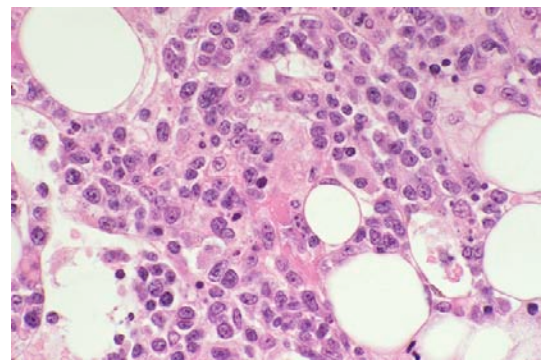


Figure B4-45
Diffuse large B-cell lymphoma: bone marrow trephine infiltrated with lymphoma cells. (H&E) (x 400)

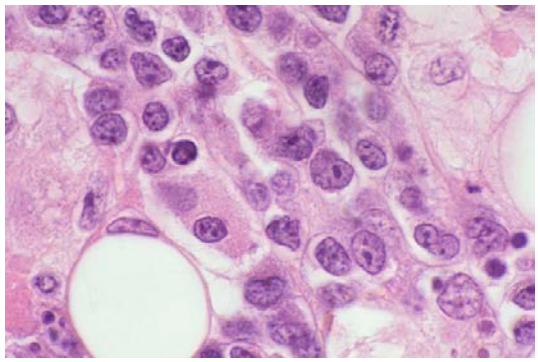


Figure B4-46
Diffuse large B-cell lymphoma: bone marrow trephine infiltrated with large lymphoma cells with round nuclei and prominent nucleoli. (H&E) (x 1000)

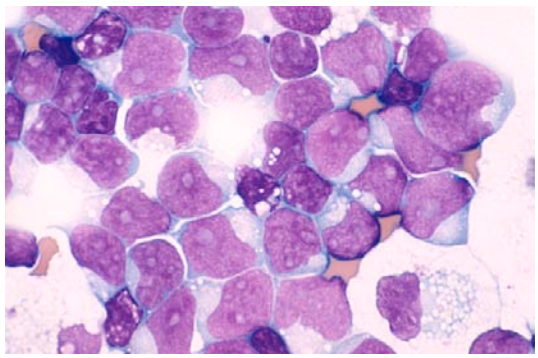


Figure B4-47
Diffuse large B-cell lymphoma: heavy infiltrate of lymphoma cells in the CSF. (x 1000)

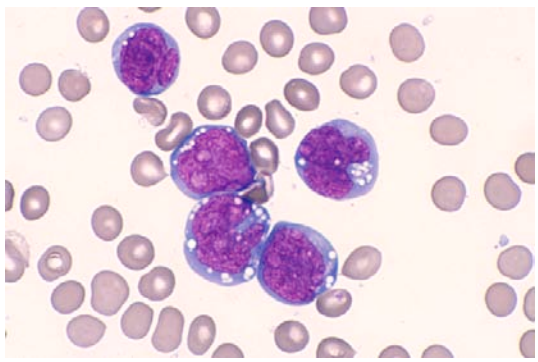


Figure B4-48
Burkitt cell lymphoma/leukaemia: peripheral blood film showing Burkitt cells with a high N/C ratio, fine chromatin pattern and nucleoli. The cytoplasm is intensely basophilic , with prominent vacuolation. The vacuoles stain positively with oil red O stain. (x 1000)

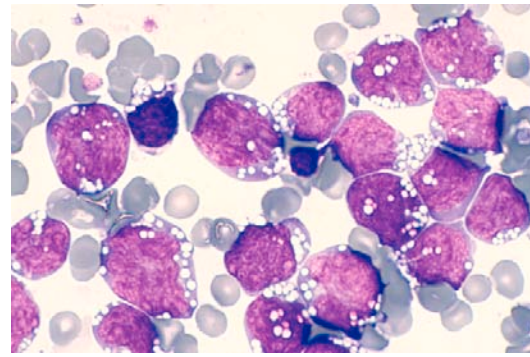


Figure B4-49
Burkitt cell lymphoma/leukaemia: bone marrow heavily infiltrated with Burkitt cells. (x 1000)

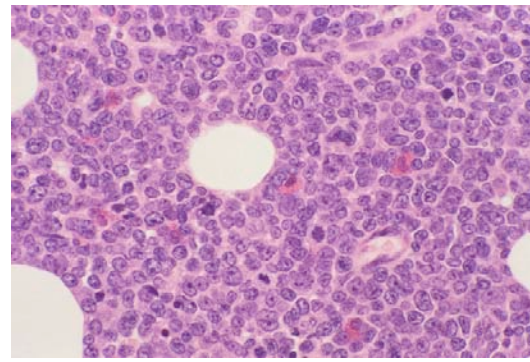


Figure B4-50
Burkitt cell lymphoma/leukaemia: bone marrow trephine showing a homogeneous infiltrate of Burkitt cells with prominent nucleoli. (H&E) (x 400)

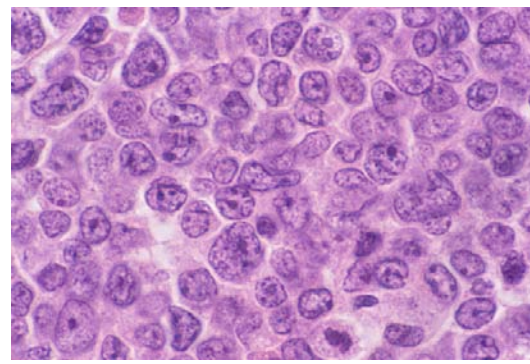


Figure B4-51
Burkitt cell lymphoma/leukaemia: bone marrow trephine showing a homogeneous infiltrate of Burkitt cells with prominent nucleoli. (H&E) (x 1000)

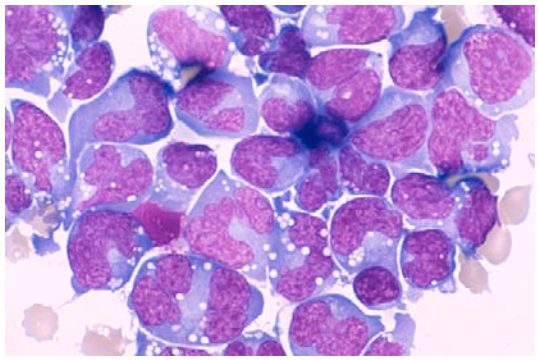


Figure B4-52
Burkitt cell lymphoma/leukaemia: pleural fluid showing a heavy infiltration with Burkitt cells. (x 1000)

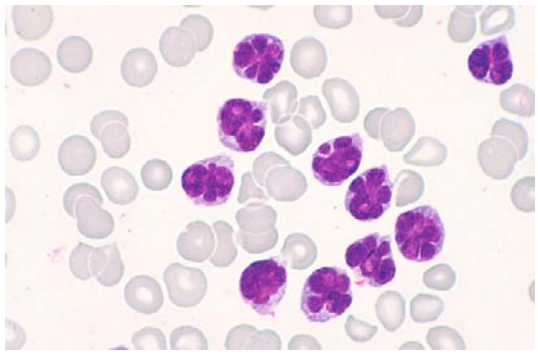


Figure B4-53
T-cell prolymphocytic leukaemia: peripheral blood showing a clonal population of prolymphocytes with characteristic cleaved nuclei and inconspicuous nucleoli. (x 1000)

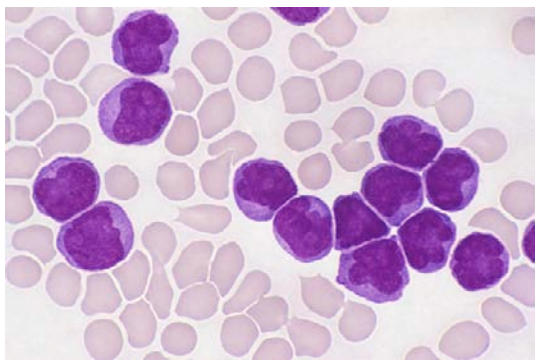


Figure B4-54
T-cell prolymphocytic leukaemia: peripheral blood showing the presence of prolymphocytes with cleaved nuclei and visible nucleoli. (x 1000)

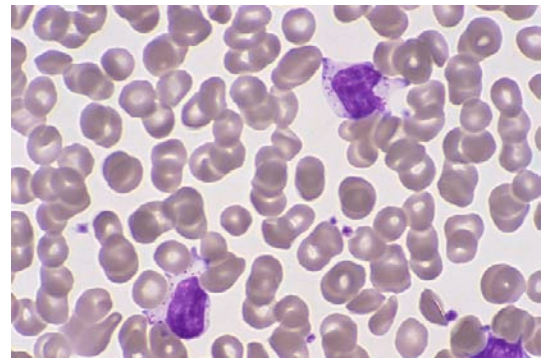


Figure B4-55
T-cell large granular lymphocytic leukaemia: peripheral blood demonstrating the presence of large granular lymphocytes with abundant cytoplasm filled with fine to coarse azurophilic granules. (x 1000)

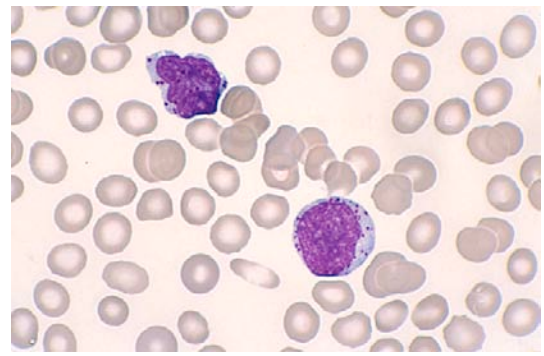


Figure B4-56
Aggressive NK-cell leukaemia: peripheral blood showing large lymphocytes with coarse azurophilic granulation. (x 1000) (Courtesy of Geoffrey Kershaw)

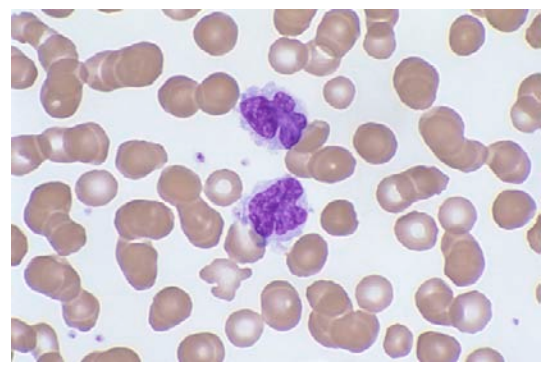


Figure B4-57
Adult T-cell leukaemia/lymphoma: peripheral blood film showing the presence of lymphocytes with convoluted nuclei. (x 1000)

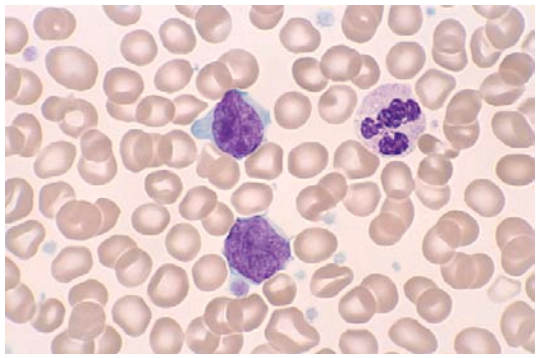


Figure B4-58
Cutaneous T-cell lymphoma: peripheral blood film showing Sézary cells with a convoluted, cerebriform nuclear appearance. (x 1000)

LYMPHOID-SPECIFIC STAINS

Periodic acid-Schiff (PAS)

The presence of glycogen within the cytoplasm of many haemopoietic cells may be demonstrated by the PAS reaction. Red to purple staining material indicates a positive reaction.

Myeloid cells all give a positive reaction, with the more mature neutrophils demonstrating the most intense positivity. Lymphocytes normally contain much less positively staining material, with only a few granules being present within the cytoplasm. Monocytes also demonstrate a fine scattering of positivity. Erythroblasts do not normally stain at any stage of development.

The PAS reaction of haemopoietic cells in disease differs from that in normal cells and the findings have some diagnostic value.

Lymphocytes in the B-lymphoproliferative disorders often contain an increased number of positively staining granules. In lymphoblasts, blocks of positively staining material may be present.

Erythroblasts may react positively in disease: deep diffuse staining has been observed in erythroleukaemia—both erythroid/myeloid and pure erythroid leukaemia.

The PAS stain is used primarily to differentiate between acute lymphoblastic and acute myelomonoblastic leukaemia. Blocks of positivity are seen in lymphoblasts while myeloblasts and monoblasts give a negative reaction. Refer to Fig B4-63.

Acid phosphatase

Acid phosphatase is present in the lysosomes of many types of haemopoietic cells, namely myeloid cells, monocytes and lymphocytes as well as megakaryocytes and platelets. Acid phosphatase is one of the few acid hydrolases that can be demonstrated in lymphoid cells and hence is of diagnostic value in the classification of lymphoproliferative neoplasms.

In the B-cell neoplasms, the reaction is often weak or negative with the exception of hairy cell leukaemia. In normal T cells, the reaction is weak or negative, in contrast to the T-cell neoplasms, where the reaction is strongly positive. The myeloid, monocyte and megakaryocyte series all react positively in varying degrees.

The acid phosphatase reaction should always be performed with and without the addition of tartaric acid since tartrate resistance is particularly helpful in the diagnosis of hairy cell leukaemia. Most positively reacting leucocytes are tartrate-sensitive and fail to react in its presence, while the lymphocytes of hairy cell leukaemia react positively with and without tartrate. This is due to the presence of a unique isoenzyme-5 found predominantly in hairy cells. Refer to Fig B4-64.

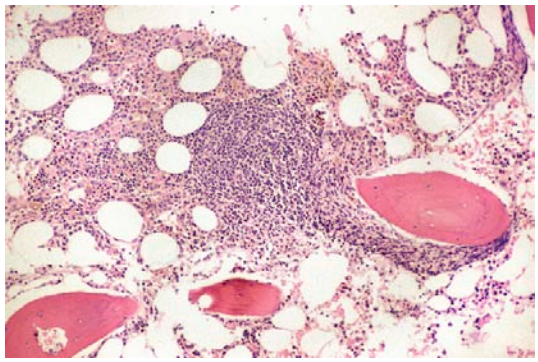


Figure B4-59
Bone marrow trephine showing a lymphoid nodule. (H&E) (x 100)

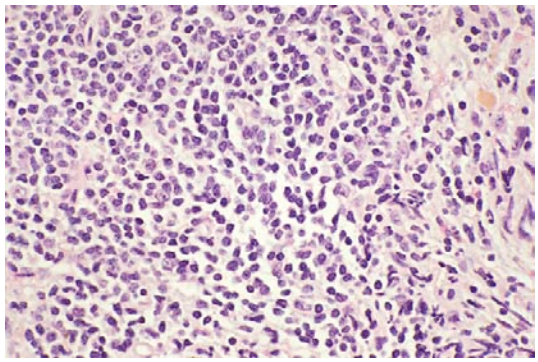


Figure B4-60
Bone marrow trephine showing a lymphoid nodule. (H&E) (x4 00)

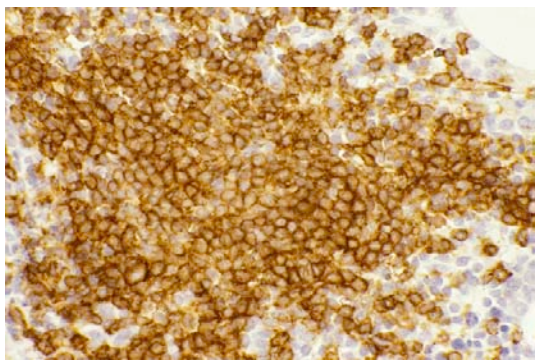


Figure B4-61
B-cell lymphoma: CD20 strongly positive. (x 400)

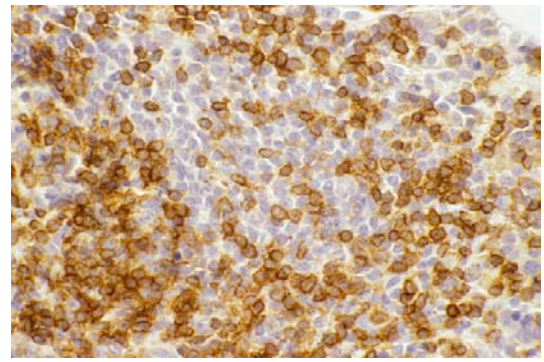


Figure B4-62
T-cell lymphoma: CD3 positive. (x 400)

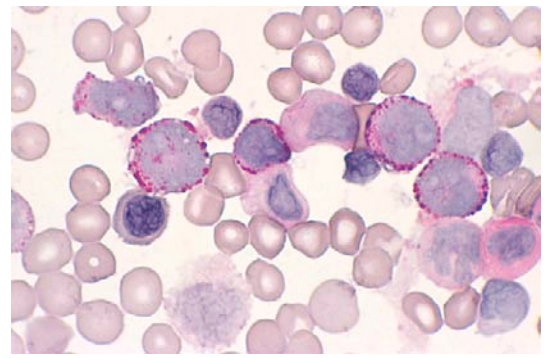


Figure B4-63
PAS stain showing block positivity on the bone marrow from a patient with B lymphoblastic leukaemia/lymphoblastic lymphoma. (x 1000)

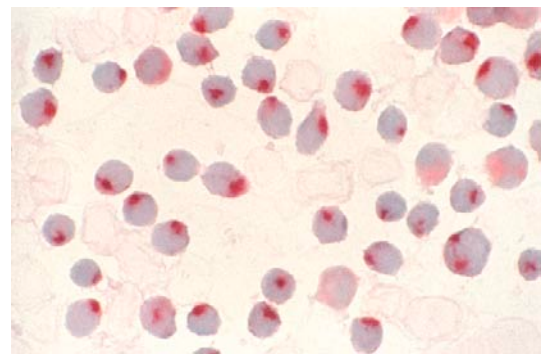
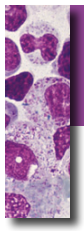


Figure B4-64
Acid phosphatase stain showing polar positivity in the T lymphocytes on the peripheral blood film from a patient with adult T-cell leukaemia/lymphoma. (x 1000)



B5 Plasma cells

PLASMA CELL MATURATION

B lymphocytes have surface membrane immunoglobulins that act as receptors for specific antigens. Antigen-antibody complexes on the B-lymphocyte membrane induce lymphocytes to undergo proliferation into plasma cells.

Plasma cell maturation is divided into three stages: the plasmablast, proplasma cell and plasma cell.

Plasmablast

The plasmablast varies from 16 to 18 μm in diameter and has a round nucleus occupying about 80% of the cell. The chromatin is fine and contains one to three nucleoli. The nucleus is usually eccentrically placed within intensely basophilic cytoplasm; a perinuclear halo, representing the Golgi apparatus, may be present. Refer to Figs B5-1 to B5-3.

Proplasma cell

The proplasma cell varies from 15 to 25 μm in diameter and has a round eccentrically placed nucleus containing one to three nucleoli. The cytoplasm is intensely basophilic, with a perinuclear halo. Refer to Figs B5-1 to B5-5.

Plasma cell

The plasma cell varies from 8 to 20 μm in diameter. It has a round eccentrically placed nucleus with dense chromatin pattern, and the cytoplasm is basophilic with a perinuclear halo. Refer to Figs B5-5 and B5-6.

PLASMA CELL NEOPLASMS

Plasma cell myeloma

Plasma cell myeloma is a malignant proliferation within the bone marrow of plasma cells that secrete abnormal amounts of monoclonal immunoglobulin. It occurs more commonly in males between the age of 60 and 70 years but may occur rarely in young adults.

Patients presenting with plasma cell myeloma initially often complain of back pain. Myeloma induces osteoporosis that may give rise to collapsed vertebrae or, in some cases, pathologic fractures.

The blood film is that of a normochromic normocytic anaemia with markedly increased rouleaux formation. The erythrocyte sedimentation rate (ESR) is usually more than 100 mm/h. The blood film has a blue hue when viewed macroscopically; this is due to increased immunoglobulin. A small number of plasma cells may be present; if the absolute number of plasma cells exceeds 2×10^9/L then the diagnosis of plasma cell leukaemia rather than plasma cell myeloma should be made. Refer to Fig B5-16.

As plasma cells are not always present in the peripheral blood, a bone marrow examination is necessary for the diagnosis of plasma cell myeloma.

The bone marrow of patients with moderately advanced disease may contain between 30% and 90% plasma cells. In severe cases, sheets of plasma cells are seen. Binucleated plasma cells are also a common feature. In some instances, the plasma cells demonstrate cytoplasmic inclusions. These may be spherical inclusions or Russell bodies representing the accumulation of immunoglobulin. Cells containing Russell bodies are called Mott cells. Inclusions may also be in the form of azurophilic rods, again representing the accumulation of immunoglobulin. In some cases, the plasma cell cytoplasm is pink. These are known as 'flaming' plasma cells or saurocytes. Refer to Figs B5-9 to B5-15.

CYTOGENETICS

Routine cytogenetic analysis may not detect anomalies; however, FISH analysis increases the abnormality detection rate up to around 90%. Typical abnormalities include hyperdiploidy with gains of chromosome 3, 5, 7, 9, 11, 15, 19, 21, translocations involving the *IGH* locus at 14q32 [t(11;14)(q13;q32), t(14;16)(q32;q23), t(4;14)(p16.3;q32), t(6;14)(p21;q32)], -13/del13q, del17p (loss of the *TP53*) and rearrangements of chromosome 1 resulting in relative gain of 1q and loss of 1p.

IMMUNOPHENOTYPE

- $CD19^{-}$, $CD20^{-}$, $CD138^{+}$, $CD38^{+}$, $CD56/58^{+}$, $CD79a^{+}$

Plasmacytoma

A plasmacytoma is a solitary tumour of plasma cells identical to those that occur in plasma cell myeloma. Plasmacytomas show up on x-ray in areas of active haemopoiesis, such as the vertebrae, ribs and skull. Refer to Figs B5-17 and Figure B5-18.

CYTOGENETICS AND IMMUNOPHENOTYPE

- The cytogenetic and immunophenotypic characteristics of plasma cells in a plasmacytoma are identical to those of plasma cell myeloma.

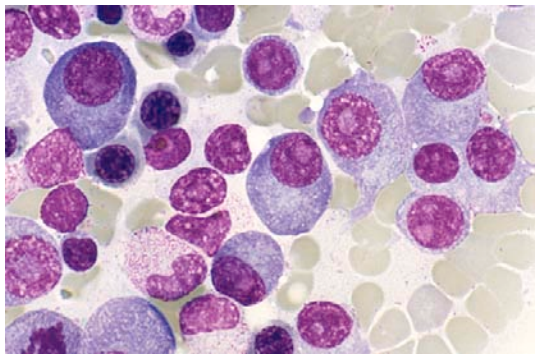


Figure B5-1
Plasma cell myeloma: bone marrow showing plasmablasts and proplasma cells. (x 1000)

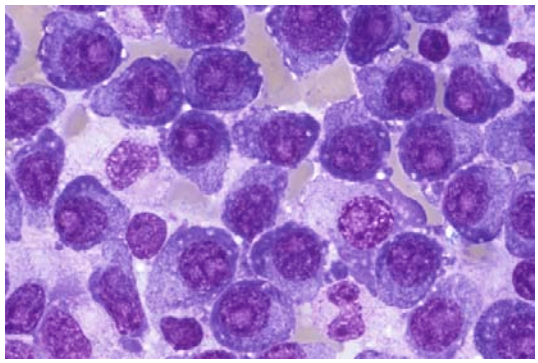


Figure B5-2
Plasma cell myeloma: bone marrow densely packed with plasmablasts and proplasma cells. (x 1000)

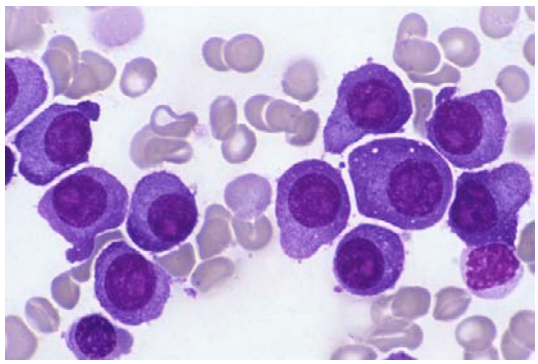


Figure B5-3
Plasma cell myeloma: bone marrow densely packed with plasmablasts and proplasma cells. (x 1000)

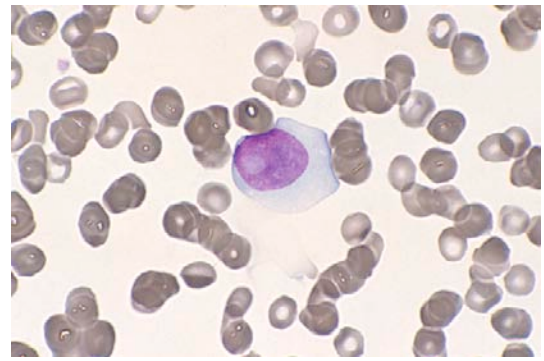


Figure B5-4
Plasma cell myeloma: proplasma cell in the peripheral blood. (x 1000)

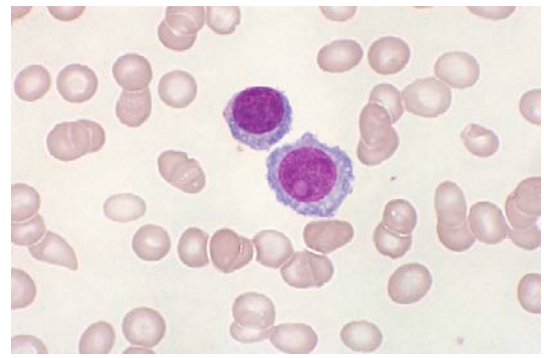


Figure B5-5
Plasma cell myeloma: proplasma cell and plasma cell in the peripheral blood. (x 1000)

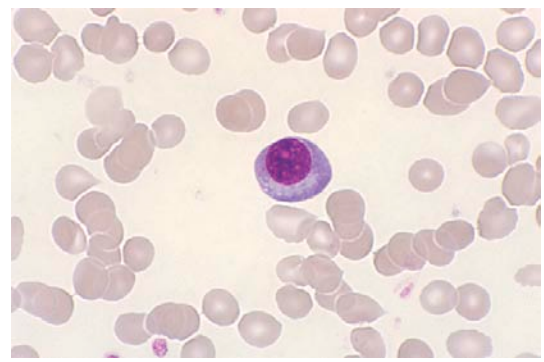


Figure B5-6
Plasma cell myeloma: plasma cell in the peripheral blood. (x 1000)

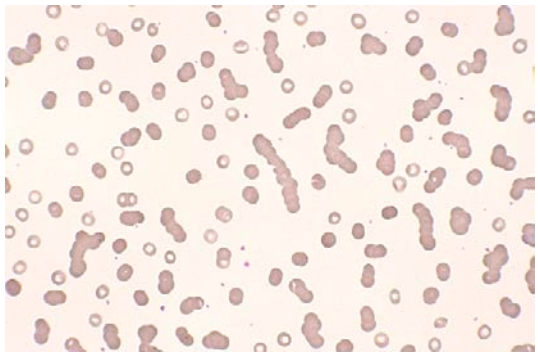


Figure B5-7
Plasma cell myeloma: peripheral blood film showing rouleaux formation. ESR >140 mm/h. (x 400)

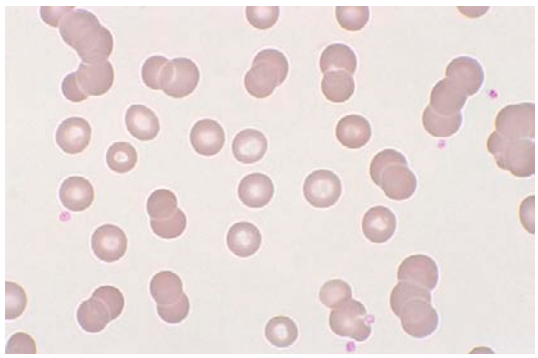


Figure B5-8
Plasma cell myeloma: peripheral blood film showing rouleaux formation. (x 1000)

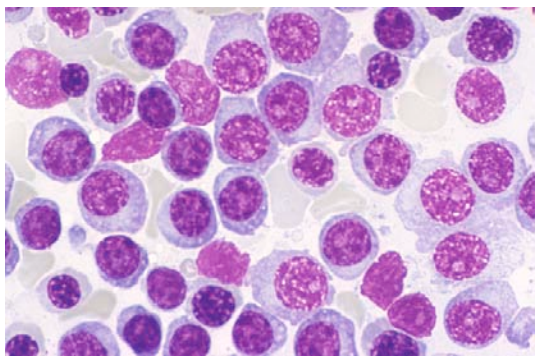


Figure B5-9
Plasma cell myeloma: bone marrow showing sheets of plasma cells and proplasma cells: note the eccentrically placed nuclei, basophilic cytoplasm and perinuclear halos. (x 1000)

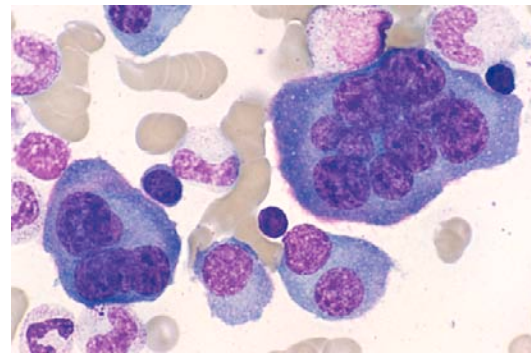


Figure B5-10
Plasma cell myeloma: bone marrow showing binucleated and multinucleated plasma cells. (x 1000)

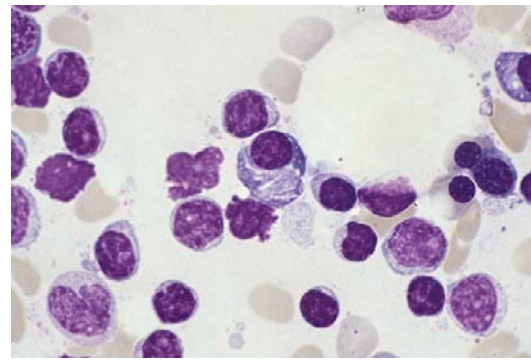


Figure B5-11
Plasma cell myeloma: bone marrow demonstrating crystals of immunoglobulin within the plasma cell cytoplasm. (x 1000)

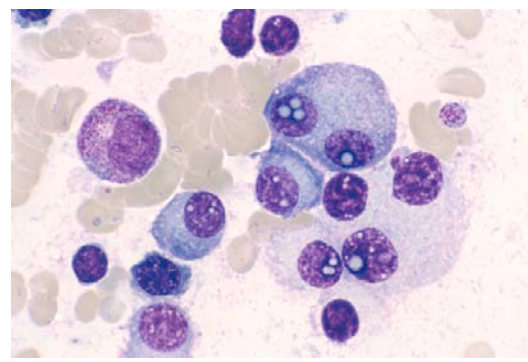


Figure B5-12
Plasma cell myeloma: plasma cells demonstrating abnormal nuclear vacuolation. (x 1000)

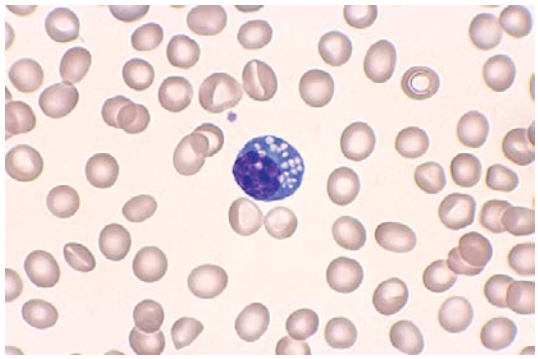


Figure B5-13
Plasma cell myeloma: Mott cell in the peripheral blood. (x 1000)

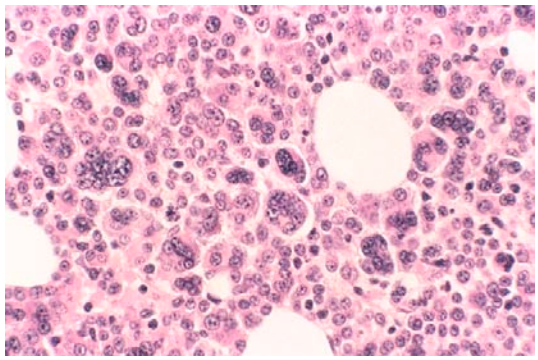


Figure B5-14
Plasma cell myeloma: sheets of plasma cells, including multinucleated forms, throughout the bone marrow trephine. (H&E) (x 400)

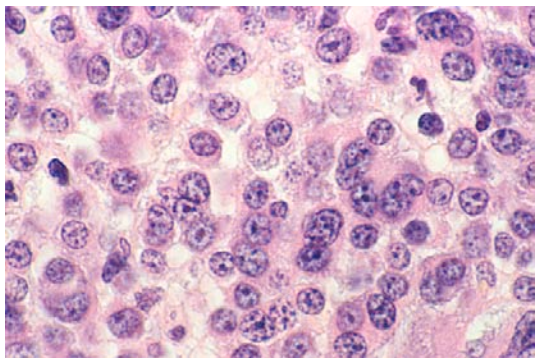


Figure B5-15
Plasma cell myeloma: bone marrow trephine showing plasma cells with classical 'clock-faced' nuclei. (H&E) (x 1000)

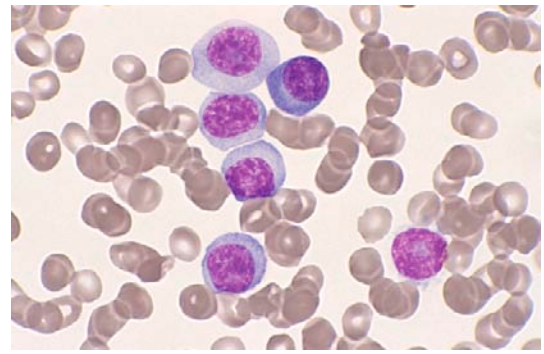


Figure B5-16
Plasma cell leukaemia: peripheral blood with an absolute plasma cell count of more than 2 x 10^9/L. (x 1000)

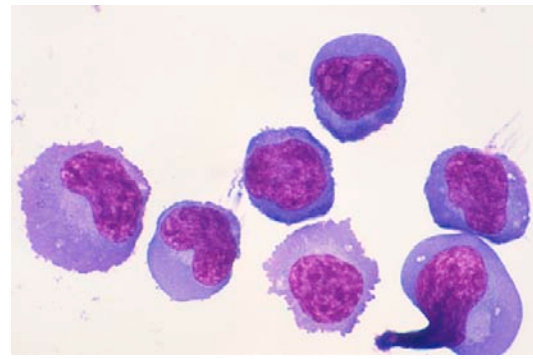


Figure B5-17
Plasmacytoma: CSF showing a heavy infiltrate of plasma cells. The bone marrow aspirate on this patient was normal. (x 1000)

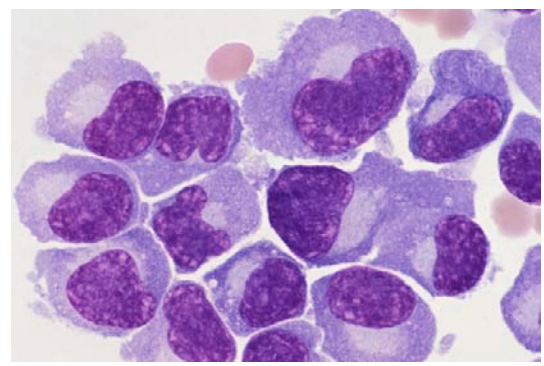
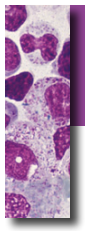


Figure B5-18
Plasmacytoma: pleural fluid showing a heavy infiltrate of plasma cells. (x 1000)



B6 Acute myeloid leukaemia (AML) and related precursor neoplasms

The WHO classification of acute leukaemia is based on cell morphology, immunophenotype and cytogenetics. Utilising this data, the acute myeloid leukaemias are divided into seven categories:

- Acute myeloid leukaemia with recurrent genetic abnormalities
- Acute myeloid leukaemia with myelodysplasia-related changes
- Therapy-related myeloid neoplasms
- Acute myeloid leukaemia, not otherwise specified
- Myeloid sarcoma
- Myeloid proliferations related to Down syndrome
- Blastic plasmacytoid dendritic cell neoplasm

In order to make a diagnosis of 'acute leukaemia' there must be at least 20% blast cells in the bone marrow.

ACUTE MYELOID LEUKAEMIA WITH RECURRENT GENETIC ABNORMALITIES

Acute myeloid leukaemia with t(8;21)(q22;q22)

AML with t(8;21)(q22;q22) usually occurs in the younger age group. The blast cells may be both large and small in size. The large blasts demonstrate a degree of maturation since they have basophilic cytoplasm containing both azurophilic granules and Auer rods. The smaller blasts tend to be found mainly in the peripheral blood. Refer to Figs B6-1 and B6-2.

CYTOCHEMISTRY/IMMUNOPHENOTYPE

- The myeloperoxidase (MPO) stain is positive.
- HLA-DR$^+$, CD13$^+$, CD33$^{-/+}$, CD34$^+$
- Coexpression of the lymphoid marker CD19 is consistently found on the smaller blasts.

Acute myeloid leukaemia with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)

AML with inv(16)(p13.1q22) and t(16;16)(p13.1;q22) occurs in all age groups; more so in the younger patient. It demonstrates both monocytic and granulocytic differentiation and is associated with a small increase in the number of eosinophils (<5%). The eosinophils are mainly at the promyelocyte and myelocyte stage and contain large immature eosinophilic granules. The peripheral blood does not usually show an eosinophilia.

The blast cells may contain Auer rods and at least 3% are MPO positive. Refer to Fig B6-3.

CYTOCHEMISTRY/IMMUNOPHENOTYPE

- The MPO stain is positive.
- CD4$^+$, CD14$^+$, CD11b$^+$, CD11c$^+$, CD13$^+$, CD15$^+$, CD33$^+$, CD65$^+$, CD117$^+$, CD34$^+$

Acute promyelocytic leukaemia (APL) with t(15;17)(q22;q12) *PML-RARA*

APL may occur at any age. The majority of cells in both the bone marrow and peripheral blood are abnormal promyelocytes. There are two types: the hypergranular or typical type and the hypogranular or microgranular type. Both are associated with a coagulopathy, namely disseminated intravascular coagulation (DIC).

The promyelocytes in the hypergranular or 'typical' type have nuclei that vary greatly in size and shape and are often bilobed or reniform. Their cytoplasm is closely packed with large azurophilic granules. Characteristically, the cells contain numerous Auer rods as well as bundles of Auer rods or 'faggots' randomly distributed within the cytoplasm. Myeloblasts with a single Auer rod may also be present.

The hypogranular or microgranular type resembles the 'typical' nuclear shape but the cytoplasm contains few or no azurophilic granules. Auer rods and bundles of Auer rods may, however, be present. Refer to Figs B6-4 to B6-6.

CYTOCHEMISTRY/IMMUNOPHENOTYPE

- The myeloperoxidase stain is strongly positive.
- HLA-DR^{-}, CD13$^{+/-}$, CD33^{+}, CD 117$^{-/+}$, CD34^{-}

Acute promyelocytic leukaemia (APL) with a variant *RARA* translocation

There is a subset of cases of acute promyelocytic leukaemia with variant translocations involving the retinoic acid receptor alpha (*RARA*) gene.

t(11;17)(q23;q12), resulting in *ZBTB16-RARA* fusion, is different morphologically in that the promyelocytes resemble normal promyelocytes with regularly shaped, round nuclei. This variant is resistant to all-trans retinoic acid (ATRA).

t(5;17)(q35;q12), resulting in *NPM1-RARA* fusion, has a minor population of hypogranular promyelocytes as well as the typical population of hypergranular promyelocytes. This variant appears to be responsive to ATRA. Refer to Fig B6-7.

Acute myeloid leukaemia with t(9;11)(p22;q23)

Acute myeloid leukaemia with t(9;11)(p22;q23) occurs in both children and adults but is more common in children. It closely resembles acute monocytic and acute myelomonocytic leukaemia. Monoblasts and promonocytes predominate. The monoblasts have round nuclei with prominent nuclei, abundant basophilic cytoplasm with fine azurophilic granules. Refer to Fig B6-8.

CYTOCHEMISTRY/IMMUNOPHENOTYPE

- The promonocytes and monoblasts show strong positivity with the non-specific esterase α-naphthyl acetate esterase. The monoblasts are mostly myeloperoxidase (MPO) negative.
- The markers vary according to the age of the patient. Children express myeloid markers while adults express monocytic markers.
- HLA-DR^{+}, CD33^{+}, CD65^{+}, CD4^{+}, CD13^{-}, CD14^{-}, CD34^{-} (myeloid markers in children)
- CD4^{+}, CD11b^{+}, CD11c^{+}, CD14^{+}, CD36^{+}, CD64^{+}, CD34^{-}, CD117^{-} (monocytic markers in adults).

Acute myeloid leukaemia with t(6;9)(p23;q34)

Acute myeloid leukaemia with t(6;9)(p23;q34) occurs in children with a median age of 13 years as well as in adults with a median age of 35 years. Monocytic features may be associated with AML t(6;9)(p23;q34) however a basophilia and multilineage dysplasia are more frequently seen. Patients usually present with a pancytopenia and a median white cell count of 12×10^{9}/L.

A peripheral blood and bone marrow basophilia occurs in 44% to 62% of cases while the majority of cases demonstrate granulocytic and erythroid dysplasia.

CYTOCHEMISTRY/IMMUNOPHENOTYPE

- The myeloblasts are consistently MPO positive.
- They also express the following:
  HLA-DR$^+$, CD13$^+$, CD33$^+$, CD38$^+$, CD15$^+$, CD117$^+$ and CD34$^+$ (myeloid markers)
  CD64+ (monocytic marker)

Acute myeloid leukaemia with inv(3)(q21q26.2) or t(3;3)(q21;q26.2)

Acute myeloid leukaemia with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) occurs most commonly in adults. Presentation with this leukaemia is either de novo or secondary to a myelodysplastic process. Peripheral blood changes include hypogranular neutrophils, pelgeroid nuclei with or without the presence of blasts. Large and giant hypogranular platelets and mild red cell changes are present. Refer to Fig B6-9.

IMMUNOPHENOTYPE

- HLA-DR$^+$, CD13$^+$, CD33$^+$, CD38$^+$, CD34$^+$
- Some cases aberrantly express CD7 and a subset may express the megakaryocytic markers CD41 and CD61

Acute myeloid leukaemia (megakaryoblastic) with t(1;22)(p13;q13)

Acute myeloid leukaemia with t(1;22)(p13;q13) shows maturation in the megakaryocyte lineage. It represents less than 1% of cases of AML and invariably occurs in infants with Down syndrome. It occurs as a de novo AML usually occurring in the first 6 months of life. Patients present with anaemia, thrombocytopenia and a raised white cell count.

The blast cells of AML t(1;22)(p13;q13) resemble the blasts seen in acute megakaryoblastic leukaemia of AML, NOS. They are medium to large in size with a round nucleus, fine chromatin pattern with one to three nucleoli. The cytoplasm is basophilic with the classical blebbing as seen in the megakaryoblasts of AML, NOS. Bone marrow aspiration usually results in a dry tap due to a hypercellular marrow with increased reticulin and collagenous fibrosis. Refer to Figs B6-10 and Fig B6-11.

CYTOCHEMISTRY/IMMUNOPHENOTYPE

- The blasts are myeloperoxidase (MPO) negative.
- CD41 (glycoprotein 11b/111a) and CD61 (glycoprotein 111a) are positive
- CD13$^+$, CD33$^+$, CD45$^-$, HLA-DR$^-$, CD36$^+$

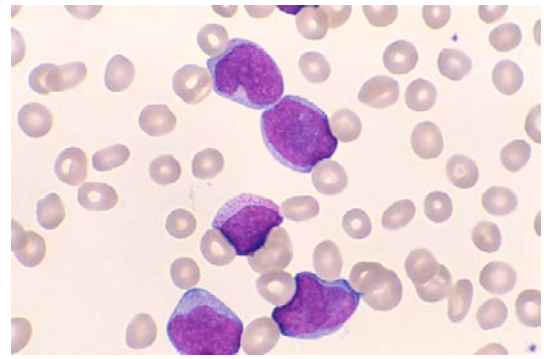


Figure B6-1
Acute myeloid leukaemia with t(8;21)(q22;q22): peripheral blood film showing blast cells with basophilic cytoplasm containing fine azurophilic granules. (x 1000)

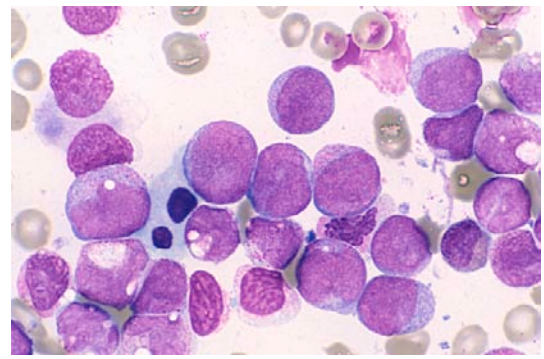


Figure B6-2
Acute myeloid leukaemia with t(8;21)(q22;q22): bone marrow from the case shown in B6–1. (x 1000)

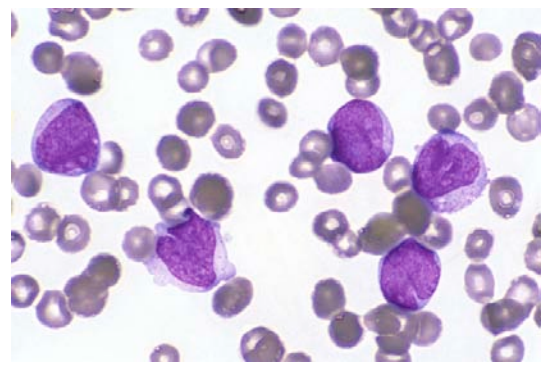


Figure B6-3
Acute myeloid leukaemia with inv(16)(p13.1q22): peripheral blood film showing the presence of blasts with a myelomonocytic appearance. They have round to folded nuclei with basophilic cytoplasm that often contains fine azurophilic granules. (x 1000)

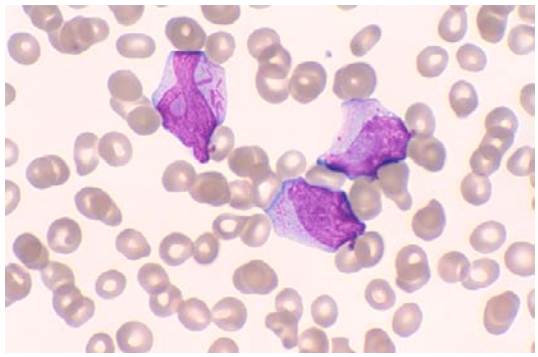


Figure B6-4
Acute promyelocytic leukaemia with t(15;17)(q22;q12): peripheral blood film showing abnormal promyelocytes whose cytoplasm is packed with large azurophilic granules and Auer rods. (x 1000)

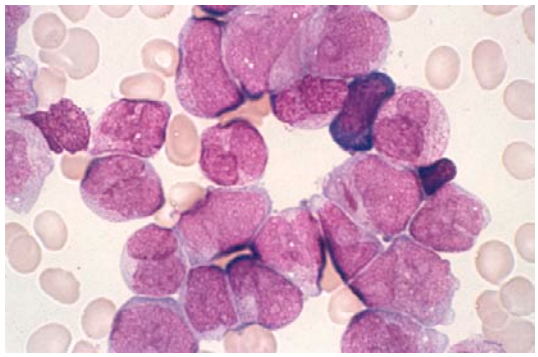


Figure B6-5
Acute promyelocytic leukaemia with t(15;17)(q22;q12): bone marrow showing a promyelocyte with bundles of Auer rods or 'faggots' within the cytoplasm. Many of the promyelocytes have the characteristic bilobed and reniform nuclear shape. (x 1000)

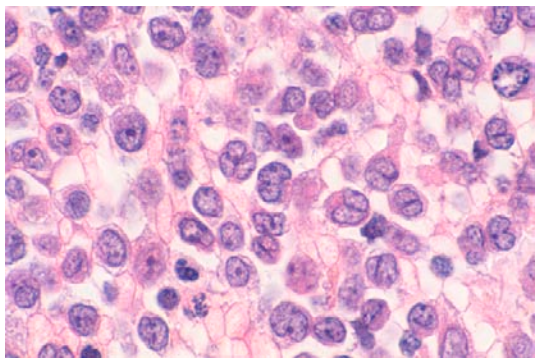


Figure B6-6
Acute promyelocytic leukaemia with t(15;17)(q22;q12): bone marrow trephine showing a heavy infiltration of abnormal promyelocytes with bilobed nuclei. (H&E) (x 1000)

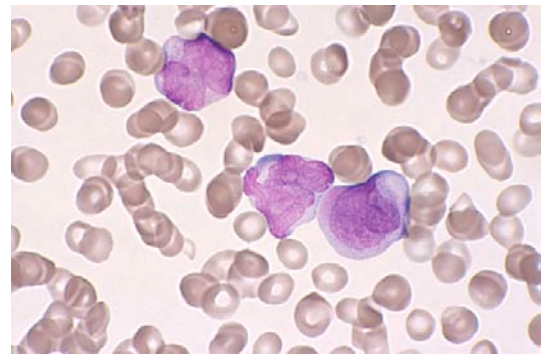


Figure B6-7
Acute promyelocytic leukaemia with t(5;17)(q35;q12) variant: peripheral blood film showing promyelocytes with the characteristic bilobed nuclei but with hypogranular cytoplasm. (X 1000)

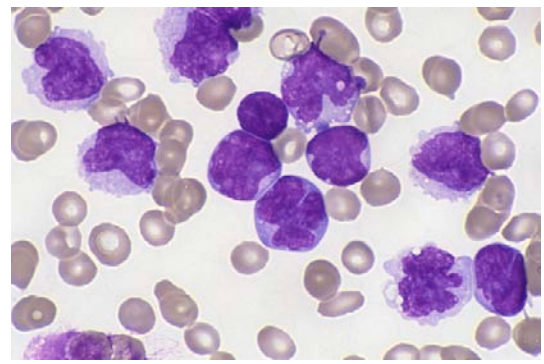


Figure B6-8
Acute myeloid leukaemia with t(9;11)(p22;q23): peripheral blood showing the presence of blasts with a myelomonocytic appearance. They have round to folded nuclei, prominent nucleoli and abundant basophilic cytoplasm. (x 1000)

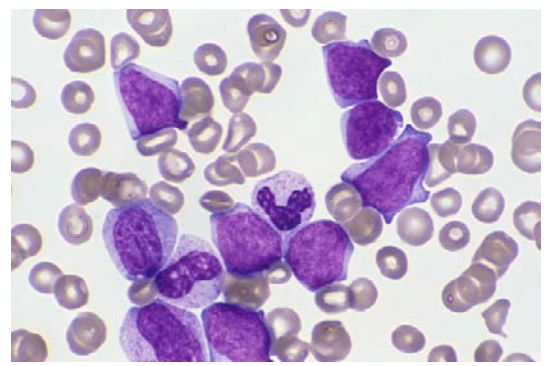


Figure B6-9
Acute myeloid leukaemia with inv(3)(q21q26.2): peripheral blood showing classical myeloblasts together with hypogranular, pelgeroid nuclei. (x 1000)

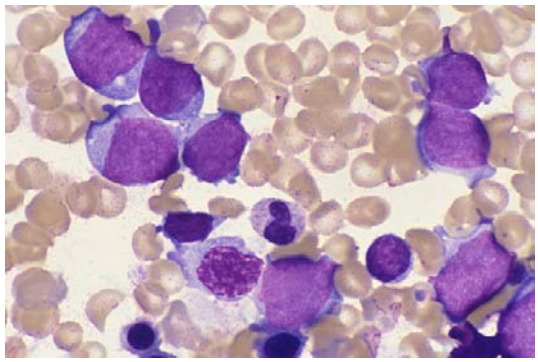


Figure B6-10
Acute myeloid leukaemia (megakaryoblastic) with t(1;22)(p13;q13): bone marrow showing an infiltrate of large blasts with fine chromatin pattern, basophilic cytoplasm with the classic budding that characterises megakaryoblasts. (x 1000)

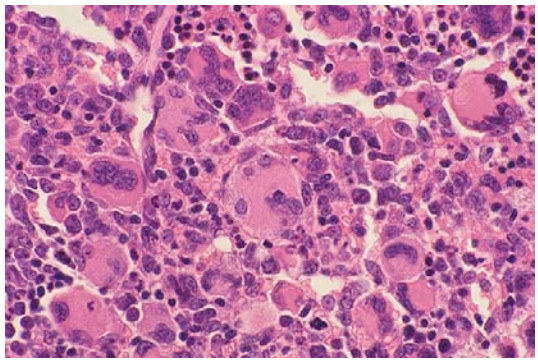


Figure B6-11
Acute myeloid leukaemia (megakaryoblastic) with t(1;22)(p13;q13): hypercellular bone marrow trephine showing a heavy infiltrate of megakaryoblasts. Such a marrow would result in a dry tap upon aspiration. (x 400)

ACUTE MYELOID LEUKAEMIA WITH MYELODYSPLASIA-RELATED CHANGES

The initial presentation of AML with multilineage dysplasia is severe pancytopenia in an elderly patient. The dysplastic features will be visible on both the peripheral blood film and the bone marrow. Dysplasia must be present in 50% or more of at least two cell lineages. Dysgranulopoiesis is characterised by hypogranular or agranular neutrophils; there are hyposegmented nuclei with pseudo Pelger-Huët or pelgeroid features as well as round unsegmented nuclei. Dyserythropoiesis is characterised by megaloblastoid red cells, nuclear fragmentation and multinucleation. Ring sideroblasts may be present in the bone marrow. Dysmegakaryopoiesis is characterised by micromegakaryocytes with hypolobulated nuclei, normal sized megakaryocytes with monolobed nuclei and large megakaryocytes with multisegmented nuclei. Refer to Fig B6-12.

CYTOGENETICS

- The cytogenetic changes involve the same chromosomes as those that occur in myelodysplastic syndrome. Karyotypes are often complex with abnormalities including -5/del(5q), -7/del(7q), del(20q), +8, i(17p), -13/del(13q), del(11q) and rearrangements of 12p.

IMMUNOPHENOTYPE

- TdT^{+}, $CD7^{+}$, $CD13^{+}$, $CD33^{+}$, $CD34^{+}$, $CD56^{-/+}$

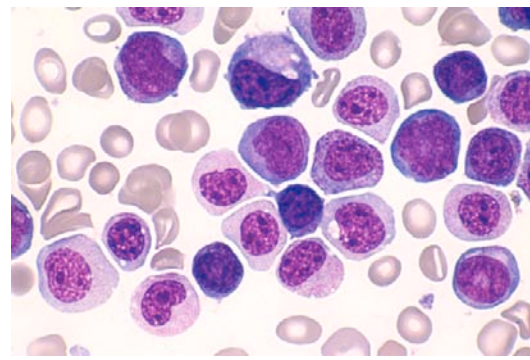


Figure B6-12
Acute myeloid leukaemia with myelodysplasia-related changes: bone marrow showing both dysgranulopoiesis and dyserythropoiesis together with an increase in blast cells. (x 1000)

THERAPY-RELATED MYELOID NEOPLASMS

Therapy-related myeloid neoplasms includes those cases of acute myeloid leukaemia (t-AML), myelodysplastic syndromes (t-MDS) and myelodysplastic/myeloproliferative neoplasms (t-MDS/MPN) that may occur following the use of cytotoxic chemotherapy and/or radiation therapy. The cytotoxic chemotherapy includes alkylating agents and topoisomerase II inhibitors, both agents being used to treat a previous neoplastic or non-neoplastic disorder. All three resultant myeloid neoplasms are considered together as a unique clinical syndrome.

Therapy-related t-AML, t-MDS and t-MDS/MPD account for approximately 10–20% of cases of AML, MDS and MDS/MPD. The incidence amongst patients receiving cytotoxic chemotherapy depends on the age of the patient, the underlying neoplasm as well as the treatment regime. The incidence of those patients receiving alkylating agents or radiation therapy increases with age whereas the incidence of those receiving topoisomerase II inhibitors is the same across all age groups.

t-AML, t-MDS and t-MDS/MPD present some 5–10 years post treatment with an alkylating agent or radiation therapy. The initial presentation is that of a single cytopenia or pancytopenia associated with trilineage dysplasia within the bone marrow. This later transforms into acute leukaemia. The neutrophils demonstrate hypolobulation of the nucleus and hypogranular cytoplasm. Increased numbers of basophils occur in 25% of cases. The red cells demonstrate megaloblastoid features together with nuclear fragmentation and multinucleation. Ring sideroblasts are present in up to 60% of cases. Dysplastic megakaryocytes, hypolobulated and hyperlobulated, occur in approximately 25% of cases. According to the number of blast cells and ringed sideroblasts present, the appropriate category within the MDS will be made. A small group of patients will present with an initial overt acute leukaemia.

Patients receiving topoisomerase II inhibitors may present with a neoplasm 1–5 years post treatment. This group of patients invariably presents with overt acute leukaemia. The epipodophyllotoxins, etoposide and teniposide are the chief agents; however, the anthracyclines, doxorubicin and epirubicin have also been reported as causative agents.

The AML that occurs following therapy with topoisomerase II inhibitors is usually monocytic or myelomonocytic and usually does not have a preliminary myelodysplastic stage. Acute promyelocytic, megakaryoblastic and lymphoblastic leukaemia rarely occur. Refer to Fig B6-13.

CYTOGENETICS

90% of cases are cytogenetically abnormal.

- *Patients receiving alkylating agent or radiation therapy*

The cytogenetic changes seen post alkylating agents and radiation therapy are similar to those that occur in AML with multi-lineage dysplasia and de novo MDS. The cytogenetic abnormalities are generally unbalanced rearrangements as part of a complex karyotype. The most common findings involve -5/del(5q), -7/del(7q) often with one or more additional abnormalities including del(13q), del(20q), del(11q), del(3p), -17, -18, -21, +8.

- *Patients receiving topoisomerase II inhibitors*

Patients exposed to topoisomerase II inhibitors are more likely to have balanced chromosomal translocations involving rearrangements of 11q23 such as t(11;19)(q23;p13) and t(9;11)(p22;q23)

Should lymphoblastic leukaemia occur, the translocation t(4;11)(q21;q23) may be identified. Rearrangements of 22q12 [such as t(8;21)(q22;q22), t(3;21)(q26.2;q22.1)] may also be seen, as well as other typical AML rearrangements such as t(15;17)(q22;q12), inv(16)(p13.1q22)

IMMUNOPHENOTYPE

- There are no specific immunophenotypic findings in therapy-related t-AML, t-MDS or t-MDS/MPN. Markers reflect the morphological changes in the peripheral blood and bone marrow in the case of MDS and MDS/MPN as well as in de novo acute leukaemia.
- $CD7^{-/+}$, $CD13^{+}$, $CD33^{+}$, $CD34^{+}$, $CD56^{-/+}$

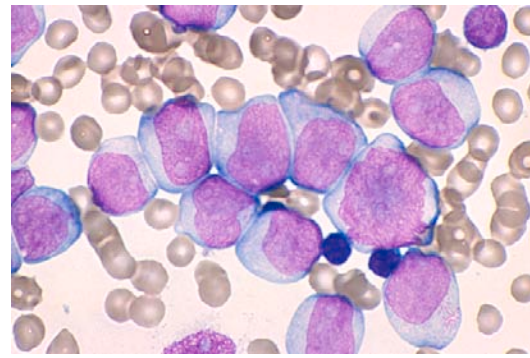


Figure B6-13
Therapy-related myeloid leukaemia: bone marrow showing a heavy infiltrate of monoblasts. (x 1000)

ACUTE MYELOID LEUKAEMIA, NOT OTHERWISE SPECIFIED

Acute myeloid leukaemia with minimal differentiation

Acute myeloid leukaemia with minimal differentiation may occur at any age however most patients are infants or older adults. The blast cells show no evidence of myeloid differentiation. Morphologically, the blasts are medium sized with a high N:C ratio. They have a slightly indented nucleus, fine chromatin pattern, and one or two nucleoli. The cytoplasm is basophilic and agranular. Less than 3% of the blasts are positive for MPO, Sudan black B and naphthol AS-D chloroacetate esterase. Refer to Figs B6-14 and B6-15.

CYTOGENETICS

- No specific chromosomal abnormality is associated with AML with minimal differentiation. Complex karyotypes with abnormalities including -5/del(5q), -7/del(7q), +8 and del(11q) may occur. Mutations of *RUNX1* and *FLT3* are found in many cases.

IMMUNOPHENOTYPE

- HLA-DR^{+}, CD13^{+}, CD33^{+}, CD38^{+}, CD34^{+}, CD117^{+}

Acute myeloid leukaemia without maturation

Acute myeloid leukaemia without maturation occurs at any age; however, the majority of patients are adults with a median age of 46 years. The blasts have a high N:C ratio, round nuclei with primitive chromatin pattern and one or more distinct nucleoli. The cytoplasm is basophilic and agranular. Auer rods are present or at least 3% of the blasts are MPO positive or Sudan black B positive. Refer to Figs B6-16 to B6-19.

CYTOGENETICS

- No specific chromosomal abnormality is associated with AML without maturation.

IMMUNOPHENOTYPE

- HLA-DR$^{+/-}$, CD13^{+}, CD33^{+}, CD34$^{+/-}$, CD117^{+}, CD15^{-}

Acute myeloid leukaemia with maturation

Acute myeloid leukaemia with maturation occurs in both young and older adults. The blast cells have nucleoli, varying amounts of cytoplasm and usually many azurophilic granules. Promyelocytes, myelocytes and neutrophils make up at least 10% of the bone marrow cells. Neutrophils show a variable degree of dysplasia, namely abnormal nuclear segmentation and hypogranulation. In rare cases, the abnormal maturation involves the eosinophils. Basophils may also be increased. Refer to Figs B6-20 to B6-23.

CYTOGENETICS

No specific chromosomal abnormality is associated with AML with maturation.

IMMUNOPHENOTYPE

HLA-DR$^{-/+}$, CD13^{+}, CD11b^{+}, CD15^{+}, CD33^{+}, CD34$^{-/+}$, CD117$^{-/+}$

Acute myelomonocytic leukaemia

Acute myelomonocytic leukaemia occurs in all age groups but most commonly in older patients with a median age group of 50 years. Acute myelomonocytic leukaemia is characterised by both granulocytic and monocytic populations present in varying proportions in the bone marrow and peripheral blood. The bone marrow is characterised by the presence of at least 20% blasts. Neutrophils and myeloid precursors as well as monocytes and monocyte precursors each comprise at least 20% of the marrow cells. The peripheral blood film has a high monocyte count namely 5×10^9/L or greater.

The monoblasts have rounded nuclei with a fine chromatin pattern and one or two prominent nucleoli. The cytoplasm is basophilic and lacks granules. The promonocytes show some differentiation in the maturation of the nucleus, which is indented and often contains a nucleolus. The cytoplasm is blue-grey and may contain a few fine azurophilic granules as well as vacuoles.

At least 3% of the blasts (myeloblasts) are MPO positive. The monoblasts, promonocytes and monocytes are a-naphthyl acetate esterase positive. Refer to Figs B6-24 to B6-28.

CYTOGENETICS

- Non-specific myeloid associated chromosome abnormalities (such as +8) are seen in the majority of cases.

IMMUNOPHENOTYPE

- $CD13^+$, $CD33^+$, $CD15^+$, $CD65^+$, $CD34^{+/-}$ (myeloid markers)
- $CD4^+$, $CD11b^+$, $CD11c^+$, $CD14^+$, $CD36^+$, $CD64^+$ (monocytoid markers)

Acute monoblastic and monocytic leukaemia

Acute monoblastic and monocytic leukaemia can occur at any age but most commonly occurs in young adults. Acute monoblastic and acute monocytic leukaemia are myeloid leukaemias in which acute monoblastic leukaemia is characterised by the presence of at least 80% monoblasts and acute monocytic leukaemia is characterised by at least 80% promonocytes and monocytes.

The monoblasts have rounded nuclei with a fine chromatin pattern and one or two prominent nucleoli. The cytoplasm is basophilic and lacks granules. The promonocytes show some differentiation in the maturation of the nucleus, which is indented and often contains a nucleolus. The cytoplasm is blue-grey and may contain a few fine azurophilic granules as well as vacuoles.

The monoblasts and promonocytes usually show α-naphthyl acetate esterase positivity. In 10–20% of cases, the α-naphthyl acetate esterase stain is negative or very weakly positive and immunophenotyping may be necessary to make a diagnosis. Refer to Figs B6-29 to B6-32.

CYTOGENETICS

- Generally non-specific chromosome abnormalities are present in the majority of cases. t(8;16)(p11.2;p13.3) may be associated with acute monocytic and acute myelomonocytic leukaemia.

CYTOCHEMISTRY/IMMUNOPHENOTYPE

- α-Naphthyl acetate esterase is positive in acute monoblastic and monocytic leukaemia.
- $CD13^+$, $CD33^+$, $CD15^+$, $CD65^+$, $CD34^-$, $CD117^+$ (myeloid markers)
- $CD4^+$, $CD11b^+$, $CD11c^+$, $CD14^+$, $CD36^+$, $CD64^+$, $CD68^+$ (monocytoid markers)

Acute erythroid leukaemia

Acute erythroid leukaemia is characterised by a significant increase in erythroid precursors. Erythroid leukaemia may occur at any age, including childhood. It is divided into two categories according to whether there is a significant myeloid component:

- Erythroleukaemia (erythroid/myeloid)
- Pure erythroid leukaemia.

ERYTHROLEUKAEMIA (ERYTHROID/MYELOID)

This category represents acute myeloblastic leukaemia with predominant erythroid differentiation. At least 50% of the nucleated cells in the bone marrow are erythroid precursors and at least 20% of the non-erythroid cells are myeloblasts. All stages of maturation in

the erythroid series are represented in this category. The red cell precursors show marked dysplastic changes. Megaloblastoid nuclei together with binucleated or multinucleated forms are present. The myeloblasts resemble the blasts seen in any of the WHO classification categories with the exception of acute promyelocytic leukaemia.

The erythroid precursors are periodic acid-Schiff (PAS) positive and glycophorin A positive while the myeloblasts are MPO positive and Sudan black B positive. Refer to Figs B6-33 to B6-36.

CYTOGENETICS

- There is no specific chromosomal abnormality.
- Commonly associated with highly complex karyotypes with multiple structural abnormalities including -5/del(5q), -7/del(7q), +8.

CYTOCHEMISTRY/IMMUNOPHENOTYPE

- The erythroblasts are glycophorin A positive and PAS positive.
- HLA-DR$^{+/-}$, CD13^{+}, CD33^{+}, CD34$^{+/-}$, CD71$^{-/+}$

PURE ERYTHROID LEUKAEMIA

This category represents a neoplastic proliferation of more than 80% erythroid precursors within the bone marrow. There is no evidence of a myeloblastic population of cells. The erythroblasts are medium to large in size, with deeply basophilic cytoplasm. The nuclei are round, with a fine chromatin pattern and one to two nucleoli. The more differentiated red cell precursors are glycophorin A positive. Refer to Figs B6-37 and B6-38.

CYTOGENETICS

- There is no specific chromosomal abnormality.
- Commonly associated with highly complex karyotypes with multiple structural abnormalities including -5/del(5q), -7/del(7q), +8.

CYTOCHEMISTRY/IMMUNOPHENOTYPE

- The differentiated erythroblasts are glycophorin A positive and PAS positive.
- HLA-DR^{-}, CD34^{-}, CD117^{+}, CD71^{+}

Acute megakaryoblastic leukaemia

Acute megakaryoblastic leukaemia is an acute leukaemia in which at least 50% of the blast cells are megakaryoblasts. It occurs in both adults and children and is associated with Down syndrome. The megakaryoblasts range from medium to large in size. They have a round nucleus with fine chromatin pattern and one to three nucleoli. The cytoplasm is basophilic and agranular with characteristic cytoplasmic blebs and buds. Occasionally, small megakaryoblasts may be present; they have a high N:C ratio and resemble lymphoblasts. Circulating megakaryocytic fragments may be seen on the blood film. Bone marrow aspiration often results in a dry tap due to increased fibrosis. Refer to Figs B6-39 and B6-40.

CYTOGENETICS

- No specific chromosomal abnormalities are associated with acute megakaryoblastic leukaemia.

IMMUNOPHENOTYPE

- HLA-DR^{-}, CD13^{-}, CD33$^{+/-}$, CD34^{-}, CD41^{+}, CD45^{-}, CD61^{+}
- Note that CD41 is glycoprotein 11b/111a and CD61 is glycoprotein 111a.

Acute basophilic leukaemia

Acute basophilic leukaemia is a very rare form of leukaemia occurring as an end-stage leukaemia in less than 1% of all cases of chronic myelogenous leukaemia. The proliferating cell is the basophil. Rarely are mature basophils present; rather there are basophil precursors with or without circulating blasts. When present, the blasts have a high N/C ratio, a round to oval or bilobed nucleus and a dispersed chromatin pattern with one to three nucleoli. The cytoplasm is basophilic and contains coarse basophilic and/or mast-cell-like large granules. Refer to Fig B6-41.

CYTOGENETICS

- There is no consistent chromosomal abnormality associated with acute basophilic leukaemia.

IMMUNOPHENOTYPE

- HLA-DR$^+$, CD13$^+$, CD33$^+$, CD123$^+$, CD11b$^+$, CD34$^+$, CD117$^-$

Acute panmyelosis with myelofibrosis

Acute panmyelosis with myelofibrosis involves abnormal proliferation of the three cell lines. It is associated with fibrosis of the bone marrow. Acute panmyelosis occurs predominantly in adults but has been known to occur in children. It differs from chronic idiopathic myelofibrosis in that patients present with a marked pancytopenia that is not associated with a splenomegaly. Few red cell teardrop poikilocytes are seen on the blood film. Myeloid precursors with dysplastic features together with atypical platelets may be present on the blood film.

Bone marrow aspiration often results in a dry tap due to the infiltration with fibrous tissue. When bone marrow is obtained, it is hypercellular with hyperplasia of the three cell lines. The megakaryocytes are prominent since they have dysplastic large and small hypolobulated nuclei in eosinophilic cytoplasm. They stain PAS positive and MPO positive. Refer to Figs B6-42 to B6-44.

CYTOGENETICS

- Often cytogenetically abnormal, however none specifically associated with this leukaemia.

IMMUNOPHENOTYPE

- CD13$^+$, CD33$^+$, CD41$^+$, CD61$^+$, CD117$^+$, CD34$^+$

Myeloid sarcoma

Myeloid sarcoma is a tumour composed of myeloid cells that occurs as a localised lesion in bone or at an extramedullary site. Its appearance may precede myeloid leukaemia or any other myeloproliferative or myelodysplastic disorder or it may occur concurrently with any of these disorders. The most commonly occurring myeloid sarcoma is the granulocytic sarcoma, composed of myeloblasts, neutrophils and neutrophil precursors; monoblastic sarcomas are seen less frequently. There are three types of myeloid sarcomas based on the degree of maturation of the myeloid cell:

- The blastic type, which is composed mainly of myeloblasts
- The immature type, which is composed mainly of myeloblasts and promyelocytes
- The differentiated type, which is composed mainly of promyelocytes, myelocytes and neutrophils.

Cytochemical stains can be performed on imprints made from the tumour to identify its cell lineage. Refer to Figs B6-45 and B6-46.

CYTOGENETICS

- Abnormalities detected in 55% of cases, including -7, +8, 11q23 (*MLL*) rearrangements, inv(16), +4, -16, 16q-, 5q-, 20q- and +11.
- t(8;21)(q22;q22) may be seen in childhood and less frequently in adults.

CYTOCHEMISTRY/IMMUNOPHENOTYPE

- A granulocytic sarcoma will stain positively with MPO and AS-D chloroacetate esterase while a monoblastic sarcoma will stain positively with the α-naphthol acetate esterase.
- $CD13^+$, CD33+, $CD117^+$ (myeloid markers)
- $CD11c^+$, $CD14^+$, $CD163^+$ (monocytoid markers)

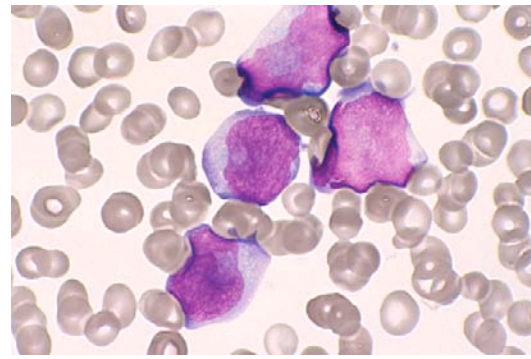


Figure B6-14
Acute myeloid leukaemia with minimal differentiation: peripheral blood film showing undifferentiated blast cells with a high N/C ratio, round nuclei with primitive chromatin pattern and nucleoli. The cytoplasm is basophilic and agranular. (x 1000)

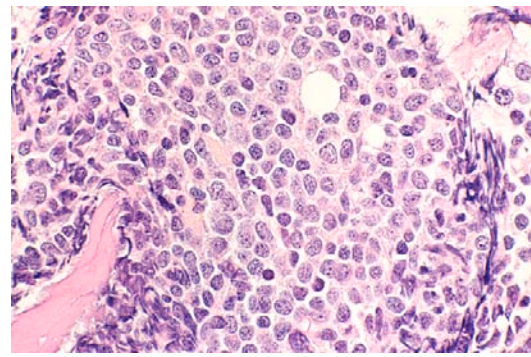


Figure B6-15
Acute myeloid leukaemia with minimal differentiation: bone marrow trephine from the above case B6-14. (H&E) (x 400)

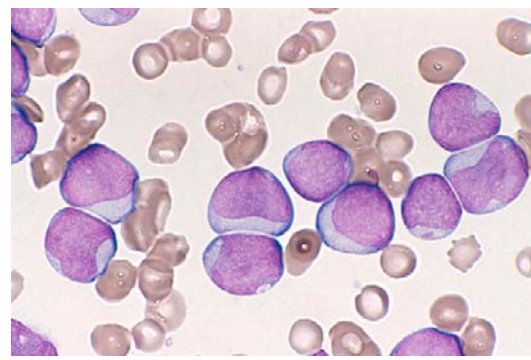


Figure B6-16
Acute myeloid leukaemia without maturation: peripheral blood film showing blast cells with a high N/C ratio, round nuclei, primitive chromatin pattern and nucleoli. The cytoplasm is basophilic and agranular, with an occasional Auer rod. Auer rods are composed of primary or azurophilic granules. They are found in myeloblasts but never in lymphoblasts; hence their presence may be used as a distinguishing feature to differentiate between myeloid and lymphoid acute leukaemia. (x 1000)

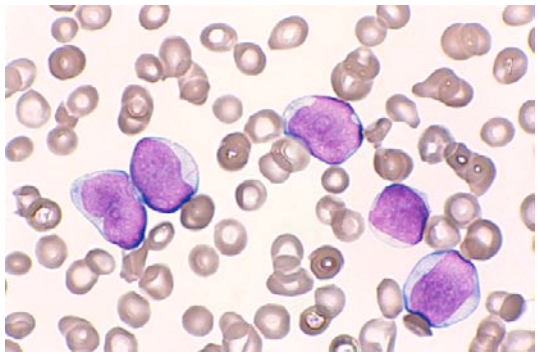


Figure B6-17
Acute myeloid leukaemia without maturation: peripheral blood film with blast cells showing the same features as seen in case B6-16. (x 1000)

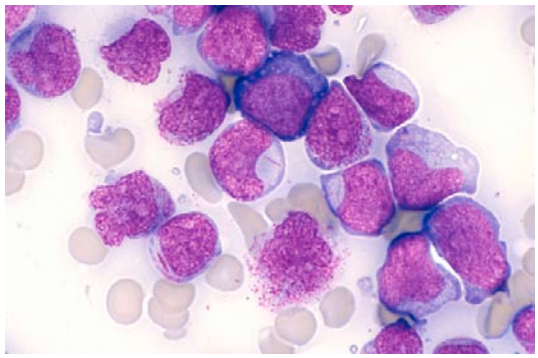


Figure B6-18
Acute myeloid leukaemia without maturation: bone marrow showing classical blast cells, some with Auer rods. (x 1000)

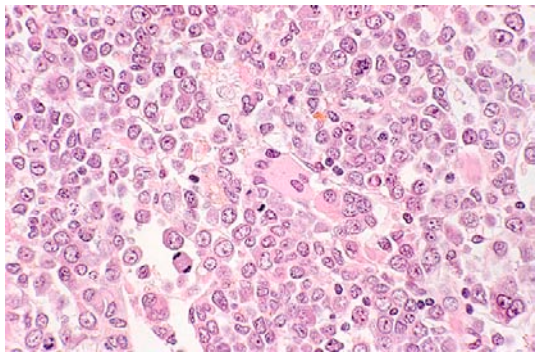


Figure B6-19
Acute myeloid leukaemia without maturation: bone marrow trephine showing a homogeneous infiltrate of myeloblasts. (H&E) (x 400)

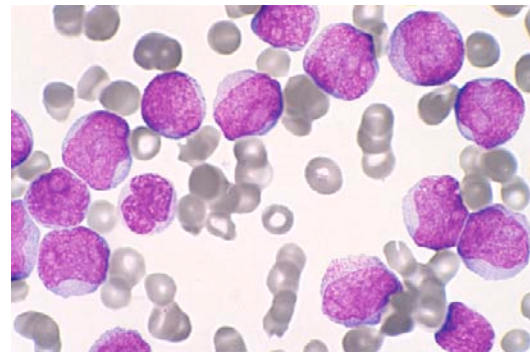


Figure B6-20
Acute myeloid leukaemia with maturation: peripheral blood film showing blast cells with and without azurophilic granules—a feature of maturation. (x 1000)

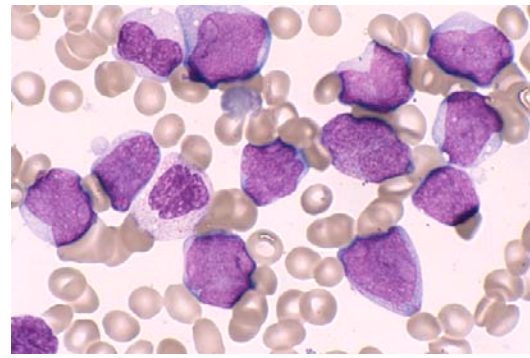


Figure B6-21
Acute myeloid leukaemia with maturation: bone marrow showing an infiltrate of myeloblasts and promyelocytes. (x 1000)

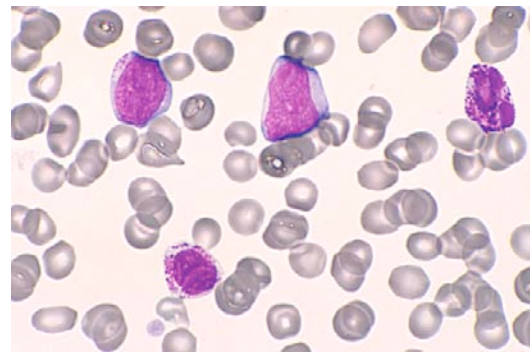


Figure B6-22
Acute myeloid leukaemia with maturation: peripheral blood film showing a myeloblast and promyelocyte with increased numbers of basophils. (x 1000)

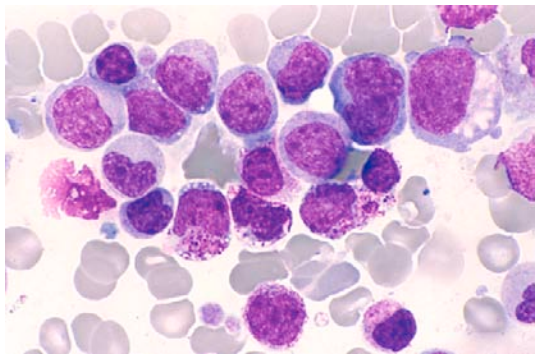


Figure B6-23
Acute myeloid leukaemia with maturation: bone marrow infiltrated with myeloblasts and increased numbers of basophils. (x 1000)

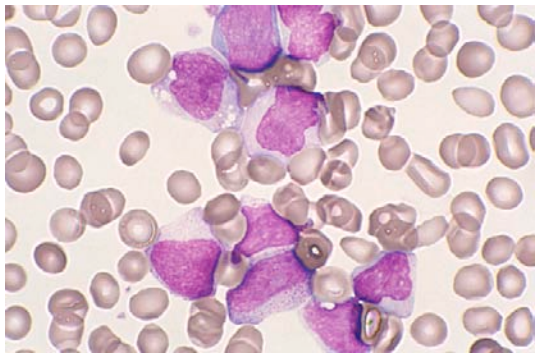


Figure B6-24
Acute myelomonocytic leukaemia: peripheral blood film showing myeloid and monocytoid precursors. (x 1000)

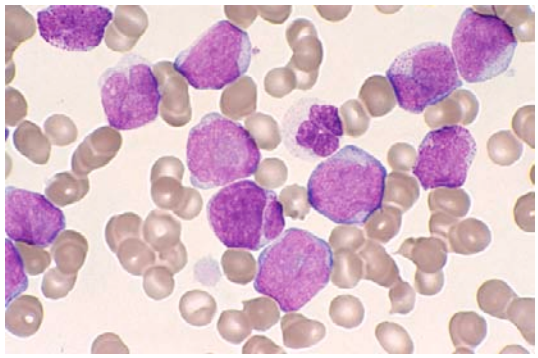


Figure B6-25
Acute myelomonocytic leukaemia: peripheral blood film showing myeloid and monocytoid precursors. (x 1000)

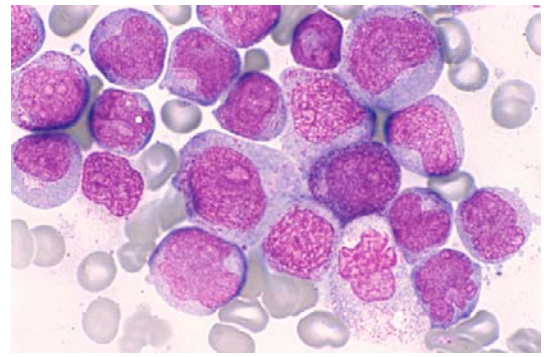


Figure B6-26
Acute myelomonocytic leukaemia: bone marrow showing myeloid and monocytoid precursors. (x 1000)

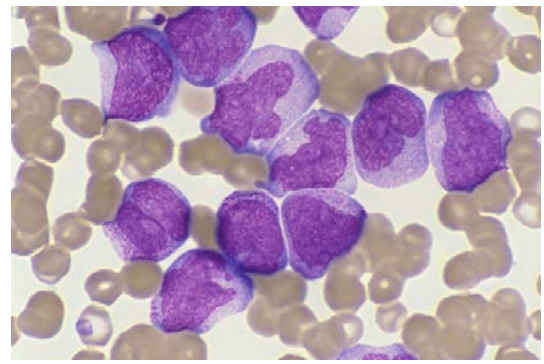


Figure B6-27
Acute myelomonocytic leukaemia: bone marrow showing both myeloid and monocytoid precursors. (x 1000)

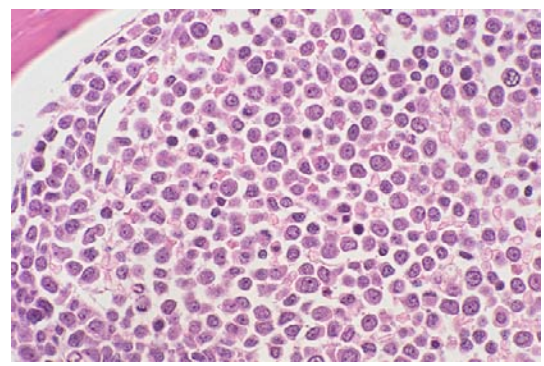


Figure B6-28
Acute myelomonocytic leukaemia: bone marrow trephine heavily infiltrated with myeloid and monocytoid precursors. (x 400)

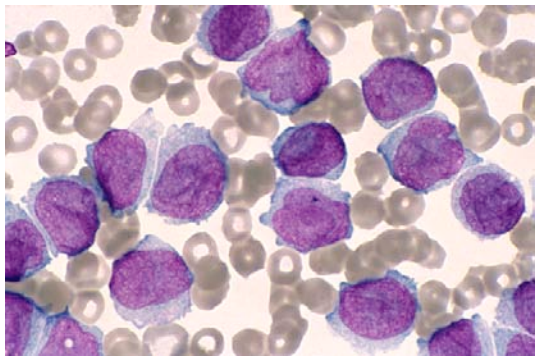


Figure B6-29
Acute monoblastic leukaemia: bone marrow showing a homogeneous population of undifferentiated monoblasts. (x 1000)

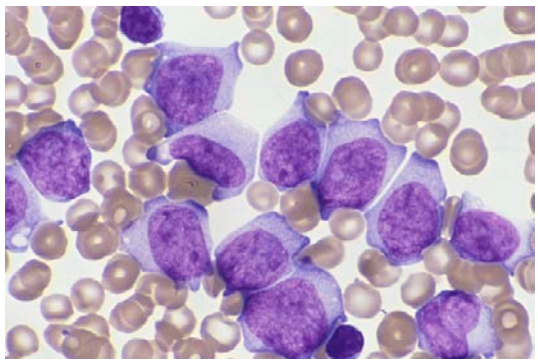


Figure B6-30
Acute monoblastic leukaemia: peripheral blood film showing monoblasts with rounded nuclei, fine chromatin pattern and prominent nucleoli. The cytoplasm is basophilic and agranular. (x 1000)

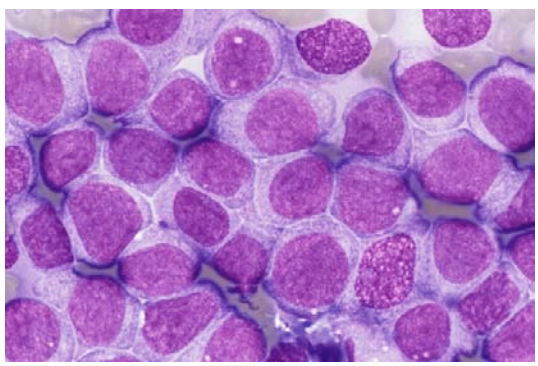


Figure B6-31
Acute monoblastic leukaemia: bone marrow showing a heavy infiltrate of monoblasts. (x 1000)

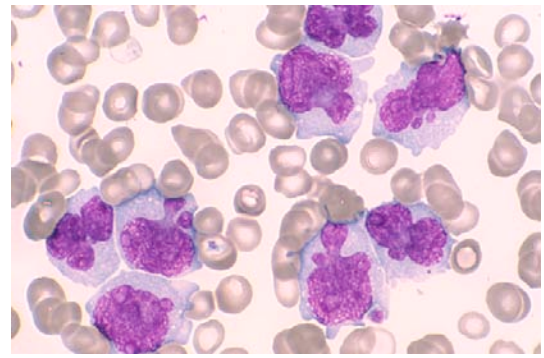


Figure B6-32
Acute monocytic leukaemia: bone marrow showing increased numbers of both monocytes and promonocytes. (x 1000)

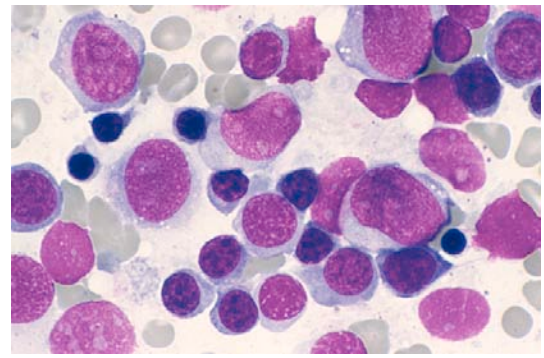


Figure B6-33
Erythroleukaemia (erythroid/myeloid): bone marrow showing increased numbers of erythroid precursors and myeloblasts. (x 1000)

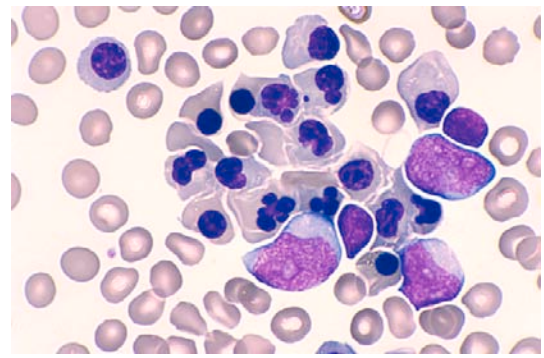


Figure B6-34
Erythroleukaemia (erythroid/myeloid): bone marrow showing marked nuclear asynchrony, nuclear budding and increased numbers of myeloblasts. (x 1000)

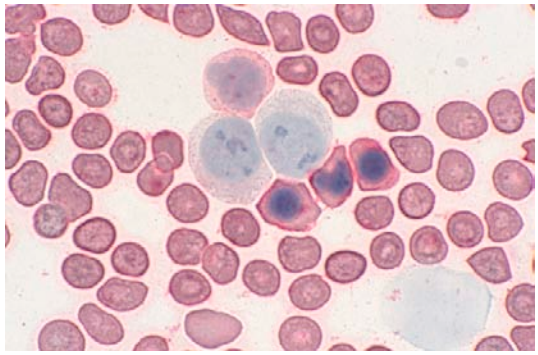


Figure B6-35
Erythroleukaemia (erythroid/myeloid): bone marrow stained with the transferrin receptor, glycophorin A: the erythroid precursors have stained positively while the myeloblasts are negative. (x 1000)

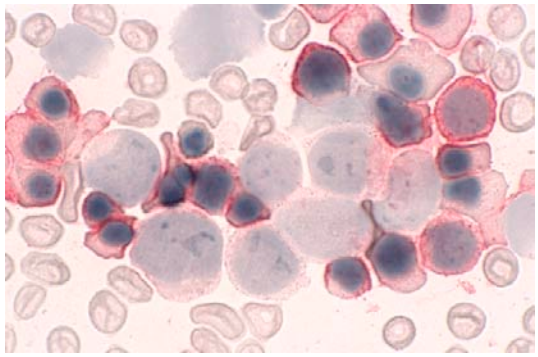


Figure B6-36
Erythroleukaemia (erythroid/myeloid): bone marrow stained with the transferrin receptor, glycophorin A: the erythroid precursors have stained positively while the myeloblasts are negative. (x 1000)

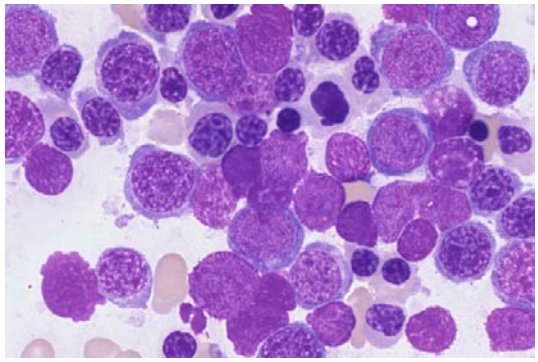


Figure B6-37
Erythroid leukaemia (pure erythroid): bone marrow infiltrated with erythroid precursors. (x 1000)

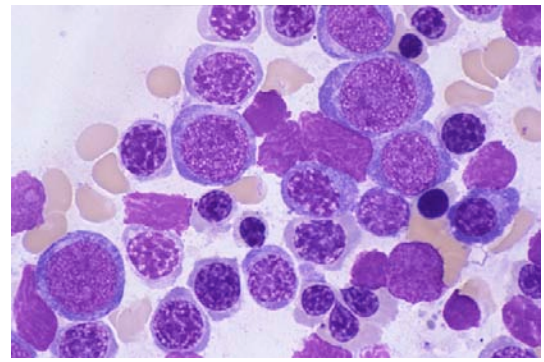


Figure B6-38
Erythroid leukaemia (pure erythroid): bone marrow infiltrated with erythroid precursors. (x 1000)

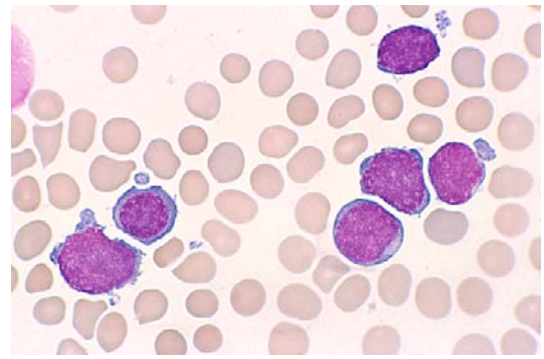


Figure B6-39
Acute megakaryoblastic leukaemia: peripheral blood film showing megakaryoblasts with cytoplasmic budding. These cytoplasmic buds will falsely elevate the platelet count should they break away from the cytoplasm. (x 1000)

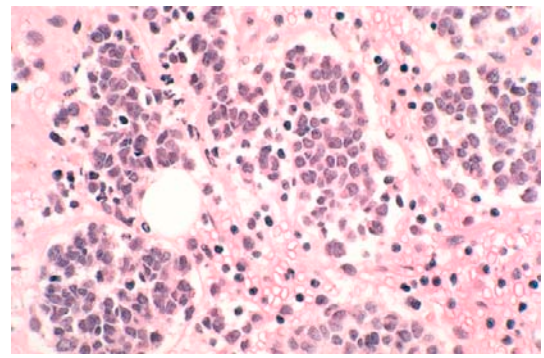


Figure B6-40
Acute megakaryoblastic leukaemia: bone marrow trephine infiltrated with megakaryoblasts. (H&E) (x 400)

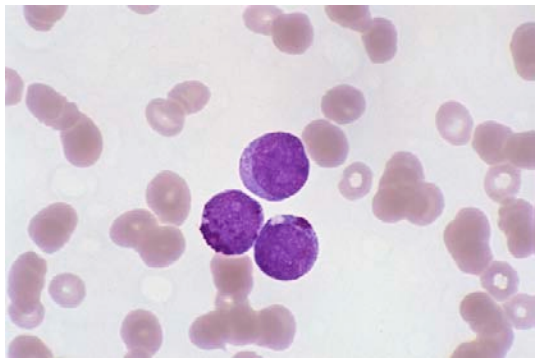


Figure B6-41
Acute basophilic leukaemia: peripheral blood film showing a myeloblast together with two basophil precursors whose cytoplasm is filled with mast-cell-like large granules. (x 1000)

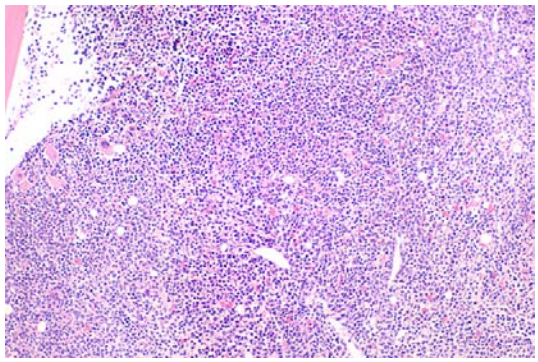


Figure B6-42
Acute panmyelosis with myelofibrosis: hypercellular bone marrow trephine with hyperplasia of all three cell lineages. (H&E) (x 100)

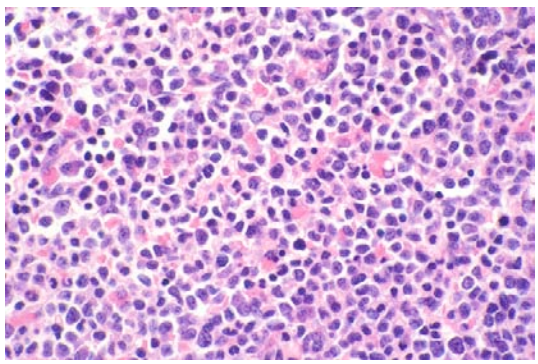


Figure B6-43
Acute panmyelosis with myelofibrosis: hypercellular bone marrow trephine with hyperplasia of all three cell lineages. (H&E) (x 400)

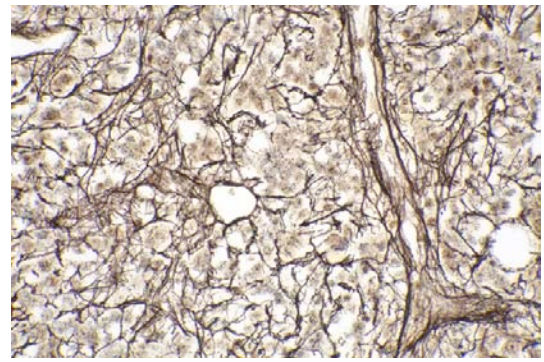


Figure B6-44
Acute panmyelosis with myelofibrosis: reticulin stain of the bone marrow trephine demonstrating dense fibrous tissue infiltrating the marrow. (x 400)

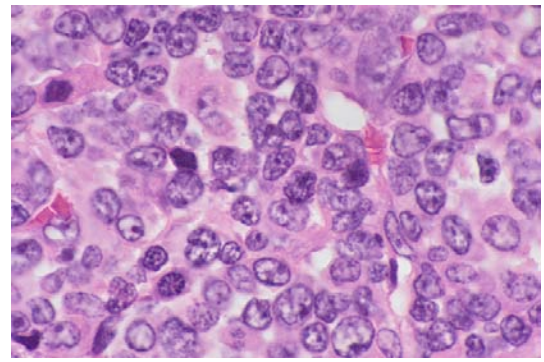


Figure B6-45
Myeloid sarcoma: a case of myeloid sarcoma (blastic type) of the kidney showing an infiltrate of myeloblasts. (H&E) (x 1000)

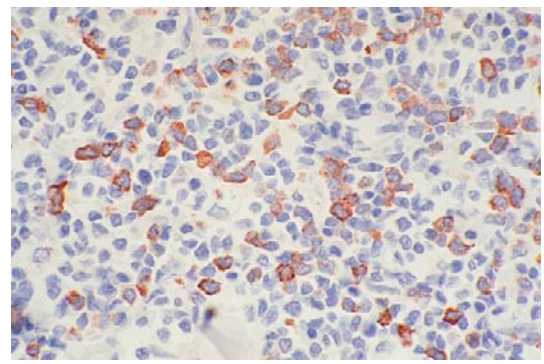


Figure B6-46
Myeloid sarcoma: myeloperoxidase stain demonstrating the presence of myeloblasts in the above case of myeloid sarcoma of the kidney. (x 400)

MYELOID PROLIFERATIONS RELATED TO DOWN SYNDROME

Transient abnormal myelopoiesis (TAM)

TAM is discussed in Part C.

Myeloid leukaemia associated with Down syndrome

Myeloid leukaemia associated with Down syndrome occurs in about 1–2% of children with Down syndrome. It occurs within the first 3 years of life and is typically a megakaryoblastic leukaemia. The children initially present with thrombocytopenia followed by a dysplastic phase similar to refractory cytopenia of childhood (RCC) proceeding to MDS with excess blasts and then to overt leukaemia.

The morphology of the blast cells is similar to that described under megakaryoblastic leukaemia. Budding of the cytoplasm is a classical feature. Dyserythropoiesis with megaloblastoid forms, bi and tri nucleated cells and nuclear fragmentation. Dysgranulopoiesis may be a feature of both the peripheral blood and bone marrow. The megakaryocytes are also dysplastic. Refer to Figs B6-47 and B6-48.

CYTOGENETICS

- In addition to trisomy 21 of Down syndrome, +8 (13–44%) and monosomy 7 (rare).

IMMUNOPHENOTYPE

- The blast cells in myeloid leukaemia associated with Down syndrome are similar to the blasts of TAM except that 50% of cases are CD34 negative.
- HLA-DR^{-}, CD34$^{-/+}$, CD56^{+}, CD117^{+}, CD13^{+}, CD33^{+}, CD4^{+}, CD7^{+}, CD45^{-}, CD41^{+}, CD61^{+}, CD71^{+}

Blastic plasmacytoid dendritic cell neoplasm

Blastic plasmacytoid dendritic cell neoplasm (BPDC) is a very aggressive neoplasm. It occurs in older patients with a median age of 64 years, and involves the skin as well as the bone marrow and peripheral blood. The neoplasm is derived from plasmacytoid dendritic cell precursors and is associated with cytopenias, especially thrombocytopenia. Approximately 10–20% of cases develop an associated acute myeloid leukaemia or a chronic myelomonocytic leukaemia (not to be confused with the myelodysplastic/myeloproliferative neoplasm CMML).

The blood film is characterised by the presence of medium-sized blast cells with a small amount of grey-blue cytoplasm. The bone marrow shows a mild to heavy infiltrate together with features of myelodysplasia, especially in the megakaryocytes.

CYTOGENETICS

- Complex karyotypes are common.
- 5q21, 5q34, 12p13, 13q13-21,15q and loss of chromosome 9.

CYTOCHEMISTRY/IMMUNOPHENOTYPE

- The MPO and α-naphthyl acetate esterase are both negative.
  TdT$^{-/+}$, CD4^{+}, CD43^{+}, CD45RA^{+}, CD56^{+}, CD123^{+}, CD68$^{+/-}$, CD34^{-}, CD117^{-}

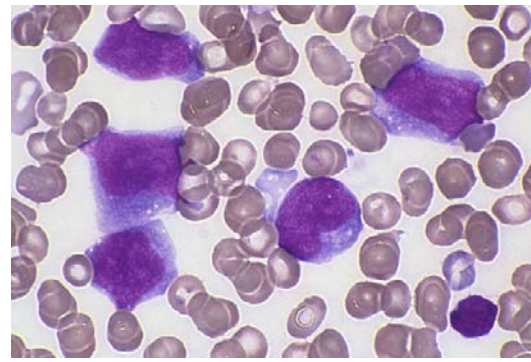


Figure B6-47
Myeloid leukaemia associated with Down syndrome: bone marrow showing an infiltrate of megakaryoblasts with fine chromatin pattern, nucleoli and agranular basophilic cytoplasm. (x 1000)

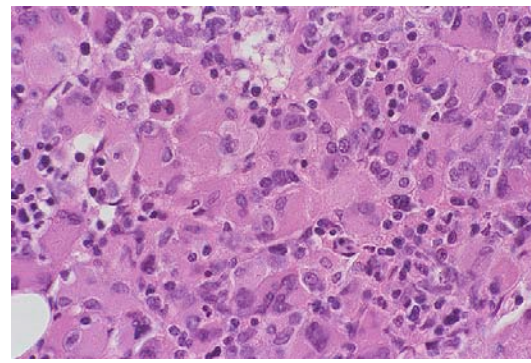


Figure B6-48
Myeloid leukaemia associated with Down syndrome: bone marrow trephine heavily infiltrated with megakaryoblasts and megakaryocytes. (H&E) (x1000)

ACUTE LEUKAEMIAS OF AMBIGUOUS LINEAGE

Acute leukaemias of ambiguous lineage are a group of leukaemias that do not demonstrate any one particular cell lineage. The ambiquous leukaemias encompass acute undifferentiated leukaemia (AUL) and mixed phenotype acute leukaemia (MPAL).

The term mixed phenotype acute leukaemia or bilineage acute leukaemia is used to describe a leukaemia where there are separate populations of blasts representing more than one lineage and the term biphenotypic leukaemia is used to describe acute leukaemia where the blasts co-express more than one lineage on the same cell.

Acute undifferentiated leukaemia (AUL)

This is a very rare form of acute leukaemia where the blasts do not express specific markers for either the myeloid or lymphoid lineage.

CYTOGENETICS

- Too few cases have been described to date to gather specific chromosomal abnormalities.

IMMUNOPHENOTYPE

- The blasts express no more than one membrane marker of any particular lineage.

Mixed phenotype acute leukaemia with t(9;22)(q34;q11.2); *BCR-ABL1*

This leukaemia meets the criteria for MPAL but also has a *BCR-ABL1* fusion gene or Ph chromosome present.

The blasts have a dimorphic population, one morphology resembling lymphoblasts and the other population resembling myeloblasts. However some cases have no distinguishing morphology between the blasts. Refer to Fig B6-49.

CYTOGENETICS

- All cases of MPAL have either a t(9;22)(q34;q11.2) or the *BCR-ABL1* fusion gene. In addition there may be other cytogenetic abnormalities and complex karyotypes.

IMMUNOPHENOTYPE

- Blast cells in the majority of cases express antigens for B and myeloid lineages, rarely do they express antigens for T and myeloid lineages.

Mixed phenotype acute leukaemia with t(v;11q23); *MLL* rearranged

This leukaemia meets the criteria for MPAL; the blasts have a translocation involving the mixed lineage leukaemia (*MLL*) gene. It is a very rare leukaemia seen mainly in infancy. The blasts have a dimorphic population, one population resembling monoblasts and the other population resembling lymphoblasts. However some cases have no distinguishing morphological features between the blasts. Refer to Figs B6-50 and B6-51.

CYTOGENETICS

- Must contain rearrangements of the *MLL* locus located at 11q23. Numerous translocation partners have been described. Additional abnormalities may also be seen.

IMMUNOPHENOTYPE

- $CD19^{+}$, $CD10^{-}$, $CD15^{+/-}$, $CD22^{+/-}$, $CD79a^{+/-}$ (lymphoid markers)
- $CD13^{+}$, $CD33^{+}$, $CD117^{+}$ (myeloid markers)
- $CD11c^{+}$, $CD14^{+}$, $CD163^{+}$ (monocytoid markers)

Mixed phenotype acute leukaemia, B/myeloid, NOS

The blasts in this leukaemia have either no distinguishing morphological features or there may be a dimorphic population of blasts, those resembling lymphoblasts and those resembling myeloblasts.

CYTOGENETICS

- Often cytogenetically abnormal however none specifically associated with this leukaemia.
- Abnormalities may include del(6q), 12p rearrangements, del(5q) and chromosome 7 anomalies. Complex karyotypes may be seen.

CYTOCHEMISTRY/IMMUNOPHENOTYPE

- MPO positive.
- $CD13^+$, $CD33^+$, $CD117^+$ (myeloid markers)
- Mature B-cell lineage markers in the presence of a B-cell population.

Mixed phenotype acute leukaemia, T/myeloid, NOS

This is a rare leukaemia occurring in both children and adults. The blasts either have no distinguishing morphological features, resembling the blasts of ALL or there may be a dimorphic population of blasts resembling both lymphoblasts and myeloblasts. Refer to Figs B6-52 and B6-53.

CYTOGENETICS

- Often cytogenetically abnormal; however, none specifically associated with this leukaemia.

CYTOCHEMISTRY/IMMUNOPHENOTYPE

- MPO positive.
- $CD13^+$, $CD33^+$, $CD117^+$ (myeloid markers)
- T-cell lineage markers including CD2, CD5 and CD7

Mixed phenotype acute leukaemia, NOS—rare types

In mixed phenotype acute leukaemia, NOS, the blasts demonstrate both the T and B lineage on the same cell. There have been some cases where the blasts demonstrate three lineages, B,T and the myeloid lineage on the same cell.

CYTOGENETICS

- No specific chromosomal abnormalities are seen in this leukaemia.

IMMUNOPHENOTYPE

- $HLA\text{-}DR^+$, $CD19^+$, $CD20^+$, $CD10^+$, $CD34^+$, TdT^+ (B lymphoid markers)
- $HLA\text{-}DR^-$, $CD2^+$, $CD5^+$, $CD7^+$, cyto$CD3^+$, TdT^+, $CD34^-$ (T lymphoid markers)

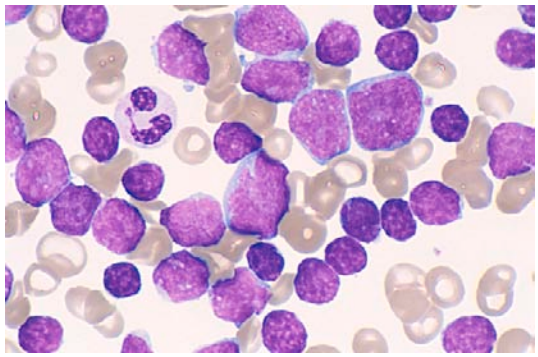


Figure B6-49
Mixed phenotype acute leukaemia with t(9;22)(q34;q11.2); *BCR-ABL1*: bone marrow showing a dimorphic population of blasts; one population resembling lymphoblasts and the other resembling myeloblasts. (x 1000)

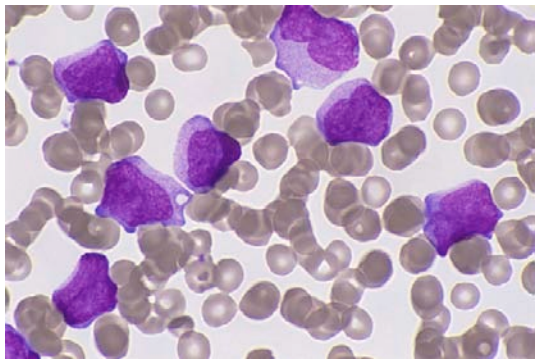


Figure B6-50
Mixed phenotype acute leukaemia with t(v;11q23);*MLL* rearranged: peripheral blood showing two populations of blast cells; lymphoblasts and monoblasts. (x 1000)

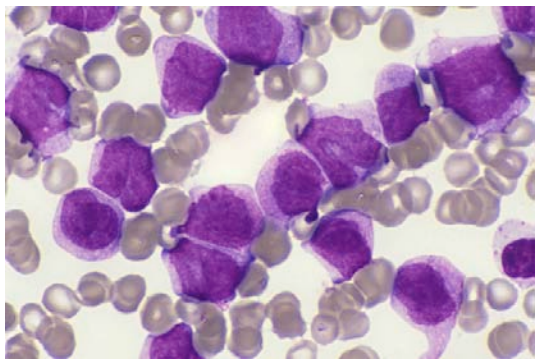


Figure B6-51
Mixed phenotype acute leukaemia with t(v;11q23);*MLL* rearranged: bone marrow showing a population of blasts which morphologically resemble monoblasts. (x 1000)

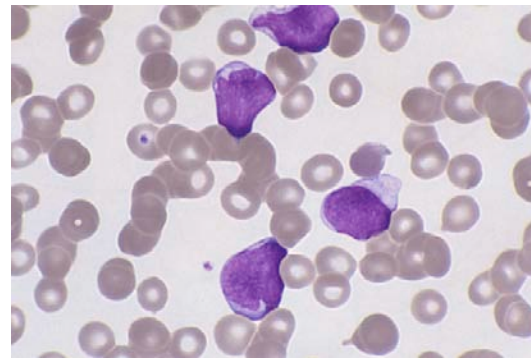


Figure B6-52
Mixed phenotype acute leukaemia, T/myeloid, NOS: peripheral blood film showing blast cells with no distinguishing features. (x 1000)

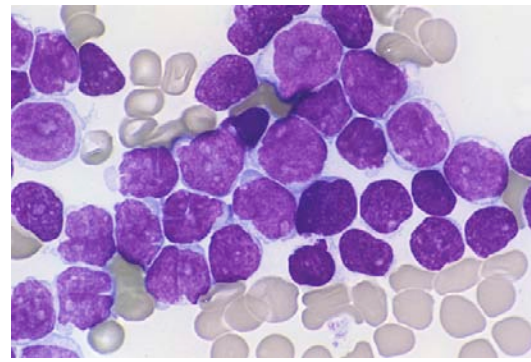


Figure B6-53
Mixed phenotype acute leukaemia, T/myeloid, NOS: bone marrow showing two populations of blast cells, lymphoblasts and myeloblasts. (x 1000)

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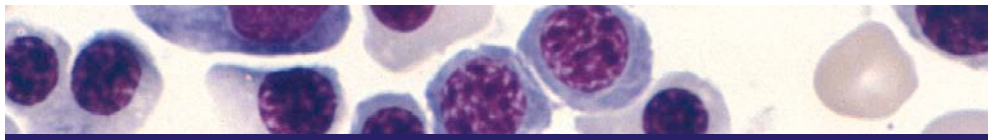


PART C

HAEMATOLOGY RELATING TO PAEDIATRICS

CORD BLOOD

Red cells

During the third trimester of pregnancy, red cell production progresses at a rate 3 to 5 times that of an adult. This rapid turnover results in fewer cell divisions; hence the red cells are macrocytic with a mean cell volume (MCV) ranging from 101 to 117 fL in diameter. This rapid turnover in production leads to increased numbers of red cell progenitors: approximately 12 to 16 times more than occurs in adult bone marrow.

The number of nucleated red cells seen in term cord blood ranges from 1 to 24 per 100 white blood cells. The reticulocyte count ranges from 3% to 7%. Refer to Fig C1-1.

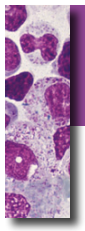
White cells

The total white cell count and differential in full-term cord blood vary according to the conduct of labour. A vaginal delivery is characterised by an absolute neutrophilia ranging from 4.4 to 21.0 × 10^9/L. The neutrophilia is the result of exertion expended by the infant during birth. A slight left shift with an occasional myelocyte and band form may be present.

Delivery by caesarian section, or the gentle lifting out of the newborn, results in a much lower white cell count and an inverted ratio of neutrophils to lymphocytes.

Platelets

The platelet count in full-term cord blood ranges from 190 to 430 × 10^9/L. The platelets are morphologically normal.



C1 Red cell disorders in the neonate and childhood

ANAEMIA AT BIRTH

Anaemia at birth may be due to obstetric accident or to occult haemorrhage prior to birth.

Obstetric accident

- The umbilical artery constricts shortly after birth, preventing blood flowing from the infant to the mother; however, the umbilical vein remains dilated, permitting blood to flow in the direction of gravity. The cord should be clamped while the infant is held within 30 cm above or below the level of the mother. If the infant is held too high, blood will flow back into the placenta, resulting in anaemia in the infant. If the infant is held too low, blood will flow from the placenta to the infant, resulting in an unacceptably high haemoglobin level in the infant.
- Accidental incision of the placenta at caesarian section can result in a massive fetal haemorrhage.

Occult haemorrhage prior to birth

- Fetomaternal haemorrhage may occur at any stage of pregnancy, particularly when there is a need to perform invasive procedures. It may also occur during labour and delivery. The presence of fetal cells in the mothers' blood may be demonstrated by performing a Kleihauer test. Refer to Fig A4-9.
- Twin-to-twin haemorrhage occurs in monozygotic multiple pregnancies, where there is a single placenta. The polycythaemic twin has a haemoglobin level as high as 300 g/L. It develops the hyperviscosity syndrome and has a high bilirubin. The anaemic twin may have a haemoglobin level as low as 35 g/L; it is pale and weak and has cardiac failure. Should the twin-to-twin haemorrhage be a gradual one, there will be a significant difference in birthweight between the twins. Refer to Figs C1-2 and C1-3.
- An intracranial haemorrhage can occur in very-low-weight premature infants. Anaemia at birth or a sudden drop in the red cell count and haemoglobin during the first few days of life are the initial clinical signs of an intracranial haemorrhage.

ERYTHROCYTOSIS AT BIRTH

A number of clinical conditions can lead to erythrocytosis at birth. These include hydrops, birth asphyxia, respiratory distress syndrome, pulmonary hypertension and meconium aspiration. The peripheral blood film in these conditions shows increased numbers of nucleated red cells and thrombocytopenia. The nucleated red cell count can often be as high as 500 to 800 nucleated red blood cells (NRBCs) per 100 white blood cells (WBCs). The haemoglobin and red cell count are usually normal. The reticulocyte count is mildly elevated and the DAT is negative. In cases of meconium aspiration, the neutrophils show toxic changes and a left shift.

A similar blood picture, together with thrombocytopenia, may be secondary to an intrauterine infection. The mechanism of this thrombocytopenia is not fully understood. Megakaryocyte numbers are normal or increased. In such cases, a TORCH (Toxoplasma, Others, Rubella, Cytomegalovirus, Herpes simplex) screen should be performed on both the infant and the mother. This group of congenital infections, together with syphilis, can cause neonatal thrombocytopenia. Refer to Fig C1-4.

DEFICIENCY ANAEMIAS

Iron-deficiency anaemia

There is a rapid accumulation of iron by the fetus during gestation. This increase occurs regardless of the iron status of the mother. Iron deficiency in the mother will have little effect on the concentration of haemoglobin or serum ferritin in the newborn infant.

Changes in iron balance and rate of erythropoiesis occur in the first few days after birth. These changes coincide with improved access to oxygen via the lungs and lead to a decrease in erythropoietin production and hence a reduction in the rate of erythropoiesis. The haemoglobin falls, as do the MCV and the reticulocyte count. Iron released from this red cell breakdown is salvaged by the reticuloendothelial system, thus increasing neonatal iron stores. The reduction in erythropoiesis continues for the next 6 to 8 weeks. After this period, there is an increase in the reticulocyte response as the haemoglobin concentration gradually increases to a value of about 125 g/L. The iron stored during this period is sufficient to provide adequate stores for the doubling of body weight occurring during the first 6 months of life. After this period, absorption of dietary iron is necessary to maintain a normal iron balance. The period from 6 months to at least 5 years of age is characterised by a microcytic hypochromic blood picture and a low ferritin. The ferritin remains low until replenished by an adequate diet.

Since haematological measurements are frequently used as screening procedures to detect abnormalities within a population, recognition of these variables in the paediatric age group will prevent needless laboratory investigation.

Megaloblastic anaemia

Megaloblastic anaemia in infancy is due almost invariably to vitamin B_{12} (cobalamin) deficiency. Vitamin B_{12} deficiency is seen mainly in infants born of strict vegan nursing mothers. Such infants can develop neurological symptoms if vitamin B_{12} is not replaced promptly.

Folic acid deficiency is very rare in infants. This is especially so because the folic acid levels in both serum and red cells are increased in the newborn. As the red cell number and haemoglobin drop in the first few days of life, so does the level of folic acid. In premature infants, this drop may be even greater, especially if the infant has feeding difficulties. Thus it is common practice to give premature infants folic acid supplements to prevent deficiency.

HAEMOGLOBIN DISORDERS

These are discussed in Chapter A4.

ALLO-IMMUNE HAEMOLYTIC ANAEMIA

ABO and Rhesus (Rh) haemolytic disease of the newborn are examples of allo-immune haemolytic anaemia.

ABO haemolytic disease of the newborn

ABO haemolytic disease of the newborn occurs most commonly in group O mothers and affects babies of groups A and B. The chief manifestation of ABO haemolytic disease is jaundice. It usually appears within the first 24 hours of life and is generally mild, especially when compared with the jaundice seen in Rh haemolytic disease of the newborn. The haemoglobin concentration is usually normal and the blood film shows increased numbers of spherocytes that stand out against a background of normochromic round macrocytes. The DAT may be negative or weakly positive. Refer to Fig C1-5.

Rh haemolytic disease of the newborn

When anti-D antibody present in a sensitised Rh-negative mother enters the fetal circulation, Rh-positive fetal cells are destroyed, giving rise to a haemolytic process known as Rh haemolytic disease of the newborn. This haemolytic process is characterised by jaundice, a low haemoglobin level (usually <140 g/L), a raised reticulocyte count (>7% and sometimes as high as 30–40%) and an increase in the number of NRBCs, that is, more than 24 NRBCs/100 WBCs.

In severe cases of Rh haemolytic disease of the newborn, extremely high numbers of NRBCs will be seen. The DAT is strongly positive. Refer to Fig C1-6.

OTHER HAEMOLYTIC ANAEMIAS

These are discussed in Chapter A3.

RED CELL ENZYME DEFICIENCIES

Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency

G-6-PD is the enzyme controlling the first step in a chain of reactions that constitute the pentose phosphate pathway. Patients with G-6-PD deficiency have a normal blood picture until subjected to an oxidative challenge either in the form of an oxidant drug or after ingestion of fava beans (favism). Either of these challenges induces a haemolytic anaemia. Within 6–24 hours, the patient passes dark urine and the haemoglobin level drops significantly. The blood film shows spherocytes, bite cells and blister cells. A supravital stain such as methyl violet, cresyl blue or brilliant cresyl blue will demonstrate a reticulocytosis as well as the presence of Heinz bodies.

G-6-PD deficiency is prevalent in Africa, Southeast Asia and the countries bordering the Mediterranean. Refer to Figs A3.23 and C1-7.

Pyruvate kinase (PK) deficiency

PK is an erythrocyte glycolytic enzyme involved in the Embden-Myerhof pathway of metabolism. Deficiency of this enzyme is associated with chronic haemolysis and thus anaemia, jaundice and splenomegaly. There is a great variation in the clinical severity of PK deficiency. Anaemia may be profound, presenting in early infancy and requiring frequent blood transfusions for survival, or it may be so mild that the deficiency may not be discovered until late childhood or even adulthood. Anaemia, when present, is lifelong and varies little in intensity except during pregnancy.

The blood film shows a red cell picture consistent with that of a haemolytic anaemia with the presence of occasional prickle cells (cells with irregular projections). The number of prickle cells is strikingly increased following splenectomy. Refer to Figs C1-8 and C1-9.

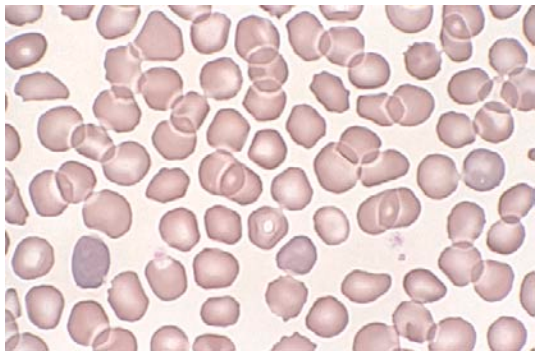


Figure C1-1
Cord blood from a full-term newborn showing normochromic macrocytes and reticulocytes. An occasional target cell, spherocyte and nucleated red cell may also be present. (x 1000)

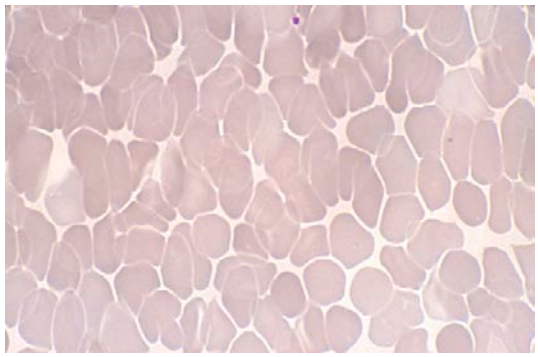


Figure C1-2
Twin-to-twin transfusion: blood film from the polycythaemic twin with a haemoglobin of 260 g/L. (x 1000)

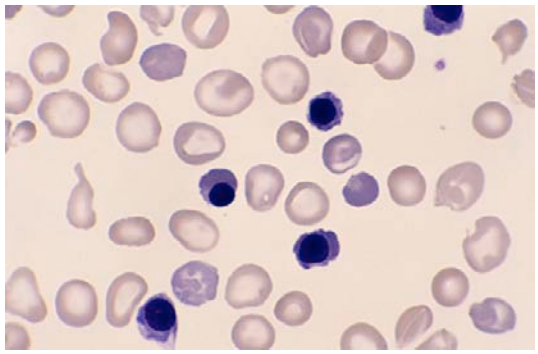


Figure C1-3
Twin-to-twin transfusion: blood film from the anaemic twin with a haemoglobin of 39 g/L showing increased reticulocytes and NRBCs. (x 1000)

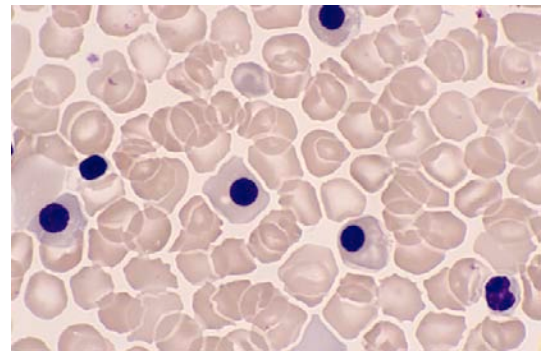


Figure C1-4
Erythrocytosis: peripheral blood film from a newborn showing 1086 NRBCs/100 WBCs and a reticulocyte count of 7.0%. A DAT performed on this infant was negative. (x 1000)

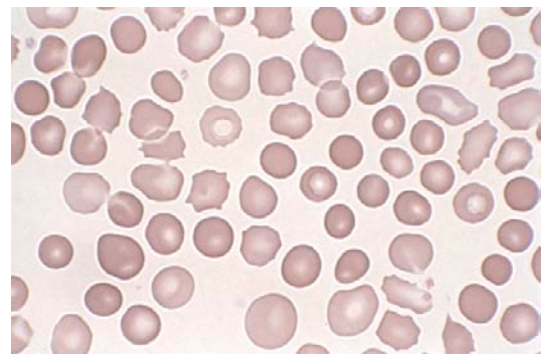


Figure C1-5
ABO haemolytic disease of the newborn showing increased numbers of spherocytes. (x 1000)

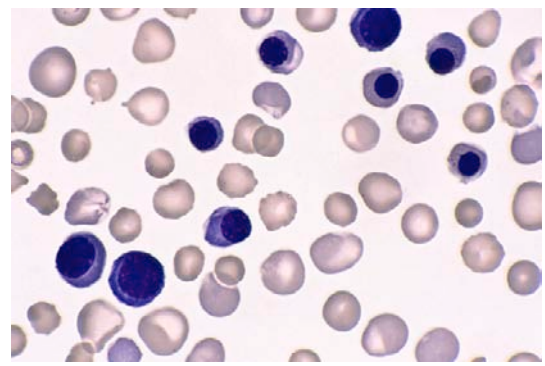


Figure C1-6
Rh haemolytic disease of the newborn showing increased numbers of nucleated red cells and reticulocytes. (x 1000)

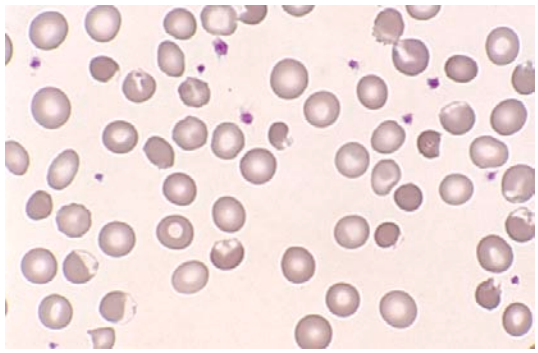


Figure C1-7
G-6-PD deficiency: peripheral blood film showing both bite and blister cells following ingestion of fava beans (favism). (x 1000)

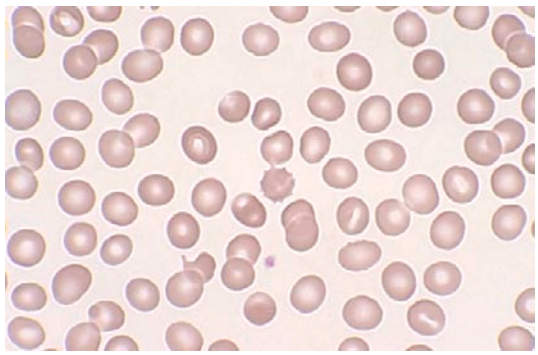


Figure C1-8
PK deficiency: peripheral blood film showing a single prickle cell in the centre of the field. (x 1000)

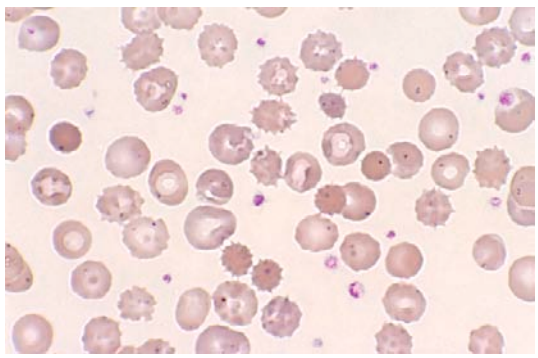
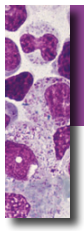


Figure C1-9
PK deficiency post-splenectomy: peripheral blood film showing increased numbers of prickle cells as well as post-splenectomy features. (x 1000)



C2 Bone marrow failure

Bone marrow failure is characterised by reduced production of all three lineages within the bone marrow: erythrocytes, granulocytes and platelets. This gives rise to pancytopenia in the peripheral blood.

Bone marrow failure has been divided into four groups:

- Aplastic anaemia
- Disorders characterised by a pancytopenia
- Disorders characterised by a single cytopenia
- Disorders characterised by a leucoerythroblastic blood picture.

APLASTIC ANAEMIA

Aplastic anaemia is a prime example of bone marrow failure. It is characterised by reduced bone marrow production of all three cell lineages: erythrocytes, granulocytes and platelets. This reduction causes peripheral blood pancytopenia and may be either acquired or inherited. In severe aplastic anaemia, the granulocyte or neutrophil count is $<0.5 \times 10^9$/L, the platelet count is $<20 \times 10^9$/L and the reticulocyte count is <1%. The anaemia may be normocytic or macrocytic. The bone marrow is hypocellular, with empty fragments and increased fat spaces.

Aplastic anaemia may be inherited or acquired. Acquired aplastic anaemia may be secondary to certain drugs, chemicals and viruses (parvovirus, Epstein-Barr virus, cytomegalovirus and human immunodeficiency virus). Inherited aplastic anaemia includes disorders such as Fanconi anaemia, dyskeratosis congenita and Shwachman-Diamond syndrome. Refer to Fig C2-1.

PANCYTOPENIAS

Fanconi anaemia (FA)

FA is an autosomal-recessive disorder encompassing a group of patients with familial aplastic anaemia and physical anomalies. There is clinical variability amongst patients with FA. Three groups of patients have been described: those with physical anomalies and normal haematological findings, those with no physical anomaly but with abnormal haematological findings and those with both a physical anomaly and abnormal haematological findings.

The haematological features in Fanconi anaemia lead to the gradual onset of bone marrow failure. Patients present initially with thrombocytopenia that is followed by neutropenia and then anaemia. The red cells are often macrocytic with an MCV of more than 100 fL. There is an increase in HbF production. Erythropoietin levels are also increased. The bone marrow is hypercellular in early FA (before the development of pancytopenia). When the patient becomes aplastic, the bone marrow is hypocellular and fatty with few haemopoietic elements. There is an increase in the number of lymphocytes, reticulum cells, mast cells and plasma cells. FA is characterised by abnormal chromosome fragility. Spontaneous

breaks, gaps, rearrangements, exchanges and endoreduplications occur. This abnormal chromosome fragility can be exacerbated by the addition of diepoxybutane (DEB) in chromosome culture. FA homozygotes have a mean of 8.96 breaks per cell with DEB culture compared with 0.06 breaks in normal subjects.

Dyskeratosis congenita (DC)

DC is a disorder of the mucocutaneous and haemopoietic systems. The mucocutaneous aspect of the disorder is characterised by a triad of events: pigmentation of the upper body, mucosal leucoplakia and nail dystrophy. The haemopoietic aspect of the disorder is characterised by aplastic anaemia occurring in the second decade of life and in approximately 50% of cases. A small percentage of cases have a predisposition to develop cancer in the third to fourth decade of life.

DC has three patterns of inheritance: X-linked recessive, autosomal-recessive and autosomal-dominant.

The haematological features of DC are anaemia, leucopenia and thrombocytopenia. The bone marrow is initially hypercellular but gradually becomes hypocellular, culminating in aplastic anaemia. The red cells are macrocytic and HbF is increased. Chromosome fragility in DC is variable when DEB is added to culture. Refer to Fig C2-2.

Shwachman-Diamond syndrome (SDS)

SDS is an autosomal-recessive disorder characterised by pancreatic dysfunction, malabsorption, steatorrhoea, failure to thrive, short stature and (in some cases) mental retardation. Fatty infiltration of the pancreas leads to pancreatic insufficiency with low or absent duodenal trypsin, amylase and lipase.

The main haematological feature of SDS is neutropenia. Anaemia and thrombocytopenia may occur in some cases. The neutropenia is intermittent rather than constant and is associated with skin infections and pneumonia. Abnormal neutrophil mobility, migration and chemotaxis lead to recurrent infections. An increase in HbF, even without anaemia, is common. Bone marrow hypoplasia with maturation arrest in the myeloid line is present.

Chromosomes are normal and there is no increased breakage following clastogenic stress with DEB.

SINGLE CYTOPENIAS

Diamond-Blackfan anaemia (DBA)

DBA is a congenital pure red cell aplasia diagnosed in children usually less than 1 year old; rarely is it diagnosed after 6 years. Specific physical anomalies are associated with DBA. Craniofacial abnormalities include a flat nasal bridge, wide-set eyes and a thick upper lip. Deafness and short stature are also features of the disease.

The haemoglobin levels at birth have a mean value of 70 g/L and can be as low as 26 g/L. The red cells are invariably macrocytic. There is reticulocytopenia. The white cell and platelet counts are normal. Platelet function is also normal. Fetal haemoglobin is usually increased and is distributed heterogeneously.

The bone marrow shows erythroid hypoplasia. The myeloid cells and megakaryocytes are normal in number while the lymphocytes are increased.

Inheritance is sporadic. Some cases are inherited in an autosomal-dominant manner and others in an autosomal-recessive manner. Refer to Fig C2-3.

Transient erythroblastopenia of childhood (TEC)

TEC is an acquired red cell aplasia occurring in previously healthy children usually between the ages of 1 and 3 years. It is frequently preceded by a viral infection some 2

months prior to presentation. It is a self-limiting disease with patients often presenting during the recovery phase of the infection.

Haemoglobin levels range from 24 to 110 g/L, with a mean of 58 g/L. The reticulocyte count is less than 1%. The white cell count is usually normal although neutropenia may occur. The platelet count is normal except in those cases preceded by a viral infection such as parvovirus B19 infection, where the count may be significantly reduced.

The bone marrow shows erythroid hypoplasia. There is often a maturation arrest in the erythroid lineage. Should the bone marrow be sampled during the recovery phase, erythropoiesis will be markedly increased.

Spontaneous and complete recovery usually occurs within 2 months of diagnosis.

Refer to Figs C2-4 and C2-5.

Congenital dyserythropoietic anaemia (CDA)

CDA is an inherited anaemia characterised by abnormal, ineffective erythropoiesis. It is associated with mild to moderate anaemia and an ineffective reticulocyte response.

Specific morphological changes occur within the red cell series in both the bone marrow and the peripheral blood. The red cell changes within the bone marrow include megaloblastoid erythropoiesis, binucleated and budding nuclei and cytoplasmic connections or bridging. The peripheral blood is macrocytic with punctate basophilia.

CDA has been classified into three separate groups according to morphological and serological findings. Refer to Fig C2-6.

TYPE I

Type I CDA is inherited in an autosomal-recessive manner. Patients have splenomegaly and mild to moderate anaemia. The red cells are macrocytic with an average MCV of 100 fL. Anisocytosis, poikilocytosis and basophilic stippling are features of the peripheral blood film. The bone marrow changes affect mainly the early erythroblasts. There are megaloblastoid changes, binucleated forms and cytoplasmic bridging.

The acidified serum test and the sucrose lysis test are negative.

TYPE II

Type II CDA is often referred to as HEMPAS (Hereditary Erythroblastic Multinuclearity with a Positive Acidified Serum test). Inheritance is autosomal-recessive. Patients have splenomegaly as well as hepatomegaly and jaundice; gallstones are common. The anaemia is mild to moderate, with an average MCV of 93 fL. Anisocytosis, poikilocytosis, teardrops and basophilic stippling are features of the peripheral blood film. The bone marrow changes affect mainly the late erythroblasts. Binucleated and multinucleated erythroblasts are present but cytoplasmic bridging is not seen.

The acidified serum test is positive and the sucrose lysis test is negative.

TYPE III

Type III CDA is inherited in an autosomal-dominant manner. Splenomegaly is only seen occasionally. The anaemia is usually mild with a normal to slightly increased MCV. Anisocytosis and poikilocytosis are features of the peripheral blood film. The bone marrow shows multinuclearity, sometimes with up to 12 nuclei in one erythroblast.

The acidified serum test and the sucrose lysis test are negative.

Congenital sideroblastic anaemia

Congenital sideroblastic anaemia is inherited as an X-linked recessive disorder. It presents in childhood, predominantly but not exclusively in males. It is characterised by a dimorphic blood picture. Female carriers show a mild microcytic hypochromic picture.

The initial presentation is that of anaemia. The bone marrow is mildly hypercellular, with erythroid hyperplasia. Some of the erythroblasts show micronormoblastic maturation as well as defective haemoglobinisation with ragged and vacuolated cytoplasm. The Perl's Prussian blue stain shows the presence of ring sideroblasts. These ring sideroblasts represent the presence of perinuclear mitochondria containing large deposits of inorganic iron. Haem production is impaired and there is ineffective erythropoiesis. Iron stores are usually increased. Neutropenia and abnormal neutrophil function as well as thrombocytopenia and abnormal platelet function suggest that congenital sideroblastic anaemia is a broad-spectrum haemopoietic stem cell defect. Refer to Figs C2-7 and C2-8.

Parvovirus B19 infection

Infection with parvovirus B19 may lead to transient erythroblastopenia in a variety of genetically inherited anaemias such as hereditary spherocytosis, PK deficiency, sickle cell disease, autoimmune haemolytic anaemia and thalassaemia. Parvovirus B19 may also cause thrombocytopenia and/or neutropenia in these patients as well as in immunosuppressed patients and in otherwise healthy children.

The virus infects the erythroid progenitors using the P antigen as a receptor. It prevents replication and maturation of the infected cell. The bone marrow in these infected patients shows a relative absence of erythroid precursors as well as the presence of giant proerythroblasts. The aplasia is transient and the marrow recovers, usually within 1–2 weeks. Refer to Figs C2-9 and C2-10.

LEUCOERYTHROBLASTOSIS

Osteopetrosis

Osteopetrosis or Albers-Schönberg disease is often referred to as 'marble bone' disease. The osteoclasts in osteopetrosis, although normal in number and morphology, are functionally abnormal in that they are unable to reabsorb and remodel bone. This defect results in loss of haemopoietic tissue due to obliteration of the marrow cavity.

Two forms of osteopetrosis occur depending on the mode of inheritance. The milder form is inherited as an autosomal-dominant disorder. It is diagnosed in late childhood and is characterised by the presence of dense sclerotic bones that fracture easily. There are no specific haematological findings associated with the milder form.

The severe or malignant form of osteopetrosis is inherited as an autosomal-recessive disorder. It is diagnosed in infancy or early childhood and is also characterised by the presence of dense sclerotic bones. This more severe form is associated with clinical abnormalities such as microcephaly, blindness, deafness and cranial nerve palsies.

Haematological complications are severe in the malignant form of osteopetrosis. They include anaemia, leucopenia and thrombocytopenia. The red cells are macrocytic. The blood film is leucoerythroblastic, with teardrop poikilocytes. Refer to Figs C2-11 and C2-12.

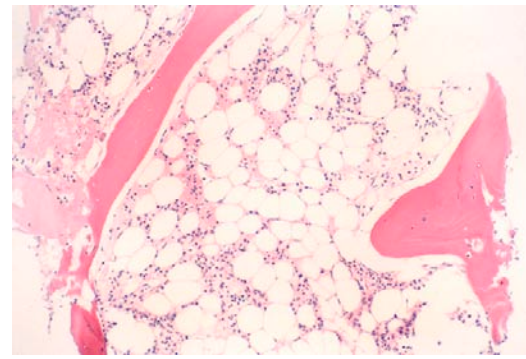


Figure C2-1
Aplastic anaemia: hypocellular bone marrow trephine from a 2-year-old child showing increased fat spaces. (x 100)

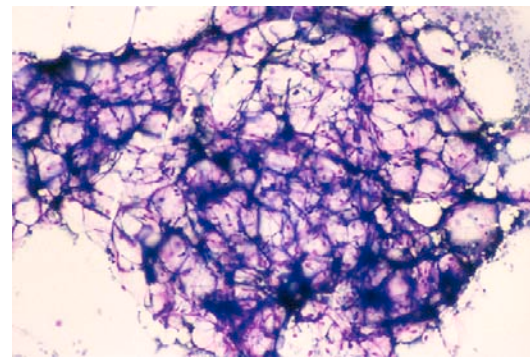


Figure C2-2
Dyskeratosis congenita: hypocellular bone marrow fragment in a 12-year-old child. (x 100)

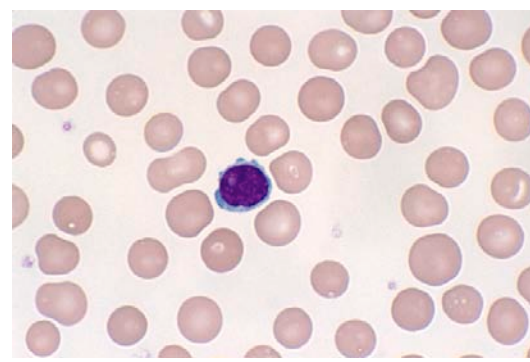


Figure C2-3
Diamond-Blackfan anaemia: peripheral blood film from a 6-month-old child with a haemoglobin of 46 g/L and an MCV of 124 fL. (x 1000)

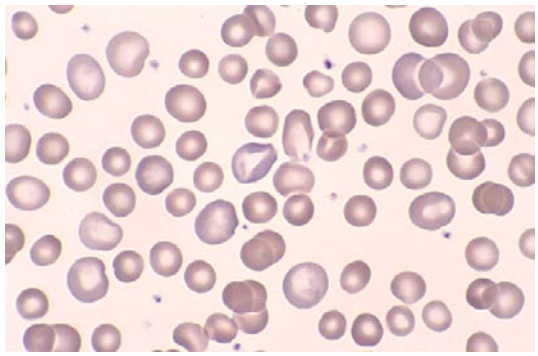


Figure C2-4
Transient erythroblastopenia of childhood (TEC): peripheral blood film from a 2-year-old child recovering from TEC with a reticulocyte count of 13.0%. (x 1000)

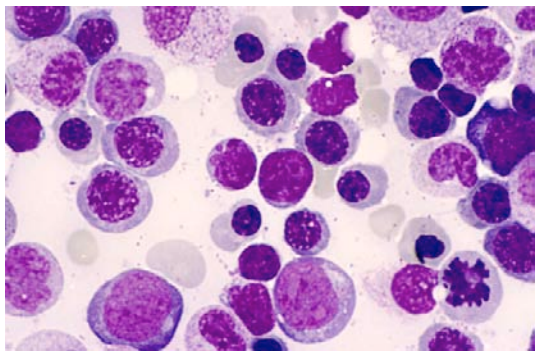


Figure C2-5
Transient erythroblastopenia of childhood: bone marrow sampled during recovery, showing markedly increased erythropoiesis. (x 1000)

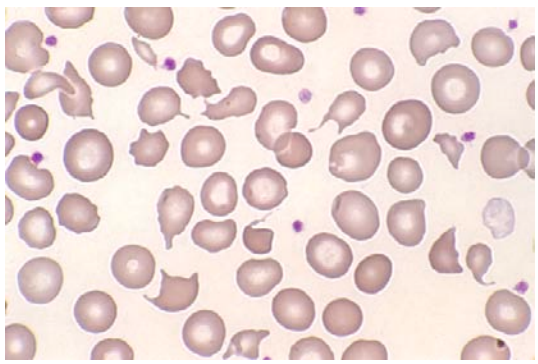


Figure C2-6
Congenital dyserythropoietic anaemia (Type 1): peripheral blood film from a 10-day-old neonate showing marked anisocytosis and poikilocytosis with many red cell fragments. (x 1000)

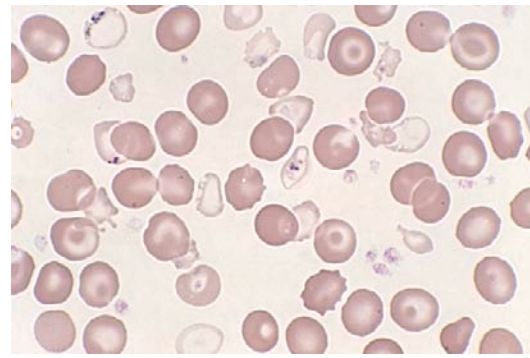


Figure C2-7
Congenital sideroblastic anaemia: peripheral blood film from a male child showing a marked dimorphic blood picture (transfusion-dependant) with Pappenheimer bodies. (x 1000)

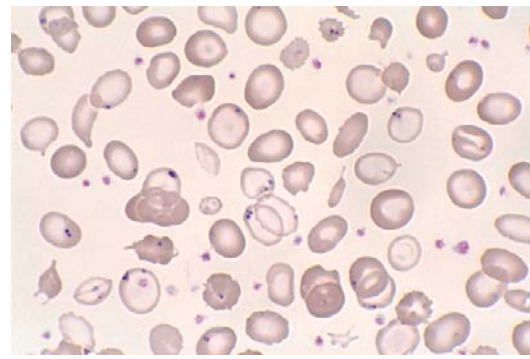


Figure C2-8
Congenital sideroblastic anaemia: peripheral blood film from a 21-year-old male with transfusion-dependent anaemia showing a microcytic blood picture with many Pappenheimer bodies. (x 1000)

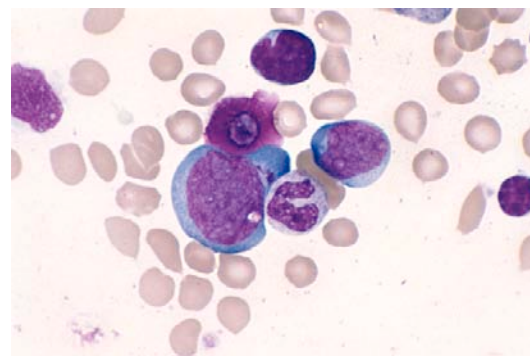


Figure C2-9
Parvovirus B19 infection: bone marrow from a 7-month-old child showing the presence of a giant proerythroblast. (x 1000)

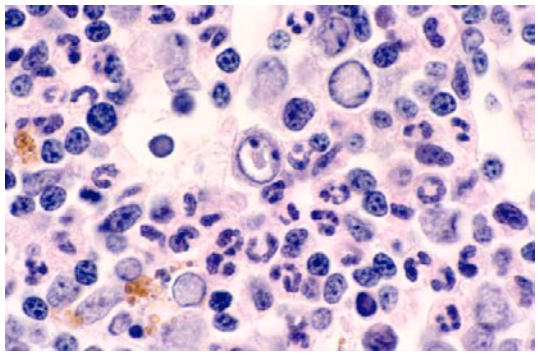


Figure C2-10
Parvovirus B19 infection: bone marrow trephine showing an intracellular viral inclusion within a giant proerythroblast. (x 1000)

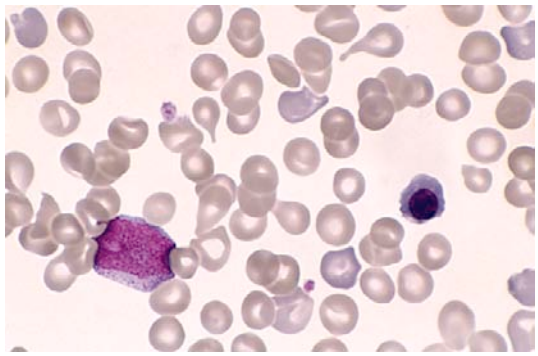


Figure C2-11
Osteopetrosis: leucoerythroblastic blood picture with teardrop poikilocytes from a 7-week-old infant with the malignant form of osteopetrosis. (x 1000)

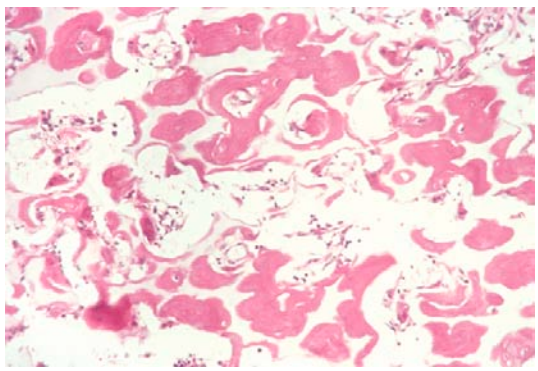
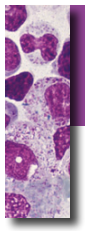


Figure C2-12
Osteopetrosis: bone marrow trephine showing the 'marble bone' effect in a young child. (H&E) (x 50). (Courtesy of Dr H Smith)



C3 Benign disorders of leucocytes in the neonate and childhood

NEUTROPHILIA

Neonatal neutrophilia

An absolute neutrophilia, present in the first few days of life, is associated with vaginal birth and hence is not present when birth is by caesarian section. The absolute neutrophil count on day 1 ranges from 4.4 to 21.0 × 10^9/L, and by day 7 falls to between 1.5 and 15.3 × 10^9/L. This early physiological neutrophilia is sometimes left-shifted, with an occasional myeloid precursor (e.g. myelocyte, metamyelocyte or band form) present on the peripheral blood film. Neutrophilia persisting beyond the first few days of life may be indicative of a bacterial infection.

Sepsis in the neonate

Diagnosis of neonatal septicaemia is one of the most difficult tasks in clinical medicine. In the neonate, bacterial infections have a high mortality rate despite the use of antibiotics. An increase in the number of neutrophils, the presence of toxic granulation and a left shift with band forms are all features suggestive of septicaemia. The presence of band forms may be the only indicator of sepsis; hence it is important to count every band form on a neonatal blood film. A ratio of band forms to total neutrophils of 0.2 or higher is suggestive of sepsis.

A band form has no nuclear segmentation. The width of the nucleus at any constriction point is not less than one-third of the width at its widest point.

Toxic granulation in isolation is not specific for sepsis and may be seen in other conditions such as Alder granulation, Chédiak-Higashi anomaly, cytokine (granulocyte colony-stimulating factor, G-CSF) therapy, inflammatory bowel disease (Crohn's disease and ulcerative colitis), Stevens-Johnson syndrome and Kawasaki disease. Refer to Figs C3-1 and C3-2.

Kawasaki disease

Kawasaki disease is an acute febrile vasculitis of unknown aetiology affecting children from 2 months to 5 or more years of age. It is characterised by a non-exudative conjunctivitis, erythematous lips and a strawberry tongue, erythematous and oedematous hands and feet, and an erythematous truncal and desquamating perineal rash.

The blood picture is that of a normochromic normocytic/hypochromic microcytic anaemia (according to the age) with an absolute neutrophilia, thrombocytosis and a raised erythrocyte sedimentation rate (ESR). The neutrophils show toxic granulation, vacuolation and cytoplasmic swelling of the neutrophil membrane.

Recognition of these morphological changes, in conjunction with the clinical characteristics, aids in making a diagnosis of Kawasaki disease. This is vital since Kawasaki disease may lead to coronary artery aneurysms in early adulthood if not treated appropriately. Refer to Fig C3-3.

NEUTROPENIA

Cyclic neutropenia

Cyclic neutropenia is a rare disorder characterised by severe neutropenia lasting 3–6 days in a 21-day cycle. During this neutropenic phase, the absolute neutrophil count is $<0.2 \times 10^9$/L and the bone marrow shows a maturation arrest at the myelocyte stage. Monocytes, lymphocytes, eosinophils, platelets and reticulocytes also demonstrate cycling. The majority of patients experience oral ulcers, stomatitis and pharyngitis with lymphadenopathy during the neutropenic phase. The severity of the infection parallels the degree of neutropenia. Prior to the use of recombinant growth factors or the cytokine G-CSF, approximately 10% of patients suffered infectious complications in the neutropenic phase and died. G-CSF is now widely used in cyclic neutropenia. It increases the neutrophil count, thus preventing transient infections during the neutropenic phase. Evidence for genetic transmission has been noted in about 25% of families with cyclic neutropenia. The mode of inheritance is autosomal-recessive. However, in other cases, the disease occurs spontaneously.

Kostmann syndrome

Kostmann syndrome is a severe congenital neutropenia occurring in early childhood. Absolute neutrophil counts of $<0.2 \times 10^9$/L are characteristic, while the bone marrow shows a maturation arrest at the promyelocyte/myelocyte stage. A compensatory monocytosis as well as eosinophilia may be present.

Some children with Kostmann syndrome develop bacterial infections in the first few weeks of life and almost all cases develop infections by the age of 6 months. Skin abscesses, fungal infections and septicaemia are commonly seen. Kostmann syndrome is responsive to cytokines. G-CSF raises the neutrophil count to such a level that infection and septicaemia are rarely seen. This results in a dramatic improvement in the quality of life of patients with Kostmann syndrome.

The mode of inheritance is autosomal-recessive. Refer to Figs C3-4 and C3-5.

EOSINOPHILIA

Eosinophilia in the neonate

Eosinophilia can occur in the neonatal period. The duration of the eosinophilia can be in excess of 6 weeks, especially in those infants with high absolute eosinophil counts. The majority of neonatal cases of eosinophilia are associated with gram-negative infections such as necrotising enterocolitis.

Eosinophilia in early childhood

Eosinophilia occurs in allergic reactions such as hay fever, asthma and milk-protein colitis. The intake of cow's milk may lead to allergic eosinophilia.

Eosinophilia also occurs in parasitic infections of the gastrointestinal tract. *Toxocara canis* infection may result in a marked systemic reaction with persistent eosinophilia lasting many years. No causative agent has been found in a significant number of cases of eosinophilia. Many of these cases resolve slowly over a period of time. Refer to Fig C3-6.

BASOPHILIA/MASTOCYTOSIS

An absolute basophilia is associated with hypersensitivity reactions to food substances or drugs; it may also occur in conjunction with acute urticaria. Refer to Fig C3-7.

Mastocytosis is discussed in Chapter B1.

LYMPHOCYTOSIS

Bordetella pertussis

Bordetella pertussis (whooping cough) is a highly infectious disease occurring primarily in infants younger than 2 years of age. Infection results from inhaling droplets contaminated with *B. pertussis*.

The blood film shows a marked increase in the number of T lymphocytes. The total leucocyte count rises to a level of 15 to 50 × 10^9/L, with the number of lymphocytes ranging from 70% to 90%. The persistent lymphocytosis in the blood is due to a 'lymphocyte promoting factor' produced by *B. pertussis*. This factor inhibits lymphocyte migration from the blood to the lymphoid tissues. Refer to Fig C3-8.

Infectious mononucleosis (IM)

Some difficulty may be experienced in making a diagnosis of infectious mononucleosis in children less than 2 years of age. This is because the immune system of children in this age group is still underdeveloped and the commercial screening tests may give a false-negative result in this age bracket. A definitive diagnosis using viral serology (Epstein Barr virus) must be made in such cases. Refer to Figs B4-5 to B4-7.

Infectious mononucleosis is discussed in Chapter B4.

Acute infectious lymphocytosis

Acute infectious lymphocytosis occurs in children between the ages of 1 and 14 years, with the highest incidence in the first 10 years of life. It may be associated with a low-grade fever and diarrhoea. The absolute lymphocyte count is very high. It can reach 50 × 10^9/L and the majority of lymphocytes are $CD4^+$ T lymphocytes. The condition resolves in 2–4 weeks without treatment.

Acute infectious lymphocytosis is thought to be associated with a viral infection. Refer to Fig C3-9.

MONOCYTOSIS

Reactive haemophagocytic syndrome

Langerhans cell histiocytosis (LCH)

These monocytic disorders are discussed in Chapter B2.

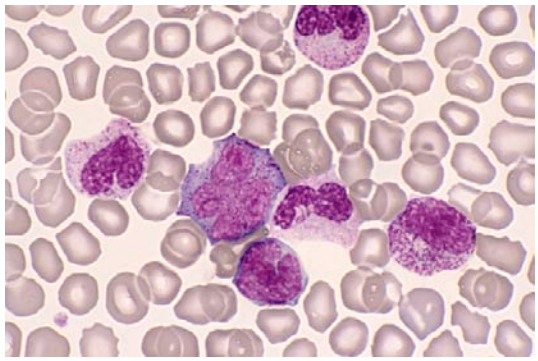


Figure C3-1
Neonatal sepsis: peripheral blood film showing a left shift in the myeloid series following meconium aspiration. (x 1000)

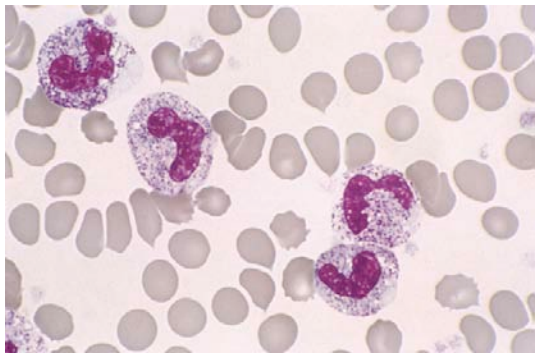


Figure C3-2
Sepsis at 2 months of age: peripheral blood film showing the presence of band forms together with toxic granulation and vacuolation. In a case such as this, every band form must be enumerated in the differential. (x 1000)

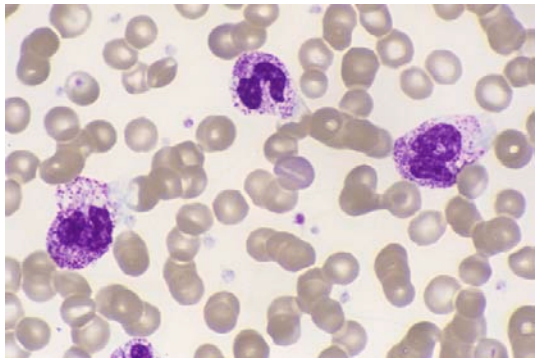


Figure C3-3
Kawasaki disease: peripheral blood film from a 3-year-old child with a prolonged high fever and rash. The neutrophils show the presence of primary granules, vacuolation and cytoplasmic swelling of the neutrophil membrane. (x 1000)

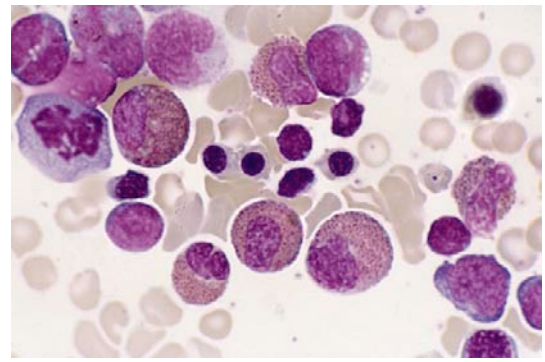


Figure C3-4
Kostmann syndrome: bone marrow showing a reduction in the myeloid lineage as well as a marked eosinophilia. (x 1000)

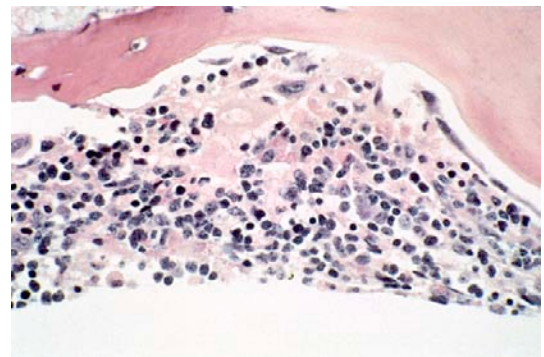


Figure C3-5
Kostmann syndrome: bone marrow trephine showing marked neutropenia (H&E) (x 400)

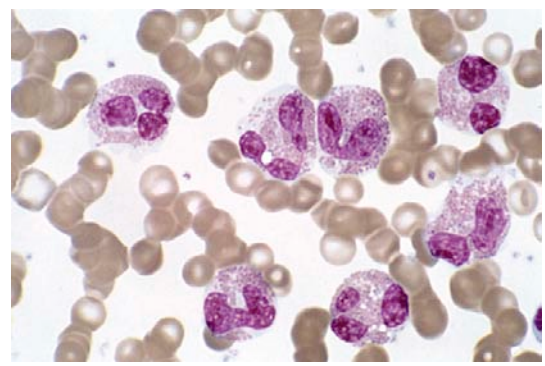


Figure C3-6
Parasitic infection: peripheral blood film from a child infected with Toxocara canis showing a marked eosinophilia. The absolute eosinophil count on this child was 46.4 × 10^9/L. (x 1000)

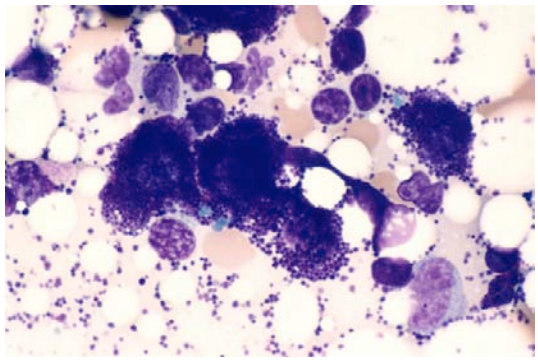


Figure C3-7
Cutaneous mastocytosis: bone marrow from an 18-month-old child suffering from an itchy red rash since birth. The bone marrow is heavily infiltrated with mast cells. Some of the squashed mast cells have released histamine granules. (x 1000)

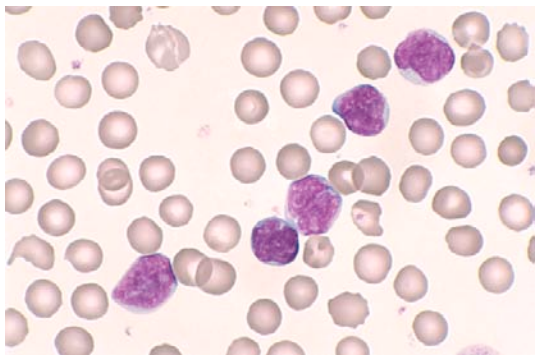


Figure C3-8
Bordetella pertussis: peripheral blood film showing T lymphocytes with cleaved nuclei. (x 1000)

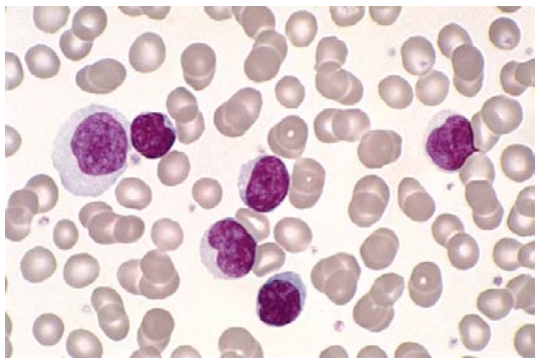
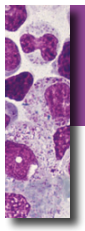


Figure C3-9
Acute infectious lymphocytosis: morphologically normal T lymphocytes present in the peripheral blood film from a 3-year-old child with an absolute lymphocyte count of 39 x 10^9/L. (x 1000)



C4 Myeloproliferative neoplasms in the neonate and childhood

TRANSIENT ABNORMAL MYELOPOIESIS (TAM)

Transient abnormal myelopoiesis may occur in the neonatal period. It is often associated with Down syndrome (trisomy 21) and morphologically closely resembles AML. TAM is characterised by an uncontrolled proliferation of blasts that regress spontaneously within weeks or months. This myeloproliferative disorder is associated with a hepatosplenomegaly. The haemoglobin is usually normal while the platelet count is reduced.

TAM may be confused with congenital leukaemia in the initial stages or until spontaneous remission occurs within the first 3 months of life. Twenty-five per cent of cases of TAM will go on to develop acute megakaryoblastic leukaemia by the age of 3 years. In approximately 20–30% of cases with Down syndrome, the blast cells will persist and a true congenital leukaemia will develop. Acute lymphoblastic as well as acute myeloblastic leukaemia may occur in children with Down syndrome. The most frequently occurring myeloblastic leukaemia is megakaryoblastic leukaemia. Refer to Figs C4-1 to C4-5.

CYTOGENETICS

- In addition to the trisomy 21 of Down syndrome, no specific chromosomal abnormalities are associated with transient abnormal myelopoiesis.

IMMUNOPHENOTYPE

- The markers expressed by TAM resemble those expressed by megakaryoblastic leukaemia: HLA-DR$^{-/+}$, CD34^{+}, CD56^{+}, CD117^{+}, CD13^{+}, CD33^{+}, CD7^{+}, CD45^{-}, CD41^{+}, CD61^{+}

JUVENILE MYELOMONOCYTIC LEUKAEMIA (JMML)

This is discussed in Chapter B1.

MONOSOMY 7 MYELOPROLIFERATIVE DISEASE (MPD)

Monosomy 7 MPD has been referred to as a 'subleukaemic' disorder occurring in young children, usually less than 5 years of age. It affects boys more frequently than girls. These children have a long history of recurrent infections and abnormal neutrophil function. They have a leucocytosis with a left shift in the myeloid lineage as well as an absolute monocytosis, anaemia and thrombocytopenia. The bone marrow is hypercellular and often dysplastic. HbF is either normal or slightly raised.

Monosomy 7 often progresses to acute myeloblastic leukaemia. Refer to Fig C4-6.

CYTOGENETICS
- –7/del(7q)

Myelodysplastic syndromes (MDS)

MDS are rarely seen in childhood. They are usually associated with other conditions such as Down syndrome, Fanconi anaemia, Kostmann syndrome, Diamond-Blackfan anaemia and Shwachman-Diamond syndrome.

MDS may also occur as de novo or be secondary to chemotherapy given for some other malignancy. Refer to Figs C4-7 and C4-8.

They are discussed in Chapter B1.

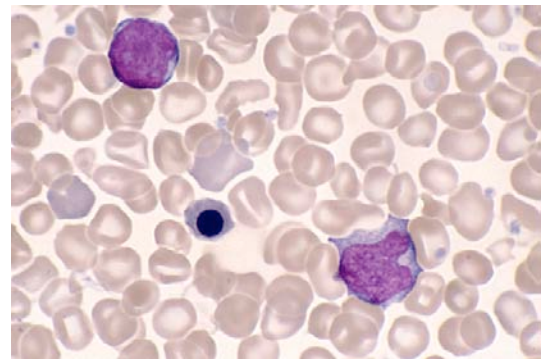


Figure C4-1
Transient abnormal myelopoiesis (TAM): peripheral blood film from a newborn with Down syndrome. Two blast cells and a nucleated RBC are present. The white cell count is 15.8 x 10^9/L and the absolute blast cell count is 5.2 x 10^9/L. (x 1000)

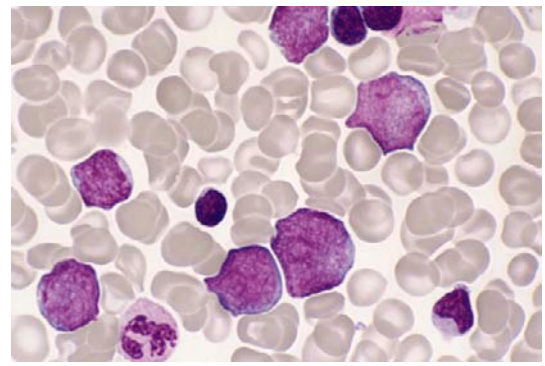


Figure C4-2
Transient abnormal myelopoiesis (TAM): bone marrow from the above case showing a proliferation of blast cells. The blast cells are pleomorphic with some cytoplasmic budding, suggestive of megakaryoblastic leukaemia. (x 1000)

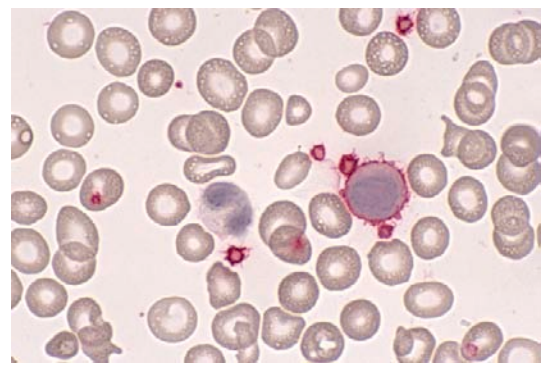


Figure C4-3
Transient abnormal myelopoiesis (TAM): bone marrow from the above case. The blast cells have been shown to be megakaryoblastic with the monoclonal antibody CD61 using the alkaline phosphatase-anti-alkaline phosphatase (AP-AAP) technique. The platelets and megakaryoblast, together with its cytoplasmic protrusions, have stained positively. (x 1000)

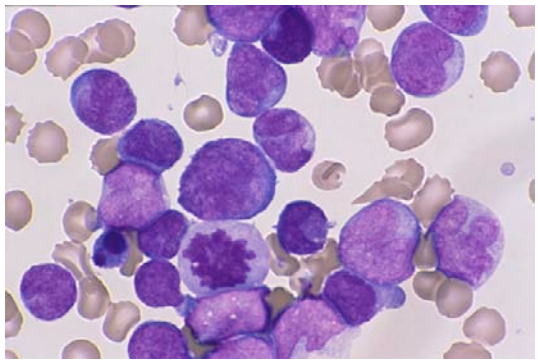


Figure C4-4
Congenital leukaemia: peripheral blood showing a proliferation of blast cells including a mitotic figure. (x 1000)

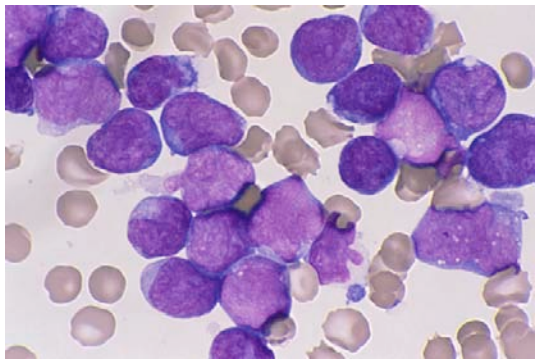


Figure C4-5
Congenital leukaemia: peripheral blood showing a proliferation of blast cells. (x 1000)

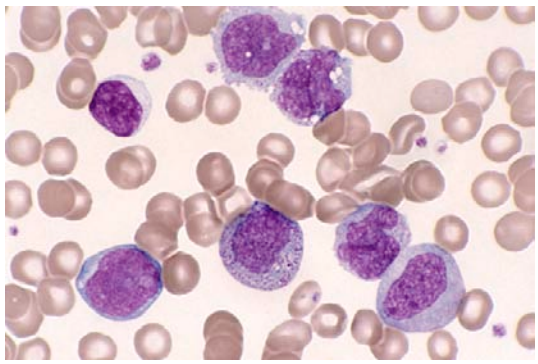


Figure C4-6
Monosomy 7 myeloproliferative disease (MPD): peripheral blood film from a 5-year-old child showing myeloid precursors together with a monocytosis. (x 1000)

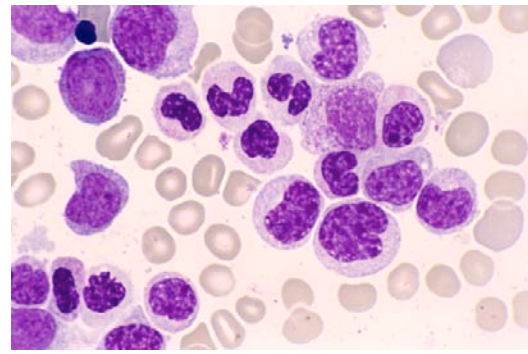


Figure C4-7
Myelodysplastic syndrome (MDS): bone marrow from a child successfully treated for acute lymphoblastic leukaemia. The blood picture shows a drug-induced MDS with many pelgeroid and hypogranular neutrophils and myelocytes. (x 1000)

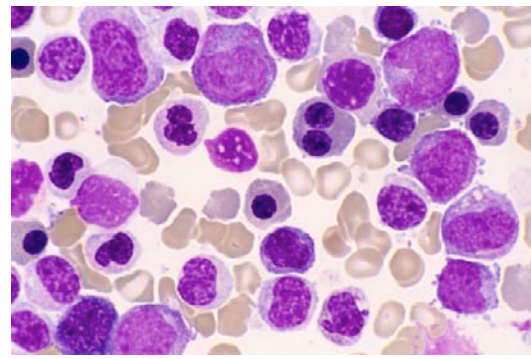
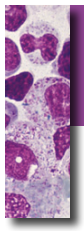


Figure C4-8
Myelodysplastic syndrome (MDS): bone marrow from the above case showing many pelgeroid and hypogranular neutrophils as well as dysplastic and bilobed erythroid precursors. (x 1000)



C5 Non-haemopoietic malignancies in the neonate and childhood

NEUROBLASTOMA

Neuroblastoma is a tumour of developing sympathetic nervous tissue. It is an embryonal tumour and usually presents in the first 5 years of life. It occurs in any site where sympathetic nerve tissue is found, namely the adrenal medulla and the paraspinal sympathetic ganglia. In about 50% of cases, neuroblastoma is found infiltrating the bone marrow.

Neuroblastoma cells have a tendency to form clumps and rosettes on bone marrow films. The process of aspirating the bone marrow may result in physical disruption of these clumps, leaving bare nuclei and cytoplasmic or stromal debris over the marrow film.

Neuroblastoma is associated with a normocytic normochromic anaemia, but in cases where there has been bleeding into the tumour, the red cells may be microcytic and hypochromic. The platelet count is usually increased, as is the ESR (>50 mm/h). Tumour cells are rarely seen on the peripheral blood film. Refer to Figs C5-1 to C5-6.

CYTOGENETICS

Chromosomes exhibit ploidy changes and can be divided into two major groups:

- The near triploid group—exhibits mainly numerical changes rather than structural abnormalities such as *NMYC* amplification, del(1p), del(11q) and relative gain of 17q.
- The diploid/tetraploid group—is more likely to be associated with structural abnormalities such as *NMYC* amplification (often seen as double minutes), del(1p), del(11q) and relative gain of 17q.

IMMUNOPHENOTYPE

- The mouse monoclonal antibody NB84 directed against human neuroblastoma tissue stains 90% of neuroblastomas in paraffin section. The staining pattern of NB84 is cytoplasmic, showing strong positivity in the areas of fibrillary or rosette formation.
- Ewing sarcoma (see below) is the only other small round cell tumour reacting with the NB84 antibody.

RHABDOMYOSARCOMA

Rhabdomyosarcoma is the most common soft tissue malignancy occurring in children. It a tumour of striated muscle and only involves the bone marrow in 25–30% of cases.

Rhabdomyosarcoma cells in the marrow appear as large cells with prominent vacuolation. The vacuoles often coalesce into elongated lakes and stain positively with periodic acid-Schiff (PAS) reagent.

Rhabdomyosarcoma may arise anywhere in the body: the head, the neck, the retroperitoneum, the genitourinary tract and the extremities. It has two age peaks, the first being between the ages of 2 and 6 years and the second between 14 and 18 years. Refer to Figs C5-7 to C5-12.

CYTOGENETICS

- Multiple numerical abnormalities may occur, often containing t(2;13)(q35;q14) or variants t(1;13)(p36;q14), t(1;3)(p36;q14) along with rearrangements of chromosome 1.

IMMUNOPHENOTYPE

- The mouse monoclonal antibody D33 reacts with the intermediate filament protein desmin in tumours of myogenic origin: both those arising from smooth muscle (leiomyosarcomas) and those from striated muscle (rhabdomyosarcoma). The reaction with desmin present in these tumour cells stains a yellow-brown colour.

EWING SARCOMA

Ewing sarcoma (EWS) is a tumour of bone seen predominantly in the young (less than 20 years of age). It occurs in the femur, the ribs and also the vertebrae and pelvic bones. Ewing sarcoma cells are small, round and undifferentiated. They have prominent vacuoles and tend to form clumps or small aggregates. Approximately 64% of cases with EWS show focal positivity with the NB84 antibody. EWS also stains positively with PAS reagent. Refer to Figs C5-13 to C5-16.

CYTOGENETICS

- 90% of cases contain t(11;22)(q24;q12) resulting in fusion of EWS(22q12) and *FLI1*(11q24). Variants, involving EWS may also be seen in 5% of cases i.e. t(21;22) or t(7;22).
- Other abnormalities may also include +8, +2, +5, +7, +9, +12, +14, +20 and trisomy 1q which may result from the presence of der(6)t(1;16).

IMMUNOPHENOTYPE

- There is no specific monoclonal antibody able to demonstrate the presence of Ewing sarcoma apart from demonstrating the lack of desmin positivity.

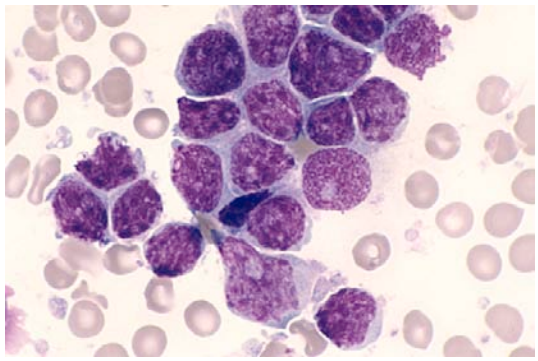


Figure C5-1
Neuroblastoma: a clump of tumour cells in the bone marrow characterised by a relatively high N/C ratio and a tendency to form clumps. (x 1000)

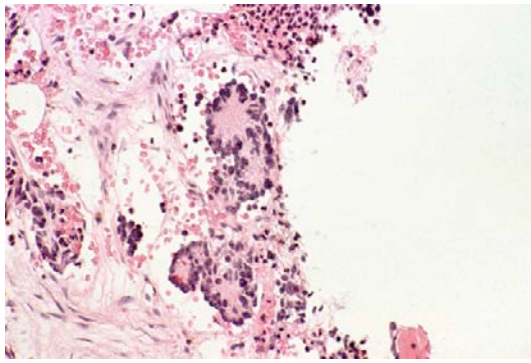


Figure C5-2
Neuroblastoma: bone marrow trephine showing rosettes of neuroblastoma cells. (H&E) (x 200)

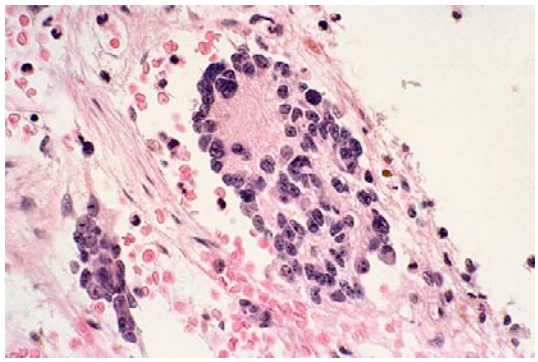


Figure C5-3
Neuroblastoma: bone marrow trephine showing a single rosette of neuroblastoma cells. (H&E) (x 400)

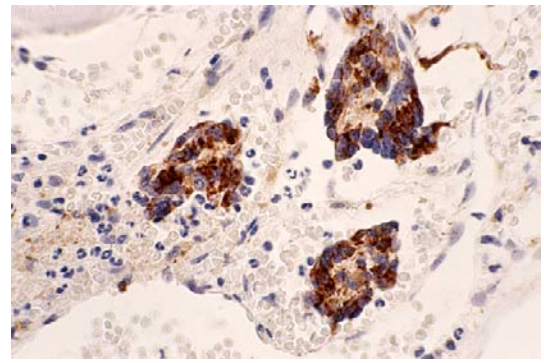


Figure C5-4
Neuroblastoma: bone marrow trephine showing rosettes of neuroblastoma cells stained with the mouse monoclonal antibody NB84. (x 200)

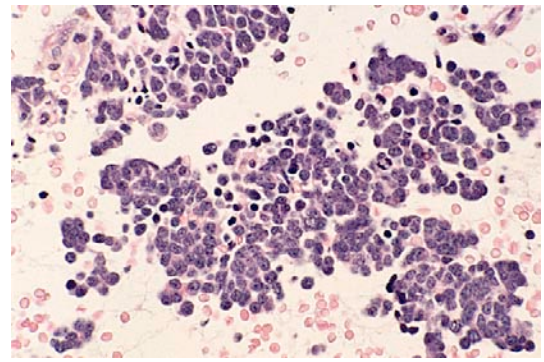


Figure C5-5
Neuroblastoma: bone marrow trephine showing sheets rather than rosettes of neuroblastoma cells. (H&E) (x 400)

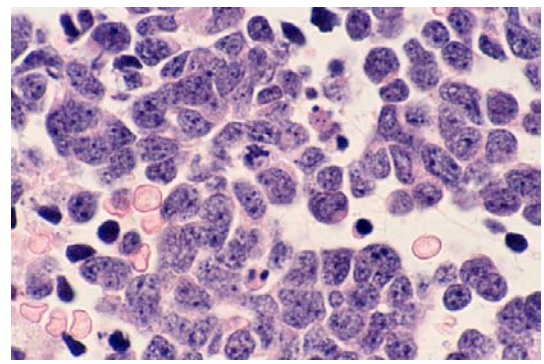


Figure C5-6
Neuroblastoma: bone marrow trephine showing sheets of neuroblastoma cells with prominent nucleoli. (H&E) (x 1000)

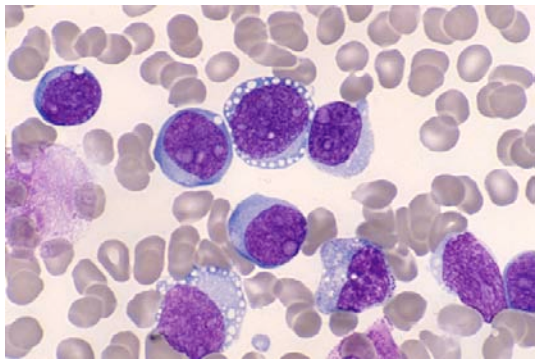


Figure C5-7
Rhabdomyosarcoma: bone marrow showing individual tumour cells with prominent vacuolation. (x 1000)

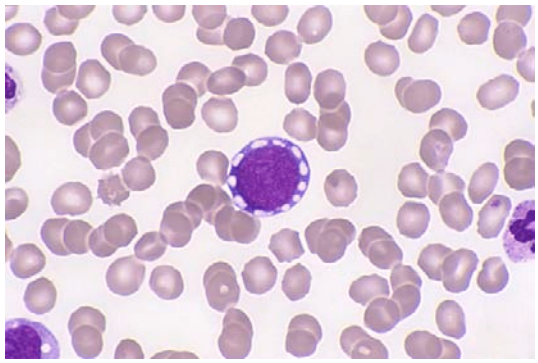


Figure C5-8
Rhabdomyosarcoma: bone marrow showing a classical tumour cell with prominent vacuolation. (x 1000)

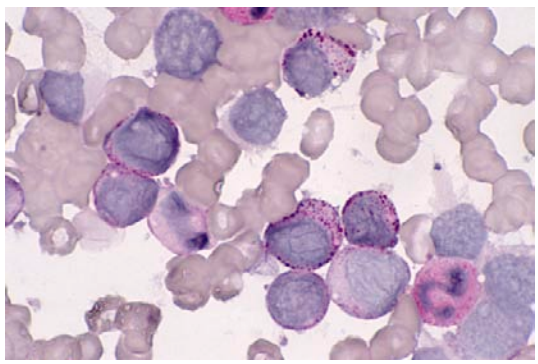


Figure C5-9
Rhabdomyosarcoma: PAS stain of the bone marrow showing tumour cells containing blocks of PAS positive material. A similar pattern may be seen in Ewing sarcoma. (x 1000)

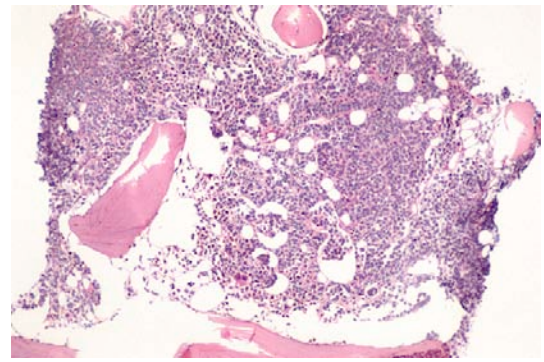


Figure C5-10
Rhabdomyosarcoma: bone marrow trephine heavily infiltrated with rhabdomyosarcoma cells. (H&E) (x 100)

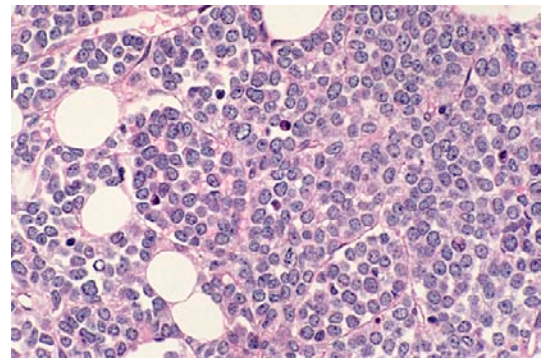


Figure C5-11
Rhabdomyosarcoma: bone marrow trephine showing infiltrate of rhabdomyosarcoma cells. (H&E) (x 400)

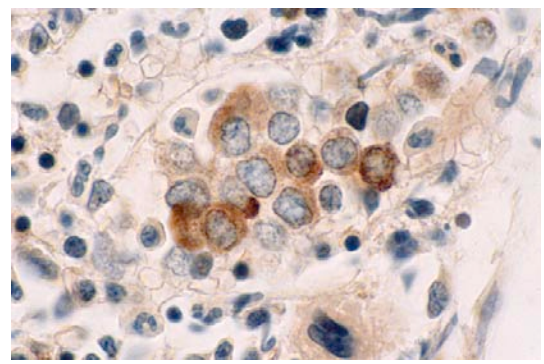


Figure C5-12
Rhabdomyosarcoma: bone marrow trephine showing the presence of desmin (yellow-brown colour) within the tumour cells, indicative of myogenic origin. The presence of desmin confirms the diagnosis of rhabdomyosarcoma. (x 1000)

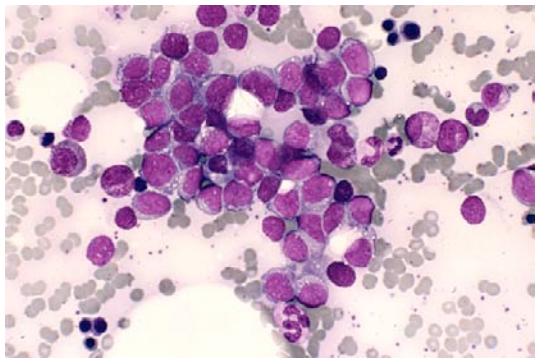


Figure C5-13
Ewing sarcoma: bone marrow showing sheets of Ewing sarcoma cells. (x 400)

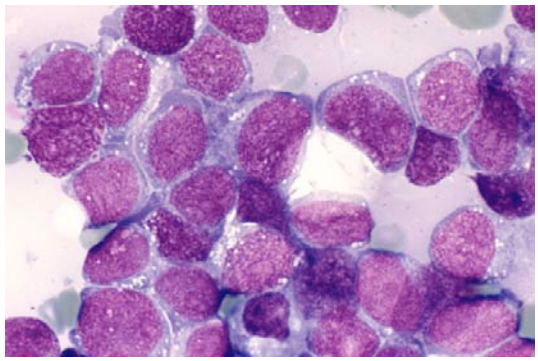


Figure C5-14
Ewing sarcoma: bone marrow showing sheets of Ewing sarcoma cells with characteristic vacuolated cytoplasm. (x 1000)

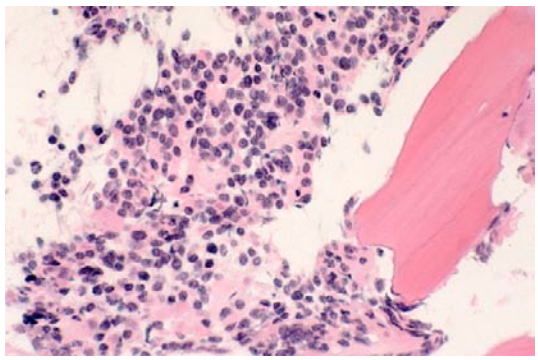


Figure C5-15
Ewing sarcoma: bone marrow trephine showing a heavy infiltrate of Ewing sarcoma. (H&E) (x 400)

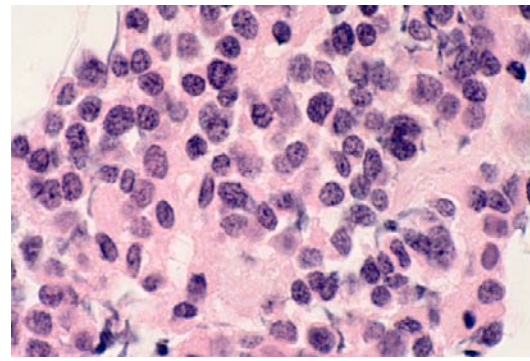
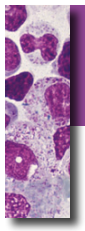


Figure C5-16
Ewing sarcoma: bone marrow trephine showing a heavy infiltrate of Ewing sarcoma. (H&E) (x 1000)



C6 Storage disorders in the neonate and childhood

Storage disorders are difficult to diagnose in routine diagnostic laboratories. The changes in the peripheral blood include vacuolated lymphocytes, neutrophils, and monocytes. Foamy macrophages are present in the bone marrow. In some storage disorders, namely the mucopolysaccharidoses, metachromatic granules occur within lymphocyte vacuoles.

The clinical symptoms include pancytopenia, hepatosplenomegaly and lymphadenopathy. The central nervous system may also be affected.

GAUCHER DISEASE AND NIEMANN-PICK DISEASE

These are discussed in Chapter B2.

α-MANNOSIDOSIS

α-Mannosidosis is an oligosaccharide storage disease characterised by deficiency of the enzyme α-mannosidase. There are two phenotypes: the infantile phenotype, which presents in the first 12 months of life and has a short survival, and the juvenile/adult phenotype, presenting later in life and having a longer survival.

The clinical features include skeletal changes, coarse facial features and mental retardation. Hepatosplenomegaly is present in the early stage of the disease. Abnormal chemotaxis of the neutrophils leads to recurrent bacterial infections.

The circulating lymphocytes have vacuolated cytoplasm and the bone marrow is packed with foamy macrophages. The lymphocyte vacuoles show periodic acid Schiff (PAS) positivity.

Increased levels of mannose-containing oligosaccharides are found in the tissues as well as in the urine. Refer to Fig C6-1.

MUCOPOLYSACCHARIDOSES

Mucopolysaccharide storage disorders lead to morphological changes in white cells in both the peripheral blood and the bone marrow. Mucopolysaccharides are complex carbohydrates found in various types of connective tissue, including cartilage and bone. The presence of excessive amounts of mucopolysaccharide leads to coarse facial features, skeletal dysplasia and limitation of joint movement (Hurler syndrome).

Mucopolysaccharidosis results from a lysosomal enzyme deficiency.

Hurler syndrome (Gasser lymphocytes)

Hurler syndrome, a mucopolysaccharide storage disorder, is characterised by the presence of lymphocytes whose cytoplasm contains clear vacuoles filled with coarse metachromatic granules of mucopolysaccharide. These lymphocytes have been described by Gasser and are known as Gasser lymphocytes. Refer to Fig C6-2.

Cystinosis

Cystinosis is an autosomal-recessive disorder that first presents at 6–12 months of age. The clinical characteristics of the disorder include failure to thrive, progressive renal failure, loss of muscle function, photophobia and fair hair.

The bone marrow is characterised by the presence of macrophages packed with rectangular shaped crystals of cystine. Rarely can these crystals be seen in the peripheral blood.

Diagnosis of cystinosis is by way of a biochemical assay rather than by bone marrow examination. The cystine content of the white cells is measured. This is because the aqueous staining solutions used to stain the crystals tend to dissolve much of the cystine, leaving few crystals visible under the light microscope. Refer to Figs C6-3 and Fig C6-4.

Wolman disease

Wolman disease is an autosomal-recessive disorder. It is characterised by an abnormality in the lysosomal acid lipase (LIPA) gene resulting in a deficiency of the acid cholesteryl ester hydrolase enzyme. Deficiency of this enzyme leads to an accumulation of cholesterol and triglycerides within the body organs and tissues.

The clinical features of Wolman disease include steatorrhoea, hepatosplenomegaly, intestinal malabsorption, abdominal protuberance, poor weight gain and diffuse punctate adrenal calcification. Xanthomatous changes are seen in the liver, the adrenal glands, the spleen, the lymph nodes, the small intestine, the lungs, the thymus and, to a lesser extent, the skin.

The peripheral blood film is characterised by the presence of vacuolated lymphocytes and the bone marrow by the presence of foamy macrophages. The vacuoles in both the lymphocytes and bone marrow macrophages stain positively with the oil red O stain and the Sudan black B stain. Normal to moderately elevated levels of plasma lipids and hypercholesterolaemia are present.

Death occurs in early infancy. Refer to Figs C6-5 and C6-6.

Sialic acid storage disease

Sialic acid storage disease is a rare inherited autosomal-recessive neurodegenerative disorder resulting from a block in sialic acid release from cell lysosomes. The accumulation of sialic acid in many cells, including lymphocytes, leads to cytoplasmic vacuolation.

Sialic acid storage disease is diagnosed by the presence of increased amounts of sialic acid in blood, urine and lymphocytes. The disease presents in two forms: a severe form occurring in early life, named infantile sialic acid storage disease (ISSD), and a less aggressive form, named SALLA disease after the region in Finland where it was first recognised. The infantile form is characterised by coarse facial features, lack of skin pigmentation, hepatosplenomegaly and anaemia progressing to fluid retention, ascites, heart failure and early death. SALLA disease has similar features as well as mental retardation and cerebella ataxia. SALLA disease stops progressing after early childhood, and affected subjects have a near-normal life span. Refer to Fig C6-7.

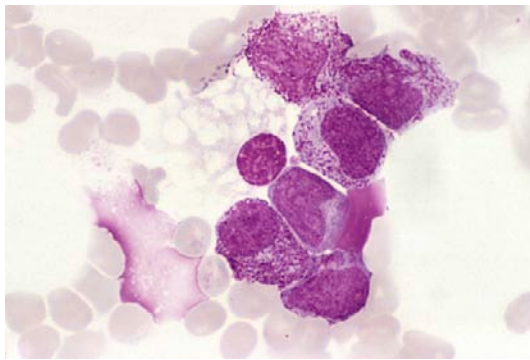


Figure C6-1
α-Mannosidosis: bone marrow showing the presence of a foamy macrophage. (x 1000)

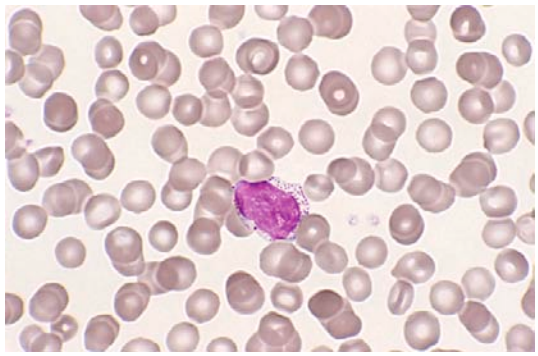


Figure C6-2
Hurler syndrome: peripheral blood film from a child with Hurler syndrome showing a classical Gasser lymphocyte whose cytoplasm is filled with clear vacuoles containing large coarse metachromatic granules of mucopolysaccharide. (x 1000)

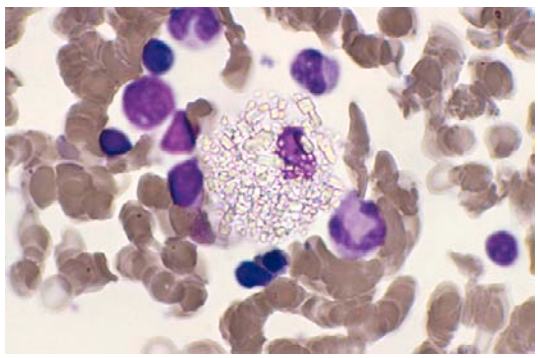


Figure C6-3
Cystinosis: bone marrow showing the presence of a macrophage whose cytoplasm is packed with rectangular shaped crystals of cystine. (x 1000)

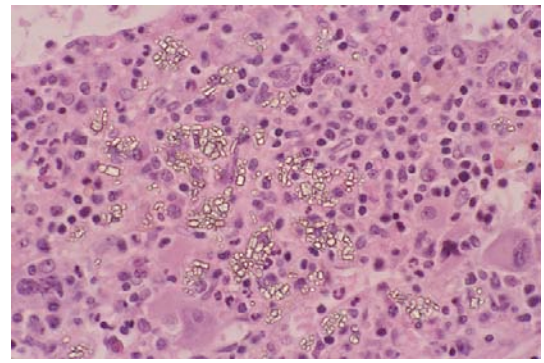


Figure C6-4
Cystinosis: bone marrow trephine infiltrated with crystals of cystine. (x 400)

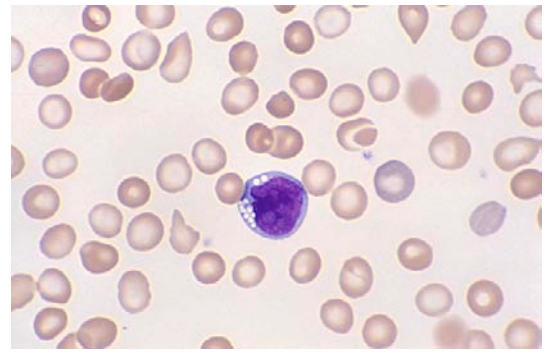


Figure C6-5
Wolman disease: peripheral blood film from a 4-week-old neonate showing characteristic vacuolation in a lymphocyte. (x 1000)

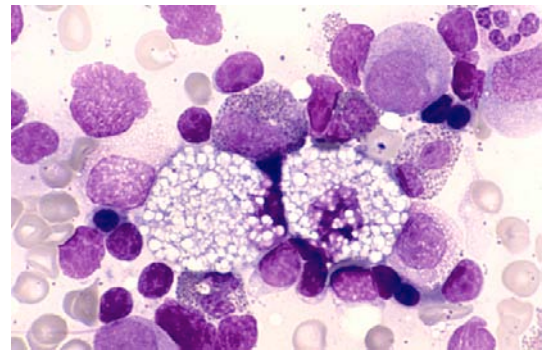


Figure C6-6
Wolman disease: foamy macrophages present in the bone marrow of the above neonate. (x 1000)

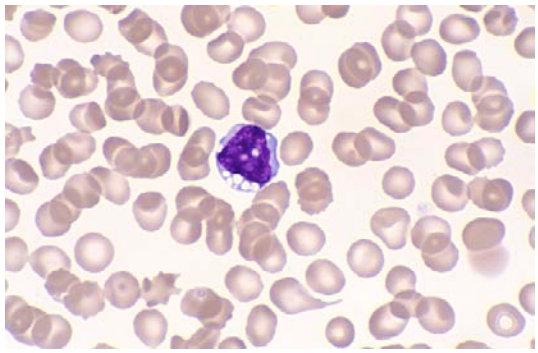
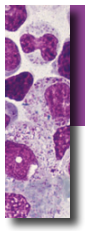


Figure C6-7
Sialic acid storage disease: peripheral blood film from a 2-month-old child showing a characteristic vacuolated lymphocyte. (x 1000)



C7 Platelet abnormalities in the neonate and childhood

THROMBOCYTOPENIA

Thrombocytopenia is a frequent occurrence amongst ill newborns, being found in 20–30% of all admissions to a neonatal intensive care ward. It is more common among infants with neonatal asphyxia, respiratory distress syndrome, pulmonary hypertension and meconium aspiration. The aetiology of the thrombocytopenia is not understood. The platelet life span is decreased while the megakaryocyte numbers are normal or increased. These features are also seen in infection, disseminated intravascular coagulation and immune/mechanical destruction.

Most infants with thrombocytopenia come to the attention of the neonatologist because of haemorrhagic symptoms: petechiae, purpura, epistaxis or mucous membrane bleeding.

Mechanisms of thrombocytopenia in neonates and children fall into two basic categories:

- Increased destruction
- Impaired or ineffective thrombopoiesis.

THROMBOCYTOPENIA DUE TO INCREASED DESTRUCTION

Idiopathic thrombocytopenic purpura (ITP)

ITP is one of the most frequently seen examples of increased platelet destruction. A typical history is bruising and petechiae appearing suddenly in a child who is otherwise in excellent health. The peak age for the diagnosis of ITP is 2 to 4 years. In childhood ITP, males and females are affected with equal frequency—in contrast to adult ITP, in which females dominate by a 3:1 ratio. Often there is a history of a viral illness or vaccination 1 to 3 weeks prior to presentation. The platelet count in these children is commonly $<10 \times 10^9$/L. The blood film should be carefully scanned for the presence of reactive lymphocytes as well as large platelets when a case of ITP is suspected.

In the majority of cases, ITP in children is an acute self-limiting disease resolving within 6 months whether or not therapy is given. Refer to Fig C7-1.

THROMBOCYTOPENIA DUE TO IMPAIRED OR INEFFECTIVE THROMBOPOIESIS

Amegakaryocytic thrombocytopenia (AMEGA)

AMEGA occurs in infancy and early childhood. The mode of inheritance is either X-linked or autosomal-recessive. AMEGA is a very rare disorder in which patients present with a petechial rash, bruising or bleeding in the first year of life. Mild to moderate

anaemia and leucopenia may be present. Megakaryocytes in the bone marrow are either reduced in number or absent. Granulopoiesis and erythropoiesis are normal. The red cells may be macrocytic and the HbF increased.

Between 20% and 40% of cases may develop aplastic anaemia in the first few years of life.

Patients with AMEGA have a predisposition to develop acute leukaemia. Refer to Figs C7-2 and C7-3.

Bernard-Soulier syndrome (BSS)

BSS is inherited as an autosomal-recessive disorder. It is characterised by a moderate thrombocytopenia. The platelet counts range between 50 and 60 × 10^9/L, with giant forms present, many being the size of small lymphocytes.

The platelets in BSS demonstrate both qualitative and quantitative defects in the glycoprotein 1b-V-1X complex within the platelet membrane. BSS platelets demonstrate normal aggregation in the presence of adenosine diphosphate (ADP), epinephrine (adrenaline), collagen and arachidonic acid, but do not aggregate in the presence of ristocetin. Refer to Fig C7-4.

Gray platelet syndrome (GPS)

GPS is inherited as an autosomal-dominant disorder. It is characterised by a moderate thrombocytopenia, with large pale staining platelets on the Romanowsky-stained film. The platelets are deficient in α-granules. There is also a deficiency in storage proteins, platelet factor 4, thrombospondin, platelet-derived growth factor and β-thromboglobulin. These storage proteins may be increased in the serum, suggesting a defect in α-granule protein packaging within the megakaryocyte. The megakaryocytes are normal in number but poorly granulated.

Patients with GPS have a prolonged skin bleeding time and hence a mild bleeding tendency. Refer to Fig C7-5.

May-Hegglin anomaly (MHA)

MHA is inherited as an autosomal-dominant disorder characterised by the presence of giant platelets, variable thrombocytopenia and RNA inclusion bodies within the cytoplasm of the neutrophils.

Platelet function is also variable; it may be normal in some cases but impaired in others. Refer to Figs B1.24 and C7-6.

Thrombocytopenia with absent radii (TAR)

TAR syndrome is inherited as an autosomal-recessive disorder. It is characterised by a thrombocytopenia and bilateral radial aplasia.

The bone marrow shows decreased to absent numbers of megakaryocytes. Myelopoiesis and erythropoiesis are normal although a leucocytosis with counts of more than 100 × 10^9/L as well as the presence of immature myeloid forms may be seen at birth. This leukaemoid reaction is transient and subsides spontaneously. Anaemia due to blood loss resulting from the thrombocytopenia may also be present. Refer to Fig C7-7.

Wiskott-Aldrich syndrome (WAS)

WAS is inherited as an X-linked recessive disorder. It is characterised by thrombocytopenia, with small platelets that have a slightly reduced survival time. Thrombopoiesis is ineffective while megakaryocyte numbers range from normal to high. Immunodeficiency is also a feature of WAS. An inability to produce antibodies makes children with WAS

susceptible to both bacterial and viral infections (e.g. herpes and warts). Eczema is also commonly seen.

A positive DAT haemolytic anaemia may also occur in conjunction with WAS. Refer to Fig C7-8.

THROMBOCYTOSIS

The most frequently occurring causes of thrombocytosis in neonates and children are reactive. They include infection (bacterial and viral), surgery or trauma, haemorrhage, stress, inflammatory disease (rheumatoid arthritis), malignancies, chronic blood loss, the use of certain drugs and the use of cytokines. With any of the above, the degree of thrombocytosis usually parallels the degree of activity of the underlying condition.

Refer to Fig C7-9.

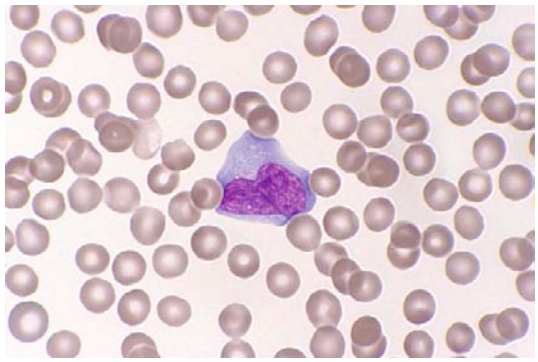


Figure C7-1
Idiopathic thrombocytopenic purpura (ITP): peripheral blood film showing a reactive T lymphocyte in a 3-year-old child with post viral ITP. The platelet count is less than 1 x 10^9/L. (x 1000)

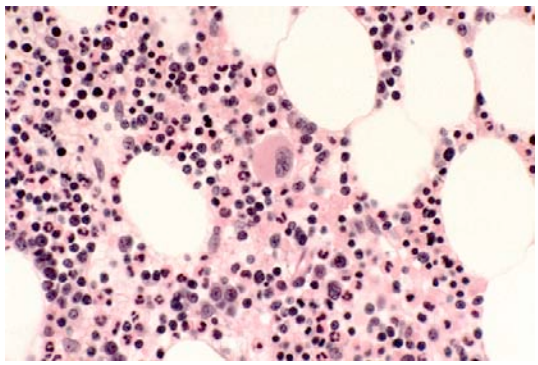


Figure C7-2
Amegakaryocytic thrombocytopenia: bone marrow trephine showing a markedly reduced number of megakaryocytes. (H&E) (x 400)

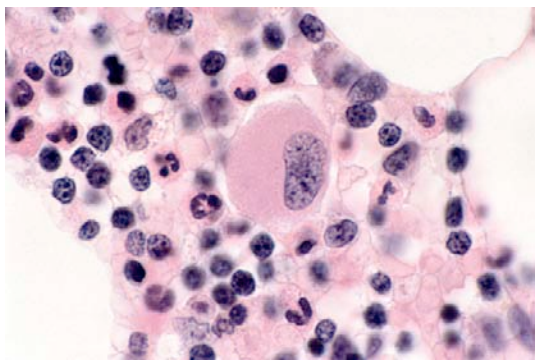


Figure C7-3
Amegakaryocytic thrombocytopenia: bone marrow trephine showing a hypolobulated megakaryocyte in the presence of normal granulopoiesis and erythropoiesis. (H&E) (x 1000)

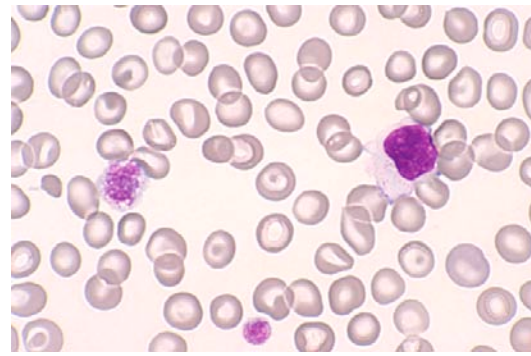


Figure C7-4
Bernard-Soulier syndrome: peripheral blood film showing thrombocytopenia and giant platelets. Often the platelets are the size of small lymphocytes. (x 1000)

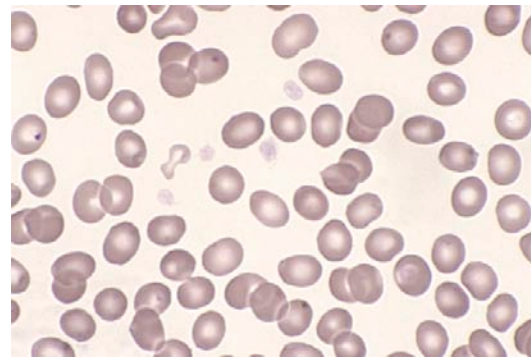


Figure C7-5
Gray platelet syndrome: peripheral blood film showing large pale-staining and poorly granulated platelets. (x 1000)

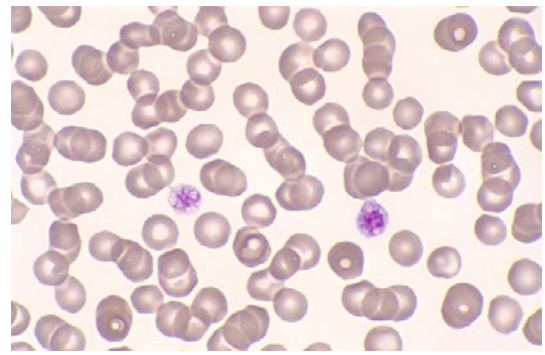


Figure C7-6
May-Hegglin anomaly: peripheral blood film showing thrombocytopenia and large and giant platelets. (x 1000)

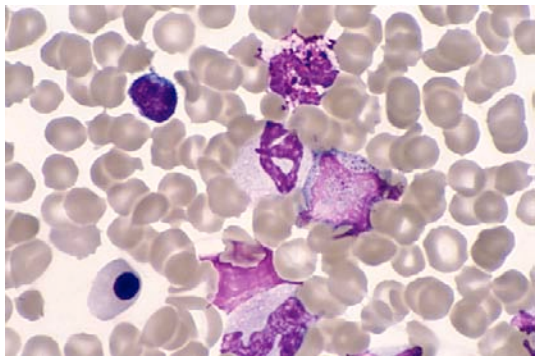


Figure C7-7
Thrombocytopenia with absent radii (TAR) syndrome: peripheral blood film from a newborn showing a leukaemoid reaction and thrombocytopenia. (x 1000)

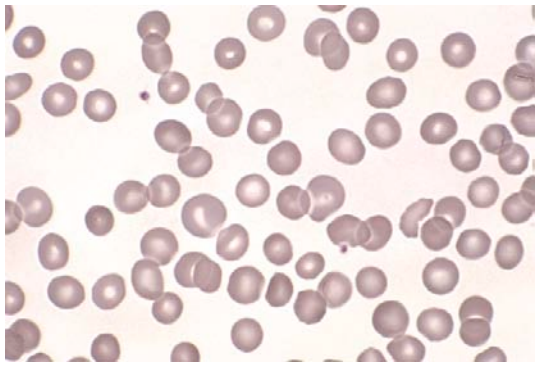


Figure C7-8
Wiscott-Aldrich syndrome: peripheral blood film showing small platelets and thrombocytopenia. (x 1000)

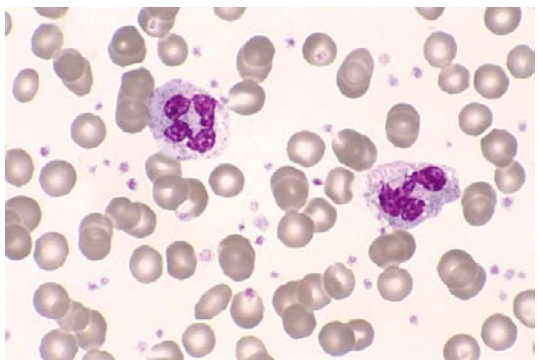
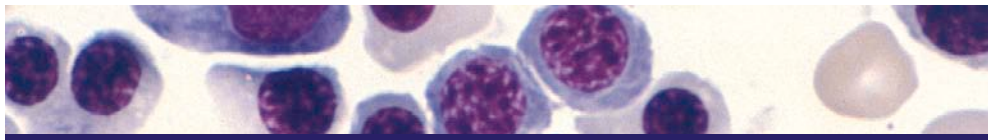
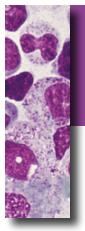


Figure C7-9
Reactive thrombocytosis: peripheral blood film from an 8-month-old child with a platelet count of 1046 x 10^9/L. (x 1000)



PART D

BLOOD PARASITES



D1 Malarial parasites

DIAGNOSTIC CHARACTERISTICS

Four species of human malaria are seen: *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. *Plasmodium knowlesi*, known to infect long-tailed macaques (*Macaca fascicularis* and *M. nemestrina*) in South-East Asia is now recognised as a fifth species of malaria infecting humans.

Plasmodium falciparum

- Infected red cells are not enlarged.
- Only early trophozoites are seen in the red cells unless the infection is very heavy; this is because the intermediate stages migrate to the fine capillaries of the body.
- Early trophozoites are small, with a thin thread of cytoplasm. Often two or three chromatin dots are present.
- Multiple infection of red cells is common, especially in those of moderate to heavy parasite density.
- Accolé or marginal forms are common.
- Late trophozoites are round in shape and have abundant pigment. They occupy about two-thirds of the red cell.
- Maurer's dots or clefts may be present. They represent areas of pseudopodal attachment that, deprived of haemoglobin, react differently to Romanowsky stains.
- Schizonts contain between 16 and 36 merozoites although there may be as few as 8 and as many as 40.
- Gametocytes are crescent-shaped with a central chromatin mass. The male gametocytes stain pink while the females stain blue.

Refer to Figs D1-1 to D1-7 and Fig D1-18.

Plasmodium vivax

- Infected red cells are enlarged.
- Parasites at all stages of maturation are seen within the red cells.
- Early trophozoites are larger and thicker than their *P. falciparum* counterparts. They rarely have two chromatin dots.
- Multiple infection of red cells may occur, but only when the parasite density is high.
- Late trophozoites are irregular in outline and occupy most of the red cell.
- Schüffner's dots are present when stained at pH 7.2. The nature of these dots is unknown; however Schüffner believed them to be related to changes in the host cells that make them susceptible to the specific red of the Romanowsky stains.
- Schizonts contain between 12 and 24 merozoites.
- Gametocytes are round and fill the red cell. The male gametocytes stain pink while the females stain blue.

Refer to Figs D1-8 to D1-18.

Plasmodium malariae

- Infected red cells are not enlarged.
- Parasites at all stages of maturation may be present within the red cell.
- Early trophozoites are approximately the same size as their *P. vivax* counterparts and have single chromatin dots.
- Multiple infection of red cells is rare.
- Late trophozoites are compact and heavily pigmented and band forms are common.
- Schizonts contain between 6 and 12 merozoites and may take on the appearance of a 'daisy head'.
- This parasite closely resembles *P. vivax*.
- Infected red cells are enlarged but not to the same degree as in *P. vivax*.
- Parasites at all stages of maturation may be present within the red cell.
- Early trophozoites are approximately the same size as their *P. vivax* counterparts and have large chromatin dots.
- Multiple infection of red cells rarely occurs.
- Late trophozoites are compact and occupy about two-thirds of the red cell.
- Schüffner's dots are present at pH 7.2. They stain more intensely than in *P. vivax*.
- Schizonts contain between 6 and 18 merozoites.
- Gametocytes are similar to those of *P. vivax* except that they are more compact and do not fill the infected cell.

Refer to Figs D1-19 to D1-23.

Plasmodium ovale

- This parasite closely resembles *P vivax*.
- Infected red cells are enlarged but not to the same degree as in *P. vivax*.
- Parasites at all stages of maturation may be present within the red cell.
- Early trophozoites are approximately the same size as their *P. vivax* counterparts and have large chromatin dots.
- Multiple infection of red cells rarely occurs.
- Late trophozoites are compact and occupy about two-thirds of the red cell.
- Schüffner's dots are present at pH7.2. They stain more intensely than in *P. vivax*.
- Schizonts contain between 6 and 18 merozoites.
- Gametocytes are similar to those of *P. vivax* except that they are more compact and do not fill the infected cell.

Refer to Figs D1-24 to D1-28.

Plasmodium knowlesi

- Infected red cells are not enlarged.
- Parasites at all stages of maturation may be present within the red cell.
- Early trophozoites are approximately the same size as their *P. malariae* counterparts. They may have two chromatin dots.
- Multiple infection of red cells is rare.
- Late trophozoites are compact and heavily pigmented; band forms are common.
- Schizonts contain between 8 and 10 merozoites.
- Gametocytes are compact and fill the red cell. They are similar to the gametocytes of *P. malariae*.

Refer to Figs D1-29 to D1-32.

DIAGNOSTIC TECHNIQUES

Blood specimens that are to be examined for malarial parasites should be tested by the laboratory as follows:

1. A full blood count should be performed; in the majority of malaria cases, leucopenia and thrombocytopenia will be present.
2. An additional thin film should be stained with Giemsa at pH 7.2; Schüffner's dots will only stain at this pH.
3. A thick film should be stained with Field's stain. Thick films are made by placing some blood on a glass slide and spreading it out to cover about four times its original area. The correct thickness will have been achieved if newsprint can be viewed through the film. Thick films concentrate malarial parasites, which is particularly helpful when the parasite density is low, while thin films are used to assess parasite morphology and stage of development.

Staining of malarial parasites

THIN FILM

The thin film is stained with a 10% solution of Gurr's R66 Giemsa in a pH 7.2 buffer for 30 minutes. This is the best stain for Schüffner's dots.

THICK FILM

Field's stain, a water-based stain composed of methylene blue (solution A) and eosin (solution B), is used to stain thick films. The thick film is dried for 20 minutes at 37°C. The unfixed slide is then dipped into solution A for 2 seconds and gently rinsed in buffered water (pH 6.8–7.0) to wash off excess stain; it is then dipped into solution B for 4 seconds and again the excess stain is washed off in buffered water. The slide is examined under oil immersion.

Parasite density count

Parasite counts are a necessary part of the microscopic diagnosis of infections with *P. falciparum*. They give a guide to parasite density and are used to assess the effect of specific treatment.

The most accurate method to determine the parasite density is to compare the number of parasites with the number of white cells on the thick film. This is done by counting 100 white cells in consecutive fields and then counting the number of malarial parasites that occur in those same fields. The number of parasites x 10^9/L is expressed as follows:

$$\frac{\text{number of parasites}}{\text{100 white cells}} \times \text{WCC} = \text{parasite density} \times 10^9 / \text{L}$$

Relating parasite numbers to red blood cells is not recommended since anaemia and multiple infections of red cells will introduce errors. With that in mind, clinicians still prefer to have a result reflecting the percentage of red cells infected.

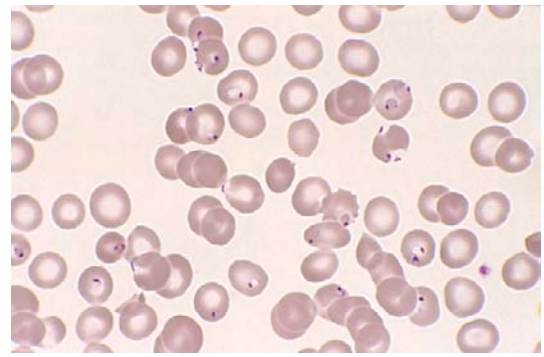


Figure D1-1
Plasmodium falciparum: peripheral blood film showing early trophozoites. (x 1000)

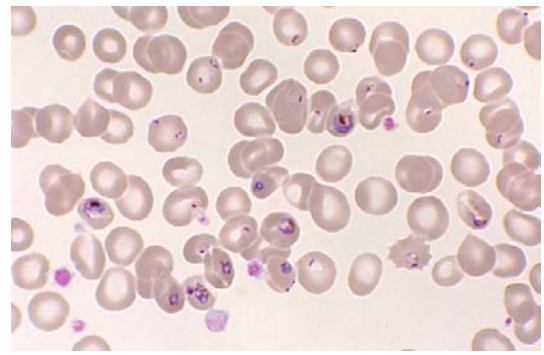


Figure D1-2
Plasmodium falciparum: peripheral blood film showing early and late trophozoites. (x 1000)

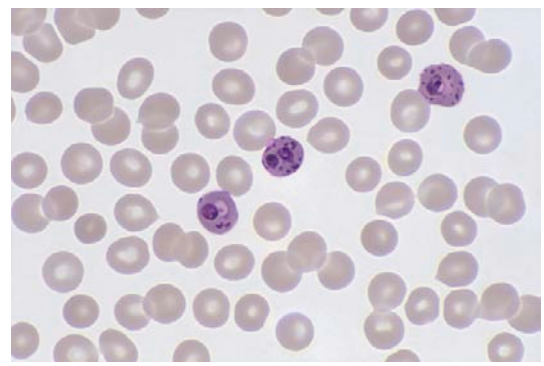


Figure D1-3
Plasmodium falciparum: peripheral blood film showing the presence of Maurer's dots and clefts. (x 1000)

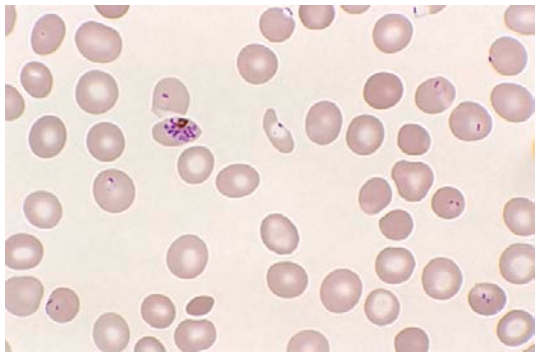


Figure D1-4
Plasmodium falciparum: peripheral blood film showing early trophozoites and a single schizont. (x 1000)

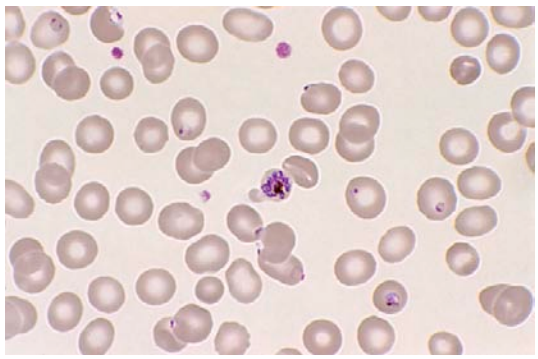


Figure D1-5
Plasmodium falciparum: peripheral blood film showing early and late trophozoites and a single schizont. (x 1000)

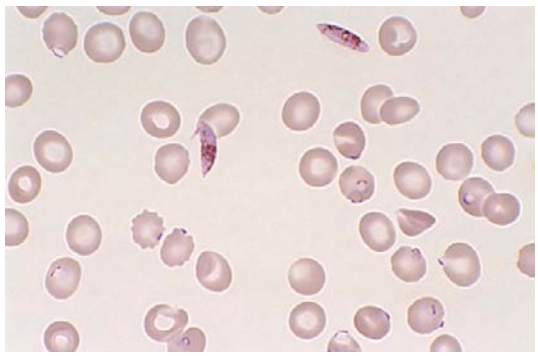


Figure D1-6
Plasmodium falciparum: peripheral blood film showing early trophozoites and gametocytes. (x 1000)

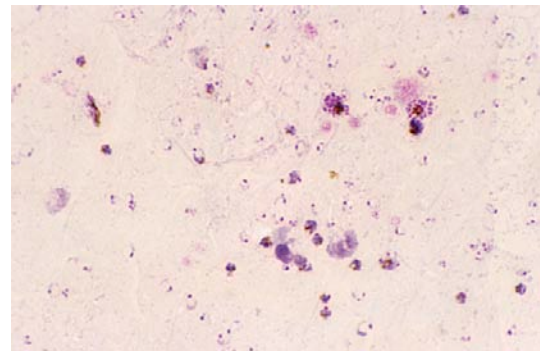


Figure D1-7
Plasmodium falciparum: thick film showing early and late trophozoites, schizonts and a single gametocyte. (x 1000)

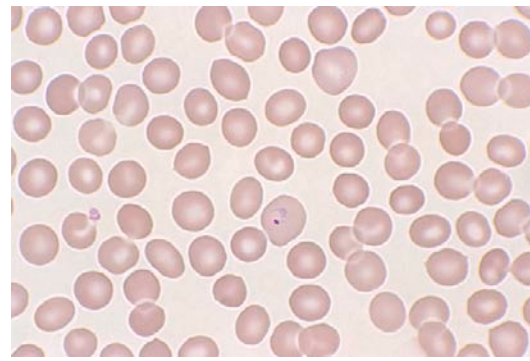


Figure D1-8
Plasmodium vivax: peripheral blood film showing an early trophozoite. (x 1000)

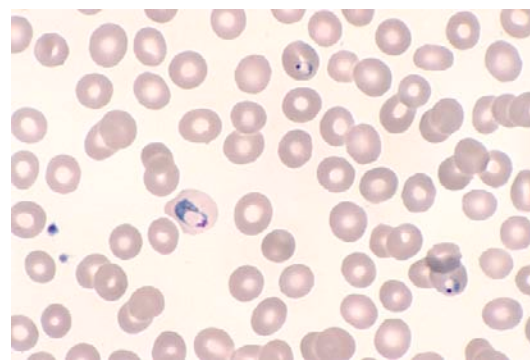


Figure D1-9
Plasmodium vivax: peripheral blood film showing early trophozoites. (x 1000)

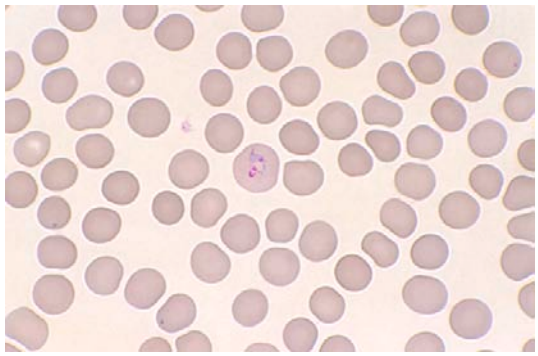


Figure D1-10
Plasmodium vivax: peripheral blood film showing a late trophozoite. (x 1000)

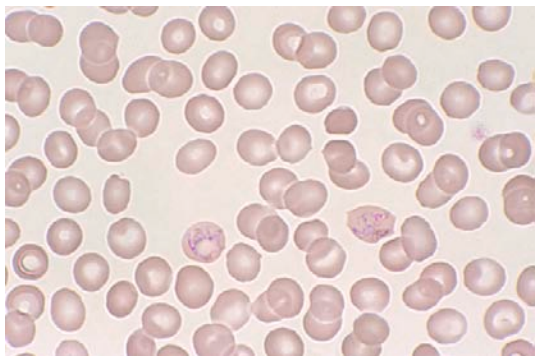


Figure D1-11
Plasmodium vivax: peripheral blood film showing late trophozoites. (x 1000)

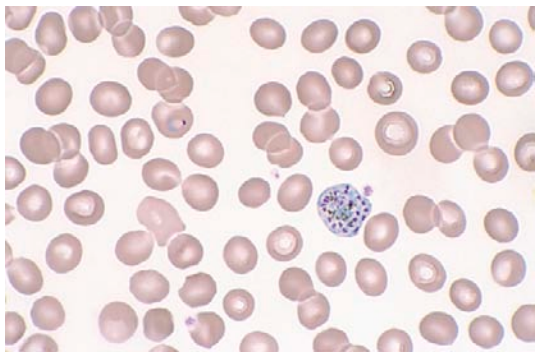


Figure D1-12
Plasmodium vivax: peripheral blood film showing an early trophozoite and schizont. (x 1000)

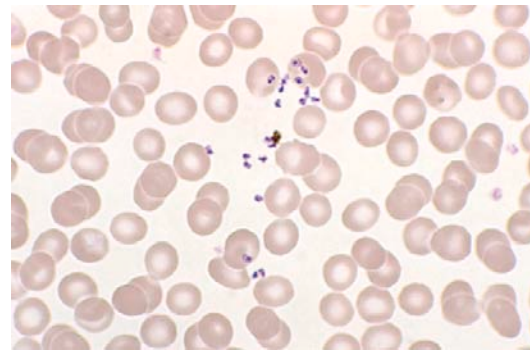


Figure D1-13
Plasmodium vivax: peripheral blood film showing individual merozoites released from a ruptured schizont. (x 1000)

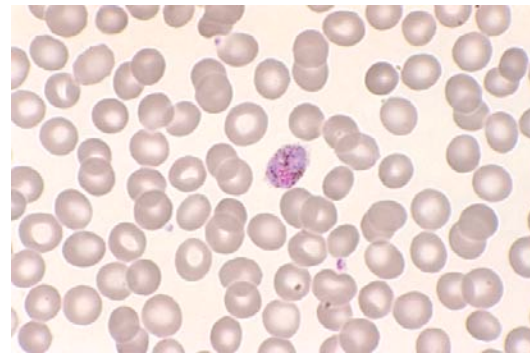


Figure D1-14
Plasmodium vivax: peripheral blood film showing a single gametocyte. (x 1000)

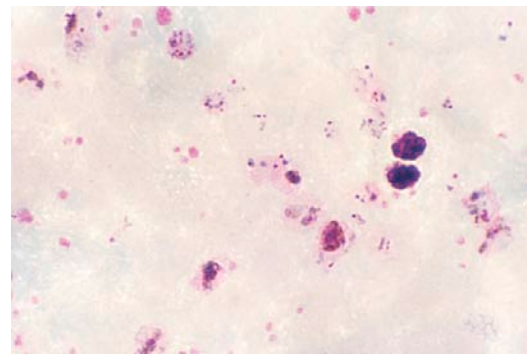


Figure D1-15
Plasmodium vivax: thick film showing early and late trophozoites and gametocytes. (x 1000)

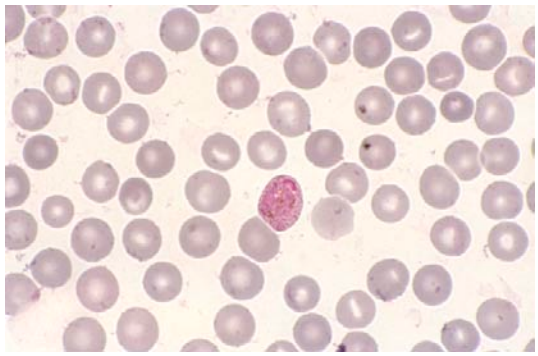


Figure D1-16
Plasmodium vivax: peripheral blood film showing a late trophozoite with Schüffner's dots (pH 7.2). (x 1000)

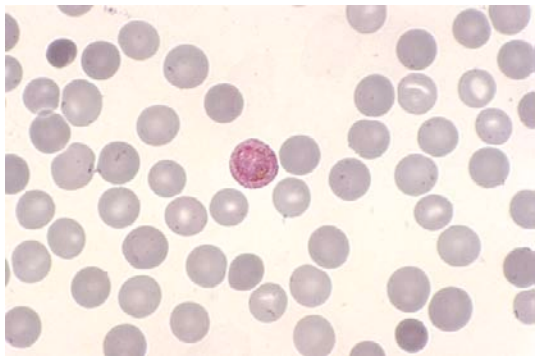


Figure D1-17
Plasmodium vivax: peripheral blood film showing a gametocyte with Schüffner's dots (pH 7.2). (x 1000)

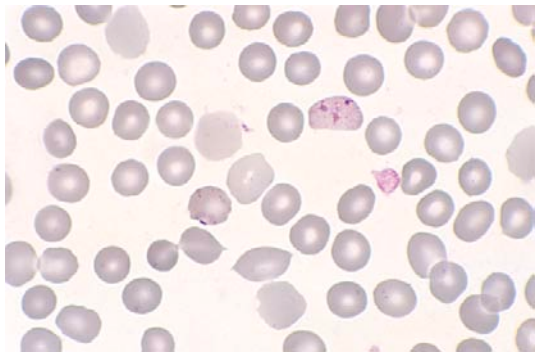


Figure D1-18
Mixed infection: peripheral blood film showing a late trophozoite of *P. vivax* with Schüffner's dots as well as an accolé trophozoite of P. falciparum (pH 7.2). (x 1000)

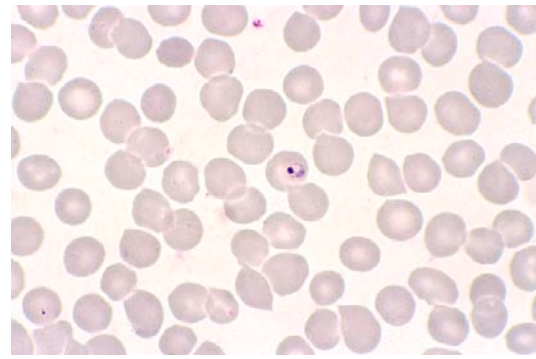


Figure D1-19
Plasmodium malariae: peripheral blood film showing an early trophozoite. (x 1000)

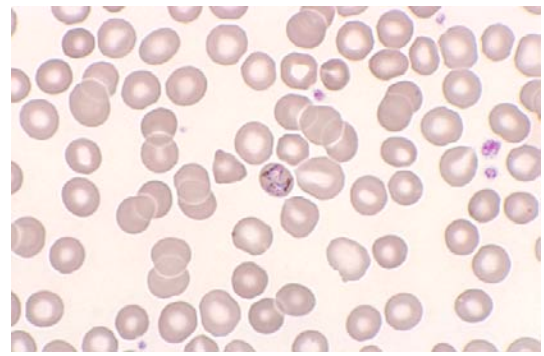


Figure D1-20
Plasmodium malariae: peripheral blood film showing a late trophozoite. (x 1000)

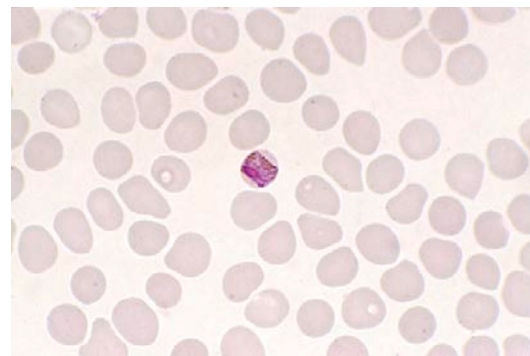


Figure D1-21
Plasmodium malariae: peripheral blood film showing a band-form trophozoite. (x 1000)

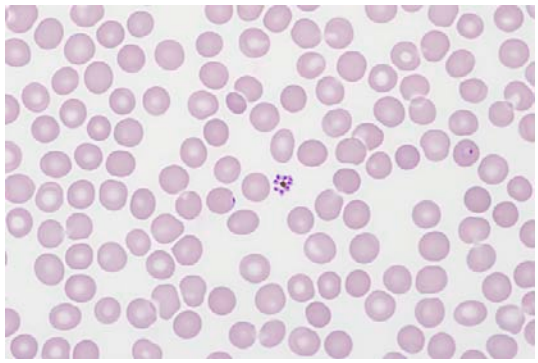


Figure D1-22
Plasmodium malariae: peripheral blood film showing a single schizont. (x 1000)

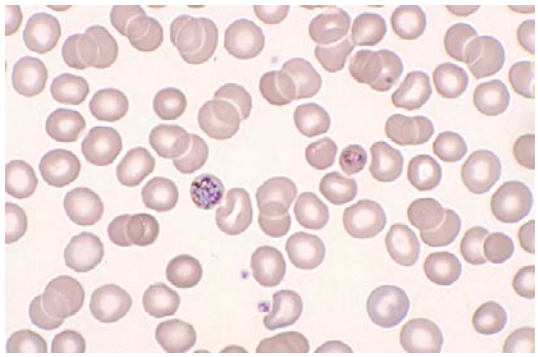


Figure D1-23
Plasmodium malariae: peripheral blood film showing a late trophozoite and gametocyte. (x 1000)

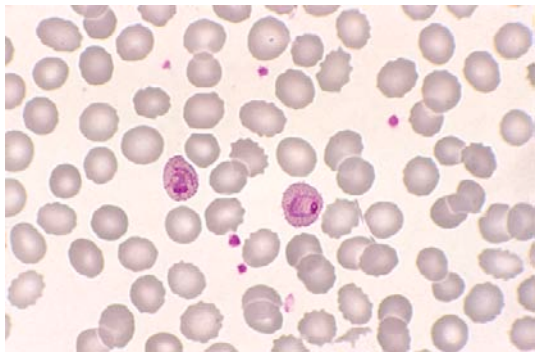


Figure D1-24
Plasmodium ovale: peripheral blood film showing an early and late trophozoite (pH 7.2). (x 1000)

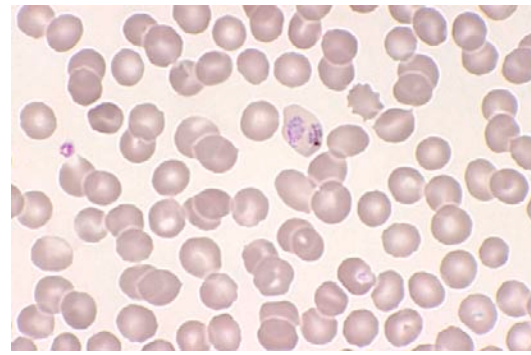


Figure D1-25
Plasmodium ovale: peripheral blood film showing a late trophozoite. (x 1000)

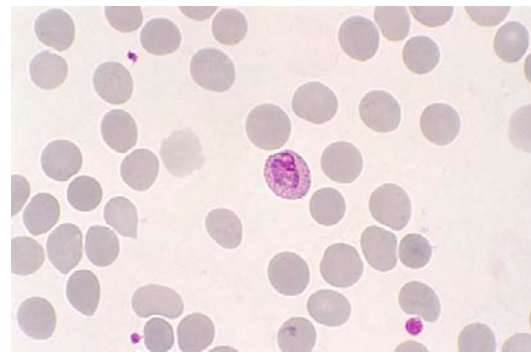


Figure D1-26
Plasmodium ovale: peripheral blood film showing a late trophozoite (pH 7.2). (x 1000)

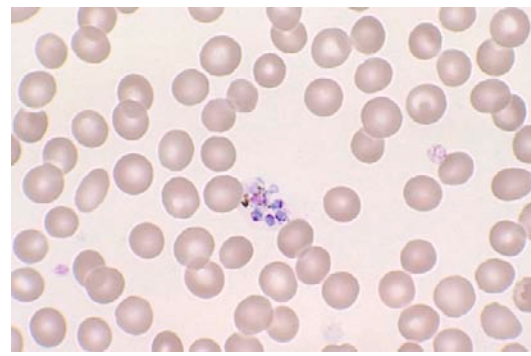


Figure D1-27
Plasmodium ovale: peripheral blood film showing a single schizont. (x 1000)

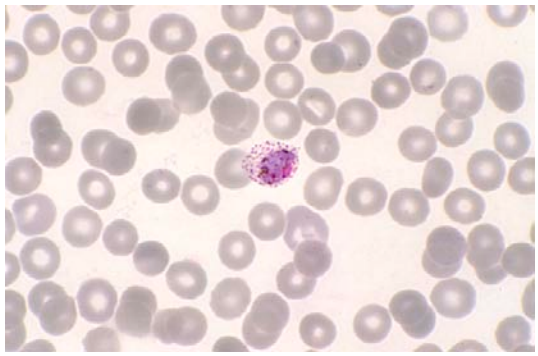


Figure D1-28
Plasmodium ovale: peripheral blood film showing a single gametocyte (pH 7.2). (x 1000)

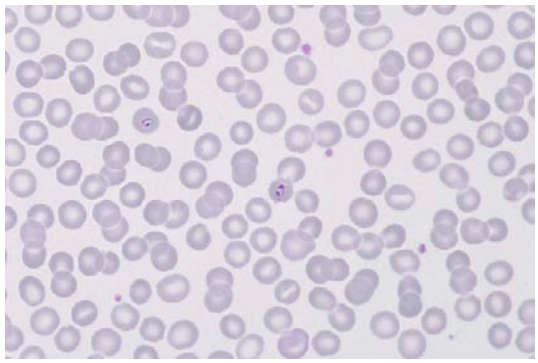


Figure D1-29
Plasmodium knowlesi: peripheral blood film showing early trophozoites (pH 7.2). (x 1000)

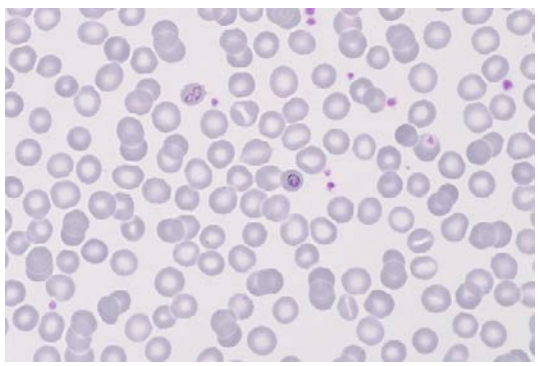


Figure D1-30
Plasmodium knowlesi: peripheral blood film showing late trophozoites (pH 7.2). (x 1000)

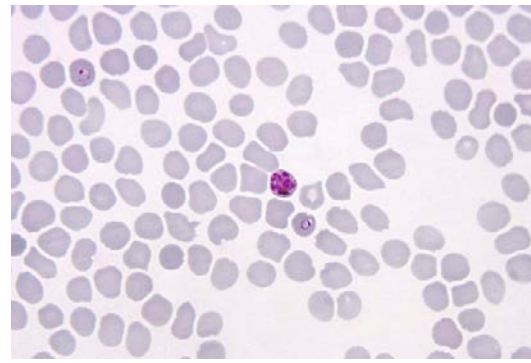


Figure D1-31
Plasmodium knowlesi: peripheral blood film showing a single schizont (pH 7.2). (x 1000) (Courtesy of Robyn Wells)

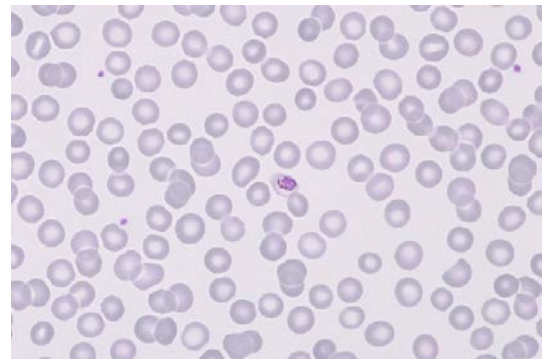
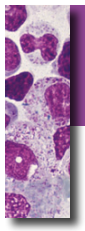


Figure D1-32
Plasmodium knowlesi: peripheral blood film showing a single gametocyte (pH 7.2). (x 1000)



D2 Non-malarial blood parasites

MICROFILARIA (*WUCHERERIA BANCROFTI*)

Microfilaria are threadworms (Nematoda). They are transmitted to humans by flies and mosquitoes, lodging in the lymphatic vessels where they cause lymphangitis and elephantiasis. Microfilaria are approximately 280 µm in length and 7 µm wide and are covered by an outer sheath. The nuclei of the body cells do not extend to the end of the gradually tapering tail. Microfilaria are found in the blood stream only during the night. Refer to Fig D2-1.

MICROFILARIA (*LOA LOA*)

Loa loa microfilaria is transmitted to humans by deer flies and mango (mangrove) flies namely *Chrysops silicea* and *Chrysops dimidiata*. The geographical distribution of *Loa loa* is restricted to the rainforest and swamp forest areas of West Africa. *Loa loa* (also known as the African eye worm) migrates throughout the subcutaneous tissues, occasionally crossing into the subconjunctival tissue where it can be easily observed. Adult *Loa loa* worms are sexual; the female worms measure 400 to 700 µm in length and 5 µm in diameter while the males measure 300 to 340 µm in length and 4.3 µm in diameter. *Loa loa* microfilaria can be morphologically recognised from other species of microfilaria as they are sheathed and contain body nuclei that extend to the tip of the tail. *Loa loa* are found in the peripheral blood during the day. Refer to Fig D2-2.

TRYPANOSOME (*TRYPANOSOMA BRUCEI GAMBIENSE*)

Trypanosomes are protozoa and belong to the Flagellata. *Trypanosoma brucei gambiense*, together with *T brucei rhodesiense*, are the causative agents of acute sleeping sickness and occur only in Africa. They are approximately 26 µm in length and 1–3 µm wide and have a central nucleus and, at the forward-moving end, a flagellum ending in a kinetoplast that (like the nucleus) stains red with Romanowsky stains.

Trypanosomes are transmitted by the tsetse fly. Refer to Fig D2-3.

HISTOPLASMOSIS (*HISTOPLASMA CAPSULATUM*)

Histoplasma capsulatum is a dimorphic fungus that grows as a mould in many areas of the world. It grows well in moist surface soil enriched with bird and bat droppings. The hyphae of *H. capsulatum* produce unencapsulated spores, both small and large. These spores, when inhaled, are ultimately phagocytosed by neutrophils and may be seen on the blood film and bone marrow of immunosuppressed patients, especially those with AIDS. Refer to Fig D2-4.

LEISHMANIASIS (*LEISHMANIA INFANTUM*)

Leishmaniasis refers to a range of clinical states caused by the protozoa of the genus *Leishmania*. Leishmaniasis occurs in diverse ecological settings throughout the world; it is not found in Australia.

The amastigotes of *Leishmania* stain with any of the Romanowsky stains. They measure 2–4 µm in diameter and have a nucleus and a rod-shaped kinetoplast. Leishmaniasis is transmitted by the phlebotomine sandfly (genus *Phlebotomus*). Refer to Figs D2-5 to D2-7.

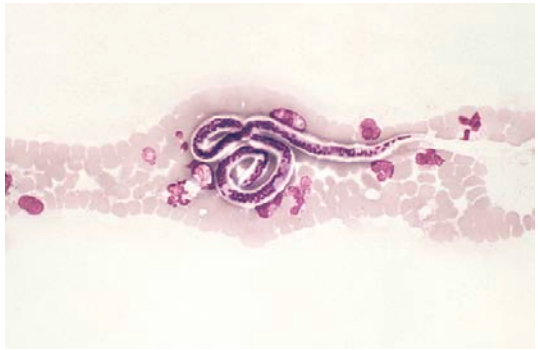


Figure D2-1
Microfilaria (*Wuchereria bancrofti*). (x 1000)

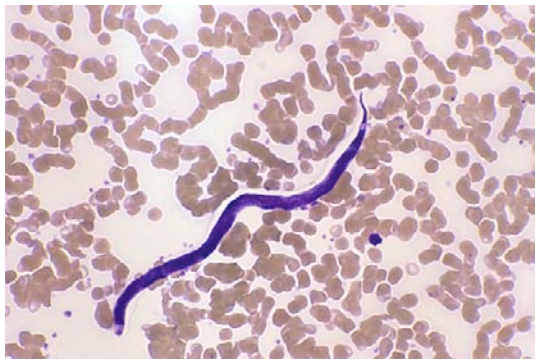


Figure D2-2
Microfilaria (*Loa loa*). (x 1000)

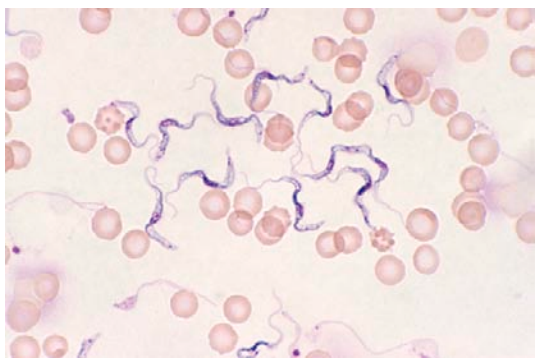


Figure D2-3
Trypanosomes (*Trypanosoma brucei gambiense*). (x 1000)

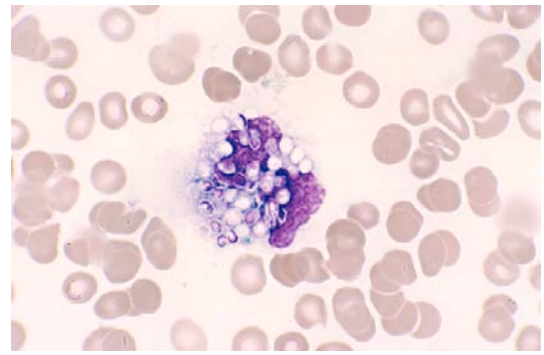


Figure D2-4
Histoplasmosis (*Histoplasma capsulatum*). (x 1000)

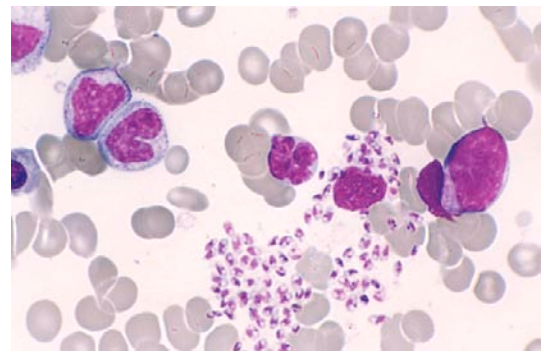


Figure D2-5
Leishmaniasis (*Leishmania infantum*). (x 1000)

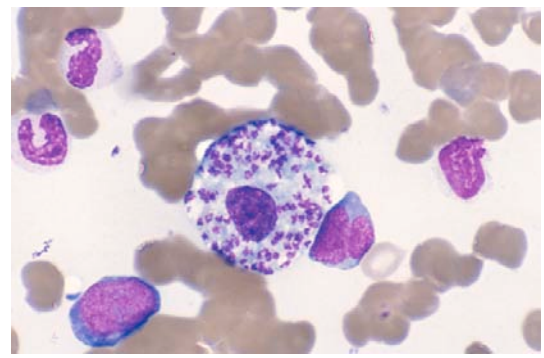


Figure D2-6
Leishmaniasis (*Leishmania infantum*) contained within a bone marrow macrophage. (x 1000)

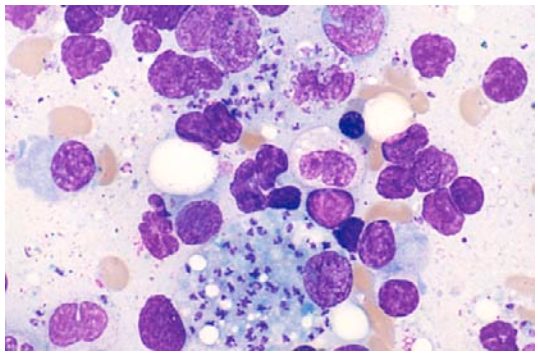


Figure D2-7
Leishmaniasis (*Leishmania infantum*) infiltrating the bone marrow. (x 1000)

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