

THE ESSENTIAL RAPID REFERENCE FOR  
ALL HAEMATOLOGISTS

# OXFORD HANDBOOK OF CLINICAL HAEMATOLOGY

Drew Provan | Trevor Baglin  
Inderjeet Dokal | Johannes de Vos

A concise, clinical handbook covering the whole of haematology

Includes rare disorders as well as common conditions affecting both adults and children

Contains all the latest guidelines and a brand new chapter on rare diseases

Covers all major advances in the specialty including malignant haematology and transfusion medicine

FOURTH EDITION  
**4**  
FOURTH EDITION

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Oxford Handbook of Rheumatology 3/e  
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Oxford Handbook of Urology 3/e



# Oxford Handbook of Clinical Haematology

FOURTH EDITION

## **Drew Provan**

Reader in Haematology  
Barts & The London School of Medicine and Dentistry  
Queen Mary University of London, UK

## **Trevor Baglin**

Consultant Haematologist  
Addenbrooke's NHS Trust  
Cambridge, UK

## **Inderjeet Dokal**

Chair of Child Health and Centre Lead  
Barts & The London School of Medicine and Dentistry  
Queen Mary University of London  
Honorary Consultant  
Barts Health NHS Trust  
London, UK

## **Johannes de Vos**

Consultant Haematologist  
Royal Surrey County Hospital NHS Foundation Trust  
Guildford, UK

**OXFORD**  
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# Preface to the fourth edition

The world of haematology has been exciting over the past few years, and we have seen major advances since the third edition of the *Oxford Handbook of Clinical Haematology* was published. These are most obvious in haemato-oncology, with the development of new agents and regimens for treating malignant haematology disorders. But there have also been advances in haemostasis and red cell haematology with the arrival of the novel oral anticoagulation drugs and new oral chelators for the treatment of iron overload, in addition to a number of other advances.

This edition sees a change of editorial team and we are very happy to have John de Vos, haemato-oncologist, on board. He has overhauled the haemato-oncology sections bringing them thoroughly up to date. We have also sought the advice of Shubha Allard, consultant in transfusion medicine, to make sure the blood transfusion section is accurate, in addition to Banu Kaya, a red cell haematologist who has brought the red cell material up to date.

For the first time, we have incorporated a new chapter on rare disorders which we hope readers will find useful.


We are very grateful to the editorial team at Oxford University Press for their patience and hard work, especially Liz Reeve and Michael Hawkes.

There may be errors or omissions from the book and we would welcome any comments or feedback (email [drewprovan@mac.com](mailto:drewprovan@mac.com)). We will try to incorporate these in future editions.

DP  
TB  
ID  
JdV

January 2014

# Preface to the third edition

It is hard to believe that at least three years have passed since the second edition of the handbook. As with all medical specialties, Haematology has seen major inroads with new diagnostic tests, treatments and a plethora of guidelines. In fact, Haematology has the largest collection of guidelines covering all aspects of haematology care ( <http://www.bcshguidelines.com>) and was the first specialty to design guidelines in the 1980s.

The book underwent a major revision with the second edition, most notably the sections dealing with malignant disease. For the new edition these have been brought right up to date by Charles Singer. Coagulation has been entirely rewritten by Trevor Baglin and now truly reflects the current investigation and management of coagulation disorders. Following the retirement of Professor Sir John Lilleyman we needed to find a new author for the Paediatric Haematology component of the book. Thankfully, we were able to persuade Professor Inderjeet Dokal to take on this mantle and he has revised this section thoroughly.

In addition to these significant changes, we have gone through the entire book and attempted to ensure that obsolete tests have been removed and that the Handbook, in its entirety, reflects contemporary haematology practice.

As ever, we are very keen to hear about errors or omissions, for which we are entirely responsible! We would also very much like readers to contact us if there are topics or subject areas which they would like to see included in the fourth edition. We also need more trainee input so if there are any volunteer proof-readers or accuracy checkers among the haematology trainee community we would very much like to hear from you.

DP  
CRJS  
TB  
ISD  
2008

# Foreword to the fourth edition

The *Concise Oxford English Dictionary* defines a handbook as 'a short manual or guide'. Modern haematology is a vast field which involves almost every other medical speciality and which, more than most, straddles the worlds of the basic biomedical sciences and clinical practice. Since the rapidly proliferating numbers of textbooks on this topic are becoming denser and heavier with each new edition, the medical student and young doctor in training are presented with a daunting problem, particularly as they try to put these fields into perspective. And those who try to teach them are not much better placed; on the one hand they are being told to decongest the curriculum, while on the other they are expected to introduce large slices of molecular biology, social science, ethics, and communication skills, not to mention a liberal sprinkling of poetry, music, and art.

In this over-heated educational scene the much maligned 'handbook' could well stage a comeback and gain new respectability, particularly in the role of a friendly guide. In the past this genre has often been viewed as having little intellectual standing, of no use to anybody except the panic-stricken student who wishes to try to make up for months of mis-spent time in a vain, one-night sitting before their final examination. But given the plethora of rapidly changing information that has to be assimilated, the carefully prepared précis is likely to play an increasingly important role in medical education. Perhaps even that ruination of the decent paragraph and linchpin of the pronouncements of medical bureaucrats, the 'bullet point', may become acceptable, albeit in small doses, as attempts are made to highlight what is really important in a scientific or clinical field of enormous complexity and not a little uncertainty.

In the fourth edition of this short account of blood diseases the editors have continued to provide an excellent service to medical students, as well as doctors who are not specialists in blood diseases, by summarizing in simple terms the major features and approaches to diagnosis and management of most of the blood diseases that they will encounter in routine clinical practice or in the tedious examinations that face them. And, of equal importance, they have been able to update and summarize some of the major advances that have been made in this rapidly moving field since the appearance of the early editions of this handbook. As in previous editions they have managed to avoid one of the major pitfalls of this type of teaching: in trying to reduce complex issues down to their bare bones it is all too easy to introduce inaccuracies.

One word of warning from a battle-scarred clinician however. A précis of this type suffers from the same problem as a set of multiple-choice questions. Human beings are enormously complex organisms, and sick ones are even more complicated; during a clinical lifetime the self-critical doctor will probably never encounter a 'typical case' of anything. Thus the

outlines of the diseases that are presented in this book must be used as approximate guides, and no more. But provided they bear this in mind, students will find that it is a very valuable summary of modern haematology; the addition of the Internet sources is a genuine and timely bonus.

D. J. Weatherall  
Oxford, June 2014

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# Acknowledgements

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We would like to acknowledge the patience and forbearance of our wives and families for the months of neglect imposed by the work on this edition. Warm thanks, as ever, are extended to Oxford University Press, and in particular Catherine Barnes, Senior Commissioning Editor for Medicine, Elizabeth Reeve, Commissioning Editor, Beth Womack, Managing Editor, and Kate Wilson, Production Manager. We fell behind schedule with this edition and are grateful to the whole OUP team for bearing with us so patiently and not harassing us! We apologize for anyone omitted but this is entirely unintentional.



# Contributors

**Shubha Allard**

Consultant Haematologist  
Barts Health NHS Trust, and NHS Blood & Transplant, UK

**Banu Kaya**

Consultant Haematologist  
Barts Health NHS Trust, UK

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# Symbols and abbreviations

↔	cross-reference
↓	decreased
↑	increased
▶	important
▶▶	very important
↔	normal
🌐	website
♀	female
♂	male
1°	primary
2°	secondary
2,3 DPG	2,3 diphosphoglycerate
2-CDA	2-chlorodeoxyadenosine
A <sub>2</sub> -M	alpha-2 microglobulin
6-MP	6-mercaptopurine
<sup>99m</sup> Tc-MIBI	<sup>99m</sup> Tc methoxyisobutyl-isonitride or <sup>99m</sup> Tc-MIBI scintigraphy
AA	aplastic anaemia or reactive amyloidosis
Ab	antibody
ABVD	adriamycin (doxorubicin), bleomycin, vinblastine, dacarbazine
ACD	acid-citrate-dextrose or anaemia of chronic disease
ACE	angiotensin converting-enzyme
ACL	anticardiolipin antibody
ACML	atypical chronic myeloid leukaemia
ADA	adenosine deaminase
ADE	cytosine arabinoside (Ara-C) daunorubicin etoposide
ADP	adenosine 5-diphosphate
AFB	acid-fast bacilli
Ag	antigen
AIDS	acquired immunodeficiency syndrome
AIHA	autoimmune haemolytic anaemia
AIN	autoimmune neutropenia
AITL	angio-immunoblastic T-cell lymphoma
AL	(1°) amyloidosis
ALB	serum albumin

ALCL	anaplastic large cell lymphoma
ALG	antilymphocyte globulin
ALIPs	abnormal localization of immature myeloid precursors
ALL	acute lymphoblastic leukaemia
ALS	advanced life support
ALT	alanine aminotransferase
AML	acute myeloid leukaemia
AMP	adenosine monophosphate
ANA	antinuclear antibodies
ANAE	alpha naphthyl acetate esterase
ANCA	antineutrophilic cytoplasmic antibody
ANH	acute normovolaemic haemodilution
APC	activated protein C
APCR	activated protein C resistance
APL	antiphospholipid antibody
APML	acute promyelocytic leukaemia
APS	antiphospholipid syndrome
APTR	activated partial thromboplastin ratio
APTT	activated partial thromboplastin time
ARDS	adult respiratory distress syndrome
ARF	acute renal failure
ARMS	amplification refractory mutation system
ASCT	autologous stem cell transplantation
AST	aspartate aminotransferase
AT (ATIII)	antithrombin III
ATCML	adult-type chronic myeloid (granulocytic) leukaemia
ATG	antithymocyte globulin
ATLL	adult T-cell leukaemia/lymphoma
ATP	adenosine triphosphate
ATRA	all-trans retinoic acid
A-V	arteriovenous
AvWS	acquired von Willebrand syndrome
$\beta_2$ -M	beta-2-microglobulin
BAL	broncho-alveolar lavage
B-CLL	B-cell chronic lymphocytic leukaemia
bd	bis die (twice daily)
BEAC	BCNU (Carmustine), etoposide, cytosine, cyclophosphamide
BEAM	BCNU (Carmustine), etoposide, cytarabine (ara-C), melphalan

BFU-E	burst-forming unit-erythroid
BJP	Bence Jones protein
BL	Burkitt lymphoma
BM	bone marrow
BMJ	<i>British Medical Journal</i>
BMM	bone marrow mastocytosis
BMT	bone marrow transplantation
BNF	<i>British National Formulary</i>
BP	blood pressure
BPL	BioProducts Laboratory
BSS	Bernard–Soulier syndrome
BTG	$\beta$ -thromboglobulin
BU	Bethesda units
C/I	consolidation/intensification
Ca	carcinoma
Ca <sup>2+</sup>	calcium
CABG	coronary artery bypass graft
cALL	common acute lymphoblastic leukaemia
CAMT	congenital amegakaryocytic thrombocytopenia
CaPO <sub>4</sub>	calcium phosphate
CBA	collagen binding activity
CBV	cyclophosphamide, carmustine (BCNU), etoposide
CCF	congestive cardiac failure
CCR	complete cytogenetic response
CD	cluster differentiation or designation
CDA	congenital dyserythropoietic anaemia
cDNA	complementary DNA
CEL	chronic eosinophilic leukaemia
CGL	chronic granulocytic leukaemia
CHAD	cold haemagglutinin disease
CHOP	cyclophosphamide, doxorubicin, vincristine, prednisolone
CJD	Creutzfeldt–Jakob disease (v = variant)
Cl <sup>-</sup>	chloride
CLD	chronic liver disease
CLL	chronic lymphocytic ('lymphatic') leukaemia
CM	cutaneous mastocytosis
CMC	chronic mucocutaneous candidiasis
CML	chronic myeloid leukaemia
CMML	chronic myelomonocytic leukaemia
CMV	cytomegalovirus

CNS	central nervous system
COAD	chronic obstructive airways disease
COC	combined oral contraceptive
CR	complete remission
CRF	chronic renal failure
CRP	C-reactive protein
CRVT	central retinal venous thrombosis
CsA	ciclosporin A
CSF	cerebrospinal fluid
CT	computed tomography
CTLp	cytotoxic T-lymphocyte precursor assays
CTZ	chemoreceptor trigger zone
CVA	cerebrovascular accident
CVP	cyclophosphamide, vincristine, prednisolone; central venous pressure
CVS	chorionic villus sampling
CVS	cardiovascular system
CXR	chest x-ray
CyA	ciclosporin A
CytaBOM	cytarabine, bleomycin, vincristine, methotrexate
d	day
DAGT	direct antiglobulin test
DAT	direct antiglobulin test daunorubicin, cytosine (Ara-C),
dATP	deoxy ATP
DBA	Diamond–Blackfan anaemia
DC	dyskeratosis congenita
DCS	dendritic cell system
DCT	direct Coombs' test
DDAVP	desamino D-arginyl vasopressin
DEAFF	detection of early antigen fluorescent foci
DEB	diepoxy butane
DFS	disease-free survival
DHAP	dexamethasone, cytarabine, cisplatin
DI	delayed intensification
DIC	disseminated intravascular coagulation
dL	decilitre
DLBCL	diffuse large B-cell lymphoma
DLI	donor leucocyte/lymphocyte infusion
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid

DOB	date of birth
DPG	diphosphoglycerate
DRVVT	dilute Russell's viper venom time/test
DTT	dilute thromboplastin time
DVT	deep vein thrombosis
DXT	radiotherapy
EACA	epsilon aminocaproic acid
EBV	Epstein–Barr virus
EBVP	etoposide, bleomycin, vinblastine, prednisolone
ECG	electrocardiograph
ECOG	European Co-operative Oncology Group
EDTA	ethylenediamine tetraacetic acid
EEC	endogenous erythroid colonies
EFS	event-free survival
EGF	epidermal growth factor
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EMH	extramedullary haemopoietic
EMU	early morning urine
EPO	erythropoietin
EPOCH	etoposide, vincristine, doxorubicin, cyclophosphamide, prednisone
EPS	electrophoresis
ESHAP	etoposide, methylprednisolone, cytarabine, platinum
ESR	erythrocyte sedimentation rate
ET	essential thrombocythaemia or exchange transfusion
ETTL	enteropathy type T-cell lymphoma
FAB	French–American–British
FACS	fluorescence-activated cell sorter
FBC	full blood count
FCM	fludarabine, cyclophosphamide, melphalan
FDG-PET	<sup>18</sup> fluoro-D-2-deoxyglucose positron emission tomography
FDP	fibrin degradation products
Fe	iron
FEIBA	factor eight inhibitor bypassing activity
FEL	familial erythrophagocytic lymphohistiocytosis
FeSO <sub>4</sub>	ferrous sulfate
FFP	fresh frozen plasma
FFS	failure-free survival
Fgn	fibrinogen

FH	family history
FISH	fluorescence <i>in situ</i> hybridization
FITC	fluorescein isothiocyanate
FIX	factor IX
fL	femtolitre
FL	follicular lymphoma
FNA	fine needle aspirate
FNHTR	febrile non-haemolytic transfusion reaction
FOB	faecal occult blood
FVIII	factor VIII
FVL	factor V Leiden
g	gram
G&S	group, screen, and save
G6PD	glucose-6-phosphate dehydrogenase
GA	general anaesthetic
GCS	graded compression stockings
G-CSF	granulocyte colony stimulating factor
GI	gastrointestinal
GIT	gastrointestinal tract
GM-CSF	granulocyte macrophage colony stimulating factor
GP	glycoprotein
GPI	glycosylphosphatidylinositol
GPS	grey platelet syndrome
GT	Glanzmann thrombasthenia
GvHD	graft-versus-host disease
GvL	graft versus leukaemia
h	hour
HAART	highly active antiretroviral therapy
HAV	hepatitis A virus
Hb	haemoglobin
HbA	haemoglobin A
HbA <sub>2</sub>	haemoglobin A <sub>2</sub>
HbF	haemoglobin F (fetal Hb)
HbH	haemoglobin H
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HC	hydroxycarbamide <i>or</i> heavy chain
HCD	heavy chain disease
HCG	human chorionic gonadotrophin
HCI	heparin cofactor II



HCL	hairy cell leukaemia
HCO <sub>3</sub>	bicarbonate
Hct	haematocrit
HCV	hepatitis C virus
HD	haemodialysis
HDM	high-dose melphalan
HDN	haemolytic disease of the newborn
HDT	high-dose therapy
HE	hereditary elliptocytosis
HELLP	haemolysis, elevated liver enzymes and low platelets
HES	hypereosinophilic syndrome
HHT	hereditary haemorrhagic telangiectasia
HI	haematological improvement
HIT(T)	heparin-induced thrombocytopenia (with thrombosis)
HIV	human immunodeficiency virus
HL	Hodgkin lymphoma (Hodgkin disease)
HLA	human leucocyte antigen
HLH	haemophagocytic lymphohistiocytosis
HMP	hexose monophosphate shunt
HMW	high molecular weight
HMWH	high molecular weight heparin
HMWK	high-molecular-weight kininogen
HPA	human platelet antigen
HPF	high power field
HPFH	hereditary persistence of fetal haemoglobin
HPLC	high-performance liquid chromatography
HPP	hereditary pyropoikilocytosis
HRT	hormone replacement therapy
HS	hereditary spherocytosis
HTC	hospital transfusion committee
HTLV-1	human T-lymphotropic virus type 1
HTO	high titre antibodies
HUMARA	human androgen receptor gene assay
HUS	haemolytic uraemic syndrome
IAGT	indirect antiglobulin test
IAHS	infection-associated haemophagocytic syndrome
ICE	ifosfamide, carboplatin, etoposide
ICH	intracranial haemorrhage
ICUS	idiopathic cytopenia of uncertain (undetermined) significance

IDA	iron deficiency anaemia
IF	involved field (radiotherapy)
IFA	intrinsic factor antibody
IFRT	involved field radiotherapy
Ig	immunoglobulin
IgA	immunoglobulin A
IgD	immunoglobulin D
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IL-1	interleukin-1
IM	intramuscular
IMF	idiopathic myelofibrosis
INR	international normalized ratio
inv	chromosomal inversion
IPC	intermittent pneumatic compression devices
IPF	immature platelet fraction
IPI	International Prognostic Index
IPSS	International Prognostic Scoring System
ISM	Indolent systemic mastocytosis
ISS	International Sensitivity Index
IST	immune suppressive therapy
IT	intrathecal
ITP	idiopathic thrombocytopenic purpura
ITU	Intensive Therapy Unit
IU	international units
IUGR	intrauterine growth retardation
IUT	intrauterine transfusion
IV	intravenous
IVI	intravenous infusion
IVIg	intravenous immunoglobulin
JCMML	juvenile chronic myelomonocytic leukaemia
JML	juvenile myelomonocytic leukaemia
JVP	jugular venous pressure
KCT	kaolin clotting time
kg	kilogram
L	litre
LA	lupus anticoagulant
LAP	leucocyte alkaline phosphatase (score)
LC	light chain

LCH	Langerhans cell histiocytosis
LDH	lactate dehydrogenase
LDHL	lymphocyte depleted Hodgkin lymphoma
LFS	leukaemia-free survival
LFTs	liver function tests
L&H	lymphocytic and histiocytic
LGL	large granular lymphocyte
LLN	lower limit of normal
LMWH	low-molecular-weight heparin
LN	lymph node(s)
LP	lumbar puncture
LPD	lymphoproliferative disorder
LRCHL	lymphocyte-rich classical HL
LSCS	lower segment Caesarian section
LTC	large transformed cells
M&P	melphalan and prednisolone
MACOP-B	methotrexate, doxorubicin, cyclophosphamide, vincristine, bleomycin, prednisolone
MAHA	microangiopathic haemolytic anaemia
MALT	mucosa-associated lymphoid tissue
m-BACOD	methotrexate, bleomycin, doxorubicin (adriamycin), cyclophosphamide, vincristine, dexamethasone
mcg	microgram
MC	mast cell(s)
MCH	mean cell haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCHL	mixed cellularity Hodgkin lymphoma
MCL	mast cell leukaemia or mantle cell lymphoma
MCP	mitoxantrone, chlorambucil, prednisolone
MCR	major cytogenetic response
MCS	mast cell sarcoma
MC&S	microscopy, culture, and sensitivity
M-CSF	macrophage colony stimulating factor
MCV	mean cell volume
MDS	myelodysplastic syndrome
MetHb	methaemoglobin
MF	myelofibrosis
mg	milligram
MGUS	monoclonal gammopathy of undetermined significance
MHC	major histocompatibility complex

MI	myocardial infarction
min	minute
mL	millilitre
MLC	mixed lymphocyte culture
MM	multiple myeloma
MMC	mitomycin C
MNC	mononuclear cell(s)
MoAb	monoclonal antibody
MP	melphalan and prednisolone
MPCM	maculopapular cutaneous mastocytosis
MPD	myeloproliferative disease
MPN	myeloproliferative neoplasm
MPO	myeloperoxidase
MPS	mononuclear phagocytic system
MPT	melphalan, prednisolone, and thalidomide
MPV	mean platelet volume
MRD	minimal residual disease
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MRSA	meticillin-resistant <i>Staphylococcus aureus</i>
MSBOS	maximum surgical blood ordering schedule
MSU	midstream urine
MT	mass: thoracic
MTX	methotrexate
MUD	matched unrelated donor (transplant)
MW	molecular weight
MZL	mantle zone lymphoma
Na <sup>+</sup>	sodium
NaCl	sodium chloride
NADP	nicotinamide adenine diphosphate
NADPH	nicotinamide adenine diphosphate (reduced)
NAIT	neonatal alloimmune thrombocytopenia
NAP	neutrophil alkaline phosphatase
NBT	nitro blue tetrazolium
NCCN	National Comprehensive Cancer Network
NEJM	<i>New England Journal of Medicine</i>
NHL	non-Hodgkin lymphoma
NLPHL	nodular lymphocyte predominant HL
NRBC	nucleated red blood cells
NS	nodular sclerosing

NS	non-secretory (myeloma)
NSAID	non-steroidal anti-inflammatory drug
NSE	non-specific esterase
NSHL	nodular sclerosing HL
OAF	OC-activating factor
OB	osteoblast
OC	osteoclast
OCP	oral contraceptive pill
od	<i>omni die</i> (once daily)
OPG	osteoprotogerin
OPG	orthopantomogram
OR	overall response
OS	overall survival
OWR	Osler–Weber–Rendu
PA	pernicious anaemia
PAI	plasminogen activator inhibitor
PaO <sub>2</sub>	partial pressure of O <sub>2</sub> in arterial blood
PAS	periodic acid–Schiff
PB	peripheral blood
PBSC	peripheral blood stem cell
PBSCH	peripheral blood stem cell harvest
PBSCT	peripheral blood stem cell transplant
PC	protein C
PCC	prothrombin complex concentrate
PCH	paroxysmal cold haemoglobinuria
PCL	plasma cell leukaemia
PCP	<i>Pneumocystis carinii</i> ( <i>jirovecii</i> ) pneumonia
PCR	polymerase chain reaction
PCV	packed cell volume
PD	peritoneal dialysis
PDGF	platelet-derived growth factor
PDW	platelet distribution width
PE	pulmonary embolism
PEP	post-exposure prophylaxis
PET	pre-eclamptic toxæmia or positron emission tomography
PF	platelet factor
PFA	platelet function analysis
PFK	phosphofructokinase
PFS	progression-free survival

PGD2	prostaglandin D2
PGE1	prostaglandin E1
PGK	phosphoglycerate kinase
Ph	Philadelphia chromosome
PIG	phosphatidylinositol glycoproteins
PIVKA	protein induced by vitamin K absence
PK	pyruvate kinase
PLL	prolymphocytic leukaemia
PML	promyelocytic leukaemia
PNET	primitive neuroectodermal tumour
PO	<i>per os</i> (by mouth)
PPH	post-partum haemorrhage
PPI	proton pump inhibitor
PPP	primary proliferative polycythaemia
PRCA	pure red cell aplasia
PRN	as required
ProMACE	prednisolone, doxorubicin, cyclophosphamide, etoposide
PRV	polycythaemia rubra vera
PS	protein S
PSA	pure sideroblastic anaemia <i>or</i> prostate-specific antigen
PT	prothrombin time
PTCL	peripheral T-cell lymphomas
PTP	pretest probability <i>or</i> post-transfusion purpura
PUVA	phototherapy with psoralen plus UV-A
PV	polycythaemia vera
PVO	pyrexia of unknown origin
qds	<i>quater die sumendus</i> (to be taken 4 times a day)
QoL	quality of life
RA	refractory anaemia
RAEB	refractory anaemia with excess blasts
RAEB-t	refractory anaemia with excess blasts in transformation
RAR	retinoic acid receptor
RARS	refractory anaemia with ring sideroblasts
RBC	red blood cell
RCC	red blood cell count
RCM	red cell mass
RCMD	refractory cytopenia with multilineage dysplasia
RDS	respiratory distress syndrome
RDW	red cell distribution width
RE	relative erythrocytosis; reticuloendothelial

REAL	Revised European American Lymphoma
RES	reticuloendothelial system
RFLP	restriction fragment length polymorphism
Rh	Rhesus
rhAPC	recombinant human activated protein C
rhG-CSF	recombinant human granulocyte colony stimulating factor
rHuEPO	recombinant human erythropoietin
RI	remission induction
RIA	radioimmunoassay
RIC	reduced-intensity conditioning
RiCoF	ristocetin cofactor
RIPA	ristocetin-induced platelet agglutination
RS	Reed–Sternberg or ringed sideroblasts
RT	reptilase time
RT-PCR	reverse transcriptase polymerase chain reaction
s	second
SAA	serum amyloid A protein
SAGM	saline adenine glucose mannitol
SaO <sub>2</sub>	arterial oxygen saturation
SAP	serum amyloid P protein
SB	Sudan
SBP	solitary plasmacytoma of bone
SC	subcutaneous
SCA	sickle cell anaemia
SCBU	special care baby unit
SCD	sickle cell disease
SCID	severe combined immunodeficiency
SCT	stem cell transplantation or silica clot time
SD	standard deviation
SE	secondary erythrocytosis
SEP	extramedullary plasmacytoma
SLE	systemic lupus erythematosus
SLL	small lymphocytic lymphoma
SLVL	splenic lymphoma with villous lymphocytes
SM	systemic mastocytosis
SM-AHNMD	systemic mastocytosis with an associated clonal haematological non-mast cell lineage disease
Smlg	surface membrane immunoglobulin
SMZL	splenic marginal zone lymphoma
SOB	short of breath

SPB	solitary plasmacytoma of bone
SPD	storage pool disorders/deficiency
stat	<i>statim</i> (immediate; as initial dose)
sTfR	soluble transferrin receptor
SVC	superior vena cava
SVCO	superior vena caval obstruction
T°	temperature
t $\frac{1}{2}$	half-life
T4	thyroxine
TA-GVHD	transfusion-associated graft-versus-host disease
TAM	transient abnormal myelopoiesis
TAR	thrombocytopenia with absent radius
TB	tuberculosis
TBI	total body irradiation
TCR	T-cell receptor
tds	<i>ter die sumendum</i> (to be taken 3 times a day)
TdT	terminal deoxynucleotidyl transferase
TEC	transient erythroblastopenia of childhood
TEG	thromboelastography
TENS	transcutaneous nerve stimulation
TF	tissue factor
TFT	thyroid function test(s)
TGF-B	transforming growth factor-B
TI	transfusion-independence
TIA	transient ischaemic attack
TIBC	total iron binding capacity
tiw	three times in a week
TKI	tyrosine kinase inhibitor
TLS	tumour lysis syndrome
TNF	tumour necrosis factor
topo II	topoisomerase II
TORCH	toxoplasmosis, rubella, cytomegalovirus, herpes simplex
TPA	tissue plasminogen activator
TPI	triphosphate isomerase
TPN	total parenteral nutrition
TPO	thrombopoietin
TPR	temperature, pulse, respiration
TRAP	tartrate-resistant acid phosphatase
TRM	treatment-related mortality
TSE	transmissible spongiform encephalopathy



TSH	thyroid-stimulating hormone
TT	thrombin time
TTP	thrombotic thrombocytopenic purpura
TXA	tranexamic acid
TXA <sub>2</sub>	thromboxane A <sub>2</sub>
U&E	urea and electrolytes
U	unit
UC	ulcerative colitis
UFH	unfractionated heparin
ULN	upper limit of normal
URTI	upper respiratory tract infection
US	ultrasound
USS	ultrasound scan
UTI	urinary tract infection
VAD	vincristine doxorubicin (adriamycin) dexamethasone regimen
VBAP	vincristine, carmustine (BCNU), doxorubicin, prednisolone
VBMCP	vincristine, carmustine, melphalan, cyclophosphamide, prednisolone
VC	vena cava
VDRL	Venereal Disease Research Laboratory
VEGF	vascular endothelial growth factor
VF	ventricular fibrillation
VIII:C	factor VIII clotting activity
Vit K	vitamin K
VMCP	vincristine, melphalan, cyclophosphamide, prednisolone
VMP	melphalan, prednisolone, bortezomib
VOD	veno-occlusive disease
VTE	venous thromboembolism
VUD	volunteer unrelated donor
vWD	von Willebrand disease
vWF	von Willebrand factor
vWFAg	von Willebrand factor antigen
WAS	Wiskott–Aldrich syndrome
WBC	white blood cell
WBRT	whole-brain radiotherapy
WCC	white cell count
WM	Waldenström macroglobulinaemia
X-match	cross-match
XDPs	cross-linked fibrin degradation products
ZPP	zinc protoporphyrin

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# Clinical approach

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## History taking in patients with haematological disease

### Approach to patient with suspected haematological disease

An accurate history combined with a careful physical examination are fundamental parts of clinical assessment. Although the likely haematological diagnosis may be apparent from tests carried out before the patient has been referred, it is nevertheless essential to assess the clinical background fully—this may influence the eventual plan of management, especially in older patients.

It is important to find out early on in the consultation what the patient may already have been told prior to referral, or what he/she thinks the diagnosis may be. There is often fear and anxiety about diagnoses such as leukaemia, haemophilia, or HIV infection.

### Presenting symptoms and their duration

A full medical history needs to be taken to which is added direct questioning on relevant features associated with presenting symptoms:

- *Non-specific symptoms* such as fatigue, fevers, weight loss.
- *Symptoms relating to anaemia*, e.g. reduced exercise capacity, recent onset of breathlessness and nature of its onset, or worsening of angina, presence of ankle oedema.
- *Symptoms relating to neutropenia*, e.g. recurrent oral ulceration, skin infections, oral sepsis.
- *Evidence of compromised immunity*, e.g. recurrent oropharyngeal infection.
- *Details of potential haemostatic problems*, e.g. easy bruising, bleeding episodes, rashes.
- *Anatomical symptoms*, e.g. abdominal discomfort (splenic enlargement or pressure from enlarged lymph nodes), CNS symptoms (from spinal compression).
- *Past medical history*, i.e. detail on past illnesses, information on previous surgical procedures which may suggest previous haematological problems (e.g. may suggest an underlying bleeding diathesis) or be associated with haematological or other sequelae, e.g. splenectomy.
- *Drug history*: ask about prescribed and non-prescribed medications.
- *Allergies*: since some haematological disorders may relate to chemicals or other environmental hazards specific questions should be asked about occupational factors and hobbies.
- *Transfusion history*: ask about whether the patient has been a blood donor and how much he/she has donated. May occasionally be a factor in iron (Fe) deficiency anaemia. History of previous transfusion(s) and their timing is also critical in some cases, e.g. post-transfusion purpura.
- *Tobacco and alcohol consumption* is essential; both may produce significant haematological morbidity.
- *Travel*: clearly important in the case of suspected malaria but also relevant in considering other causes of haematological abnormality, including HIV infection.

- *Family history* is also important, especially in the context of inherited haematological disorders.

A complete history for a patient with a haematological disorder should provide all the relevant medical information to aid diagnosis and clinical assessment, as well as helping the haematologist to have a working assessment of the patient's social situation. A well-taken history also provides a basis for good communication which will often prove very important once it comes to discussion of the diagnosis.

## Physical examination

This forms part of the clinical assessment of the haematology patient. Pay specific attention to:

**General examination**—e.g. evidence of weight loss, pyrexia, pallor (the latter is not a reliable clinical measure of anaemia), jaundice, cyanosis, or abnormal pigmentation or skin rashes.

**The mouth**—ulceration, purpura, gum bleeding, or infiltration, and the state of the patient's teeth. Hands and nails may show features associated with haematological abnormalities, e.g. koilonychia in chronic Fe deficiency (rarely seen today).

**Record**—weight, height, T<sup>o</sup>, pulse, and blood pressure; height and weight give important baseline data against which sequential measurements can subsequently be compared. In myelofibrosis, for example, evidence of significant weight loss in the absence of symptoms may be an indication of clinical progression.

**Examination**—of chest and abdomen should focus on detecting the presence of lymphadenopathy, hepatic and/or splenic enlargement. Node sizes and the extent of organ enlargement should be carefully recorded.

**Lymph node enlargement**—often recorded in centimetres e.g. 3cm × 3cm × 4cm; sometimes more helpful to compare the degree of enlargement with familiar objects, e.g. pea. Record extent of liver or spleen enlargement as maximum distance palpable from the lower costal margin.

**Erythematous margins of infected skin lesions**—mark these to monitor treatment effects.

**Bones and joints**—recording of joint swelling and ranges of movement are standard aspects of haemophilia care. In myeloma, areas of bony tenderness and deformity are commonly present.

**Optic fundi**—examination is a key clinical assessment in the haematology patient. May yield the only objective evidence of hyperviscosity in paraproteinaemias (➔ Hyperviscosity, p.646) or hyperleucocytosis (➔ Leucostasis, p.663) such as in, e.g. CML. Regular examination for haemorrhages should form part of routine observations in the severely myelosuppressed patient; rarely changes of opportunistic infection such as candidiasis can be seen in the optic fundi.

**Neurological examination**—fluctuations of conscious level and confusion are clinical presentations of hyperviscosity. Isolated nerve palsies in a patient with acute leukaemia are highly suspicious of neurological involvement or disease relapse. Peripheral neuropathy and long tract signs are well-recognized complications of B<sub>12</sub> deficiency.

## Splenomegaly

Many causes. Clinical approach depends on whether splenic enlargement is present as an isolated finding or with other clinical abnormalities, e.g. jaundice or lymphadenopathy. Mild-to-moderate splenomegaly has a much greater number of causes than massive splenomegaly (see Table 1.1).

**Table 1.1** Causes of splenomegaly

<b>Infection</b>	Viral	EBV, CMV, hepatitis
	Bacterial	SBE, miliary tuberculosis, <i>Salmonella</i> , <i>Brucella</i>
	Protozoal	Malaria, toxoplasmosis, leishmaniasis
<b>Haemolytic</b>	Congenital	Hereditary spherocytosis, hereditary elliptocytosis, sickle cell disease (infants), thalassaemia, pyruvate kinase deficiency, G6PD deficiency
	Acquired	AIHA (idiopathic or 2°)
<b>Myeloproliferative and leukaemic</b>		Myelofibrosis, CML, polycythaemia rubra vera, essential thrombocythaemia, acute leukaemias
<b>Lymphoproliferative</b>		CLL, hairy cell leukaemia, Waldenström, SLVL, other NHL, Hodgkin lymphoma, ALL and lymphoblastic NHL
<b>Autoimmune disorders and storage disorders</b>		Rheumatoid arthritis, SLE, hepatic cirrhosis, Gaucher's disease, histiocytosis X, Niemann–Pick disease
<b>Miscellaneous</b>		Metastatic cancer, cysts, amyloid, portal hypertension, portal vein thrombosis, tropical splenomegaly

Clinical approach essentially involves a working knowledge of the possible causes of splenic enlargement and determining the more likely causes in the given clinical circumstances by appropriate further investigation. There are fewer causes of massive splenic enlargement, i.e. the spleen tip palpable below the level of the umbilicus.

### Massive splenomegaly

- Myelofibrosis.
- Chronic myeloid leukaemia (CML).
- Lymphoproliferative disease—chronic lymphocytic leukaemia (CLL) and variants including splenic lymphoma with villous lymphocytes (SLVL), hairy cell leukaemia (HCL) and marginal zone lymphoma.
- Tropical splenomegaly.
- Leishmaniasis.
- Gaucher's disease.
- Thalassaemia major.

## Lymphadenopathy

Occurs in a range of infective or neoplastic conditions; less frequently enlargement occurs in active collagen disorders. May be isolated, affecting a single node, localized, involving several nodes in an anatomical lymph node grouping, or generalized, where nodes are enlarged at different sites. As well as enlargement in the easily palpable areas (cervical, axillary, and iliac), node enlargement may be hilar or retroperitoneal and identifiable only by imaging. Isolated/localized lymphadenopathy usually results from local infection or neoplasm. Generalized lymphadenopathy may result from systemic causes, especially when symmetrical, as well as infection or neoplasm (see Table 1.2). Rarely drug-associated (e.g. phenytoin).

**Table 1.2** Causes of lymphadenopathy

<b>Infective</b>	Bacterial	Tonsillitis, cellulitis, tuberculous infections, and 1° syphilis usually produce isolated or localized node enlargement
	Viral	EBV, CMV, rubella, HIV, HBV, HCV
	Other	Toxoplasma, histoplasmosis, chlamydia, cat-scratch
<b>Neoplastic</b>		Hodgkin lymphoma (typically isolated or localized lymphadenopathy), NHL isolated, generalized or localized, CLL, metastatic carcinoma, acute leukaemia (ALL especially, but occasionally AML)
<b>Collagen and other systemic disorders</b>		E.g. rheumatoid arthritis, SLE, sarcoidosis

### History and examination—points to elicit

- Age.
- Onset of symptoms, whether progressing or not.
- Systemic symptoms, weight loss (>10% body weight loss in <6 months).
- Night sweats.
- Risk factors for HIV infection.
- Local or systemic evidence of infection.
- Evidence of systemic disorder such as rheumatoid arthritis.
- Evidence of malignancy; if splenic enlargement present then lymphoreticular neoplasm is more likely.
- Specific disease-related features, e.g. pruritus and alcohol-induced lymph node pain associated with Hodgkin disease.
- Determine the duration of enlargement  $\pm$  associated symptoms, whether nodes are continuing to enlarge, and whether tender or not. Distribution of node enlargement should be recorded as well as size of node.

### Investigations

- Full blood count (FBC) and peripheral blood film examination.
- Erythrocyte sedimentation rate (ESR) or plasma viscosity.
- Screening test for infectious mononucleosis and serological testing for other viruses.



- Imaging—e.g. chest radiography; chest abdominal ± pelvic computed tomography (CT) scanning may also be helpful to define hilar, retroperitoneal, and para-aortic nodes.
- Microbiology—e.g. blood cultures, indirect testing for tuberculosis (TB), and culture of biopsied or aspirated lymph node material.
- Lymph node biopsy for definitive diagnosis especially if a neoplastic cause suspected. Aspiration of enlarged lymph nodes is generally unsatisfactory in providing effective diagnostic material. Avoid biopsy of groin nodes since these often show only non-specific features.
- Bone marrow (BM) examination should be reserved for staging in confirmed lymphoma or leukaemia cases—it is not commonly a useful 1° investigation of lymphadenopathy.

## Unexplained anaemia

Evaluate with the combined information from clinical history, physical examination, and results of investigations (see Table 1.3).


### History—focus on:

- Duration of symptoms of anaemia—short duration of dyspnoea and fatigue etc. suggests recent bleeding or haemolysis. Gradual anaemia of longer duration often associated with adaptation and fewer symptoms.
- Specific questioning on blood loss—include system-related questions, e.g. gastrointestinal tract (GIT) and gynaecological sources, ask about blood donation.
- Family history—e.g. in relation to hereditary problems such as hereditary spherocytosis (HS) or ethnic haemoglobin (Hb) disorders such as thalassaemia or HbSS.
- Past history—e.g. association of gastrectomy with later occurrence of Fe and/or B<sub>12</sub> deficiency.
- Drug history—including prescribed and non-prescribed medication.
- Dietary factors—mainly relates to folate and Fe deficiency, rarely B<sub>12</sub> in vegans. Fe deficiency always occurs because Fe losses exceed intake (*it is extremely rare in developed countries for diet to be the sole cause of Fe deficiency*).

### Examination

- May identify indirectly helpful signs, e.g. koilonychia in chronic Fe deficiency (rare), jaundice in haemolytic disorders.
- Lymphadenopathy suggesting lymphoreticular disease or viral infection.
- Hepatosplenomegaly in lymphoproliferative or myeloproliferative disorders.

### Full blood count

Laboratory investigation of anaemia is discussed fully in  Chapter 2. Anaemia in adult ♂ if Hb <13.0g/dL and in adult ♀ if Hb <11.5g/dL.

**Table 1.3** MCV useful for initial anaemia evaluation

<b>↓ MCV (&lt;76fL)</b>	Fe deficiency
	α & β thalassaemia, HbE, HbC
	Anaemia of chronic disorders
<b>Normal MCV (78–98fL)</b>	Recent bleeding
	Anaemia of chronic disorders
	Most non-haematinic deficiency causes
	Combined Fe + B <sub>12</sub> /folate deficiency
<b>↑ MCV (&gt;100fL)</b>	Folate or B <sub>12</sub> deficiency
	Haemolytic anaemia
	Liver disease
	Marrow dysplasia and failure syndromes including aplastic anaemia
	2° to antimetabolite drug therapy, e.g. hydroxyurea (now called hydroxycarbamide)

The need for film examination, reticulocyte counting, and additional tests on the FBC sample such as checking for Heinz bodies is based on the initial clinical and FBC findings. The findings from the initial FBC examination have a major influence in determining the nature and urgency of further clinical investigation.

*Serum ferritin level* will identify Fe deficiency and focus on the need for detailed investigation for blood loss which, for adult males and postmenopausal females, will frequently require large bowel examination with colonoscopy or barium enema, and gastroscopy. BM examination may occasionally be required.

*Anaemia is not a diagnosis*—it is an abnormal clinical finding requiring an explanation for its cause. There is no place for empirical use of Fe therapy for management and treatment of ‘anaemia’ in modern medical practice.

## Patient with elevated haemoglobin

Finding a raised Hb concentration requires a systematic clinical approach for differential diagnosis and further investigation. Initially it is essential to check whether the result ties in with the known clinical findings—if unexpected the FBC should be re-checked to exclude a mix-up over samples or a sampling artefact. Dehydration and diuretic therapy may ↑ the Hct and these should be excluded in the initial phase of assessment.

Having determined that the ↑ Hb concentration is genuine the issue is whether there is a genuine ↑ in red cell mass or not, and the explanation for the elevated Hb.

Anoxia is a major stimulus to RBC production and will result in an increase in erythropoietin with consequent erythrocytosis.

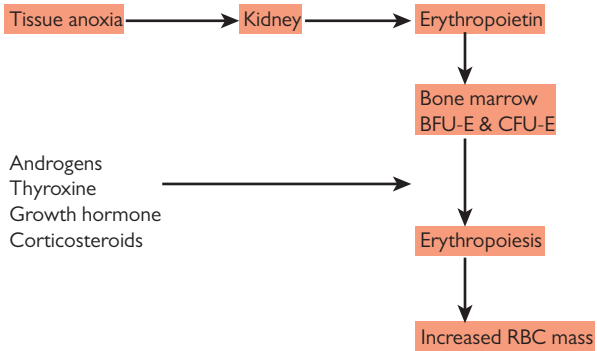
### History and examination should assess:

- Recent travel and residence at high altitude (>3000m).
- Chronic obstructive airways disease (COAD), other hypoxic respiratory conditions, cyanotic congenital heart disease, other cardiac problems causing hypoxia.
- Smoking—heavy cigarette smoking causes ↑ carboxyHb levels leading to ↑ red blood cell (RBC) mass to compensate for loss of O<sub>2</sub> carrying capacity.
- Ventilatory impairment 2° to gross obesity, alveolar hypoventilation (Pickwickian syndrome).
- Possibility of high-affinity Hb abnormalities arises if there is a family history (FH) of polycythaemia, otherwise requires assessment through Hb analysis.
- If obvious 2° causes excluded possibilities include:
  - *Spurious polycythaemia*—pseudopolycythaemia or Gaisbock's syndrome, associated features can include cigarette smoking, obesity, hypertension, and excess alcohol consumption; sometimes described as 'stress polycythaemia'.
  - *Primary proliferative polycythaemia (polycythaemia rubra vera)*—plethoric facies, history of pruritus after bathing or on change of environmental temperature, and presence of splenomegaly are helpful clinical findings to suggest this diagnosis.
  - *Inappropriate erythropoietin excess*—occurs in a variety of benign and malignant renal disorders. Rare complication of some tumours including hepatoma, uterine fibroids, and cerebellar haemangioblastoma.

Part of clinical assessment must also include an evaluation of thrombotic risk; previous thrombosis or a family history of such problems ↑ the urgency of investigation and appropriate treatment (➔ see also Chapter 7, pp.264–276).

Anaemia and hypoxia are detected by the renal sensors. This leads to ↑ production of erythropoietin (EPO) which drives the marrow (through BFU-E and CFU-E) to produce RBCs. Other factors may also drive red cell production, including androgens and growth hormone as shown in Fig. 1.1.

Anaemia  
Hypoxia



**Fig. 1.1** Erythropoietin

### Further reading

McMullin, M.F., et al. (2005) Guidelines for the diagnosis, investigation and management of polycythaemia/erythrocytosis. *Br J Haematol*, **130**, 174–95.

## Elevated white blood cell (WBC) count

Leucocytosis is defined as elevation of the white cell count (WCC)  $>2$  SD above the mean. The detection of leucocytosis should prompt immediate scrutiny of the automated WBC differential (generally accurate except in leukaemia) and the other FBC parameters. A blood film should be examined and if in doubt a manual differential count should be performed. It is important to evaluate leucocytosis in terms of the age-related absolute normal ranges for neutrophils, lymphocytes, monocytes, eosinophils, and basophils (🔍 Normal ranges (adult), p.810; Paediatric normal ranges, p.812) and the presence of abnormal cells: immature granulocytes, blasts, nucleated red cells, and 'atypical cells'.

*Leukaemoid reaction*—leucocytosis  $>50 \times 10^9/L$  defines a neutrophilia with marked 'left shift' (band forms, metamyelocytes, myelocytes, and occasionally promyelocytes and myeloblasts in the blood film). Differential diagnosis is CML and in children, juvenile CML. Primitive granulocyte precursors are also frequently seen in the blood film of the infected or stressed neonate, and any seriously ill patient, e.g. on the intensive therapy unit (ITU).

*Leucoerythroblastic blood film*—contains myelocytes, other primitive granulocytes, nucleated red cells, and often tear drop red cells; is due to BM invasion by tumour, fibrosis, or granuloma formation and is often an indication for a BM biopsy. Other causes include anorexia, haemolysis, and severe illness.

*Leucocytosis due to blasts*—suggests diagnosis of acute leukaemia and is an indication for cell typing studies and BM examination.

FBC, blood film, white cell differential count, and the clinical context in which the leucocytosis is detected will usually indicate whether this is due to a 1° haematological abnormality or reflects a 2° response.

► It is clearly important to seek a history of symptoms of infection and examine the patient for signs of infection or an underlying haematological disorder.

### Neutrophilia

- 2° to acute infection is most common cause of leucocytosis.
- Usually modest (uncommonly  $>30 \times 10^9/L$ ), associated with a left shift and occasionally toxic granulation or vacuolation of neutrophils.
- Chronic inflammation causes less marked neutrophilia often associated with monocytosis.
- Moderate neutrophilia may occur following steroid therapy, heatstroke, and in patients with solid tumours.
- Mild neutrophilia may be induced by stress (e.g. immediate postoperative period) and exercise.
- May be seen following a myocardial infarction or major seizure.
- Frequently found in states of chronic BM stimulation (e.g. chronic haemolysis, idiopathic thrombocytopenic purpura (ITP) and asplenia).
- 1° haematological causes of neutrophilia are less common. CML is often the cause of extremely high leucocyte counts ( $>200 \times 10^9/L$ ), predominantly neutrophils with marked left shift, basophilia, and occasional myeloblasts. The presence of the Ph chromosome on

karyotype analysis are usually helpful to differentiate CML from a leukaemoid reaction.

- Less common are juvenile CML, transient leukaemoid reaction in Down syndrome, hereditary neutrophilia, and chronic idiopathic neutrophilia.
- Neutrophilia is often seen after treatment with granulocyte colony stimulating factor (G-CSF).

BM examination is rarely necessary in the investigation of a patient with isolated neutrophilia. Investigation of a leukaemoid reaction, leucoerythroblastic blood film, and possible chronic granulocytic leukaemia (CGL) or juvenile CML are firm indications for a BM aspirate and trephine biopsy. BM culture, including culture for atypical mycobacteria and fungi, may be useful in patients with persistent pyrexia or leucocytosis.

### Lymphocytosis

- Lymphocytosis  $>4.0 \times 10^9/L$ .
- Normal infants and young children  $<5$  years have a higher proportion and concentration of lymphocytes than adults.
- Rare in acute bacterial infection except in pertussis (may be  $>50 \times 10^9/L$ ).
- Acute infectious lymphocytosis also seen in children, usually associated with transient lymphocytosis and a mild constitutional reaction.
- Characteristic of infectious mononucleosis but these lymphocytes are often large and atypical and the diagnosis may be confirmed with a heterophile antibody agglutination test (Monospot; Paul–Bunnell).
- Similar atypical cells may be seen in patients with CMV and hepatitis A infection.
- Chronic infection with brucellosis, TB, 2° syphilis, and congenital syphilis may cause lymphocytosis.
- Lymphocytosis is characteristic of CLL, acute lymphoblastic leukaemia (ALL), and occasionally non-Hodgkin lymphoma (NHL).

Where a 1° haematological cause is suspected, immunophenotypic analysis of the peripheral blood lymphocytes will often confirm or exclude a neoplastic diagnosis. BM examination is indicated if neoplasia is strongly suspected and in any patient with concomitant neutropenia, anaemia, or thrombocytopenia, or if there are constitutional symptoms, e.g. night sweats, weight loss.

## Reduced WBC count

It is uncommon for absolute leucopenia ( $\text{WBC} < 4.0 \times 10^9/\text{L}$ ) to be due to isolated deficiency of any cell other than the neutrophil though in marked leucopenia several cell lines are often affected.

### ► Neutropenia

Defined as a neutrophil count  $< 2.0 \times 10^9/\text{L}$ . The risk of infective complications is closely related to the absolute neutrophil count. More severe when neutropenia is due to impaired production from chemotherapy or marrow failure rather than to peripheral destruction or maturation arrest where there is often a cellular marrow with early neutrophil precursors and normal monocyte counts. Type of infection determined by the degree and duration of neutropenia (see Table 1.4). Ongoing chemotherapy further ↑ the risk of serious bacterial and fungal opportunistic infection and the presence of an indwelling IV catheter ↑ the incidence of infection with coagulase-negative staphylococci and other skin commensals. Patients with chronic immune neutropenia may develop recurrent stomatitis, gingivitis, oral ulceration, sinusitis, and peri-anal infection.

**Table 1.4** Clinical significance of neutropenia

Neutrophil count	Risk of infection
$1.0\text{--}1.5 \times 10^9/\text{L}$	No significant ↑ risk of infection
$0.5\text{--}1.0 \times 10^9/\text{L}$	Some ↑ in risk; some fevers can be treated as an outpatient
$< 0.5 \times 10^9/\text{L}$	Major ↑ in risk; treat all fevers with broad spectrum IV antibiotics as an inpatient

History and physical examination provide a guide to the subsequent management of a patient with neutropenia. Simple observation is appropriate initially for an asymptomatic patient with isolated mild neutropenia who has an unremarkable history and examination. If there has been a recent viral illness or the patient can discontinue a drug which may be the cause, follow-up over a few weeks may see resolution of the abnormality.

### Investigations

BM examination if there is concomitant anaemia or thrombocytopenia, history of significant infection, or if lymphadenopathy or organomegaly on examination. Usually unhelpful in patients with an isolated neutropenia  $> 0.5 \times 10^9/\text{L}$ . However, if neutropenia persists, perform BM aspiration, biopsy and cytogenetics, and check serology for collagen diseases, anti-neutrophil antibodies, autoantibodies, HIV, and immunoglobulins.

### Differential diagnoses

*Isolated neutropenia* may be the presenting feature of myelodysplasia, aplastic anaemia, Fanconi anaemia, or acute leukaemia but these conditions will usually be associated with other haematological abnormalities.

*Post-infectious (most usually post-viral) neutropenia* may last several weeks and may be followed by prolonged immune neutropenia.



**Severe sepsis**—particularly at the extremes of life.

**Drugs**—cytotoxic agents and many others, e.g. phenothiazines, many antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), antithyroid agents, and psychotropic drugs. Neutrophil recovery starts within a few days of stopping offending drug.

**Autoimmune neutropenia** due to antineutrophil antibodies may occur in isolation or in association with haemolytic anaemia, ITP, or systemic lupus erythematosus (SLE).

**Felty's syndrome** neutropenia is accompanied by seropositive rheumatoid arthritis and splenomegaly.

**Chronic benign neutropenia** of infancy and childhood is associated with fever and infection; resolves by age 4 years, probably has immune basis.

**Benign familial or racial neutropenia** is a feature of rare families and of certain racial groups, notably of patients of black African descent, is associated with mild neutropenia but no propensity to infection.

**Chronic idiopathic neutropenia** is a diagnosis of exclusion, associated with severe neutropenia but often a benign course.

**Cyclical neutropenia** is a condition usually of childhood onset and dominant inheritance characterized by severe neutropenia, fever, stomatitis, and other infections occurring at 4-week intervals.

**Hereditary causes (less common)** include Kostmann syndrome (↻ p.597), Shwachman–Diamond–Oski syndrome (↻ p.597), Chediak–Higashi syndrome (↻ p.603), reticular dysgenesis, and dyskeratosis congenita.

### Management

Febrile episodes should be managed according to the severity of the neutropenia (Table 1.4) and the underlying cause (BM failure is associated with more life-threatening infections). Broad-spectrum IV antibiotics may be required and empirical systemic antifungal therapy may be required in those who fail to respond to antibiotics. Prophylactic antibiotic and antifungal therapy may be helpful in some patients with chronic neutropenia as may G-CSF. Antiseptic mouthwash is of value and regular dental care is important.

### ► Lymphopenia

Lymphopenia ( $<1.5 \times 10^9/L$ ) may be seen in acute infections, cardiac failure, pancreatitis, tuberculosis, uraemia, lymphoma, carcinoma, SLE and other collagen diseases and after corticosteroid therapy, radiation, chemotherapy, and antilymphocyte globulin therapy. Most common cause of chronic severe lymphopenia in recent years is HIV infection (↻ HIV infection and AIDS, p.552).

Chronic severe lymphopenia ( $<0.5 \times 10^9/L$ ) is associated both with opportunistic infections notably *Candida* spp., *Pneumocystis jiroveci*, CMV, herpes zoster, *Mycoplasma* spp., *Cryptosporidium*, and toxoplasmosis and with an ↑ incidence of neoplasia particularly NHL, Kaposi's sarcoma and skin and gastric carcinoma.

### Further reading

Palmbad, J.E., et al. (2002) Idiopathic, immune, infectious, and idiosyncratic neutropenias. *Semin Hematol*, **39**, 113–20.

## Elevated platelet count

Thrombocytosis is defined as a platelet count  $>450 \times 10^9/L$ . May be due to a *primary* myeloproliferative neoplasm (MPN) or a *secondary* reactive feature. If the platelet count is markedly elevated a patient with a MPN has a risk of haemorrhage (due to the production of dysfunctional platelets), or thrombosis, or both. The patient's history may reveal features of the condition to which the elevated platelet count is 2°. Clinical examination may provide similar clues or reveal splenomegaly which suggests a myeloproliferative neoplasm (MPN). FBC may provide useful information: marked leucocytosis with left shift (in the absence of a history of infection), basophilia, or an elevated haematocrit and red cell count are highly suggestive of a MPN when associated with thrombocytosis. Unusual for reactive thrombocytosis to cause a platelet count  $>1000 \times 10^9/L$ . (see Table 1.5)

Note: platelet counts below this may occur in MPNs.

### Differential diagnosis

**Table 1.5** Differential diagnosis of thrombocytosis

Myeloproliferative neoplasms	Disorders associated with ↑ platelets
1° thrombocythaemia	Haemorrhage
Polycythaemia vera	Trauma
Chronic myeloid leukaemia	Surgery
Idiopathic myelofibrosis	Fe deficiency anaemia
	Malignancy (Ca lung, Ca breast, Hodgkin disease)
	Acute and chronic infection
	Inflammatory disease, e.g. rheumatoid arthritis, UC
	Post-splenectomy

### Investigation

- BM aspirate may show megakaryocyte abnormalities in MPN.
- BM trephine biopsy may show marked myeloid hyperplasia, clusters of abnormal megakaryocytes, and ↑ reticulin or fibrosis in MPN.

### Management

- In reactive thrombocytosis treat the underlying condition.
- Unusual to require treatment to ↓ the platelet count in a patient with reactive thrombocytosis.
- Consider low-dose aspirin.
- Reactive thrombocytosis is generally transient.
- If 2° to Fe deficiency—review FBC after Fe therapy: the platelet count normalizes if thrombocytosis was due to Fe deficiency.
- Fe deficiency may have masked PRV—this will be revealed by Fe therapy.

- If impossible to define the cause of thrombocytosis then a watch-and-wait policy should be followed in an asymptomatic patient.
- If MPN is suspected—➔ see Essential thrombocythaemia, p.281.

## Reduced platelet count

Thrombocytopenia is defined as a platelet count  $<150 \times 10^9/L$ . Although there is no precise platelet count at which a patient will or will not bleed, most patients with a count  $>20 \times 10^9/L$  are asymptomatic. The risk of spontaneous haemorrhage  $\uparrow$  significantly  $<10 \times 10^9/L$ . Purpura is the most common presenting symptom usually found on the lower limbs and areas subject to pressure. May be followed by bleeding gums, epistaxis, or more serious life-threatening haemorrhage. A patient with newly diagnosed severe thrombocytopenia with or without purpura is a medical emergency and may require admission for further investigation and treatment.

Confirm low platelet count by examination of the blood sample for clots (artefactual low platelets) and the blood film for platelet aggregates (causing pseudo-thrombocytopenia, or 'false' thrombocytopenia). If aggregates are seen a FBC should be repeated in a citrate sample. History and examination will determine the clinical severity of the thrombocytopenia and should also reveal the duration of symptoms, presence of any prodromal illness, causative medication, or underlying disease.

Determine whether the cause of thrombocytopenia is failure of production or  $\uparrow$  consumption (see Table 1.6). FBC may be helpful as the MPV is often elevated in the latter group (large platelets may also be seen on the blood film). May also reveal additional haematological abnormalities (normocytic anaemia or neutropenia) suggestive of a BM disorder. A coagulation screen should also be performed. Examination of the BM is the definitive investigation in all patients with moderate or severe thrombocytopenia—may reveal normal megakaryocytes or compensatory hyperplasia in peripheral destruction syndromes or marrow hypoplasia or infiltration. Tests for platelet antibodies are unreliable but an autoimmune screen may be helpful to exclude lupus.

### Management

Treat underlying condition. Most patients with a platelet count  $>30 \times 10^9/L$  require no specific therapy but should be investigated. Avoid antiplatelet agents and anticoagulants. In the event of life-threatening haemorrhage platelet transfusion should be administered to thrombocytopenic patients *with the exception of those with HITT and TTP*.

**Table 1.6** Differential diagnosis of thrombocytopenia

Failure of production	Increased consumption
Drugs and chemicals (➔ p.490)	ITP (➔ p.492)
Viral infection	Drugs (➔ p.490)
Radiation	DIC (➔ p.494)
Aplastic anaemia (➔ p.102)	Infection
Leukaemia	Massive haemorrhage and transfusion (➔ p.508)
Marrow infiltration	SLE
Megaloblastic anaemia (➔ p.46)	CLL and lymphoma (➔ p.490)
HIV (➔ p.553)	Heparin (➔ p.510)
	TTP (➔ p.538)
	Hypersplenism (➔ p.492)
	Post-transfusion purpura (➔ p.492)
	HIV (➔ p.553)

**Further reading**

Spencer, F.A. (2000). Heparin-induced thrombocytopenia: patient profiles and clinical manifestations. *J Thromb Thrombolysis*, **10**, (Suppl. 1), 21–5.

## Easy bruising

Evaluation of a patient who complains of easy bruising involves a detailed history, physical examination with particular attention to any current haemorrhagic lesions, and the performance of basic haemostatic investigations. More common in ♀ and often difficult to evaluate. Also a frequent complaint in the elderly.

### History

Careful attention to the history is essential to the diagnosis of all the haemorrhagic disorders and one must attempt to define the nature of the bruising in a patient with this complaint. *Note:* many normal healthy people believe that they have excessive bleeding or bruising. Conversely some people with haemorrhagic disorders and abnormal bleeding histories will not volunteer the information unless asked directly or indeed may consider their bleeding to be normal. Remember that excessive bruising may be a manifestation of a blood vessel disorder rather than a coagulopathy or platelet disorder.

### Ask about

*Presenting complaint*—how long and how frequently has easy bruising occurred? Is it ecchymoses or purpura? How extensive are bruises? Are they located in areas subject to trauma (e.g. limbs) or pressure (e.g. waist band)? Do petechiae occur? Are bruises painful? How long to resolution? How many currently?

### Associated symptoms

Has there been gum bleeding? Has the patient experienced prolonged bleeding after skin trauma, dental extraction, childbirth, or surgery? Has there been any other form of haemorrhage, e.g. epistaxis, menorrhagia, joint or soft tissue haematoma, haematemesis, melaena, haemoptysis, or haematuria? Is there a history of poor wound healing?

### Family history

Has any other family member a history of excessive bleeding or bruising?

### Drug history

Is the patient on any medication (remember self-medication of vitamins and food supplements), most notably aspirin, anticoagulant therapy?

### Systematic enquiry

Is there evidence of a disorder associated with a haemorrhagic tendency, e.g. hepatic or renal failure, malabsorption, leukaemia, lymphoma, connective tissue disorder, or amyloidosis?

### Physical examination

Haemorrhagic skin lesions are likely to be present in a patient with a serious problem and their distribution will often indicate the extent to which they are likely to be related to trauma. Senile purpura is almost invariably on the hands and forearms. True purpura is easily differentiated from erythema and telangiectasis by pressure. Petechiae are highly suggestive of a platelet or vascular disorder whilst palpable purpura are associated with anaphylactoid purpura. In addition there may be other physical findings which may indicate an underlying disorder, e.g. spleno-

megaly or lymphadenopathy in leukaemia or lymphoma, signs of hepatic failure, telangiectasia in hereditary haemorrhagic telangiectasia (HHT or Osler–Rendu–Weber syndrome) or hyperextensible joints and paper-thin scars in Ehlers–Danlos syndrome.

### Basic haemostatic investigations

All patients should be investigated except those in whom history and examination has given strong grounds for believing that they are normal and in whom there is a history of a normal response to a haemostatic challenge, e.g. surgery or dental extraction.

### Screening tests

- FBC and blood film.
- Activated partial thromboplastin time (APTT).
- Prothrombin time (PT).
- Thrombin clotting time and/or fibrinogen.
- Bleeding time is now an obsolete investigation of dubious value and no longer performed.

If these investigations are normal there is no indication for further haemostatic investigations unless the history provides strong grounds for believing that there is indeed a haemostatic disorder. The appropriate further investigation of the haemostatic mechanism is discussed in Chapter 10 (➔ Haemostasis and thrombosis, p.453).

### Differential diagnoses

- Common diagnoses:
  - Simple easy bruising (purpura simplex).
  - Trauma (including non-accidental injury in children).
  - Senile purpura.
- Haemostatic defects:
  - Thrombocytopenia.
  - Platelet function defects.
  - Coagulation abnormalities (rarely).
  - Patient on anticoagulant drugs or anti-platelet agents.
- Vascular defects:
  - Corticosteroid use/excess.
  - Collagen diseases.
  - Uraemia.
  - Dysproteinaemias.
  - Anaphylactoid purpura.
  - Ehlers–Danlos syndrome.
  - Scurvy.
  - Vasculitis.

## Recurrent thromboembolism

A hypercoagulable state should be suspected in all patients with recurrent thromboembolic disease, family history of thrombosis, thrombosis at a young age or at an unusual site (in addition to recurrent thromboembolism) associated with inherited thrombophilia (see Table 1.7). Further important aspects of the history are precipitating factors at the time of thrombosis and lifestyle considerations, e.g. smoking, exercise, and obesity. Clinical examination may reveal signs suggestive of an associated underlying condition.

**Table 1.7** Hypercoagulable states

<b>Inherited</b>	Activated protein C resistance (factor V Leiden)
	Protein C deficiency
	Protein S deficiency
	Prothrombin gene mutation
	Hyperhomocysteinaemia
	Sickle cell disease
	Antithrombin deficiency and some very rare abnormalities of fibrinogen, plasminogen, and plasminogen activator
<b>Acquired</b>	Immobilization
	Oral contraceptive or oestrogen therapy
	Postpartum
	Old age
	Postoperative
	Malignancy (notably Ca pancreas)
	Nephrotic syndrome
	Myeloproliferative disorders
	Hyperhomocysteinaemia
	Antiphospholipid syndrome (lupus anticoagulant)
	Hyperviscosity
	Paroxysmal nocturnal haemoglobinuria
	Thrombotic thrombocytopenic purpura
	Heparin-induced thrombocytopenia

### Laboratory investigation

➔ See Thrombophilia, p.530.

### Further reading

Kyrle, P.A., et al. (2004.) The risk of recurrent venous thromboembolism in men and women. *N Engl J Med*, **350**, 2558–63.



## Pathological fracture

Fracture in a bone compromised by the presence of a pathological process resulting in fracture following relatively minor trauma. Most commonly due to local neoplastic involvement or osteoporosis.

### Haematological causes

- Local bony damage.
- Myelomatous deposits, solitary bone plasmacytoma (SBP).
- Lymphomatous infiltration.
- Metastatic carcinoma ( $\pm$  marrow infiltration); breast, prostate, and lung are most common 1° sites.
- Gaucher disease.
- Sick cell anaemia.
- Homozygous thalassaemia.
- Osteoporosis from prolonged corticosteroid therapy, e.g. for autoimmune or respiratory disease.

### Clinically

Presentation as local pain, discomfort, and restriction of mobility.

### Diagnosis

Confirmed by x-ray or other imaging.

### Management

- Awareness of risk/possibility and early diagnosis.
- Analgesia.
- Orthopaedic—immobilization and support as appropriate for nature and site of injury, surgical intervention including pinning or other fixation.
- Radiotherapy—local management of fracture 2° to local malignancy. If patient eligible for orthopedic/spinal surgery then radiotherapy post-surgery.
- Mobilization—physiotherapy.
- Treatment of underlying condition predisposing to fracture.
- Prophylactic surgery/pinning of critical lesions.

## Raised ESR

The ESR remains an established, empirical test clinically useful as a method for identifying and monitoring the acute phase response. It is influenced by changes in fibrinogen,  $\alpha$ -macroglobulins, and immunoglobulins which enhance red cell aggregation *in vitro*. (see Table 1.8).

*Plasma viscosity (PV)* is also an effective measure of acute phase reactants and can be used as an alternative to the ESR in clinical practice;  $\uparrow$  in ESR and plasma viscosity generally parallel each other. PV is not readily available in all laboratories.

### Normal ranges

- 0–10mm/h for ♂ 18–65 years.
- 1–20mm/h for ♀ 18–65 years.
- Upper limits of normal  $\uparrow$  by 5–10mm/h for patients >65 years.
- Other factors, e.g. haematocrit (Hct) influences the ESR.
- Should be regarded as semiquantitative.
- Marked elevations are clinically significant.
- Modest elevations can be more problematic to interpret and are non-specific.

The main advantages to the ESR are its low cost and technical simplicity allied to the absence of a more accurate, inexpensive, and technically simple alternative.

**Table 1.8** Causes of raised ESR

Pregnancy	$\uparrow$ in pregnancy; maximal in 3rd trimester
Infections	Acute and chronic infections, including TB Note: $\uparrow$ ESR also occurs in HIV infection
Collagen disorders	Rheumatoid, SLE, polymyalgia rheumatica, vasculitides, etc. (including temporal arteritis); ESR useful as non-specific monitor of disease activity
Other inflammatory processes	Inflammatory bowel disease, sarcoidosis, post-MI
Neoplastic conditions	Carcinomatosis, NHL, Hodgkin lymphoma and paraproteinaemias (benign and malignant)

### Investigations

Given the wide range of situations in which a raised ESR can arise, further investigation depends on a carefully conducted history and examination. In the absence of likely causes from these, simple initial laboratory and radiology assessments to include urinalysis, FBC and blood film examination, urea, electrolytes, serum protein electrophoresis, an autoimmune profile, and CXR should represent a practical and pragmatic 1<sup>o</sup> diagnostic screen.

➡ See Haematological investigations, p.745.

### Further reading

Gabay, C., et al. (1999). Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med*, **340**, 448–54.

## Serum or urine paraprotein

### Differential diagnosis

#### Common

- Monoclonal gammopathy of undetermined significance (MGUS).
- Smoldering myeloma.
- Multiple myeloma.
- Solitary bone or extra-osseous plasmacytoma.
- Lymphoproliferative disorders, e.g. CLL, NHL, Waldenström.

#### Less common

- Autoimmune disorders, e.g. rheumatoid arthritis, SLE.
- Polymyalgia rheumatica.

#### Rare

- AL amyloid (1° amyloid).
- Plasma cell leukaemia.
- Heavy chain disease.

### Discriminating clinical features

**MGUS**—no symptoms or signs of end-organ damage, normal FBC and biochemical profile, paraprotein level <30g/L and *stable*, immunoparesis (rarely present), BM plasma cells <10%, no lytic lesions.

**Smouldering myeloma**—as for MGUS but higher stable paraprotein level >30g/L or BM plasma cells >10% without end-organ damage.

**Plasmacytoma**—localized bone pain, low paraprotein level, isolated bony lesion.

**Myeloma**—symptoms and signs of anaemia or hyperviscosity (↻ see Hyperviscosity, p.646); bone pain or tenderness, lytic bone lesions, raised  $\text{Ca}^{2+}$ , creatinine, urate; high  $\beta_2$  microglobulin and low albumin; immunoparesis; In the presence of myeloma related organ or tissue impairment, *no minimum level for serum paraprotein or BM plasma-cell percentage*.

**Plasma cell leukaemia**—as myeloma but fulminant history. Plasma cells seen on blood film.

**Heavy chain disease**—rare, characterized by a single heavy chain only in serum or urine electrophoresis. Presence of any light chain excludes.

**Amyloid**—myriad clinical features. Diagnosis on biopsy of affected site or, if inaccessible, by BM or rectal biopsy—characteristic fibrils stain with Congo Red and show green birefringence in polarized light.

**CLL and NHL**—systemic symptoms, e.g. fever, night sweats, weight loss. Lymphadenopathy or hepatosplenomegaly likely. Confirm on BM or node biopsy.

**Waldenström**—as for CLL but with symptoms or signs of hyperviscosity (↻ Waldenström macroglobulinaemia, p.368).

**Autoimmune disorders**—suggested by joint pain, skin rashes, multisystem disease. Confirm on autoimmune profile including rheumatoid factor, ANA, ANCA.

↻ See Multiple myeloma, p.336.

## Anaemia in pregnancy

Physiological changes in red cell and plasma volume occur during pregnancy.

- Red cell mass ↑ by ≤30%.
- Plasma volume ↑ ≤60%.
- Net effect to ↑ blood volume by ≤50% with lowering of the normal.
- Hb concentration to 10.0–11.0g/dL during pregnancy. MCV ↑ during pregnancy.
- Fe deficiency is a common problem and cause of anaemia in pregnancy (see Table 1.9).

**Table 1.9** Iron utilization in pregnancy

Cause of ↑ requirements	Amount of additional Fe
↑ Red cell mass	~500mg
Fetal requirements	~300mg
Placental requirements	~5mg
Basal losses over pregnancy (1.0–1.5mg/d)	~250mg

These result in a total requirement of ≤1000mg Fe requiring an average daily intake of 3.5–4.0mg/d. Average Western diet provides <4.0mg Fe/d so that balance is marginal during pregnancy. Diets with Fe mainly in non-haem form (e.g. vegetables) provide less Fe available for absorption. Thus a high risk of developing Fe deficiency anaemia which is exacerbated if preconception Fe stores are reduced.

**Folate requirements** are ↑ during pregnancy because of ↑ cellular demands; folate levels tend to drop during pregnancy.

**Prophylaxis** recommendation to give 40–60mg elemental Fe/d which will ↑ availability of dietary absorbable Fe and protect against chronic Fe deficiency; debated whether supplements required by all pregnant women or only for those in at-risk socio economic and nutritionally deficient groups. Folate supplementation is recommended for all and also appears to reduce incidence of neural tube defects.

- Dilutional anaemia—Hb seldom <10.0g/dL (requires no therapy).
- Fe deficiency—may occur with normal MCV because of ↑ MCV associated with pregnancy; check serum ferritin and give Fe replacement; assess and treat the underlying cause.
- Blood loss—sudden ↓ in Hb may signify fetomaternal bleeding or other forms of concealed obstetric bleeding.
- Folate deficiency—macrocytic anaemia in pregnancy almost invariably will be due to folate deficiency (B<sub>12</sub> deficiency is extremely rare during pregnancy).
- Microangiopathic haemolysis/disseminated intravascular coagulation (DIC) may be seen in eclampsia or following placental abruption or intrauterine death. HELLP syndrome (➡ p.96) is a rare but serious cause of anaemia.
- Anaemia may also arise during pregnancy from other unrelated causes and should be investigated.

## Thrombocytopenia in pregnancy

A normal uncomplicated pregnancy is associated with a platelet count in the normal range though up to 10% of normal deliveries may be associated with mild thrombocytopenia ( $>100 \times 10^9/L$ ). Detection of thrombocytopenia in a pregnant patient requires consideration not only of the diagnoses listed in the previous topic (➔ Anaemia in pregnancy, p.26) but also the conditions associated with pregnancy which cause thrombocytopenia. An additional important consideration is the possible effect on the fetus and its delivery.

If thrombocytopenia is detected late in pregnancy, most women will have a platelet count result from the booking visit (at 10–12 weeks) for comparison. Mild thrombocytopenia ( $100\text{--}150 \times 10^9/L$ ) detected for the first time during an uncomplicated pregnancy is not associated with any risk to the fetus nor does it require special obstetric intervention other than hospital delivery.

### Non-immune thrombocytopenia

- Thrombocytopenia may develop in association with pregnancy-induced hypertension, pre-eclampsia, or eclampsia. Successful treatment of hypertension may be associated with improvement in thrombocytopenia which is believed to be due to consumption. Treatment of hypertension, pre-eclampsia, or eclampsia may necessitate delivery of the fetus who is not at risk of thrombocytopenia. HELLP syndrome (haemolysis, elevated liver enzymes, and low platelets) may occur in pregnancy.
- A number of obstetric complications, notably retention of a dead fetus, abruptio placentae, and amniotic fluid embolism, are associated with DIC (➔ Disseminated intravascular coagulation, p.494).

**Immune thrombocytopenia** may occur in pregnancy and women with chronic ITP may become pregnant. Therapeutic considerations must include an assessment of the risk to the fetus of transplacental passage of antiplatelet antibody causing fetal thrombocytopenia and a risk of haemorrhage before or during delivery. There is no reliable parameter for the assessment of fetal risk which, although relatively low, is most significant in women with pre-existing chronic ITP. *Note:* the severity of the mother's ITP has no bearing on the fetal platelet count.

**Women with a platelet count  $<20 \times 10^9/L$**  due to ITP should receive standard prednisolone therapy or IVIg (➔ Immune thrombocytopenia, p.492). If prednisolone fails or is contraindicated, IVIg should be administered and may need to be repeated at 3-week intervals. Splenectomy should be avoided (high rate of fetal loss). Enthusiasm has waned for assessing the fetal platelet count during pregnancy by cordocentesis followed by platelet transfusion. Fetal scalp sampling in early labour is unreliable and hazardous. Delivery should occur in an obstetric unit with paediatric support and the neonate's platelet count should be monitored for several days as delayed falls in the platelet count occur.

### Further reading

BCSH Guidelines (2003). Guidelines for the investigation and management of idiopathic thrombocytopenic purpura in adults, children and in pregnancy. *Br J Haematol*, **120**, 574–96.

## Prolonged bleeding after surgery

Prolonged bleeding following surgery often requires urgent haematological opinion and investigation. The cause of the bleeding is usually surgical, rather than due to any underlying systemic bleeding disorder.

### History and clinical assessment

- Past history in relation to previous haemostatic challenges, e.g. previous surgery, dental extractions. Ask specific questions about whether blood transfusion was required.
- Presence of specific clinical problems, e.g. impaired liver or renal function.
- Recent drug history—especially aspirin or NSAIDs which can affect platelet function. Also enquire about cytotoxic drugs and anticoagulants.
- Family history of bleeding problems especially after surgery.
- Nature of the surgery and intrinsic haemorrhagic risks of procedure.
- Whether surgery was elective or emergency (in emergency surgery known risk factors are less likely to have been corrected).
- Check case record or ask surgeon/anaesthetist for information on intraoperative bleeding, technical problems, etc.
- Whether surgery involves a high risk of triggering DIC, e.g. pancreatic or major hepatobiliary surgery.
- Detailed physical examination is not usually practical but bruising, ecchymoses, or purpura should be assessed especially if remote from the site of surgery.
- What blood products have been used and over how long? Transfusion of several units of RBCs over a short period of time will dilute available clotting factors.
- Review preoperative investigation results and other information available in the record on past procedures and/or investigations.

### Investigations

- Ensure samples not taken from heparinized line.
- FBC with platelet count and blood film examination.
- PT, APTT, and fibrinogen.

With normal platelets and coagulation screen bleeding is usually surgical and the patient should be supported with blood and urgent surgical re-exploration undertaken. Platelet function abnormalities may occur with aspirin/NSAIDs, uraemia, or extracorporeal circuits. Prolongation of both PT and APTT suggests massive bleeding and inadequate replacement, DIC, underlying liver disease, or oral anticoagulants. Disproportionate, isolated ↑ in either PT or APTT are more likely to indicate previously undiagnosed clotting factor deficiencies. A low platelet count may reflect dilution and consumption from bleeding or DIC if platelets were known to be normal preoperatively.

### Treatment

- Low platelets or platelet function abnormalities: give 1–2 adult doses of platelets stat.
- DIC—give 2 adult doses of platelets and 4 units fresh frozen plasma (FFP) (10–20 units of cryoprecipitate if fibrinogen low) and recheck PT, APTT, and FBC.

- Anticoagulant effect:
  - Heparin—reverse with protamine sulfate.
  - Warfarin—reverse with FFP or PCC.
- Empirical tranexamic acid or aprotinin may be tried if bleeding continues despite the above.

### Further reading

Michel, M., et al. (2003). Intravenous anti-D as a treatment for immune thrombocytopenic purpura (ITP) during pregnancy. *Br J Haematol*, **123**, 142–6.

## Positive sickle test (HbS solubility test)

The ↓ solubility of deoxyHbS forms the basis of this test. Blood is added to a buffered solution of a reducing agent e.g. sodium dithionate. HbS is precipitated by the solution and produces a turbid appearance. *Note:* does not discriminate between sickle cell *trait* and *homozygous disease*.

### Use

This is a quick screening test (takes ~20min), often used preoperatively to detect HbS.

### Action if sickle test +ve

- Delay elective operation until established whether disease or trait.
- Ask about family history of sickle cell anaemia or symptoms of SCA.
- FBC and film (Table 1.10).
- Hb electrophoresis, or more commonly HPLC.
- Group and antibody screen serum.

### False +ve results

- Low Hb.
- Severe leucocytosis.
- Hyperproteinaemia.
- Unstable Hb.

### False -ve results

- Infants <6 months.
- HbS <20% (e.g. following exchange blood transfusion).

Sickle test not recommended as a screening test in pregnancy as it will not detect other Hb variants that interact with HbS e.g. β thalassaemia trait. Standard Hb electrophoresis of at-risk groups should be performed (and of all pregnant women if a high local ethnic population).

**Table 1.10** FBC and film features of sickle trait vs. disease

<b>Sickle cell trait</b>	FBC—normal or ↓ MCV and MCH, no anaemia Film normal (may be microcytosis or target cells)
<b>Sickle cell disease</b>	FBC—Hb ~7–8g/dL (range ~4–11g/dL) Film—sickled RBCs, target cells, polychromasia, basophilic stippling, NRBC (hyposplenic features in adults)

### Further reading

Balasubramaniam, J., et al. (2001) Evaluation of a new screening test for sickle cell haemoglobin. *Clin Lab Haematol*, **23**, 379–83.



## Red cell disorders

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## The peripheral blood film in anaemias

Basic evaluation in anaemia includes the microscopic examination of the red blood cells. These may show features suggestive of the underlying cause of the anaemia as shown in Table 2.1.

**Table 2.1** Morphological abnormalities and variants

<b>Microcytic RBCs</b>	Fe deficiency, thalassaemia trait and syndromes, congenital sideroblastic anaemia, anaemia of chronic disorders
<b>Macrocytic RBCs</b>	Alcohol/liver disease (round macrocytes), MDS, pregnancy and newborn, haemolysis, B <sub>12</sub> or folate deficiency, hydroxyurea and antimetabolites (oval macrocytes), acquired sideroblastic anaemia, hypothyroidism, chronic respiratory failure, aplastic anaemia
<b>Dimorphic RBCs</b>	Fe deficiency responding to Fe, mixed Fe and B <sub>12</sub> /folate deficiency, sideroblastic anaemia, post-transfusion
<b>Polychromatic RBCs</b>	Response to bleeding or haematinic Rx, haemolysis, BM infiltration
<b>Spherocytes</b>	HS, haemolysis, e.g. warm AIHA, delayed transfusion reaction, ABO HDN, DIC, and MAHA, post-splenectomy
<b>Pencil/rod cells</b>	Fe deficiency anaemia, thalassaemia trait and syndromes, PK deficiency
<b>Elliptocytes</b>	Hereditary elliptocytosis, MPD, and MDS
<b>Fragmented RBCs</b>	MAHA, DIC, renal failure, HUS, TTP
<b>Teardrop RBCs</b>	Myelofibrosis, metastatic marrow infiltration, MDS
<b>Sickle cells</b>	Sickle cell anaemia, other sickle syndromes (not sickle trait)
<b>Target cells</b>	Liver disease, Fe deficiency, thalassaemia, HbC syndromes
<b>Crenated red cells</b>	Usually storage or EDTA artifact. Genuine RBC crenation may be seen post-splenectomy and in renal failure
<b>Burr cells</b>	Renal failure
<b>Acanthocytes</b>	Hereditary acanthocytosis, a-β-lipoproteinaemia, McLeod red cell phenotype, PK deficiency, chronic liver disease (esp. Zieve's)
<b>Bite cells</b>	G6PD deficiency, oxidative haemolysis
<b>Basophilic stippling</b>	Megaloblastic anaemia, lead poisoning, MDS, haemoglobinopathies
<b>Rouleaux</b>	Chronic inflammation, paraproteinaemia, myeloma
<b>↑ reticulocytes</b>	Bleeding, haemolysis, marrow infiltration, severe hypoxia, response to haematinic therapy
<b>Heinz bodies</b>	Not seen in normals (removed by spleen), small numbers seen post-splenectomy, oxidant drugs, G6PD deficiency, sulfonamides, unstable Hb (Hb Zurich, Köln)
<b>Howell–Jolly bodies</b>	Made of DNA, generally removed by the spleen, dyserythropoietic states, e.g. B <sub>12</sub> deficiency, MDS, post-splenectomy, hyposplenism
<b>H bodies</b>	HbH inclusions, denatured HbH (β <sub>4</sub> tetramer), stain with methylene blue, seen in HbH disease (– –/– α), less prominent in α thalassaemia trait, not present in normals
<b>Hyposplenic blood film</b>	Howell–Jolly bodies, target cells, occasional nucleated RBCs, lymphocytosis, macrocytosis, acanthocytes

## Anaemia in renal disease

Anaemia is consistently found in the presence of chronic renal failure since the kidneys are the main source of erythropoietin. Severity generally relates to the degree of renal impairment although anaemia may occur even if modest renal impairment. The dominant mechanism is inadequate production of erythropoietin (EPO). Other contributory factors include (i) suppressive effects of uraemia and (ii) ↓ in RBC survival. Uraemia impairs platelet function → blood loss and Fe deficiency. Small amounts of blood are inevitably left in the tubing following dialysis so that blood loss and Fe deficiency are further contributory factors in dialysis patients. Folate is lost in dialysis and supplementation is required to avoid deficiency. Aluminium toxicity (from trace amounts in dialysis fluids) and osteitis fibrosa from hyperparathyroidism are rare contributory factors.

### Laboratory features

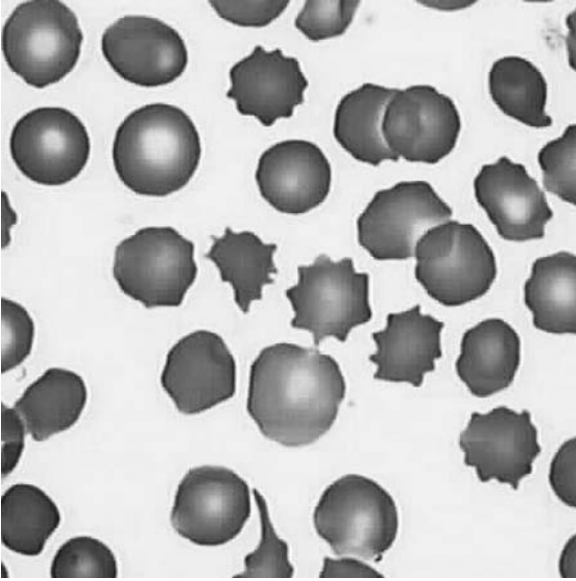
- Hb typically 5.0–10.0g/dL.
- MCV ↔.
- Blood film—mostly normochromic RBCs; schistocytes and acanthocytes present. No specific abnormalities in WBC or platelets (see Fig 2.1).
- Microangiopathic haemolytic changes present in vasculitic collagen disorders with renal failure and classically in HUS and TTP.

### Management

- Short-term treatment with RBC transfusion, based on symptoms (*not Hb concentration*).
- Correction of Fe and folic acid deficiencies.
- EPO will correct anaemia in most patients.
- Start at 50–100 units/kg SC × 3/week. Give IV Fe at same time. Response apparent <10 weeks; reduced doses required as maintenance. Renal Association guidelines have been produced for application and monitoring of EPO therapy. Although expensive it improves quality of life and avoids transfusion dependency and Fe overload.

### Side effects of EPO

- ↑ BP.
- Pure red cell aplasia.
- Thrombotic tendency.



**Fig. 2.1** Blood film: chronic renal failure with burr (irregular shaped) cells (see colour plate section).

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## Anaemia in endocrine disease

Anaemia and other haematological effects occur in various endocrine disorders. The abnormalities will usually correct as the endocrine abnormality is corrected.

### Pituitary disorders

*Deficiency/hypopituitarism* is associated with normochromic, normocytic anaemia; associated leucopenia may also occur. Abnormalities correct as normal function is restored, by replacement therapy.

### Thyroid disorders

*Hypothyroidism* may produce a mild degree of anaemia; MCV usually  $\uparrow$  but may be normal. Corrects on restoration of normal thyroid function. Menorrhagia occurs in hypothyroidism and can result in associated Fe deficiency. B<sub>12</sub> levels should be checked because of the association with other autoimmune disorders (e.g. pernicious anaemia).

*Thyrotoxicosis* may be associated with mild degrees of normochromic anaemia in 20% of cases which corrects as function is normalized. Erythroid activity is  $\uparrow$  but a disproportionate increase in plasma volume means either no change in Hb concentration or mild anaemia. Haematinic deficiencies occur and should be excluded.

### Adrenal disorders

*Hypoadrenalism* results in normochromic, normocytic anaemia; the plasma volume is  $\downarrow$  which masks the true degree of associated anaemia. The abnormalities are corrected by replacement mineralocorticoids.

*Hyperadrenalism* (Cushing's) results in erythrocytosis with a typical net increase in Hb (by 1–2g/dL). Occurs whether Cushing's is 1° or iatrogenic. Mechanism is unclear.

*Parathyroid disorders*—hyperparathyroidism may be associated with anaemia from impairment of EPO production, or in some cases from 2° marrow sclerosis.

*Sex hormones*—androgens stimulate erythropoiesis and are occasionally used to stimulate red cell production in aplastic anaemia. The influence of androgens explains the higher Hb in adult  $\sigma$  compared with  $\text{♀}$ .

*Diabetes mellitus* when poorly controlled may be associated with anaemia; however, the majority of haematological abnormalities in diabetes mellitus result from 2° disease-related complications, e.g. renal failure.

### Further reading

Spivak, J.L. (2000). The blood in systemic disorders. *Lancet*, **355**, 1707–12.



## Anaemia in joint disease

Rheumatoid arthritis, psoriatic arthropathy, and osteoarthritis may be complicated by anaemia. Various factors contribute to anaemia; commonly more than one is present, especially in rheumatoid arthritis. Some of the mechanisms that give rise to anaemia in rheumatoid also apply in other connective tissue disease, e.g. SLE, polyarteritis nodosa, etc. (see Table 2.2)

### Anaemia of chronic disorders (ACD)

ACD is a cytokine-driven suppression of red cell production. The clinical problem is being able to recognize the presence of other contributory factors in the pathogenesis of the anaemia. BM macrophages fail to pass their stored Fe to developing RBCs and a lower than expected rise in EPO suggests some inhibition in its pathway. Marrow also appears less responsive to EPO.  $\uparrow$  IL-1 has been identified. Detailed studies suggest a synergistic effect of IL-1 with T-cells to produce IFN- $\gamma$  which can suppress erythroid activity. May also be an  $\uparrow$  level of TNF- $\alpha$  which inhibits erythropoiesis through release of IFN- $\beta$  from marrow stromal cells.

### Typical features of ACD

- Hb range 7.0–11.0g/dL.
- MCV is usually  $\leftrightarrow$  but when longstanding the MCV is moderately  $\downarrow$  (may look like Fe deficiency).
- Ferritin usually  $\leftrightarrow$  but may be  $\uparrow$
- Serum Fe  $\leftrightarrow$  or  $\downarrow$ , TIBC  $\leftrightarrow$  or  $\downarrow$ . (Note: Fe and TIBC tests now obsolete).
- Serum transferrin receptor levels normal.
- $\uparrow$  zinc protoporphyrin level.
- BM Fe stores plentiful.

**Table 2.2** Additional mechanisms of anaemia in rheumatoid disease

<b>Autoimmune phenomena</b>	Warm antibody AIHA in association with rheumatoid and other collagen disorders; film will show reticulocytosis and +ve DAT  Red cell aplasia
<b>Drug-related problems</b>	Chronic blood loss (caused by medication)  Drug side effects e.g. macrocytosis from antimetabolite immunosuppressives, e.g. azathioprine and methotrexate, oxidative haemolysis 2° to dapsone or sulfasalazine (occurs in normal individuals as well as those with G6PD deficiency)  Anaemia 2° to gold therapy for rheumatoid arthritis  Idiosyncratic reactions, unexplained or unforeseeable reactions such as marrow aplasia  Rare autoimmune haemolysis due to mefenamic acid, diclofenac, or ibuprofen
<b>2° to other organ problems</b>	Hypersplenism, Felty's syndrome in rheumatoid, renal failure in SLE or polyarteritis



**Management**

Supportive transfusion in symptomatic patients; coexistent Fe deficiency should be excluded and treated. Minority may be suitable for/responsive to EPO therapy.

**Bleeding and Fe deficiency**

Usually 2° to use of NSAIDs—consider and exclude other causes of blood loss which may occur in this patient group.

**Further reading**

Bron, D. et al. (2001). Biological basis of anemia. *Semin Oncol*, **28**, 1–6.

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## Anaemia in gastrointestinal disease

Anaemia occurs in GIT disorders through mechanisms of blood loss, ACD-specific disease-related complications, or drug side effects/idiosyncrasy occurring singly or in various combinations (see Table 2.3).

**Table 2.3** Blood loss in gastrointestinal disease

<b>Acute</b>	Immediately following acute haemorrhage—RBC indices usually ↔ Normochromic anaemia
<b>Acute on chronic</b>	RBC indices show low normal or marginally ↓, especially MCV Film shows mixture of normochromic and hypochromic RBCs ('dimorphic')
<b>Chronic</b>	RBC indices show established chronic Fe deficiency features ↓ MCV, MCH, platelets often ↑

### Anaemia in GIT disorders can be simply considered against some of the commoner problems arising through the GIT

**Oesophageal**—bleeding from peptic oesophagitis, association of oesophageal web and chronic Fe deficiency.

**Gastric**—pernicious anaemia and B<sub>12</sub> deficiency, late effects of partial or total gastrectomy producing B<sub>12</sub> and/or Fe deficiency. Microangiopathic haemolytic anaemia from metastatic adenocarcinoma.

**Small bowel**—malabsorption states, e.g. Fe and/or folate deficiency 2° to coeliac disease, malabsorption from other problems including inflammatory bowel disease; hyposplenism 2° to coeliac with or without ↓ platelets.

**Large bowel**—blood loss anaemia from inflammatory bowel disorders. *Note:* these may also be associated with ACD. Rare occurrence of autoimmune haemolysis associated with ulcerative colitis.

**Pancreas**—ACD associated with carcinoma or chronic pancreatitis, DIC associated with acute pancreatitis.

**Liver**—➔ see Anaemia in liver disease, p.41.

**Drug-related anaemia arises through:**

- Upper GIT irritation causing blood loss—aspirin, NSAIDs, corticosteroids.
- Bleeding due to specific drugs, e.g. warfarin and heparin.
- Drug-induced haemolysis, e.g. oxidative (Heinz body) haemolysis due to sulfasalazine or dapsone.
- Production impairment, e.g. aplasia 2° to mesalazine.

## Anaemia in liver disease

Anaemia is common in chronic liver disorders. There are several possible causes including:

- ACD—part of marrow response to chronic inflammatory processes.
- Macrocytosis  $\pm$  anaemia: specific effects on membrane lipids cause  $\uparrow$ MCV.
- Alcohol—direct suppressive effect on erythropoiesis with  $\uparrow$ MCV.
- Folate deficiency: seen in alcoholic liver disease  $\rightarrow$  nutritional deficiency and/or direct effect of alcohol on folate metabolism.
- Blood loss from oesophageal varices  $\rightarrow$  acute or chronic anaemia.
- Hypersplenism—portal hypertension can produce marked splenic enlargement leading to hypersplenism.
- Haemolytic anaemias, e.g.:
  - Autoimmune haemolytic anaemia in association with chronic active hepatitis.
  - Zieve's syndrome (hypertriglyceridaemia + self-limiting haemolysis due to acute alcohol excess).
  - Viral hepatitis may provoke oxidative haemolysis in those with G6PD deficiency.
  - Acute liver failure—DIC and MAHA may occur.
  - Acanthocytosis: acute haemolytic anaemia with acanthocytosis (spur cell anaemia). Rare. Usually late stage liver disease, with poor prognosis.

### Alcohol

Even if no liver disease, chronic alcohol consumption may lead to  $\uparrow$ MCV (100–110fL). Anaemia may be associated with poor diet, folate deficiency, GI bleeding and Fe deficiency. Alcohol has direct toxic effects on the marrow with reversible suppression of haemopoiesis;  $\downarrow$ Hb and  $\downarrow$  platelets are most common.

### Further reading

- Savage, D., et al. (1986). Anemia in alcoholics. *Medicine* (Baltimore), **65**, 322–38.  
 Wu, A., et al. (1974). Macrocytosis of chronic alcoholism. *Lancet*, **1**, 829–31.

## Iron (Fe) deficiency anaemia

Microcytic anaemia is common and the commonest cause is chronic Fe deficiency.

### Fe physiology and metabolism

Normal (Western) diet provides ~15mg of Fe/d, of which 5–10% is absorbed in duodenum and upper jejunum. Ferrous ( $\text{Fe}^{2+}$ ) Fe is better absorbed than ferric ( $\text{Fe}^{3+}$ ) Fe. Total body Fe store ~4g. Around 1mg of Fe/d lost in urine, faeces, sweat, and cells shed from the skin and GIT. Fe deficiency is commoner in ♀ of reproductive age since menstrual losses account for ~20mg Fe/month and in pregnancy an additional 500–1000mg Fe may be lost (transferred from mother → fetus) (see Table 2.4).

**Table 2.4** Causes of Fe deficiency

<b>Reproductive system</b>	Menorrhagia
<b>GIT</b>	Oesophagitis, oesophageal varices, hiatus hernia (ulcerated; simple hiatus hernia would not cause Fe deficiency), peptic ulcer, inflammatory bowel disease, haemorrhoids, carcinoma: stomach, colorectal, (rarely angiodysplasia, hereditary haemorrhagic telangiectasia)
<b>Malabsorption</b>	Coeliac disease, atrophic gastritis ( <i>Note:</i> may also result from Fe deficiency), gastrectomy
<b>Physiological</b>	Growth spurts, pregnancy
<b>Dietary</b>	Vegans, elderly
<b>Genitourinary system</b>	Haematuria (uncommon cause)
<b>Others</b>	PNH, frequent venesection, e.g. blood donation
<b>Worldwide</b>	Commonest cause is hookworm infestation

### Assessment

*Clinical history*—review potential sources of blood loss, especially GIT loss.

*Menstrual loss*—quantitation may be difficult; ask about number of tampons used per day, how often these require changing, and duration.

*Other sources of blood loss*, e.g. haematuria and haemoptysis (these are not common causes of Fe deficiency). Ask patient if he/she has been a blood donor—regular blood donation over many years may cause chronic Fe store depletion.

*Drug therapy*, e.g. NSAIDs and corticosteroids may cause GI irritation and blood loss.

*Past medical history*, e.g. previous gastric surgery (→ malabsorption). Ask about previous episodes of anaemia and treatments with Fe.

In patients with Fe deficiency assume underlying cause is blood loss until proved otherwise. In developed countries pure dietary Fe lack causing Fe deficiency is almost unknown.

### Examination

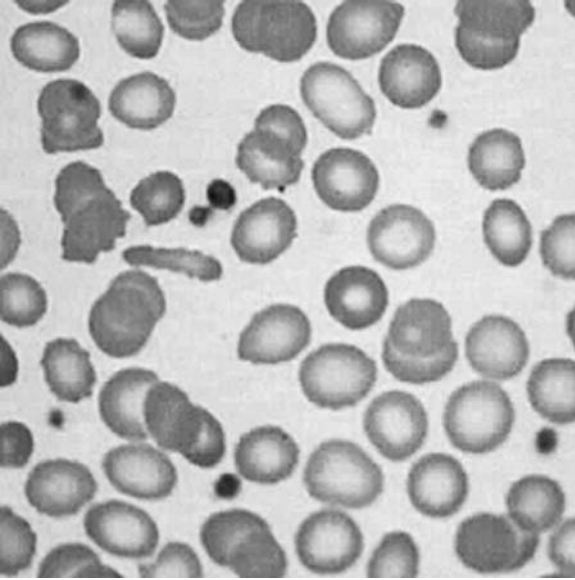
- General examination including assessment of mucous membranes (e.g. hereditary haemorrhagic telangiectasia).
- Seek possible sources of blood loss.
- Abdominal examination, rectal examination, and sigmoidoscopy mandatory.
- Gynaecological examination also required.

### Laboratory tests

- ↓ Hb.
- ↓ MCV (<76fL) and ↓ MCHC (Note: ↓ MCV in thalassaemia and ACD).
- Red cell distribution width (RDW): ↑ in Fe deficiency states with a greater frequency than in ACD or thalassaemia trait (see Fig. 2.2).
- Serum ferritin (measurement of Fe/TIBC generally unhelpful). *Ferritin assay preferred—low serum ferritin identifies the presence of Fe deficiency but as an acute phase protein it can be ↑, masking Fe deficiency. ↓ Fe and ↑ TIBC indicates Fe deficiency (though tests are obsolete).*
- The soluble transferrin assay (sTfR) is useful in cases where ↑ ESR. sTfR is ↑ in Fe deficiency but ↔ in anaemia in presence of ↑ ESR (e.g. rheumatoid, other inflammatory states). This assay is not universally available at present.
- % hypochromic RBCs—some modern analysers provide this parameter. ↑ % hypo RBCs are seen in Fe deficiency but also thalassaemia, CRF on EPO where insufficient Fe given.
- Zinc protoporphyrin (ZPP)—in the absence of Fe, zinc is incorporated into protoporphyrin. ↑ ZPP in Fe deficiency is a non-specific marker since ↑ ZPP is seen in any disorder that restricts Fe availability to developing RBCs, e.g. infection, inflammation, cancer, etc.
- Reticulocyte Hb concentration (CHR) appears to be a sensitive method for detecting early Fe deficiency.
- Examination of BM aspirate (Fe stain) is occasionally useful.
- Theoretically FOB testing may be of value in Fe deficiency but results can be misleading. False +ve results seen in high dietary meat intake.

### Treatment of Fe deficiency

Simplest, safest and cheapest treatment is oral ferrous salts, e.g. FeSO<sub>4</sub> (Fe gluconate and fumarate equally acceptable). Provide an oral dose of elemental Fe of 150–200mg/d. Side effects in 10–20% patients (e.g. abdominal distension, constipation and/or diarrhoea)—try ↓ the daily dose to bd or od. Liquid Fe occasionally necessary, e.g. children or adults with swallowing difficulties. Increasing dietary Fe intake has no routine place in the management of Fe deficiency except where intake is grossly deficient.



**Fig. 2.2** Blood film in Fe deficiency anaemia: note pale red cells with pencil cell (top left).

### Response to replacement

A rise of Hb of 2.0g/dL over 3 weeks is expected. MCV will  $\uparrow$  concomitantly with Hb. Reticulocytes may  $\uparrow$  in response to Fe therapy but is not a reliable indicator of response.

### Duration of treatment

Generally ~6 months. After Hb and MCV are normal continue Fe for at least 3 months to replenish Fe stores.

### Failure of response

- Is the diagnosis of Fe deficiency correct?
  - Consider ACD or thalassaemia trait.
- Is there an additional complicating illness?
  - Chronic infection, collagen disorder, or neoplasm.
- Is the patient complying with prescribed medication?
- Is the preparation of Fe adequate in dosage and/or formulation?
- Is the patient continuing to bleed excessively?
- Is there malabsorption?
- Are there other haematinic deficiencies (e.g. B<sub>12</sub> or folate) present?
- Reassess patient: ?evidence of continued blood loss or malabsorption.

### Parenteral Fe

Occasionally of value in genuine Fe intolerance, if compliance is a problem, or if need to replace stores rapidly, e.g. in pregnancy or prior to major surgery. *Note:* Hb will rise no faster than with oral Fe.

### Intravenous Fe

Fe may be administered IV as Fe hydroxide sucrose (ferric hydroxide with sucrose) or Fe dextran (ferric hydroxide with dextran). Facilities for cardiopulmonary resuscitation should be available though serious adverse events are uncommon.

### Further reading

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## Vitamin B<sub>12</sub> deficiency

B<sub>12</sub> deficiency presents with macrocytic, megaloblastic anaemia ranging from mild to severe (Hb <6.0g/dL). Symptoms are those of chronic anaemia: fatigue, dyspnoea on effort, etc. Neurological symptoms may also be present—classically peripheral paraesthesiae and disturbances of position and vibration sense (see Table 2.5). Occasionally neurological symptoms occur with no/minimal haematological upset. If uncorrected, the patient may develop subacute combined degeneration of the spinal cord → permanently ataxic.

### Pathophysiology

B<sub>12</sub> (along with folic acid) is required for DNA synthesis; B<sub>12</sub> is also required for neurological functioning. B<sub>12</sub> is absorbed in terminal ileum after binding to intrinsic factor produced by gastric parietal cells. Body stores of B<sub>12</sub> are 2–3mg (sufficient for 3 years). B<sub>12</sub> is found in meats, fish, eggs, and dairy produce. Strictly vegetarian (vegan) diets are low in B<sub>12</sub> although not all vegans develop clinical evidence of deficiency.

### Presenting haematological abnormalities

- Macrocytic anaemia (MCV usually >110fL). In extreme cases RBC anisopoikilocytosis can result in MCV values just within normal range.
- RBC changes include oval macrocytosis, poikilocytosis, basophilic stippling, Howell–Jolly bodies, circulating megaloblasts.
- Hypersegmented neutrophils.
- Leucopenia and thrombocytopenia common.
- BM shows megaloblastic change; marked erythroid hyperplasia with predominance of early erythroid precursors, open atypical nuclear chromatin patterns, mitotic figures and ‘giant’ metamyelocytes
- Fe stores usually ↑.
- Serum B<sub>12</sub> ↓.
- Homocysteine levels are ↑ in both B<sub>12</sub> and folate deficiency.
- Serum/red cell folate usually ↔ or ↑.
- LDH levels markedly ↑ reflecting ineffective erythropoiesis (RBC destruction within the BM and ↓ RBC lifespan).
- Autoantibody screen in pernicious anaemia: 80–90% show circulating gastric parietal cell antibodies, 55% have circulating intrinsic factor antibodies. *Note:* parietal cell antibodies are not diagnostic since found in normals; IFA is only found in 50% of patients with PA but is diagnostic.

### Management of B<sub>12</sub> deficiency

- Identify and correct cause if possible.
- Investigations are undertaken and a test of B<sub>12</sub> absorption is carried out (e.g. Schilling test). Urinary excretion of a test dose of B<sub>12</sub> labelled with trace amounts of radioactive cobalt is compared with excretion of B<sub>12</sub> bound to intrinsic factor; the test is done in two parts. B<sub>12</sub> malabsorption corrected by intrinsic factor is diagnostic of pernicious anaemia (in absence of previous gastric surgery). This test is now largely obsolete since the reagents are no longer available.
- Management—hydroxocobalamin 1mg IM and folic acid PO should be given immediately.



**Table 2.5** Causes of B<sub>12</sub> deficiency

<b>Pernicious anaemia</b>	Commonest, due to autoimmune gastric atrophy resulting in loss of intrinsic factor production required for absorption of B <sub>12</sub> . Incidence ↑ >40 years and often associated with other autoimmune problems, e.g. hypothyroidism.
<b>Following total gastrectomy</b>	May develop after major partial gastrectomy.
<b>Ileal disease</b>	Resection of ileum, Crohn's disease.
<b>Blind loop syndromes</b>	E.g. diverticulae or localized inflammatory bowel changes allowing bacterial overgrowth which then competes for available B <sub>12</sub>
<b>Fish tapeworm</b>	<i>Diphyllobothrium latum</i> .
<b>Malabsorptive disorders</b>	Tropical sprue, coeliac disease.
<b>Dietary deficiency</b>	E.g. vegans

- Supportive measures—bed rest, O<sub>2</sub>, and diuretics may be needed while awaiting response. Transfusion is best avoided but 2 units of concentrated RBCs may be used for patients severely compromised by anaemia (risk of precipitating cardiac failure); hypokalaemia is occasionally observed during the immediate response to B<sub>12</sub> and serum [K<sup>+</sup>] should be monitored.
- Response apparent in 3–5d with reticulocyte response of >10%; normoblastic conversion of marrow erythropoiesis in 12–24h. Patients frequently describe a subjective improvement within 24h.
- B<sub>12</sub> replacement therapy—initially hydroxocobalamin 5 × 1mg IM should be given during the first 2 weeks, thereafter maintenance injections are needed 3-monthly.
- If dietary deficiency seems likely and B<sub>12</sub> deficiency mild, worth trying oral B<sub>12</sub> (cyanocobalamin 50–150mcg or more, daily between meals).
- Long-term follow-up depends on the 1<sup>o</sup> cause. Pernicious anaemia patients require lifelong treatment and should be checked annually with a FBC and thyroid function; the incidence of gastric cancer is twice as high in these patients compared to the normal population.
- Broad-spectrum antibiotics should be given to suppress bacterial over-growth in blind loop syndrome ± local surgery if appropriate. Long-term IM B<sub>12</sub> may be the pragmatic solution if blind loop cannot be corrected.

### Further reading

Guidelines on the investigation and diagnosis of cobalamin and folate deficiencies. A publication of the British Committee for Standards in Haematology. BCSH General Haematology Test Force (1994). *Clin Lab Haematol*, **16**, 101–15.

Toh, B.H., van Driel, I.R., and Gleeson, P.A. (1997). Pernicious anemia. *N Engl J Med*, **337**, 1441–8.

## Folate deficiency

Folate deficiency represents the other main deficiency cause of megaloblastic anaemia; haematological features indistinguishable from those of B<sub>12</sub> deficiency. Distinction is on basis of demonstration of reduced red cell and serum folate. See Table 2.6 for causes of folate deficiency.

►► Megaloblastic anaemia patients should never receive empirical treatment with folic acid alone. *If they lack B<sub>12</sub>, folic acid is potentially capable of precipitating subacute combined degeneration of the cord.*

### Pathophysiology

Adult body folate stores comprise 10–15mg; normal daily requirements are 0.1–0.2mg, i.e. sufficient for 3–4 months in absence of exogenous folate intake. Folate absorption from dietary sources is rapid; proximal jejunum is main site of absorption. Main dietary sources of folate are liver, green vegetables, nuts, and yeast. Western diets contain ~0.5–0.7mg folate/d but availability may be lessened as folate is readily destroyed by cooking, especially in large volumes of water. Folate coenzymes are an essential part of DNA synthesis, hence the occurrence of megaloblastic change in deficiency.

### Diagnosis

Haematological findings are identical to those seen in B<sub>12</sub> deficiency—macrocytic, megaloblastic anaemia. Other findings also similar to B<sub>12</sub> except parietal cell and intrinsic factor autoantibodies usually –ve. Reduced folate levels—serum folate levels reflect recent intake, red cell folate levels give a more reliable indication of folate status (see Figs. 2.3 and 2.4).

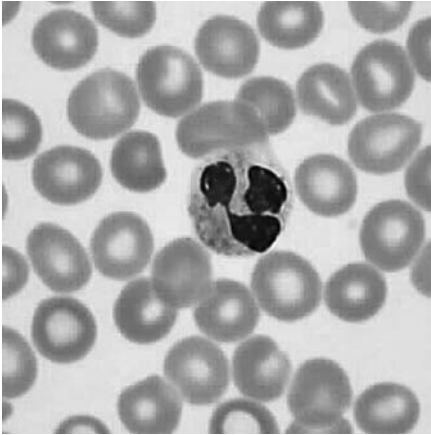
**Table 2.6** Causes of folate deficiency

↓ intake	Poor nutrition, e.g. poverty, old age, 'skid row' alcoholics
↑ requirements/losses	Pregnancy, ↑ cell turnover, e.g. haemolysis, exfoliative dermatitis, renal dialysis
Malabsorption	Coeliac disease, tropical sprue, Crohn's and other malabsorptive states
Drugs	Phenytoin, barbiturates, valproate, oral contraceptives, nitrofurantoin may induce folate malabsorption
Antifolate drugs	Methotrexate, trimethoprim, pentamidine antagonize folate cf. induce deficiency
Alcohol	Poor nutrition plus a direct depressant effect on folate levels which can precipitate clinical folate deficiency

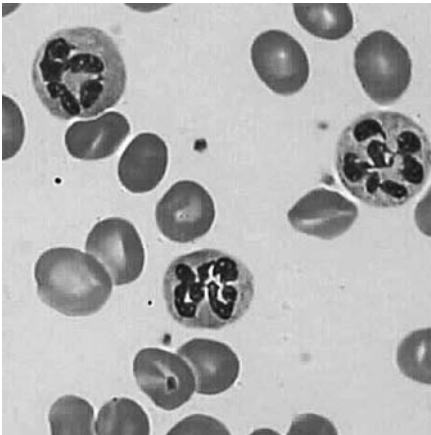
### Management

- Treatment and support of severe anaemia as for B<sub>12</sub> deficiency.
- Folic acid 5mg/d PO (never on its own, see Table 2.6), unless patient known to have normal B<sub>12</sub> level.

- Treatment of underlying cause, e.g. in coeliac disease folate levels and absorption normalize once patient established on gluten-free diet. Long-term supplementation advised in chronic haemolysis, e.g. HbSS or HS.
- Prophylactic folate supplements recommended in pregnancy and other states of ↑ demand, e.g. prematurity.



**Fig. 2.3** Blood film: normal neutrophil: usually has <5 lobes. This one has 3 lobes (➔ see colour plate section).



**Fig. 2.4** Hypersegmented neutrophils with 7–8 lobes: found in B<sub>12</sub> or folate deficiency. Note: blood films and marrow appearances are identical in B<sub>12</sub> and folate deficiencies (➔ see colour plate section).

## Other causes of megaloblastic anaemia

Megaloblastic anaemia not due to actual deficiency of either B<sub>12</sub> or folate is uncommon, but may occur in the following situations.

### Congenital

- Transcobalamin II deficiency—absence of the key B<sub>12</sub> transport protein results in severe megaloblastic anaemia (corrects with parenteral B<sub>12</sub>).
- Congenital intrinsic factor deficiency—autosomal recessive, results in failure to produce intrinsic factor. Presents as megaloblastic anaemia up to age of 2 years and responds to parenteral B<sub>12</sub>.
- Inborn errors of metabolism—errors in folate pathways, also occurs in orotic aciduria and Lesch–Nyhan syndrome.
- Megaloblastosis commonly present in the congenital dyserythropoietic anaemias (➔ see Congenital dyserythropoietic anaemias, p.588).

### Acquired

- MDS—often present in sideroblastic anaemia (RARS).
- Acute leukaemia—megaloblastic-like erythroid dysplasia in AML M6.
- Drug induced—2° to antimetabolite drugs including 6-mercaptopurine, cytosine arabinoside, zidovudine, and hydroxyurea.
- Anaesthetic agents—transient megaloblastic change after nitrous oxide.
- Alcohol excess—may result in megaloblastic change in absence of measurable folate deficiency.
- Vitamin C deficiency—occasionally results in megaloblastic change.

## Anaemia in other deficiency states

Fe, folate, or vitamin B<sub>12</sub> deficiencies account for the majority of clinically significant deficiency syndromes resulting in anaemia. Anaemia is recognized as a complication in other vitamin deficiencies and in malnutrition.

### Vitamin A deficiency

Produces a chronic disorder like Fe deficiency anaemia with ↓ MCV and MCH.

### Vitamin B<sub>6</sub> (pyridoxine) deficiency

Can produce hypochromic microcytic anaemia; sideroblastic change may occur. Pyridoxine is given to patients on antituberculous therapy with isoniazid which is known to interfere with vitamin B<sub>6</sub> metabolism and cause sideroblastic anaemia.

### Vitamin C deficiency

Occasionally associated with macrocytic anaemia ( $\pm$  megaloblastic change in 10%); since the main cause of vitamin C deficiency is inadequate diet or nutrition there may be evidence of other deficiencies.

### Vitamin E deficiency

Occasionally seen in the neonatal period in low birth weight infants—results in haemolytic anaemia with abnormal RBC morphology.

### Starvation

Normochromic anaemia  $\pm$  leucopenia occurs in anorexia nervosa; features are not associated with any specific deficiency; BM is typically hypocellular.

## Haemolytic syndromes

### Definition

Any situation in which there is a reduction in RBC life-span due to ↑RBC destruction. Failure of compensatory marrow response results in anaemia. Predominant site of RBC destruction is red pulp of the spleen.

### Classification—3 major types

1. Hereditary vs acquired.
2. Immune vs non-immune (see Table 2.7).
3. Extravascular vs intravascular.

*Hereditary* cause suggested if history of anaemia refractory to treatment in infancy ± FH, e.g. other affected members, anaemia, gallstones, jaundice, splenectomy. *Acquired* haemolytic anaemia is suggested by sudden onset of symptoms/signs in adulthood. *Intravascular* haemolysis—takes place in peripheral circulation cf. *extravascular* haemolysis which occurs in RES.

### Hereditary

- Red cell membrane disorders, e.g. HS and hereditary elliptocytosis.
- Red cell enzymopathies, e.g. G6PD and PK deficiencies.
- Abnormal Hb, e.g. thalassaemias and sickle cell disease, unstable Hbs.

### Acquired—immune

**Table 2.7** Acquired: immune

Alloimmune	Autoimmune
<ul style="list-style-type: none"> <li>• HDN</li> <li>• RBC transfusion incompatibility</li> </ul>	<ul style="list-style-type: none"> <li>• Warm AIHA—1° or 2° to SLE, CLL, drugs</li> <li>• Cold—<i>Mycoplasma</i> or EBV infection</li> <li>• Cold haemagglutinin disease (CHAD)</li> <li>• Lymphoproliferative disorders</li> <li>• Paroxysmal cold haemoglobinuria (PCH)</li> </ul>

### Acquired—non-immune

- MAHA.
- TTP/HUS.
- Hypersplenism.
- Prosthetic heart valves.
- March haemoglobinuria.
- Sepsis.
- Malaria.
- Paroxysmal nocturnal haemoglobinuria.

### Clinical features

Symptoms of anaemia, e.g. breathlessness, fatigue. Urinary changes, e.g. red or dark brown of haemoglobinuria. Symptoms of underlying disorder.

### Confirm haemolysis is occurring

- Check FBC.
- Peripheral blood film—polychromasia, spherocytosis, fragmentation (schistocytes), helmet cells, echinocytes.
- ↑ reticulocytes.
- ↑ serum bilirubin (unconjugated).
- ↑ LDH.
- Low/absent serum haptoglobin (bind free Hb).
- Schumm's test (for intravascular haemolysis).
- Urinary haemosiderin (implies chronic intravascular haemolysis, e.g. PNH).

### Discriminant diagnostic features

*Establish whether immune or non-immune—check DAT.*

?*Immune*—if DAT +ve check IgG and C3 specific reagents—suggest warm and cold antibody respectively. Screen serum for red cell alloantibodies.

?*Cold antibody present*—examine blood film for agglutination, check MCV on initial FBC sample and again after incubation at 37°C for 2h. High MCV at room temperature due to agglutinates falls to normal at 37°C. Check anti-I and anti-i titres for confirmation. Check *Mycoplasma* IgM and EBV serology, and for presence of Donath–Landsteiner antibody (cold reacting IgG antibody with anti-P specificity).

?*Warm antibody present*—IgG +ve DAT only suggestive—examine film for spherocytes (usually prominent), lymphocytosis or abnormal lymphs to suggest LPD. Examine patient for nodes.

?*Intravascular haemolysis*—check for urinary haemosiderin, Schumm's test.

?*Sepsis*—check blood cultures.

?*Malaria*—examine thick and thin blood films for parasites.

?*Renal/liver abnormality*—examine for hepatomegaly, splenomegaly, LFTs and U&E.

?*Low platelets*—consider TTP/HUS.

?*Haemoglobinopathy*—check Hb electrophoresis.

?*Red cell membrane abnormality*—check family history and perform red cell fragility test.

?*Red cell enzyme disorder*—check family history and do G6PD and PK assay. Note: enzymes may be falsely normal if reticulocytosis since increased levels are present in young RBCs.

?*PNH*—check immunophenotyping for CD55 + CD59 (Ham's acid lysis test now largely obsolete).

### Treatment

Treat underlying disorder. Give folic acid and Fe supplements if low.

### Further reading

Gehrs, B.C. and Friedberg, R.C. (2002). Autoimmune hemolytic anemia. *Am J Hematol*, **69**, 258–71.

## Genetic control of haemoglobin production

Hb comprises 4 protein subunits (e.g. adult Hb =  $2 \times \alpha + 2 \times \beta$  chains,  $\alpha_2\beta_2$ ) each linked to a haem group. Production of different globin chains varies from embryo  $\rightarrow$  adult to meet the particular environment at each stage. Globin genes are located on chromosomes 11 and 16. All globins related to  $\alpha$  globin are located on chromosome 16; all those related to  $\beta$  globin are on chromosome 11. The sequence in which they are produced during development reflects their physical order on chromosomes such that  $\zeta$  is the first  $\alpha$ -like globin to be produced in life. After  $\zeta$  expression stops,  $\alpha$  production occurs ( $\zeta \rightarrow \alpha$  switch). On chromosome 11 the arrangement of  $\beta$ -like globin genes follows the order (from left  $\rightarrow$  right)  $\varepsilon \rightarrow \gamma \rightarrow \delta \rightarrow \beta$  mirroring the  $\beta$ -like globin chains produced during development. As embryo develops into fetus,  $\zeta$  production stops and  $\alpha$  is produced. The  $\alpha$  globin combines with  $\gamma$  chains and produces  $\alpha_2\gamma_2$  (fetal Hb, HbF). After birth  $\gamma$  production  $\downarrow$  and  $\delta$  and  $\beta$  chains are produced. Adults have predominantly HbA ( $\alpha_2\beta_2$ ) although small amounts of HbA<sub>2</sub> ( $\alpha_2\delta_2$ ) and HbF are produced (see Table 2.8).

Hb switching is physiological but the mechanism is unclear. HbF ( $\alpha_2\gamma_2$ ) binds O<sub>2</sub> more tightly than adult haemoglobin (higher oxygen affinity), ensuring adequate O<sub>2</sub> delivery to the fetus which must extract its O<sub>2</sub> from mother's circulation. After birth the lungs expand and the O<sub>2</sub> is derived from the air, with  $\beta$  production replacing that of  $\gamma$ , leading to an increase in adult haemoglobin ( $\alpha_2\beta_2$ ). (See Figs. 2.5 and 2.6.)

**Table 2.8** Structure and quantity of normal haemoglobins

Haemoglobin	Globin chains	Amount
<b>Embryo</b>		
Hb Gower 1	$\zeta_2\varepsilon_2$	42% <sup>a</sup>
Hb Gower 2	$\alpha_2\varepsilon_2$	24% <sup>a</sup>
Hb Portland	$\zeta_2\gamma_2$	
<sup>a</sup> by 5th week		
<b>Fetus</b>		
HbF	$\alpha_2\gamma_2$	85%
HbA	$\alpha_2\beta_2$	5–10%
<b>Adult</b>		
HbA	$\alpha_2\beta_2$	97%
HbA <sub>2</sub>	$\alpha_2\delta_2$	2.5%
HbF	$\alpha_2\gamma_2$	0.5%

### Haemoglobin abnormalities

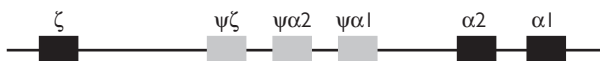
Fall into 2 major groups: *structural abnormalities* of Hb due to alterations in DNA coding for the globin protein leading to an abnormal amino acid in the globin molecule, e.g. sickle haemoglobin ( $\beta^S$ ). 2nd group of Hb disorders results from *imbalanced globin chain production*—globins produced



are structurally normal but their relative amounts are incorrect and lead to the thalassaemias.

Haemoglobinopathies result in significant morbidity and mortality on a worldwide scale. Patients with these disorders are also seen in Northern Europe and the UK, especially in areas with significant Greek, Italian, Afro-Caribbean and Asian populations.

### $\alpha$ -like genes on chromosome 16

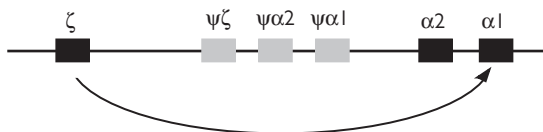


### $\beta$ -like genes on chromosome 11

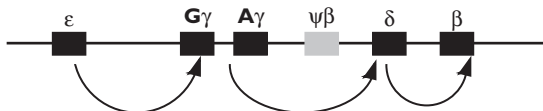


**Fig. 2.5** Arrangement of  $\alpha$ -like and  $\beta$ -like globin genes ( $\psi$  indicates pseudogene).

### $\alpha$ globin switching



### $\beta$ globin switching



**Fig. 2.6** Globin gene switching during development.

### Further reading

Rund, D., et al. (2001). Pathophysiology of alpha- and beta-thalassaemia: therapeutic implications. *Semin Hematol*, **38**, 343–9.

## Sickling disorders

Sickle cell anaemia refers to homozygous SS,  $\beta^S\beta^S$ , but compound HbSC ( $\beta^S\beta^C$ ), HbS/ $\beta^+$  or  $\beta^0$  thalassaemia, and HbSD ( $\beta^S\beta^D$ ) are all sickling disorders. Clinical symptoms can be quite variable between individuals but HbSS is generally the most severe. The gene has remained at high frequency due to conferred resistance to malaria in heterozygotes. Inheritance is autosomal recessive.

### Sickle cell anaemia (SCA, HbSS)

#### Pathogenesis

Widespread throughout Africa, Middle East, parts of India and Mediterranean. Single base change in  $\beta$  globin gene, amino acid 6 (glu  $\rightarrow$  val). Heterozygosity rates: West Africa 10–30%, African Americans and UK AfroCaribbeans 10%. Patients with SCA are the offspring of parents both of whom are carriers of the  $\beta^S$  gene, i.e. they both have sickle cell trait, and homozygotes for the abnormal  $\beta^S$  gene demonstrate features of chronic red cell haemolysis and tissue infarction. RBCs containing HbS deform (elongate) under conditions of reduced oxygenation, and form characteristic sickle cells—do not flow well through small vessels, and are more adherent than normal to vascular endothelium, leading to vascular occlusion and sickle cell crises. The vaso-occlusive process is complex and, in addition to the polymerization of globin, there is interaction of sickle cells with other types of cell and proteins. Sickle cells are more adherent to vascular endothelium than normal red cells. During vascular crises there is endothelial damage with exposure of molecules such as thrombospondin, laminin, and fibronectin. There is activation of blood coagulation with enhanced thrombin generation and hyperreactivity of platelets. Chronic haemolysis depletes the body of nitric oxide (NO) which is needed for vasoregulation resulting in chronic complications such as pulmonary hypertension and leg ulceration.

#### Clinical features

- Highly variable. Many have few symptoms whilst others have severe and frequent crises, marked haemolytic anaemia, and chronic organ damage. HbF level plays role in ameliorating symptoms ( $\uparrow$ HbF  $\rightarrow$  fewer and milder crises). Likely impact of inherited and environmental factors. Spectrum of *haemolytic* and *vasoocclusive* phenotype.
- *Newborns*—have higher HbF level than normal adult, protected during first 8–20 weeks of life. Symptoms start when HbF level falls.
- *Infection*—high morbidity and mortality due to bacterial and viral infection due to functional hyposplenism. Pneumococcal septicaemia (*Streptococcus pneumoniae*) well recognized. Other infecting organisms: meningococcus (*Neisseria meningitidis*), *Escherichia coli*, and *Haemophilus influenzae* (hyposplenic).  $\uparrow$  malaria risk with higher complication rate (prophylaxis encouraged)
- *Steady state anaemia*—children and adults often severely anaemic (Hb  $\sim$ 6.0–9.0 g/dL). The steady state Hb of each patient should be noted at the annual review. Anaemia is chronic (haemolytic) and patients generally well-adapted until episode of decompensation (e.g. severe infection) occurs. Causes of acute-on-chronic anaemia include

↑ haemolysis (usually 1–2g/dL) due to infection including malaria, vaso-occlusive crisis, transfusion reaction. G6PD deficiency can contribute to ↑ haemolysis. Sequestration syndromes including acute chest syndrome, splenic and hepatic sequestration and acute aplasia usually due to parvovirus B19 infection. Apart from aplastic crisis all others should be accompanied by ↑ reticulocyte count. In some cases transfusion therapy can be life saving but decision to transfuse should be made following discussion with an experienced clinician.

#### *Acute and chronic sickle complications*

- **Vaso-occlusive crisis:** presents with severe bone, joint, and muscle pain. Bone pain affects long bones and spine, and is due to occlusion of small vessels. Triggers: infection, dehydration, alcohol, menstruation, cold, and temperature changes—often identifiable precipitant found. Dactylitis is a subform mainly children age <6 years. Metacarpals, metatarsals, backs of hands and feet become swollen and tender (small vessel occlusion and infarction). Recurrent, can result in permanent radiological abnormalities in bones of the hands and feet (rare) with digit shortening.
- **Sepsis:** fever without source, commence broad-spectrum antibiotics without delay and organize septic screen.
- **Pulmonary complications:** acute chest syndrome—common cause of death, seen in 40% of patients, children > adults (more severe in adults). Chest wall pain, sometimes with pleurisy, fever, and SOB. Resembles infection, infarction, or embolism. May follow simple pain crisis and common postoperatively. Early recognition important. Requires prompt and vigorous treatment. Transfer to ITU if  $pO_2$  cannot be kept >70mmHg on air. Consider CPAP. Treat infection vigorously, cover for *S. pneumoniae*, *H. influenzae*, *Mycoplasma*, *Legionella*. Exchange transfusion can be life saving. Pulmonary hypertension recognized chronic complication. Screening ECHO recommended. TRV max. jet velocity >2.5m/s associated with ↑ risk of death. Chronic lung disease also seen, restrictive defect on PFT.
- **Sequestration crises**—mainly children (30%). Pooling of large volumes of blood in spleen and/or liver. Severe hypotension and profound anaemia may result in death. Splenic sequestration is seen predominantly in children <6 years of age; often occurs following a viral infection.
- **Abdominal pain and hepatobiliary complications:** gallstones common due to chronic haemolysis. RUQ pain may represent biliary colic, cholangitis, sickle chronic hepatopathy. Laparoscopic cholecystectomy may help prevent recurrent acute biliary complications.
- **Neurological complications:** silent infarcts common due to small vessel vasculopathy. ↑ risk of ischaemic stroke (young children > adults but increases in older adults). Haemorrhagic stroke more common in young adults. Significant cause of death. Cognitive deficits recognized in children and adults. TCD screening recommended for children from age 2 with transfusion therapy for 1<sup>st</sup> stroke prevention. Acute stroke should be managed with exchange transfusion.

- *Eye complications*: proliferative retinopathy (in 30%; more common in SC disease, affecting ~50% adults), blindness (esp. HbSC), retinal artery occlusion, retinal detachment. Refer to ophthalmology if sudden-onset visual impairment, eye trauma.
- *Renal and genitourinary*: haematuria common, likely due to medullary sickling. Frank haematuria—acute papillary necrosis. Renal reserve is impaired. Risk of acute kidney injury. Risk factors for chronic sickle nephropathy: hypertension, proteinuria, severe anaemia, haematuria. Avoid NSAIDs, start ACE inhibitor, good BP control. Postrenal transplant exchange transfusion recommended. Hyposthenuria (inability to concentrate urine) common cause of nocturnal enuresis. Priapism common (40%) men. Form of compartment syndrome so fulminant (>4h) priapism is medical emergency. Give oral alpha agonist, urgent penile aspiration, washout, and intracorporal alpha agonist injection (urology).

#### *Other problems*

- *Growth retardation*: common in children, but adult may have normal height (weight tends to be lower than normal). Sexual maturation delayed.
- *Acute fat embolism syndrome*: bone marrow infarction and necrosis with embolization of fat droplets. Triggers acute systemic illness. Progressive anaemia, thrombocytopenia, DIC, reticulocytopenia. Can lead to multiorgan failure.
- *Locomotor*: avascular necrosis of the head of the femur or humerus, arthritis, and osteomyelitis (*Salmonella* infection).
- *Chronic leg ulceration* is a complication of many haemoglobinopathies including sickle cell anaemia. Ischaemia is main cause. Rare in SC disease.
- *CVS*: murmurs (anaemia), tachycardia.
- *Psychosocial*: depression, socially withdrawn.

#### *Anaesthesia and surgery*

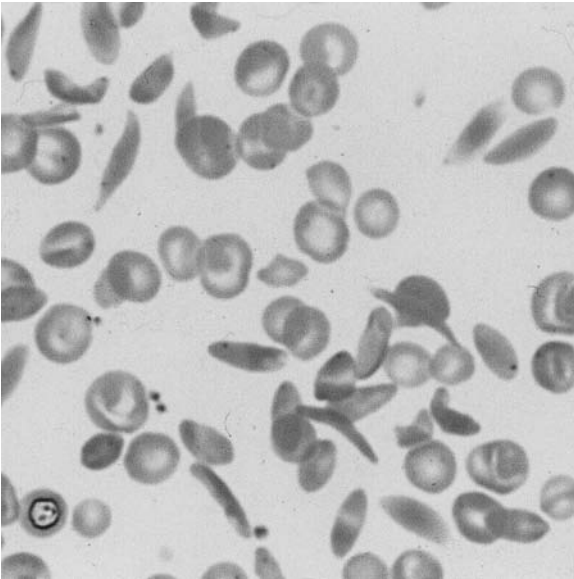
- Data from the TAPS trial demonstrated a benefit from pre-operative transfusion in patients with HbSS/HbS/ $\beta$  thalassaemia having low/medium-risk procedures (e.g. adenotonsillectomies, lap cholecystectomies). All patients should have IV hydration the evening prior to procedure under GA. Attention should be paid to oxygenation, hydration, and temperature control pre-/peri- and postoperatively. Early mobilization, incentive spirometry, and VTE prophylaxis should be considered.

#### *Obstetric care*

- Pregnant patients should be managed by team of experienced haematologists/obstetricians.
- Continue penicillin/folic acid. Add aspirin from 12 weeks' gestation. Stop NSAIDs. Prophylactic LMWH should be considered during antenatal admissions/post delivery. No evidence for routine transfusions.

### Laboratory features

Anaemia usual (Hb ~6.0–9.0g/dL in HbSS although may be much lower; HbSC have higher Hb). Reticulocytes may be ↑ (to ~10–20%) reflecting intense BM production of RBCs. Anaemic symptoms usually mild since HbS has reduced O<sub>2</sub> affinity with O<sub>2</sub> dissociation curve shifted to the right. MCV and MCH are normal, unless also thalassaemia trait (25% cases). Blood film shows marked variation in red cell size with prominent sickle cells and target cells; basophilic stippling, Howell–Jolly bodies, and Pappenheimer bodies (hyposplenic features after infancy) (see Fig 2.7). Sickle solubility test (e.g. sodium dithionate) will be positive. Does *not* discriminate between sickle cell trait and homozygous disease. Serum bilirubin often ↑ (due to excess red cell breakdown).



**Fig. 2.7** Blood film in homozygous sickle cell disease. Note the elongated (sickled) red cells.

### Confirmatory tests

Hb electrophoresis or HPLC shows 80–99% HbS with no normal HbA. HbF may be elevated to about 15%. Parents will have features of sickle cell trait.

### Laboratory screening tests

Acceptable tests include high-performance liquid chromatography (HPLC), generally used as 1st-line screening as less labour intensive and cost effective. Also quantitates HbS %. Isoelectric focusing (IEF), capillary electrophoresis (CE), and cellulose acetate electrophoresis (CAE)

at alkaline pH can be used for screening or confirmation. Sickle solubility can be used as confirmation. Also examine the blood film.

#### *Antenatal screening*

Pregnant women should be offered screening as part of their antenatal care in early pregnancy. The family origins questionnaire should be used for low-prevalence regions. If both parents of fetus are carriers offer prenatal/neonatal diagnosis.

#### *Prenatal diagnosis*

May be carried out from 1st trimester (chorionic villus sampling from 10 weeks' gestation) or 2nd trimester (fetal blood sampling from umbilical cord or trophoblast DNA from amniotic fluid). DNA may be analysed using restriction enzyme digestion with *Mst* II and Southern blotting, RFLP analysis assessing both parental and fetal DNA haplotypes, oligonucleotide probes specific for sickle globin point mutation, or PCR amplification followed by restriction enzyme digestion of amplified DNA. ARMS (amplification refractory mutation system) PCR is useful in ambiguous cases. In late pregnancy fetal blood sampling may be used to confirm diagnosis.

#### *Newborn screening programme*

Detects infants in the neonatal period to allow early intervention with penicillin prophylaxis, initiation of parental education and awareness. Dry blood spots used with HPLC or IEF. As well as the routine childhood vaccinations additional pneumococcal vaccination (prevenar + pneumovax) recommended and timely hepatitis B and annual influenza vaccination.

#### *Management—general*

- Health maintenance: avoid dehydration, extremes of temperature, over exertion. Compliance with penicillin prophylaxis (current UK guidelines recommend lifelong), folate replacement. Pneumococcal vaccination, screening for complications—transcranial Doppler (TCD) age 2–16, urinalysis for proteinuria, ECHO for pulmonary hypertension (TRV jet velocity  $>2.5\text{m/s}$ ). Education around analgesic use, life style adaptation. At least annual review in specialist clinic.
- Acute and chronic pain management: most patients with sickle cell disease suffer painful crises. Chronic pain increases with age. May be clear precipitant of acute pain—infection, temperature changes (hot or cold), or stress. Chronic pain requires multidisciplinary team input (pain specialists, psychology).
- Fluid replacement—good daily hydration. During acute crisis IV maybe required. Hyperhydration should be avoided.
- Management of pain crisis ►►➡ see Haematological emergencies: sickle pain crisis p. 666.

#### *Management of painful crises*

- Regular parental opiates are useful initially but should be tapered and replaced with oral analgesia.
- Red cell transfusion is generally not required although is indicated in sudden anaemia in children (e.g. splenic sequestration), parvovirus B19 infection (with associated transient red cell aplasia), or acute chest syndrome (hypoxia).

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## HbS—sickle-modifying therapies

### Standard therapies

**Hydroxycarbamide**—it has been recognized for some time that ↑ HbF levels ameliorate sickle cell disease as HbF reduces HbS polymerization and hence sickling. Hydroxycarbamide has been evaluated in a large number of clinical trials. Mechanism of action: initially believed to ameliorate crises through elevation of HbF levels but impact on inflammation/endothelial dysfunction may also be contributory. Clinical indications include recurrent pain, acute chest syndrome, frequent hospital admissions. Could be considered for chronic organ dysfunction. Regular monitoring required.

**Transfusion**—can be life saving for acute complications (acute chest syndrome, acute ischaemic stroke, sequestration, aplastic crisis). All patients should have extended red cell phenotype to minimize alloimmunization. Chronic transfusion programme (exchange preferred) for 1<sup>o</sup> stroke prevention (high-risk TCD), 2<sup>o</sup> stroke prevention. Considered in some patients for chronic organ dysfunction, e.g. renal impairment.

**Erythropoietin**—leads to ↑ HbF and HbS. Not widely used in the management of haemoglobinopathies as EPO levels normally not low. Evidence suggests that rHuEPO provides an additive effect when alternated with hydroxycarbamide (especially if renal impairment). Dose required is high (1000–3000 IU/kg × 3d/week) with co-administration of Fe supplements.

### Newer therapies

Aimed at ameliorating or preventing clinical complications of SCD.

**Modulators of HbF** can act by inhibiting DNA methylation (decitabine, azacytidine) or histone deacetylation (dimethylbutyrate, vorinostat and panobinostat) that work by changing chromatin structure and enhance  $\gamma$  globin gene expression and also haematopoietic differentiation.

**Membrane-active drugs**—these help reverse cellular dehydration (sickle Hb polymerization is concentration-dependent). Some drugs block cation transport channels. Includes clotrimazole and magnesium salts.

**Anti-sickling agents**—act by increasing oxygen affinity (Aes-103), preventing HbS polymerization (PEG cardoxyhaemoglobin), or blocking adenosine signaling (adenosine 2B receptor antagonists).

Modulators of endothelial dysfunction (selectin inhibitors), platelet aggregation (prasugrel, eptifibatid), inflammation (regadenoson, statins), oxidant damage (glutamine).

### Curative interventions

**BM transplantation**—sibling donor transplants for sickle cell disease have been carried out in a number of centres. Mostly restricted to children and considered for severe complications and/or requirement for transfusion programme, e.g. cerebrovascular disease. Recent data using RIC transplants in adults promising.



**Gene therapy**—potentially curative but experimental. Globin gene transfer has been attempted with variable results. Expression of exogenous gene has been at levels too low to be of benefit.

### Further reading

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## Sickle cell trait (HbAS)

Asymptomatic carriers have one abnormal  $\beta^S$  gene and one normal  $\beta$  gene (with 30 million carriers worldwide).

### Clinical features

- Carriers are not anaemic and have no abnormal clinical features.
- Sickling rare unless  $O_2$  saturation falls  $<40\%$ . Crises have been reported with severe hypoxia (anaesthesia, unpressurized aircraft).
- Associated with  $\uparrow$  risk of VTE.
- $\uparrow$  exercise-associated sudden death.
- Renal complications (haematuria, renal medullary carcinoma, may exacerbate other conditions that cause ESRF).

### Laboratory features

- Hb, MCV, MCH, and MCHC  $\leftrightarrow$  (unless also  $\alpha$  thalassaemia trait).
- HbS level 40–55% (if  $<40\%$  then also  $\alpha$  thalassaemia trait).
- Film may be  $\leftrightarrow$  or show microcytes and target cells.
- Sickle cell test will be +ve (HbSS and HbAS).

### Carrier detection

Neither FBC nor film can be used for diagnostic purposes. Detection of the carrier state relies on HbS quantitation (HPLC):  $<50\%$ , HbA  $>50\%$ , in contrast to HbS/ $\beta^+$  thalassaemia (HbS  $>50\%$ , HbA  $<50\%$ ).

▶▶ Care needed during anaesthesia (*avoid hypoxia*).

## Other sickling disorders

### HbSC

Milder than SCA but resembles it. Patients have fewer and milder crises. Retinal damage (microvascular, proliferative retinopathy) and blindness are major complications (30–35%). Arrange regular ophthalmological review by specialist. Aseptic necrosis of femoral head and recurrent haematuria are common. ↑ risk of splenic infarcts and abscesses.

▶▶ Beware thrombosis and PE especially in pregnancy.

### Clinical

Mild anaemia (Hb 8–14g/dL) and splenomegaly common. Less haemolysis, fewer painful crises, fewer infections, and less vaso-occlusive disease than SCA. Growth and development normal. Lifespan normal. Pregnancy may be hazardous.

### Film

Prominent target cells with fewer NRBC than seen in SCA. Howell–Jolly and Pappenheimer bodies (hyposplenism). Occasional C crystals may be seen.

### Diagnosis

Hb electrophoresis and family studies. MCV and MCH are much lower than in HbSS.

### HbSD, HbS<sub>Arab</sub>

Milder than HbSS. Both rare. Interactions of these globins with HbS results in reduced polymerization. HbD<sub>Punjab</sub><sup>(β<sup>121</sup> glu→gln)</sup> and HbO<sub>Arab</sub><sup>(β<sup>121</sup> glu→lys)</sup> cause little disease on their own although there may be mild haemolysis in the homozygote. These haemoglobins cause sickle cell disease when present with HbS.

### HbS/β thalassaemia

Caused by inheritance of β<sup>S</sup> from one parent and β thalassaemia from the other. Sickle/β<sup>o</sup> thalassaemia is severe since no normal β globin chains are produced. Sickle/β<sup>+</sup> thalassaemia is much milder having β globin in 5–15% of their Hb. Microcytosis and splenomegaly are characteristic. Family screening will confirm microcytosis and ↑ HbA<sub>2</sub> in one of the parents. See UK Standards.

### Management

Essentially as for HbSS with prompt treatment of crises (➡ Sickle crisis, p.666).

### Further reading

<http://www.sicklecellsociety.org/app/webroot/files/files/CareBook.pdf>

## Other haemoglobinopathies

### HbC disease ( $\beta^6 \text{glu} \rightarrow \text{lys}$ )

West Africa. Patients have benign compensated haemolysis. Development is normal, splenomegaly is common. Gallstones are recognized complication. The Hb may be mildly  $\downarrow$ . MCV and MCH  $\downarrow$  and reticulocytes  $\uparrow$ . Blood film shows prominent target cells and occasional HbC crystals. Hb electrophoresis shows mainly HbC with some HbF. HbA is absent. Red cells said to be 'stiff'. Care with anaesthesia.

### HbC trait ( $\beta^6 \text{glu} \rightarrow \text{lys}$ )

Asymptomatic. Hb is  $\leftrightarrow$ . Film may be  $\leftrightarrow$  or show presence of target cells. HbC 30–40%.

### HbD disease (e.g. DPunjab $\beta^{121} \text{glu} \rightarrow \text{lys}$ )

Found in North West India, Pakistan, and Iran. Film shows target cells.

### HbD trait (e.g. DPunjab $\beta^{121} \text{glu} \rightarrow \text{gln}$ )

Of little consequence other than interaction with HbS. Hb and MCV  $\leftrightarrow$ . Film normal or shows target cells.

### HbE disease ( $\beta^{26} \text{glu} \rightarrow \text{lys}$ )

South East Asia (commonest Hb variant), India, Burma, and Thailand. This Hb is moderately unstable when exposed to oxidants. May produce thalassaemic syndrome when mRNA splice mutants. There is mild anaemia, MCV and MCH  $\downarrow$ , reticulocytes  $\leftrightarrow$ . Film shows target cells, hypochromic and microcytic red cells. There are few symptoms; underlying compensated haemolysis, mild jaundice. Liver and spleen size are normal. Treatment is not usually required.

### HbE trait ( $\beta^{26} \text{glu} \rightarrow \text{lys}$ )

Asymptomatic. Indices similar to  $\beta$  thalassaemia trait. Hb usually  $\leftrightarrow$ .

### HbE/ $\beta$ thalassaemia

Compound heterozygote. Clinical picture variable from thalassaemia minor to thalassaemia major. Most patients have moderate disease. Hb is lower than HbEE; MCV and MCH are lower than HbE trait. Reticulocytes are raised slightly at 4–6%.



## Unstable haemoglobins

Congenital Heinz body haemolytic anaemia caused by point mutations in globin genes. Hb precipitates in RBCs when there is oxidative stress → Heinz bodies. In normal Hb there are non-covalent bonds maintaining the Hb structure; loss of bonds leads to Hb denaturation and precipitation. Production of Heinz bodies leads to less deformable red cells with reduced lifespan. The degree of haemolysis varies depending on the mutation, but may be more severe if there is fever or if the patient ingests oxidant drugs (see Table 2.9).

Predominantly autosomal dominant; most patients are heterozygotes. Mainly affects  $\beta$  globin chain, e.g. Hb Hammersmith (mutation involves amino acid in contact with haem pocket); Hb Bristol (replacement of non-polar by polar amino acid with distortion of protein).

**Table 2.9** Examples of unstable haemoglobins

Haemoglobin	Mutation
Hb Koln	$\beta_{98}$ val → met
Hb Zurich	$\beta_{63}$ his → arg
Hb Tacoma	$\beta_{30}$ arg → ser
Hb Bibba	$\beta_{136}$ leu → pro
Hb Hammersmith	$\beta_{42}$ phe → ser
Hb Bristol	$\beta_{67}$ val → asp
Hb Poole	$\beta_{130}$ trp → gly

### Clinical features

- Well compensated haemolysis.
- Hb may be ↔ if unstable Hb has high O<sub>2</sub> affinity.
- Haemolysis exacerbated by infection and oxidant drugs.
- Jaundice and splenomegaly are common.
- Some Hbs are unstable *in vitro* but show little haemolysis *in vivo*.

### Investigation

- Hb ↔ or ↓.
- MCV often ↓.
- Film shows hypochromic RBCs, polychromatic RBCs, basophilic stippling.
- Heinz bodies seen post-splenectomy.
- Reticulocytes are ↑.
- Demonstrate unstable Hb using, e.g. heat or isopropanol stability tests.
- Electrophoresis may be normal.
- Brilliant cresyl blue will stain Heinz bodies—falsely +ve in neonate due to high HbF levels.
- Estimation of P<sub>50</sub> may be helpful.
- DNA analysis of value in some cases.

**Management**

Most cases run benign course. Treatment seldom required. Gallstones common. Recommend regular folic acid supplementation. Splenectomy of value in some patients, e.g. severe haemolysis and splenomegaly. Avoidance of precipitants of haemolysis advised.

## Thalassaemias

Arise as a result of diminished or absent production of one or more globin chains. Net result is imbalanced globin chain production. Globin chains in excess form tetramers and precipitate within RBCs → chronic apoptosis in BM and chronic haemolysis in the peripheral blood. Occur at high frequency in parts of Africa, the Mediterranean, Middle East, India, and Asia. Found in high frequency in areas where malaria is endemic and thalassaemia trait offers some protection.

Named after affected gene, e.g. in  $\alpha$  thalassaemia the  $\alpha$  globin gene is altered in such a way that either  $\alpha$  globin synthesis is reduced ( $\alpha^+$ ) or abolished ( $\alpha^0$ ) from RBCs. Severity varies depending on type of mutation or deletion of the  $\alpha$  or  $\beta$  globin gene.





## $\alpha$ thalassaemia

Two  $\alpha$  globin genes on each chromosome 16, with total of 4  $\alpha$  globin genes per cell (normal person is designated  $\alpha\alpha/\alpha\alpha$ ) making  $\alpha$  thalassaemia more heterogeneous than  $\beta$  thalassaemia. Like sickle cell anaemia, patients can either have mild  $\alpha$  thalassaemia ( $\alpha$  thalassaemia trait,  $- -/\alpha\alpha$  or  $-\alpha/-\alpha$  or  $-\alpha/\alpha\alpha$ ) where 1 or 2  $\alpha$  globin genes are affected or they may have severe  $\alpha$  thalassaemia if 3 or 4 of the genes are affected.  $\alpha$  thalassaemia is generally the result of large deletions within  $\alpha$  globin complex. High prevalence of  $\alpha$  thalassaemia in Africa, Afro-Caribbeans, South and SE Asia ( $-\alpha/-\alpha$  or  $-\alpha/\alpha\alpha$ ).  $\alpha^0$  thalassaemia (i.e.  $- -/\alpha\alpha$ ) found most commonly in E Mediterranean and SE Asian ethnic groups.  $\alpha$  thalassaemia occurs from loss of linked  $\alpha$  globin genes on 1 chromosome (i.e.  $- -/\alpha\alpha$ ). Deletions in  $\alpha$  gene HS40 region (upstream regulatory region) account for most  $\alpha^0$  thalassaemia mutations.  $\alpha^+$  results from deletion of 1 of the linked  $\alpha$  genes ( $-\alpha/\alpha\alpha$ ) or inactivation due to point mutation ( $\alpha^T\alpha/\alpha\alpha$ ).

### $\alpha$ thalassaemia trait ( $-\alpha/\alpha\alpha$ , or $\alpha\alpha/- -$ or $-\alpha/-\alpha$ )

1 gene deleted. Asymptomatic.  $\downarrow$  MCV and MCH in minority.

### $\alpha$ thalassaemia trait ( $\alpha\alpha/- -$ or $-\alpha/-\alpha$ )

2 common  $\alpha^+$  deletions are  $-\alpha^{3.7}$  and  $-\alpha^{4.2}$ . The common  $\alpha^0$  deletions are ( $- -/MED$ ), ( $-\alpha/(20.5)$ ), and ( $- -/SEA$ ), ( $- -/THAI$ ), ( $- -/FIL$ ). Requires PCR for diagnosis. Will not be picked up on HPLC or electrophoresis. Asymptomatic carrier—recognized once other causes of microcytic anaemia are excluded (e.g. Fe deficiency). Hb may be  $\leftrightarrow$  or minimally  $\downarrow$ . MCV and MCH are  $\downarrow$ . Absence of splenomegaly or other clinical findings. Requires no therapy.

### Haemoglobin H disease ( $- -/-\alpha$ )

Three  $\alpha$  genes deleted; only 1 functioning copy of the  $\alpha$  globin gene/cell. Clinical features variable. May be moderate anaemia with Hb 8.0–9.0g/dL. MCV and MCH are  $\downarrow$ . Hepatosplenomegaly, chronic leg ulceration, and jaundice (reflecting underlying haemolysis). Infection, drug treatment, and pregnancy may worsen anaemia.

Blood film shows hypochromia, target cells, NRBC and  $\uparrow$  reticulocytes. Brilliant cresyl blue stain will show HbH inclusions (tetramers of  $\beta$  globin,  $\beta_4$ , that have polymerized due to lack of  $\alpha$  chains). Hb pattern consists of 2–40% HbH ( $\beta_4$ ) with some HbA, A<sub>2</sub> and F.

### Treatment

Not usually required but prompt treatment of infection advisable. Give regular folic acid especially when pregnant. Splenectomy of value in some patients with HbH disease. Needs monitoring and may require blood transfusion.

**Haemoglobin Bart's hydrops fetalis (- - /- -)**

Common cause of stillbirth in South East Asia. All 4  $\alpha$  globin genes affected.  $\gamma$  chains form tetramers (HbBart's,  $\gamma_4$ ) which bind oxygen very tightly, with resultant poor tissue oxygenation. Fetus is either stillborn (at 34–40 weeks' gestation) or dies soon after birth. They are pale, distended, jaundiced, and have marked hepatosplenomegaly and ascites. Haemoglobin is  $\sim 6.0$ g/dL and the film shows hypochromic red cells, target cells,  $\uparrow$  reticulocytes and nucleated red cells. Haemoglobin analysis shows mainly HbBart's ( $\gamma_4$ ) with a small amount of HbH ( $\beta_4$ ); HbA, A<sub>2</sub>, and F are absent. *In utero* transfusions can aid survival. Long-term transfusions will be required.

## $\beta$ thalassaemia

There are only 2 copies of  $\beta$  globin gene per cell. Abnormality in 1  $\beta$  globin gene results in  *$\beta$  thalassaemia trait*; if both  $\beta$  globin genes are affected the patient has  *$\beta$  thalassaemia major* or  *$\beta$  thalassaemia intermedia*. Prevalent in the Mediterranean region, Middle East, India, Pakistan, and SE Asia; less common in Africa (apart from Liberia and some regions of N. Africa). Unlike  $\alpha$  thalassaemia, most  $\beta$  thalassaemias are due to single point mutations (>200 identified to date; rarely due to deletions). Results in reduced  $\beta$  globin synthesis ( $\beta^+$ ) or absent  $\beta$  globin production ( $\beta^0$ ). In  $\beta$  thalassaemia major, patients have severe anaemia requiring lifelong support with blood transfusion (with resultant Fe overload). There is ineffective erythropoiesis. The  $\beta$  thalassaemia phenotype is heterogeneous since several factors influence the disease. Recently identified  $\alpha$  globin stabilizing protein binds free  $\alpha$  chains, blocking production of reactive oxygen species thereby modulating the clinical picture of  $\beta$  thalassaemia in mice. Not yet confirmed in humans. Not obvious at birth due to presence of HbF ( $\alpha_2\gamma_2$ ) but as  $\gamma$  chain production diminishes and  $\beta$  globin production increases effects of the mutation become obvious. Children fail to thrive, and development is affected. Hepatosplenomegaly (due to production and destruction of red cells by these organs) is typical. Children also develop facial abnormalities as the flat bones of the skull and other bones attempt to produce red cells to overcome the genetic defect. Skull radiographs show 'hair on end' appearances reflecting the intense marrow activity in the skull bones.

### Investigation and management

#### *$\beta$ thalassaemia trait*

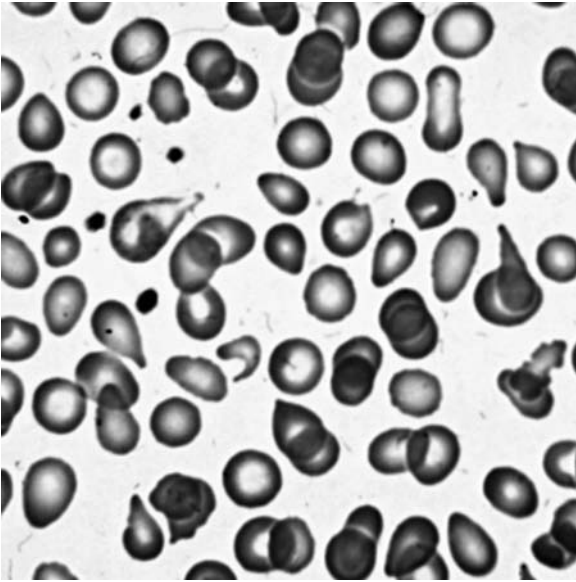
- Carrier state.
- Hb may be  $\downarrow$  but is not usually  $<10.0\text{g/dL}$ .
- MCV  $\downarrow$  to  $\sim 63\text{--}77\text{fL}$ .
- Blood film: microcytic, hypochromic RBCs; target cells often present. Basophilic stippling especially in Mediterraneans (see Fig 2.8).
- RCC  $\uparrow$ .
- HbA<sub>2</sub> ( $\alpha_2\delta_2$ )  $\uparrow$ —provides useful diagnostic test for  $\beta$  thalassaemia trait.
- Occasionally confused with Fe deficiency anaemia, however, in thalassaemia trait the serum Fe and ferritin are  $\leftrightarrow$  (or  $\uparrow$ ) whereas in IDA they are  $\downarrow$ .
- BM shows  $6 \times$  the number of erythroid precursors as  $\leftrightarrow$  though this is not required to make the diagnosis of thalassaemia major.

#### Treatment

Not usually required. Usually detected antenatally or on routine FBC preop.

#### *$\beta$ thalassaemia intermedia (non-transfusion dependent phenotype)*

- Regular blood transfusion not required; more severe than  $\beta$  thalassaemia trait but milder than  $\beta$  thalassaemia major.
- May arise through several mechanisms, e.g.
  - Inheritance of mild  $\beta$  thalassaemia mutations (e.g. homozygous  $\beta^+$  thalassaemia alleles, compound heterozygote for 2 mild  $\beta^+$  thalassaemia alleles, compound heterozygotes for mild plus severe  $\beta^+$  thalassaemia alleles).



**Fig. 2.8** Blood film in thalassaemia trait.

- Elevation of HbF.
- Coinheritance of  $\alpha$  thalassaemia.
- Coinheritance of  $\beta$  thalassaemia trait with, e.g. HbLepore.
- Severe  $\beta$  thalassaemia trait.

#### Clinical

- Variable presentation with only moderate degree of anaemia.
- Hepatosplenomegaly.
- Fe overload is a feature.
- Some patients are severely anaemic (Hb ~6g/dL) although not requiring regular blood transfusion, have impaired growth and development, skeletal deformities and chronic leg ulceration.
- Others have higher Hb (e.g. 10–12g/dL) with few symptoms.

#### Management

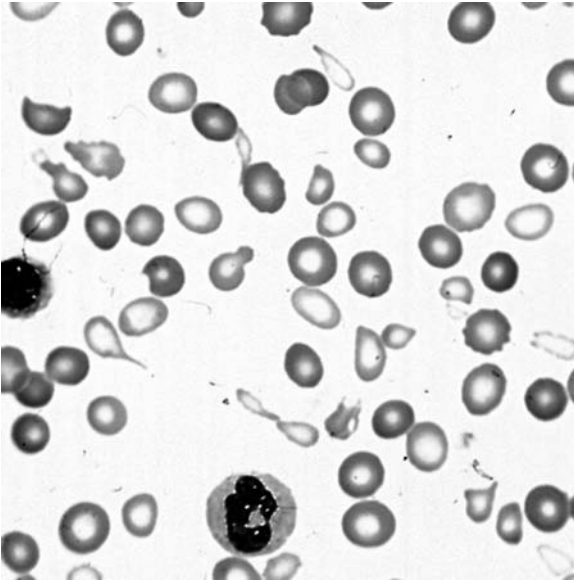
Depends on severity. May require intermittent blood transfusion (especially during growth spurts and pregnancy), Fe chelation, folic acid supplementation, prompt treatment of infection, as for  $\beta$  thalassaemia major. Hydroxycarbamide sometimes used to increase HbF. Occasionally splenectomy can be helpful to maintain higher Hb.

*$\beta$  thalassaemia major (Cooley's anaemia)*

Patients have abnormalities of both  $\beta$  globin genes. Presents in childhood with anaemia and failure to thrive. Untreated children have extramedullary haemopoiesis with hepatosplenomegaly and skeletal deformities.

*Clinical*

- Moderate/severe anaemia (Hb ~3.0–9.0g/dL).
- ↓ MCV and MCHC.
- ↑ Reticulocytes.
- Blood film: marked anisopoikilocytosis, target cells, and nucleated red cells (see Fig 2.9).
- Methyl violet stain shows RBC inclusions containing precipitated  $\alpha$  globin.
- Hb electrophoresis or HPLC shows mainly HbF ( $\alpha_2\gamma_2$ ). In some  $\beta$  thalassaemias there may be a little HbA ( $\alpha_2\beta_2$ ) if some  $\beta$  globin is produced.
- HbA<sub>2</sub> may be ↔ or mildly elevated.



**Fig. 2.9**  $\beta$  thalassaemia major. Note: bizarre red cells with marked anisopoikilocytosis.

*Management*

- Regular lifelong blood transfusion (every 2–4 weeks) to maintain pre-transfusion Hb 9.5–10g/dL and suppress ineffective erythropoiesis allowing normal growth and development in childhood. This will help to prevent hepatosplenomegaly and facial bone changes.

- Fe overload (transfusion haemosiderosis) is major problem—damages liver, heart, endocrine glands including pancreas. Compliance with iron chelation therapy determines prognosis, and can be challenging to manage especially in younger patients.
- Splenectomy is rarely required with timely transfusion therapy but can be of value (e.g. if massive splenomegaly or increasing transfusion requirements) but best avoided until after the age of 5 years due to  $\uparrow$  risk of infection.
- BM transplantation has been carried out using sibling donor HLA-matched transplants with good results in young patients with  $\beta$  thalassaemia major. The procedure carries a significant procedure-related morbidity and mortality, along with GvHD (➔ BMT section pp.424–431).
- $\uparrow$  HbF levels using 5-azacytidine or hydroxycarbamide should ameliorate  $\beta$  thalassaemia.

### Fe chelation therapies

- Choice of iron chelation therapy (mono or combination therapy) should be determined by pattern, extent of Fe loading, patient characteristics, and patient choice.

#### Available chelators

- *Desferrioxamine*: parenteral, painful, compliance problems but good efficacy. Should be given IV continuously in decompensated organ dysfunction.
- *Deferiprone*: oral, given in 3 divided doses. Side effects include: arthralgia, nausea, other GI symptoms, LFT disturbance, agranulocytosis (weekly FBC recommended),  $Zn^{2+}$  deficiency. Particularly good for cardiac iron loading.
- *Deferasirox*: new oral chelator, once daily (suspension), similar efficacy to desferrioxamine. Side effects include GI upset,  $\uparrow$  creatinine, cytopenias.
- Newer agents undergoing clinical trials.

#### Monitoring

- Ferritin 3-monthly. Annual assessment—Cardiac T2\* MRI, liver R2 or T2\* MRI, endocrine tests. Audiology/ophthalmology (chelation side effects).

#### Acute complications

- Cardiac damage—can present with cardiac arrhythmia and cardiac failure, older patients prone to AF even when myocardial T2\* satisfactory. Need urgent ECG, ECHO, CXR, cardiac monitoring and management on coronary care ward.
- Acute severe sepsis— $\uparrow$  risk of overwhelming bacterial sepsis ( $\uparrow$  further if splenectomy). Beware central venous catheter infection. Particularly *Klebsiella*, *Yersinia enterocolitica*, encapsulated organisms. Give broad-spectrum IV antibiotics, consider HDU input.
- Liver failure—cirrhosis, portal hypertension, acute hepatic decompensation. Early liver unit input, careful fluid balance.
- Endocrine dysfunction—hypocalcaemia with tetany due to hypoparathyroidism. Need cardiac monitoring, IV calcium. Diabetic complications.

### Screening

Screen mothers at first antenatal visit. If mother is thalassaemic carrier, screen father. If both carriers for severe thalassaemia offer prenatal diagnostic testing. Fetal blood sampling can be carried out at 18 weeks' gestation and globin chain synthesis analysed. Chorionic villus sampling at 10+ weeks' gestation provides a source of fetal DNA that can be analysed in a variety of methods: Southern blotting, oligonucleotide probes, or RFLP analysis may determine genotype of fetus. Moving towards PCR-based techniques; likely to improve carrier detection. Preimplantation diagnosis is performed using embryonic biopsies.

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## Other thalassaemias

### Heterozygous $\delta\beta$ thalassaemia

Produces a picture similar to  $\beta$  thalassaemia trait with  $\uparrow$  HbF (5–20%) and microcytic RBCs; HbA<sub>2</sub> is  $\leftrightarrow$  or  $\downarrow$ .

### Homozygous $\delta\beta$ thalassaemia

Homozygous condition is uncommon. There is failure of production of both  $\delta$  and  $\beta$  globins. Milder than  $\beta$  thalassaemia major, i.e.  $\beta$  thalassaemia intermedia. Represents a form of thalassaemia intermedia. Hb 8–11g/dL. Absence of HbA and HbA<sub>2</sub>; only HbF is present (100%).

### Heterozygous $\beta$ thalassaemia/ $\delta\beta$ thalassaemia

Similar to  $\beta$  thalassaemia major (but less severe). Hb produced is mainly HbF with small amount of HbA<sub>2</sub>.

### $\gamma\delta\beta$ thalassaemia

Homozygote is not viable. Heterozygous condition is associated with haemolysis in neonatal period and thalassaemia trait in adults with  $\leftrightarrow$  HbF and HbA<sub>2</sub>.

### HbLepore

This abnormal Hb is the result of unequal crossing over of chromosomes. Affects  $\beta$  and  $\delta$  globin genes with generation of a chimeric globin with  $\delta$  sequences at NH<sub>2</sub> terminal and  $\beta$  globin at COOH terminal. Production of  $\delta\beta$  globin is inefficient; there is absence of normal  $\delta$  and  $\beta$  globins. The phenotype of the heterozygote is thalassaemia trait; the homozygote picture is thalassaemia intermedia.

### HbE/ $\beta$ thalassaemia

Compound heterozygote (coinheritance of HbE and  $\beta$  thalassaemia mutation). Varied clinical course (mostly thalassaemia intermedia phenotype) with mild-moderate anaemia, splenomegaly. Some patients require regular transfusion.

### Further reading

BCSH haemoglobinopathy diagnosis guidelines: [www.bcsguidelines.com/pdf/bjh809.pdf](http://www.bcsguidelines.com/pdf/bjh809.pdf)

## Hereditary persistence of fetal haemoglobin

Heterogeneous groups of rare disorders caused by deletions or crossovers involving  $\beta$  and  $\gamma$  chain production, or non-deletional forms due to point mutations upstream of the  $\gamma$  globin gene, with high levels of HbF production in adult life. There is  $\downarrow$   $\delta$  and  $\beta$  chain production with enhanced  $\gamma$  chain production. Globin chain imbalance is much less marked than in  $\beta$  thalassaemia, resulting in milder disorder. There are few clinical effects even when 100% Hb is HbF.

May be pancellular (very high levels of HbF haemoglobin synthesis with uniform distribution in RBCs) or heterocellular ( $\uparrow$  numbers of F cells).

### Ethnic differences

- Black individuals with heterozygous pancellular HPFH have HbF levels of 15–30%.
- Greek individuals with pancellular HPFH have HbF ~10–20% (most HbF is  $\text{A}\gamma$ ).

### Mechanism

Like  $\delta\beta$  thalassaemia, HPFH frequently arises from deletions of DNA, which remove or inactivate the  $\beta$  globin gene (*note*: heterocellular HPFH may be result of mutations outside the  $\beta$  globin gene). There are also many transcription factors and epigenetic regulators that can account for the variation in HbF in adults. Transcription factors identified include BCL 11A, KLF1, MYB and chromatin modifiers (histone deacetylases, DNA methyltransferases, and nuclear receptors TR2/TR4).

### Heterozygous HPFH

Anaemia may be mild or absent. Haematological indices are normal. There is balanced  $\alpha$ /non- $\alpha$  globin chain synthesis. HbF level ~25%.

## Hb patterns in haemoglobin disorders

See Table 2.10.

**Table 2.10** Hb patterns in haemoglobin disorders

% Haemoglobin	A	F	A <sub>2</sub>	S	Other
<b>Normal</b>	<b>97</b>	<b>&lt;1</b>	<b>2–3</b>		
β thalassaemia trait	80–95	1–5	3–7		
β thalassaemia intermedia	30–50	50–70	0–5		
β thalassaemia major	0–20	80–100	0–13		
HPFH (black heterozygote)	60–85	15–35	1–3		
HPFH (black homozygote)		100			
α thalassaemia trait	85–95				Bart's 0–10% at birth H 5–30%
HbH disease	60–95				Bart's 20–30% at birth
HbBart's hydrops					Bart's 80–90%
HbE trait	60–65	1–2	2–3		E 30–35
HbE disease	0	5–10	5		E 95
HbE/β thalassaemia	0	30–40	–		E 60–70
HbE/α thalassaemia	13				E 80
HbD trait	50–65	1–5	1–3		D 45–50
HbD disease	1–5	1–3			D 90–95
HbD/β thalassaemia	0–7	1–7			D 80–90
HbC trait	60–70				C 30–40
HbC disease		slight ↑			C 95
Sickle trait	55–70	1	3	30–45	
Sickle cell anaemia	0	7	3	90	
Sickle/β <sup>+</sup> thalassaemia	5–30	5–15	–	60–85	
Sickle/β <sup>o</sup> thalassaemia	0	5–30	4–8	70–90	
Sickle/D	0	1–5		50	D 50%
Sickle/C	0	1	2–3	50–65	C 50%
HbLepore trait	80–90	1–3	2.0–2.5		Lepore 9–11%
HbLepore disease	0	70–90	0		Lepore 8–30%
HbLepore/β thalassaemia		70–90	2.5		Lepore 5–15%

## Non-immune haemolysis

### 4 major groups

- Infections.
- Vascular (mechanical damage).
- Chemical damage.
- Physical damage.

### Infection

- *Malaria*—especially falciparum. Causes anaemia through marrow suppression, hypersplenism, and RBC sequestration. In addition there is haemolysis due to destruction of parasitized RBCs by RES and intravascular haemolysis when sporozoites released from infected RBCs. Blackwater fever refers to severe acute intravascular haemolysis with haemoglobinaemia, ↓Hb, haemoglobinuria, and ARF.
- *Babesiosis*—*Babesia* (RBC protozoan). Rapid onset of vomiting, diarrhoea, rigors, jaundice, ↑T°. Haemoglobinaemia, haemoglobinuria, ARF and death.
- *Clostridium perfringens*—septicaemia and acute intravascular haemolysis.
- *Viral*—especially viral haemorrhagic fevers, e.g. dengue, yellow fever.

### Mechanical

- *Cardiac*—turbulence and shear stress following mechanical valve replacement. General features of haemolysis: ↑ reticulocytes, ↑ LDH, ↑ plasma Hb, with ↓ haptoglobins ± ↓ platelets. Urinary haemosiderin +ve.
- *MAHA*—➔ see Microangiopathic haemolytic anaemia, p.96.
- *HUS/TTP*—➔ see Thrombotic thrombocytopenic purpura, pp.538–540.
- *March haemoglobinuria*—with severe strenuous exercise, e.g. running. Destruction of RBCs in soles of feet. Worse with hard soles and uneven hard ground. Mild anaemia. No specific features on film. May be associated GIT bleeding and ↓ ferritin (lost in sweat).

### Chemical and physical

- *Oxidative haemolysis*—chronic Heinz body intravascular haemolysis with dapsone or sulafasalazine in G6PD deficient people or unstable Hb (and normals if dose high enough). Film: bite cells (RBC). Heinz bodies not prominent if intact spleen. Haemolysis well compensated.
- *MetHb*—➔ see Methaemoglobinaemia, p.95.
- *Lead poisoning*—moderate ↓RBC lifespan. Anaemia mainly due to block in haem synthesis although lead also inhibits 5' nucleotidase (NT). Basophilic stippling on film. Ring sideroblasts in BM.
- *O<sub>2</sub>*—haemolysis in patients treated with hyperbaric O<sub>2</sub>.
- *Insect bites*—e.g. spider, bee-sting (not common with snake bites).
- *Heat*—e.g. burns → severe haemolysis due to direct RBC damage.
- *Liver disease*—reduced RBC lifespan in acute hepatitis, cirrhosis, Zieve's syndrome is an uncommon form of haemolysis—intravascular associated with acute abdominal pain (➔ see Anaemia in liver disease, p.41).

- *Wilson's disease*—autosomally inherited disorder of copper metabolism, with hepatolenticular, hepatocerebral degeneration.
- *PNH*—➔ see Paroxysmal nocturnal haemoglobinuria, p.104.
- *Hereditary acanthocytosis*— $\alpha$ - $\beta$ -lipoproteinaemia. Rare, inherited. Associated with retinitis pigmentosa, steatorrhoea, ataxia, and mental retardation.

## Hereditary spherocytosis

Most common inherited RBC membrane defect characterized by variable degrees of haemolysis, spherocytic RBCs with ↑ osmotic fragility.

### Pathophysiology

Abnormal RBC cytoskeleton: partial deficiency of spectrin, ankyrin, band 3 or protein 4.2 (leads to ↓ binding to band 4.1 protein and ankyrin). Loss of lipid from RBC membrane → spherical (cf. biconcave) RBCs with reduced surface area → get trapped in splenic cords and have reduced lifespan. RBCs use more energy than normal in attempt to maintain cell shape. RBC membrane has ↑ Na<sup>+</sup> permeability (loses intracellular Na<sup>+</sup>) and energy required to restore Na<sup>+</sup> balance. Red cells are less deformable than normal.

### Epidemiology

In Northern Europeans 1:5000 people are affected. In most cases inheritance is autosomal dominant although autosomal recessive inheritance has been reported.

### Clinical features

Presents at any age. Highly variable clinical expression from asymptomatic to severely anaemic, but usually there are few symptoms. *Note:* phenotype is fairly uniform *within* a family. Well-compensated haemolysis; other features of haemolytic anaemia may be present, e.g. splenomegaly, gallstones, mild jaundice. Occasional aplastic crises occur, e.g. with parvovirus B19 infection.

### Diagnosis

- +ve family history of HS in many cases.
- Blood film shows ↑↑ spherocytic RBCs (see Fig 2.10).
- Anaemia, ↑reticulocytes, ↑LDH, unconjugated bilirubin, urinary urobilinogen with ↓ haptoglobins.
- DAT–ve.
- If typical family history, clinical and laboratory findings, additional tests may not be necessary
- If additional tests needed, screening tests recommended are EMA binding and cryohaemolysis test (good predictive value). Gel electrophoresis of red cell membrane for atypical cases. Osmotic fragility is supportive.
- Eosin-5-maleimide binding test (EMA binding)—uses flow cytometry. EMA is bound to some transmembrane proteins and is lost if there is deficiency of certain proteins. Better specificity and sensitivity than osmotic fragility.

**Osmotic fragility test**—RBCs incubated in saline at various concentrations. Results in cell expansion and eventually rupture. Normal RBCs can withstand greater volume increases than spherocytic RBCs. +ve result (i.e. confirms HS) when RBCs lyse in saline at near to isotonic concentration, i.e. 0.6–0.8g/dL (whereas normal RBCs will simply show swelling with little lysis). Osmotic fragility more marked in patients who have not undergone splenectomy, and if the RBCs are incubated at 37°C

for 24h before performing the test (see Fig 2.11). *Note:* a normal result does not exclude HS and may occur in 10–20% cases. Not routine for diagnosis.

**Autohaemolysis test**—since spherocytic RBCs use more glucose than normal RBCs (to maintain normal shape) red cells incubated in buffer or serum for 48h show lysis and release of Hb into solution, which can be measured. In HS RBCs release greater amounts of Hb cf. normal RBCs (3% vs 1% in normal).

**Cryohaemolysis test, osmotic gradient, ektacytometry and EMA** (eosin-5-maleimide) tests have higher predictive value but tests are not specific for HS (e.g. +ve result seen in SE Asian ovalocytosis).

**SDS-PAGE** form of gel electrophoresis that can be used to detect deficiency in RBC membrane proteins. Should be considered when clinical features worse than predicted.

**Genetic analyses**—e.g. DNA-based assays to detect mutations within genes for RBC membrane proteins—not routinely available.

### Complications

- Aplastic crisis (e.g. parvovirus B19 infection, but may be any virus); see temporary ↓↓ reticulocytes, Hb and Hct.
- Megaloblastic changes in folate deficiency.
- ↑ haemolysis during intercurrent illness, e.g. infections.
- Gallstones (in 50% patients; occur even in mild disease).
- Leg ulceration.
- Extramedullary haemopoiesis.
- Fe overload if multiply transfused.

### Exclude

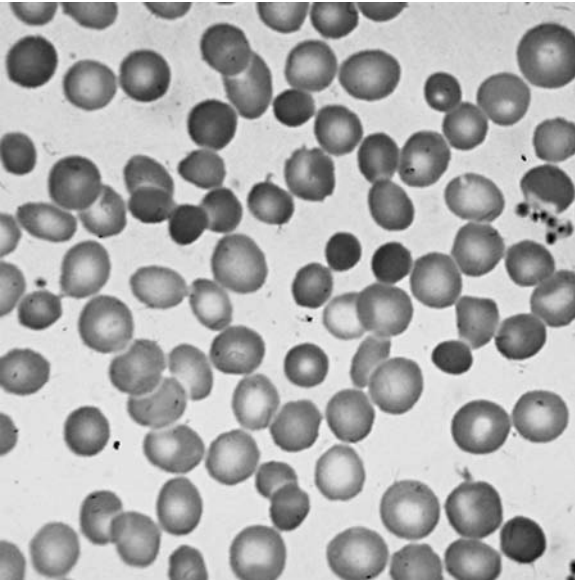
Other causes of haemolytic anaemia, e.g. immune-mediated, unstable Hbs and MAHA, which can give rise to spherocytic RBCs. CDA type II should be excluded particularly if splenectomy considered.

### Treatment

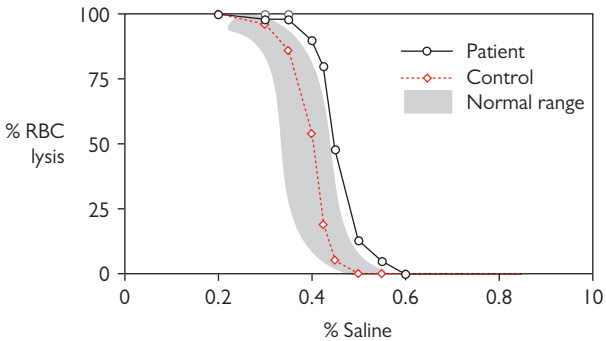
Supportive treatment is usually all that is required, e.g. folic acid (5mg/d) (moderate and severe cases). In parvovirus crisis Hb drops significantly and blood transfusion may be required. Splenectomy is 'curative' but is reserved for patients who are severely anaemic or who have symptomatic moderate anaemia. Best avoided in patients <6 years old due to risk of ↑ fatal infection post-splenectomy. Those who do need splenectomy should be considered for cholecystectomy too.

### Further reading

Bolton-Maggs, P.H., et al. (2011) Guidelines for the diagnosis and management of hereditary spherocytosis. *Br J Haematol*, **126**, 455–74.



**Fig. 2.10** Blood film in hereditary spherocytosis. Note: large numbers of dark spherical red cells (see colour plate section).



**Fig. 2.11** Osmotic fragility assay: note control red cells (red) lyse at lower % saline since they are able to take up more water than spherocytic red cells before lysis occurs.



## Hereditary elliptocytosis

Heterogeneous group of disorders with elliptical RBCs.

### 3 major groups

- Hereditary elliptocytosis.
- Spherocytic HE.
- South East Asian ovalocytosis.

### Pathophysiology

Mutations in  $\alpha$  or  $\beta$  spectrin. There may be partial, complete deficiency, or structural abnormality of protein 4.1, or absence of glycophorin C.

### Epidemiology

In Northern Europeans 1:2500 are affected. Inheritance is autosomal dominant. More common in areas where malaria is endemic.

### Clinical features

Most are asymptomatic. Well-compensated haemolysis. A few patients have chronic symptomatic anaemia. Homozygote more severely affected.

### Diagnosis

- May have +ve family history.
- Blood film shows  $\uparrow\uparrow$  elliptical or oval RBCs.
- Anaemia,  $\uparrow$  reticulocytes,  $\uparrow$  LDH, unconjugated bilirubin, urinary urobilinogen with  $\downarrow$  haptoglobins.
- DAT is -ve.
- Osmotic fragility usually  $\leftrightarrow$  (unless spherocytic HE).
- Transient increase in haemolysis if intercurrent infection.

### Complications

Usual complications of haemolytic anaemia, e.g. gallstones, folate deficiency, etc.

### Treatment

Supportive care: folic acid (5mg/d). Most patients require no treatment. In more severe cases consider splenectomy.

### Spherocytic HE

Elliptical and spherical 'sphero-ovalocytes' in peripheral blood. Haemolysis and  $\uparrow$  osmotic fragility distinguish it from common hereditary elliptocytosis. Molecular basis is unknown.

### Southeast Asian ovalocytosis

Caused by abnormal band 3 protein. RBCs are oval with 1–2 transverse ridges. Cells have  $\uparrow$  rigidity and  $\downarrow$  osmotic fragility. RBCs are more resistant to malaria than normal RBCs.

## Glucose-6-phosphate dehydrogenase (G6PD) deficiency

G6PD is involved in pentose phosphate shunt → generates NADP, NADPH, and glutathione (for maintenance of Hb and RBC membrane integrity, and reverse oxidant damage to RBC membrane and RBC components). G6PD deficiency is X-linked and clinically important cause of oxidant haemolysis. Affects ♂ predominantly; ♀ carriers have 50% normal G6PD activity. Occurs in West Africa, Southern Europe, Middle East, and South East Asia. >300 variants identified.

### Features

- Haemolysis after exposure to oxidants or infection.
- Chronic non-spherocytic haemolytic anaemia.
- Acute episodes of haemolysis with fava beans (termed favism).
- Methaemoglobinaemia.
- Neonatal jaundice.

### 3 main forms of the disease, those associated with:

1. Acute intermittent haemolytic anaemia.
2. Chronic haemolytic anaemia.
3. No risk of haemolytic anaemia.

### Mechanism

Oxidants → denatured Hb → methaemoglobin → Heinz bodies → RBC less deformable → destroyed by spleen.

### 2 main forms of the enzyme

- Normal enzyme is G6PD-B, most prevalent form worldwide.
- 20% of Africans are type A.
- A and B differ by 1 amino acid.
- Mutant enzyme with normal activity = G6PD A(+), find only in black individuals.
- G6PD A(-) is main defect in African origin; ↓ stability of enzyme *in vivo*; 5–15% normal activity.
- 400+ variants but only 2 are relevant clinically:
  - Type A(-) = Africans (10% enzyme activity).
  - Mediterranean (with 1–3% activity).

### Drug-induced haemolysis in G6PD deficiency

- Begins 1–3d after ingestion of drug.
- Anaemia most severe 7–10d after ingestion.
- Associated with low back and abdominal pain.
- Urine becomes dark (black sometimes).
- Red cells develop Heinz body inclusions (cleared later by spleen).
- Haemolysis is typically self-limiting.
- Implicated drugs shown in Table 2.11.
- But heterogeneous; variable sensitivity to drugs.
- Risk and severity are dose related.

### Haemolysis due to infection and fever

- 1–2d after onset of fever.
- Mild anaemia develops.
- Commonly seen in pneumonic illnesses.

### Favism

- Hours/days after ingestion of fava beans (broad beans).
- Beans contain oxidants vicine and convicine → free radicals → oxidize glutathione.
- Urine becomes red or very dark.
- Shock may develop—*may be fatal*.

**Table 2.11** Risk of haemolysis in G6PD-deficient individuals

Definite risk	Possible risk
<i>Antimalarial drugs</i>	Aspirin (1g/d acceptable in most cases)
Primaquine	Chloroquine
Pamaquine (not available in UK)	Probenecid
	Quinine and quinidine (acceptable in acute malaria)
<hr/>	
<i>Analgesic drugs</i>	
Aspirin	
Phenacetin	
<hr/>	
<i>Others</i>	
Dapsone	
Methylthionium chloride (methylene blue)	
Nitrofurantoin	
4-quinolones (e.g. ciprofloxacin, nalidixic acid)	
Sulfonamides (e.g. co-trimoxazole)	

### Neonatal jaundice

- May develop kernicterus (possible permanent brain damage).
- Rare in A(–) variants.
- More common in Mediterranean and Chinese variants.

### Laboratory investigation

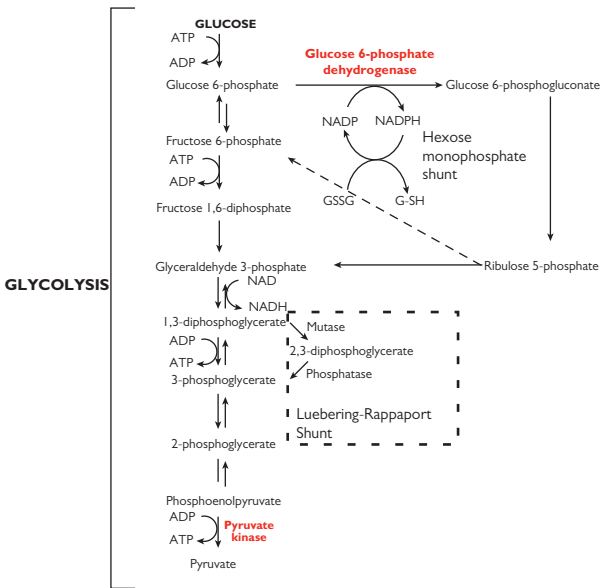
- In steady state (i.e. no haemolysis) the RBCs appear normal.
- Heinz bodies in drug-induced haemolysis (methyl violet stain).
- Spherocytes and RBC fragments on blood film if severe haemolysis.
- ↑ reticulocytes.
- ↑ unconjugated bilirubin, LDH, and urinary urobilinogen.
- ↓ haptoglobins.
- DAT –ve.

## Diagnosis

Demonstrate enzyme deficiency. In suspected RBC enzymopathy, assay G6PD and PK first, then look for unstable Hb. Diagnosis is difficult during haemolytic episode since reticulocytes have  $\uparrow\uparrow$  levels of enzyme and may get erroneously normal result; wait until steady state (~6 weeks after episode of haemolysis). Family studies are helpful (see Fig 2.12).

## Management

- Avoid oxidant drugs—see *BNF*<sup>1</sup>.
- Transfuse in severe haemolysis or symptomatic anaemia.
- IV fluids to maintain good urine output.
- $\pm$  exchange transfusion in infants.
- Splenectomy may be of value in severe recurrent haemolysis.
- Folic acid supplements (?proven value).
- Avoid Fe unless definite Fe deficiency.



**Fig. 2.12** Glycolytic pathway showing key enzymes in red.

## Reference

1. British National Formulary. [www.bnf.org](http://www.bnf.org)

## Further reading

Mason, P.J. et al. (2007). G6PD deficiency: the genotype-phenotype association. *Blood Rev* **21**, 267–83.

## Pyruvate kinase deficiency

Congenital non-spherocytic haemolytic anaemia, caused by deficiency of PK enzyme (involved in glycolytic pathway), leading to unstable enzyme with reduction in ATP generation in RBCs. O<sub>2</sub> curve is shifted to the right due to ↑ 2,3-DPG production.

### Epidemiology

Autosomal recessive. Affected persons are homozygous or double heterozygotes.

### Clinical features

Highly variable, with chronic haemolytic syndrome. May be apparent in neonate (if severe) or may present in later life.

### Diagnosis

- Variable anaemia.
- Reticulocytes ↑↑.
- DAT –ve.
- LDH ↑.
- Serum haptoglobin ↓.
- Definitive diagnosis requires assay of PK level.

### Complications

Aplastic crisis may be seen in viral infection (e.g. parvovirus B19).

### Treatment

Dependent on severity. General supportive measures include daily folic acid (5mg/d). Transfusion may be required. Splenectomy may be of value if high transfusion requirements. In aplastic crisis (e.g. viral infection) support measures should be used.

### Further reading

Prchal, J.T. et al. (2005). Red cell enzymes. *Hematology Am Soc Hematol Educ Program*, 19–23.  
Zanella, A. and Bianchi, P. (2000). Red cell pyruvate kinase deficiency: from genetics to clinical manifestations. *Baillieres Best Pract Res Clin Haematol*, **13**, 57–81.

## Other red cell enzymopathies

### Glycolytic pathway

- Hexokinase deficiency.
- Glucose phosphate isomerase deficiency.
- Phosphofructokinase (PFK) deficiency.
- Aldolase deficiency.
- Triosephosphate isomerase (TPI) deficiency.
- Phosphoglycerate kinase (PGK) deficiency.


#### Epidemiology

Incidence  $<1$  in  $10^6$ . Inheritance is autosomal recessive (most double heterozygote) except for phosphoglycerate kinase deficiency (X-linked recessive).

#### Clinical features

Similar to PK deficiency although most are more severely affected for the degree of anaemia (glycolytic block results in  $\downarrow$  2,3-DPG and left shift of  $O_2$  dissociation curve). PFK deficiency is associated with myopathy. TPI and PGK deficiencies are associated with progressive neurological deterioration.

#### Diagnosis

-  See Pyruvate kinase deficiency, p.91.
- Non-specific morphology with anisocytosis, macrocytosis, and polychromasia.
- Definitive diagnosis requires assay of deficient enzyme ( $\rightarrow$ reference lab).

#### Complications

-  see Pyruvate kinase deficiency, p.91.

#### Treatment

Folic acid (5mg/d). Transfusion may be required (beware Fe overload if high transfusion requirement). Role of splenectomy controversial.

#### Natural history

Similar to PK except TPI and PGK—TPI present in childhood and cause progressive paraparesis, most die  $<5$  years old due to cardiac arrhythmias. PGK can cause exertional rhabdomyolysis and consequential renal failure. Those affected show progressive neurological deterioration.

### Nucleotide metabolism—pyrimidine 5' nucleotidase deficiency

#### Epidemiology

Autosomal recessive. Note: lead poisoning causes acquired pyrimidine 5' nucleotidase deficiency.

*Clinical features*

- Moderate anaemia (Hb ~10g/dL).
- ↑ reticulocytes.
- ↑ bilirubin.
- Splenomegaly.

*Diagnosis*

- RBCs show prominent basophilic stippling.
- Pyrimidine 5' nucleotidase assay.

*Treatment*

Symptomatic, splenectomy is of limited value.

## Drug-induced haemolytic anaemia

Large number of drugs shown to cause haemolysis of RBCs. Mechanisms variable. May be immune or non-immune.

- Some drugs interfere with lipid component of RBC membrane.
- Oxidation and denaturation of Hb: seen with, e.g. sulfonamides, especially in G6PD-deficient subjects, but may occur in normal subjects if drugs given in large doses, e.g:
  - Dapsone.
  - Sulfasalazine.
- *Hapten mechanism* describes the interaction between certain drugs and the RBC membrane components generating antigens that stimulate antibody production. DAT +ve.
  - Penicillins.
  - Cephalosporins.
  - Tetracyclines.
  - Tolbutamide.
- *Autoantibody mediated haemolysis* is associated with warm antibody mediated AIHA. DAT +ve.
  - Cephalosporins.
  - Mefenamic acid.
  - Methyldopa.
  - Procainamide.
  - Ibuprofen.
  - Diclofenac.
  - Interferon alfa.
- *Innocent bystander mechanism* occurs when drugs form immune complexes with antibody (IgM commonest) which then attach to RBC membrane. Complement fixation and RBC destruction occurs.
  - Quinine.
  - Quinidine.
  - Rifampicin.
  - Antihistamines.
  - Chlorpromazine.
  - Melphalan.
  - Tetracycline.
  - Probenecid.
  - Cefotaxime.

### Laboratory features

As for autoimmune haemolytic anaemia, ↓ Hb, ↑ reticulocytes, etc.

### Differential diagnosis

- Warm/cold autoimmune haemolytic anaemia.
- Congenital haemolytic disorders, e.g. HS, G6PD deficiency, etc.

### Treatment

- Discontinue offending drug.
- Choose alternative if necessary.
- If DAT +ve with methyldopa no need to stop unless haemolysis.
- Corticosteroids generally unnecessary and of doubtful value.
- Transfuse in severe or symptomatic cases only.
- Outlook good with complete recovery usual.



## Methaemoglobinaemia

The normal O<sub>2</sub> dissociation curve requires Fe to be in the ferrous form (i.e. reduced, Fe<sup>2+</sup>). Hb containing the ferric (oxidized, Fe<sup>3+</sup>) form is termed methaemoglobin (MetHb). MetHb is unable to bind O<sub>2</sub> leading to poor tissue oxygenation. In health metHb should be ≤3% total Hb. May be congenital or acquired. (See Table 2.12.)

**Table 2.12** Methaemoglobinaemia

<b>Methaemoglobinaemia</b>	
<i>Congenital</i>	
HbM	α or β globin mutation in vicinity of Fe. Fe becomes stabilized in Fe <sup>3+</sup> form. Heterozygote has 25% HbM
MetHb reductase def.	Due to deficiency of NADH-cytochrome b <sub>5</sub> reductase. Autosomal recessive inheritance; symptoms mainly in homozygote
Clinical features	Cyanosis from infancy. PaO <sub>2</sub> is normal. General health is good
<i>Acquired</i>	
	Occurs when RBCs are exposed to oxidizing agents, producing HbM. Implicated agents include: phenacetin, local anaesthetics (e.g. lignocaine), inorganic nitrates (NO <sub>2</sub> ). Patients may experience severe tissue hypoxia. HbM binds O <sub>2</sub> tightly and fails to release to tissues ▶▶ HbM = 50% requires urgent medical attention.

### Diagnosis

May be history of exposure to oxidant drugs or chemicals. Spectrophotometry or haemoglobin electrophoresis will demonstrate HbM. Assays for MetHb reductase are available.

### Treatment

In patients with congenital symptomatic HbM give ascorbate or methylthioninium chloride (methylene blue). In acquired disorder remove oxidant, if present, and administer methylthioninium chloride (methylene blue).

▶ If severely affected consider exchange blood transfusion.

### Further reading

Prchal, J.T., et al. (2005) Red cell enzymes. *Hematology Am Soc Hematol Educ Program*, 19–23.

## Microangiopathic haemolytic anaemia

### Definition

↑ RBC destruction caused when mechanical forces disrupt the physical RBC membrane integrity. Caused by trauma or vascular endothelial abnormalities.

### Causes

- TTP/HUS—see Haemolytic uraemic syndrome, p.540; Thrombotic thrombocytopenic purpura, p.538.
- PET/HELLP (haemolysis, elevated liver enzymes, and low platelets).
- Malignant tumour circulations.
- Renal abnormalities, e.g. acute glomerulonephritis, transplant rejection, malignant hypertension.
- Vasculitides, e.g. Wegener's, PAN, SLE.
- DIC.
- Anticancer and other drugs, e.g. mitomycin C, tacrolimus, cyclosporin, ticlopidine, cocaine
- Cardiac—prosthetic heart valves, grafts or patches, aortic stenosis and regurgitant jets.
- March haemoglobinuria.
- A-V malformations, e.g. Kasabach–Merritt syndrome, A-V shunts.
- Burns.

### Clinical

- Varying degree of anaemia—most severe in DIC, TTP/HUS, and HELLP.
- Often associated with ↓ platelets.
- Blood film shows marked RBC fragmentation, stomatocytes, and spherocytes.
- Reticulocytosis often very marked.
- Signs of underlying disease should be sought.

### Treatment

- Diagnose and treat underlying disease.
- Give folic acid and Fe supplements if deficient.
- TTP/HUS may require plasma exchange using FFP.

### Further reading

Antman, K.H. et al. (1979). Microangiopathic hemolytic anemia and cancer: a review. *Medicine* (Baltimore), **58**, 377–84.

George, J.N. (2000). How I treat patients with thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. *Blood*, **96**, 1223–9.

Moake, J.L. (2002) Thrombotic microangiopathies. *N Engl J Med*, **347**, 589–600.

## Acanthocytosis

Abnormal RBC shape (thorn-like surface protrusions) seen in a number of conditions, inherited or acquired, affecting RBC membrane lipid structure. RBCs develop normally in marrow but once in plasma adopt characteristic shape. RBCs lose membrane and become progressively less elastic.

### Inherited conditions resulting in significant acanthocytosis

- A- $\beta$ -lipoproteinaemia.
- McLeod phenotype (lacking Kell antigen).
- In(Lu) phenotype.
- In association with abnormalities of band 3 protein.
- Hereditary hypo- $\beta$ -lipoproteinaemia.

### Acquired conditions resulting in significant acanthocytosis

- Severe liver disease.
- Myelodysplastic syndromes.
- Neonatal vitamin E deficiency.

### Inherited conditions resulting in mild acanthocytosis

- McLeod phenotype heterozygote.
- PK deficiency.

### Acquired conditions resulting in mild acanthocytosis

- Post-splenectomy and hyposplenic states.
- Starvation including anorexia nervosa.
- Hypothyroidism.
- Panhypopituitarism.

### A- $\beta$ -lipoproteinaemia

Autosomal recessive. Congenital absence of  $\beta$  apolipoprotein.  $\uparrow$  cholesterol:phospholipid ratio. RBC precursors normal. Usually obvious in early life with associated malabsorption of fat (including vitamins A, D, E, and K). Sphingomyelin accumulates.

### Haematological abnormalities

- Mild haemolytic anaemia.
- 50–90% circulating RBCs are acanthocytic.
- Reticulocytes mildly  $\uparrow$ .

### McLeod phenotype

- $\downarrow$  expression of Kell antigen on RBC.
- Mild (compensated) haemolytic anaemia.
- 10–85% acanthocytic RBCs in peripheral blood.

### Further reading

Cooper, R.A. (1980). Hemolytic syndromes and red cell membrane abnormalities in liver disease. *Semin Hematol*, **17**, 103–12.

McBride, J.A., et al. (1970). Abnormal kinetics of red cell membrane cholesterol in acanthocytes: studies in genetic and experimental abetalipoproteinaemia and in spur cell anaemia. *Br J Haematol*, **18**, 383–97.

## Autoimmune haemolytic anaemia

RBCs react with autoantibody  $\pm$  complement  $\rightarrow$  premature destruction of RBCs by reticuloendothelial system.

### Mechanism

RBCs opsonized by IgG, recognized by Fc receptors on RES macrophages  $\rightarrow$  phagocytosis. If phagocytosis incomplete remaining portion of RBC continues to circulate as *spherocyte* (*note*: phagocytosis usually complete if complement involved).

### Seen in:

- Haemolytic blood transfusion reactions.
- Autoimmune haemolytic anaemia (see Table 2.13).
- Drug-induced haemolysis (some).

**Table 2.13** Types of autoimmune haemolytic anaemia.

<b>Warm antibody induced</b>	Idiopathic
	2° to lymphoproliferative disease, e.g. CLL, NHL
	2° to other autoimmune diseases, e.g. SLE
<b>Cold antibody induced</b>	Idiopathic
	Cold haemagglutinin disease (CHAD)
	2° to <i>Mycoplasma</i> infection
	Infectious mononucleosis
	Lymphoma
<b>Paroxysmal cold haemoglobinuria</b>	Idiopathic
	2° to viral infection
	Congenital or tertiary syphilis

### Warm antibody induced haemolysis

Extravascular RBC destruction by RES mediated by warm-reacting antibody. Most cases are idiopathic with no underlying pathology, but may be 2° to lymphoid malignancies, e.g. CLL, or autoimmune disease such as SLE.

#### Epidemiology

Affects predominantly individuals >50 years of age.

#### Clinical features

- Highly variable symptoms, asymptomatic or severely anaemic.
- Chronic compensated haemolysis.
- Mild jaundice common.
- Splenomegaly usual.

### Diagnosis

- Anaemia.
- Spherocytes on peripheral blood film.
- ↑↑ reticulocytes.
- Neutrophilia common.
- RBC coated with IgG, complement, or both (detect using DAT).
- Autoantibody—often pan-reacting but specificity in 10–15% (Rh, mainly anti-e, anti-D, or anti-c).
- ↑ LDH.
- ↓ serum haptoglobin.
- Consider underlying lymphoma (BM, blood, and marrow cell markers).
- Autoimmune profile—to exclude SLE or other connective tissue disorder.

### Treatment

Prednisolone 1mg/kg/d PO tailing off after response noted (usually 1–2 weeks). If no response consider immunosuppression, e.g. azathioprine or cyclophosphamide. Splenectomy should be considered in selected cases. IVlg (0.4g/kg/d for 5d) useful in refractory cases, or where rapid response required. Rituximab (anti-CD20) is emerging as a useful agent for a range of refractory autoimmune disorders, including AIHA. Regular folic acid (5mg/d) is advised.

### Further reading

Gehrs, B.C. and Friedberg, R.C. (2002). Autoimmune hemolytic anemia. *Am J Hematol*, **69**, 258–71.

Zecca, M. et al. (2003). Rituximab for the treatment of refractory autoimmune hemolytic anemia in children. *Blood*, **101**, 3857–61.

## Cold haemagglutinin disease

Describes syndrome associated with acrocyanosis in cold weather due to RBC agglutinates in blood vessels of skin. Caused by RBC antibody that reacts most strongly at temperatures  $<32^{\circ}\text{C}$ . Complement is activated  $\rightarrow$  RBC lysis  $\rightarrow$  haemoglobinaemia and haemoglobinuria. May be idiopathic ( $1^{\circ}$ ) or  $2^{\circ}$  to infection with *Mycoplasma* or EBV (infectious mononucleosis).

### Clinical features

- Elderly.
- Acrocyanosis (blue discoloration of extremities, e.g. fingers, toes) in cold conditions.
- Chronic compensated haemolysis.
- Splenomegaly usual.

### Diagnosis

- Anaemia.
- $\uparrow\uparrow$  reticulocytes.
- Neutrophilia common.
- +ve DAT—C3 only.
- $\pm$  Autoantibodies—IgG or IgM:
  - Monoclonal in NHL.
  - Polyclonal in infection-related CHAD.
- IgM antibodies react best at  $4^{\circ}\text{C}$  (thermal amplitude  $4\text{--}32^{\circ}\text{C}$ ).
- Specificity:
  - Anti-I (*Mycoplasma*).
  - Anti-i (infectious mononucleosis)—causes little haemolysis in adults since RBCs have little anti-i (cf. newborn i  $\gg$  I).
- $\uparrow$  LDH.
- $\downarrow$  serum haptoglobin.
- Exclude underlying lymphoma (BM, blood, and marrow cell markers).
- Autoimmune profile to exclude SLE or other connective tissue disorder.

### Treatment

- Keep warm.
- Corticosteroids generally of little value.
- Chlorambucil or cyclophosphamide (greatest value when there is underlying B-cell lymphoma, occasionally helpful in  $1^{\circ}$  CHAD).
- Plasma exchange may help in some cases.
- If blood transfusion required use in-line blood warmer.
- Splenectomy occasionally useful (*note*: liver is main site of RBC sequestration of C3b-coated RBCs)
- Infectious CHAD generally self-limiting.

### Natural history

Prolonged survival, spontaneous remissions not unusual, with periodic relapses.

## Leucoerythroblastic anaemia

### Definition

A form of anaemia characterized by the presence of immature white and RBCs in the peripheral blood. Mature white cells and platelets are also often reduced.

### Causes

*Marrow infiltration by:*

- 2° malignancy: commonly breast, lung, prostate, thyroid, kidney, colon.
- Myelofibrosis (a 1° myeloproliferative disorder, ↻ see Primary myelofibrosis, p.292).
- Other haematological malignancy, e.g. myeloma and Hodgkin disease.
- Rarely, severe haemolytic or megaloblastic anaemia.

*Marrow stimulation by:*

- Infection, inflammation, hypoxia, trauma (common in ITU patients).
- Massive blood loss.

When due to marrow infiltration, there is often associated neutropenia ± thrombocytopenia. In cases with marrow stimulation there is often neutrophilia and thrombocytosis.

### Investigations

- FBC and blood film. Typical film appearances are of ↑ polychromasia due to reticulocytosis, nucleated RBCs, poikilocytosis (tear drop forms common in infiltrative causes), myelocytes and band forms, occasionally even promyelocytes and blast cells.
- Clotting screen—where cause is 2° malignancy or infective, DIC may occur.

### BM is usually diagnostic

- Hypercellular BM with normal cell maturation, typical of marrow stimulation causes.
- Infiltration with neoplastic cells of a 2° malignancy may be identified as abnormal clumps with characteristic morphology—immunohistochemistry may identify the 1° source, e.g. PSA for prostate.
- Increase in reticulin fibres running in parallel bundles identifies fibrotic infiltrative cause—usually myelofibrosis, but may occur with other haematological malignancy.

### Treatment

- Diagnose and treat underlying cause if possible.
- Supportive transfusions as required, management of BM failure.

### Further reading

Oster, W. et al. (1990). Erythropoietin for the treatment of anemia of malignancy associated with neoplastic bone marrow infiltration. *J Clin Oncol*, **8**, 956–62.

## Aplastic anaemia

### Definition

A gross reduction or absence of haemopoietic precursors in all 3 cell lineages in BM resulting in pancytopenia in peripheral blood. Although this encompasses all situations in which there is myelosuppression, the term is generally used to describe those in which spontaneous marrow recovery is unusual.

### Incidence

Rare ~5 cases per million population annually. Wide age range, slight increase around age 25 years and >65 years. 10 × more common in Asian people.

### Causes

Divided into categories where aplasia is regarded as:

- *Inevitable:*
  - TBI dose of >1.5Gy (note: >8Gy always fatal in absence of graft rescue).
  - Chemotherapy, e.g. high-dose busulfan.
- *Hereditary:*
  - Fanconi syndrome—stem cell repair defect resulting in abnormalities of skin, facies, musculo-skeletal system, and urogenital systems.
  - BM failure often delayed until adulthood.
- *Idiosyncratic:*
  - Chronic benzene exposure.
  - Drug-induced, but not dose related—mainly gold, chloramphenicol, phenylbutazone, NSAIDs, carbamazepine, phenytoin, mesalazine.
  - Genetic predisposition demonstrated for chloramphenicol.
- *Post-viral:*
  - Parvoviral infections—classically red cell aplasia but may be all elements. Devastating in conjunction with chronic haemolytic anaemia, e.g. aplastic sickle crisis.
  - Hepatitis viruses A, B, and C, CMV and EBV.
- *Idiopathic:*
  - Constitute the majority of cases.

### Classification

- According to severity most clinically useful (see Table 2.14).
- Defines highest risk groups.

**Table 2.14** Classification of severity in aplastic anaemia

<b>Severe</b>	2 of the following:	
	Neutrophils	<0.5 × 10 <sup>9</sup> /L
	Platelets	<20 × 10 <sup>9</sup> /L
	Reticulocytes	<1%
<b>Very severe</b>	Neutrophils	<0.2 × 10 <sup>9</sup> /L and infection present



### Clinical features

Reflects the pancytopenia. Bleeding from mucosal sites common, with purpura, ecchymoses. Infections, particularly upper and lower respiratory tracts, skin, mouth, peri-anal. Bacterial and fungal infections common. Anaemic symptoms usually less severe due to chronic onset.

### Diagnosis and investigation

- FBC and blood film show pancytopenia, MCV may be ↑, film morphology unremarkable.
- Reticulocytes usually absent.
- BM aspirate and trephine show gross reduction in all haemopoietic tissue replaced by fat spaces—important to exclude hypocellular MDS or leukaemia—the main differential diagnoses.
- Flow cytometry using anti-CD55 and anti-CD59 will show lack of both membrane proteins. Ham's acid lysis test is now largely obsolete.
- Specialized cytogenetics on blood to exclude Fanconi syndrome (↪ see Fanconi's anaemia, p.594).

### Complications

- Progression to more severe disease.
- Evolution to PNH—occurs in 7%.
- Transformation to acute leukaemia occurs in 5–10%.

### Treatment

- Mild cases need careful observation only. More severe will need supportive treatment with red cell and platelet transfusions and antibiotics as needed. Blood products should be CMV –ve, and preferably leucodepleted to reduce risk of sensitization.
- Specific treatment options are between allogeneic transplant and immunosuppression.
- Sibling allogeneic transplant treatment of choice for those <50 years with sibling donor. Should go straight to transplant avoiding immunosuppression and blood products if possible.
- Matched unrelated donor transplant should be considered in <25 years age group.
- Immunosuppressive options include antilymphocyte globulin (ALG) ± ciclosporin. Response to ALG may take 3 months. Refractory or relapsing patients may respond to a second course of ALG from another animal.
- Ciclosporin post-ALG looks promising.
- Androgens or danazol may be useful in some cases.

### Further reading

- Abkowitz, J.L. (2001). Aplastic anemia: which treatment? *Ann Intern Med*, **135**, 524–6.
- Young, N.S. and Barrett, A.J. (1995). The treatment of severe acquired aplastic anemia. *Blood*, **85**, 3367–77.
- Young, N.S. et al. (1997). The pathophysiology of acquired aplastic anemia. *N Engl J Med*, **336**, 1365–72.

## Paroxysmal nocturnal haemoglobinuria

### Definition

Rare acquired clonal abnormality of the haemopoietic stem cell leading to chronic haemolysis, ↑ risk of VTE and BM suppression rendering them more sensitive to complement-mediated lysis, most noticeable in RBCs. Cells lack phosphatidylinositol glycoproteins (PIG) transmembrane anchors. Caused by mutation in X-linked *PIG-A* gene.

### Incidence

Rare. Aplastic anaemia is closely related.

### Clinical features

- Chronic intravascular haemolytic anaemia particularly overnight (?due to lower blood pH). Infections trigger acceleration of haemolysis.
- WBC and platelet production also often ↓.
- Chronic haemolysis may induce nephropathy.
- Haemoglobinuria usually results in Fe deficiency.
- ↑↑ tendency to venous thrombosis particularly at atypical sites, e.g. hepatic vein (Budd–Chiari syndrome), sagittal sinus thrombosis.
- Fatigue, dysphagia, and impotence occasionally seen.
- BM failure often accompanies PNH.

### Diagnosis and treatment

- FBC, blood film—polychromasia and reticulocytosis (cf. AA).
- BM aspirate and trephine biopsy—usually hypoplastic with ↑ fat space but with erythropoietic nests or islands distinct from AA.
- Ham's test (acidified serum lysis) is invariably +ve though non-specific and seldom used now.
- Cellular immunophenotype shows altered PIG proteins, CD55, and CD59.
- Urinary haemosiderin +ve.

### Complications

- May progress to more severe aplasia.
- Transforms to acute leukaemia in 5%.
- Serious thromboses in up to 20%.

### Treatment

- Chronic disease—supportive care may be satisfactory in mild cases.
- Fe replacement usually required.
- Trial of steroid/androgens/danazol may ↓ symptoms and transfusion need.
- ALG/ciclosporin may be indicated for more severe cases as for aplastic anaemia.
- Acute major thromboses should be treated aggressively with urgent thrombolysis and 10d of heparin. Long-term warfarin mandatory. Consider warfarin prophylaxis after any one clotting episode.
- Severe cases <50 years should be considered for sibling allogeneic transplant if they have a donor—consider MUD in <25 years age group if no sibling donor.

- Eculizumab—humanized monoclonal antibody against complement C5. Inhibits terminal complement activation. Recent TRIUMPH study showed that eculizumab able to stabilize Hb and ↓ transfusion requirements in classical PNH. ↑ risk of neisserial infection and patients require vaccination against *Neisseria meningitidis* 2 weeks before receiving study drug.

### Prognosis

Median survival from diagnosis is 9 years. Major cause of mortality is thrombosis and marrow failure. Molecular genetic basis now established—could be a candidate disease for gene transplantation.

### Further reading

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- Rosse, W.F. (1997). Paroxysmal nocturnal hemoglobinuria as a molecular disease. *Medicine* (Baltimore), **76**, 63–93.

## Pure red cell aplasia

### Definition

A group of disorders characterized by reticulocytes <1% in PB, <0.5% mature erythroblasts in BM but with normal WBC and platelets. Incidence rare. May be caused by abnormal stem cells or 2° to autoimmune disease, viral infection, or chemicals. Congenital and acquired forms exist (see Table 2.15).

**Table 2.15** Classification of red cell aplasia

<b>Congenital</b>	Diamond–Blackfan anaemia (DBA), ↻ see Congenital red cell aplasia, p.590
<b>Acquired</b>	Childhood: transient erythroblastopenia of childhood (TEC)
	Adults: 1° autoimmune or idiopathic
	2° chronic: thymoma, haematological malignancies especially CLL, pernicious anaemia, some solid tumours, SLE, RA, malnutrition with riboflavin deficiency
	2° transient: infections especially parvovirus B19, CMV, HIV, many drugs e.g. chloramphenicol, gold, dapsone, rifampin, sulfasalazine
	Recent interest following red cell aplasia in renal patients treated with SC EPO

### Clinical features

- Lethargy usually only symptom of the anaemia since slow onset.
- No abnormal physical signs except of any underlying disease.

### Diagnosis and investigations

- FBC shows severe normochromic, normocytic anaemia with reticulocytes <1%. WBC and platelets ↔.
- BM shows absence of erythroblasts but is normocellular (distinguishes from aplastic anaemia).

### Treatment

- Treat underlying cause first if identified.
- Remove thymoma.
- If due to parvovirus B19, try IVIg (if congenital haemolytic anaemia transfusion may be required).
- Assume immune origin if no other cause found and give prednisolone 60mg od PO as starter dose ~40% response. Failure of response, try ciclosporin or ALG or azathioprine.

### Prognosis

- 15% spontaneous remission. 65% will respond to immunosuppression.
- 50% will relapse but 80% of relapsers will respond again.
- A few progress to AA or AML.

### Further reading

Raghavachar, A. (1990). Pure red cell aplasia: review of treatment and proposal for a treatment strategy. *Blut*, **61**, 47–51.

## Iron (Fe) overload

Fe is an essential metal but overload occurs when intake of Fe exceeds requirements and occurs due to the absence in humans of a physiological mechanism to excrete excess Fe. Sustained ↑ Fe intake (dietary or parenteral) may result in Fe accumulation, overload, and potentially fatal tissue damage. Fe absorption is regulated at the enterocyte level by HFE and other proteins.

Timing and pattern of tissue damage is determined by rate of accumulation, the quantity of total body Fe and distribution of Fe between reticuloendothelial (RE) storage sites and vulnerable parenchymal tissue. Fe accumulation in parenchymal cells of the liver, heart, pancreas, and other organs is the major determinant of clinical sequelae.

### Haemochromatosis

- Inherited (autosomal recessive) occurring in up to 0.5% population (N Europe).
- 10–13% N European Caucasians are heterozygotes.
- Haemochromatosis locus is tightly linked to the HLA locus on chromosome 6p and up to 10% of the population are heterozygous.
- Single missense mutation found in the homozygous state in 80% of patients.
- The gene designated *HFE* is an MHC class Ib gene.
- 2 mutations in *HFE* cause most cases of HH. C282Y is the most important. Most HH patients have this mutation (individuals homozygous for C282Y account for ~90% cases of HH). Heterozygotes do not develop Fe overload providing there are no additional risk factors (e.g. alcohol, hepatitis). The second is H63D; this is less penetrant. A small proportion develop Fe overload. Compound heterozygotes (C282Y/H63D) may develop mild Fe overload. A third (uncommon) mutation is S65C.
- Homozygotes develop symptomatic Fe overload.

Caused by failure to regulate Fe absorption from bowel causing progressive increase in total body Fe. Parenchymal accumulation occurs initially in liver then pancreas, heart, skin, and other organs rather than RE sites. Symptoms do not usually develop until middle age when body Fe stores of ≥15–20g have accumulated. Environmental factors (e.g. alcohol use in males and menstruation in females) affect rate of accumulation and age at presentation. Clinical expression of haemochromatosis is seen 10 × more commonly in ♂. Only 25% of heterozygotes show evidence of minor increases in Fe stores and clinical problems do not occur.

Clinical manifestations of Fe overload only occur in homozygotes and presentation as 'bronze diabetes' is characteristic (➔ see Table 2.16).

### Evaluation of Fe status

- Indirect measure of Fe stores is serum ferritin estimation (useful surrogate marker of total body Fe stores). Rises to maximum concentration of 4000mcg/L and may underestimate extent of Fe overload in some patients. *Note:* may be spuriously ↑ by infection, inflammation, or neoplasia.

**Table 2.16** Clinical features of Fe overload (homozygous haemochromatosis)

• Skin pigmentation	• Slate grey or bronze discolouration
• Hepatic dysfunction	• Hepatomegaly, chronic hepatitis, fibrosis, cirrhosis, hepatocellular carcinoma (20–30%)
• Diabetes mellitus	• Retinopathy, nephropathy, neuropathy, vascular complications
• Gonadal dysfunction	• Hypogonadism, impotence
• Other endocrine dysfunction	• Hypothyroidism, hypoparathyroidism, adrenal insufficiency
• Abdominal pain	• Unknown aetiology (25%)
• Cardiac dysfunction	• Cardiomyopathy, heart failure, dysrhythmias (10–15%)
• Chondrocalcinosis	• Arthropathy

- The % transferrin saturation provides confirmatory evidence but no measure of the extent of Fe overload. Useful screening test. If >60% in ♂ or 45–50% in ♀ repeat and investigate further.
- MRI useful tool for assessment of Fe overload in heart (T2\*) and liver (T2\*/R2). Useful for monitoring response to treatment (e.g., venesection).
- Liver biopsy provides a direct albeit invasive measure of Fe stores (% Fe concentration by weight) and visual assessment of Fe distribution, and the extent of tissue damage. Only indicated if histological investigation also planned, e.g., staging of cirrhosis.
- MRI T2\* useful tool for assessment of Fe overload in heart, liver and other sites.
- SQUID (superconducting quantum interference device) uses high-power magnetic field and detectors that measure the interference of Fe within the field. Linear correlations have been demonstrated between SQUID measurements and liver biopsy Fe levels but restricted availability.

### Diagnosis

May be difficult to differentiate haemochromatosis from Fe overload 2° to other causes, particularly that associated with chronic liver disease. Recent identification of *HFE* gene will provide a tool for more definitive diagnosis and screening of relatives (previously performed by serum ferritin estimation).

### Management

- Aim to reduce ferritin to <50mcg/L and prevent complications of overload.
- Achieved by regular venesection (500mL blood = 200–250mg Fe) on weekly basis until Fe deficiency develops (may take many months).

- Hb should be measured prior to each venesection and response to therapy can be monitored by intermittent measurement of the serum ferritin.
- R2 liver (some individuals also require T2\* heart) 6–12-monthly.
- Once Fe deficiency develops a maintenance regimen can be commenced with venesection every 3–4 months.
- Avoid alcohol, exogenous Fe.
- Liver cirrhosis and hepatoma risk are not reversible.

### Natural history

Cirrhosis and hepatocellular carcinoma are the most common causes of death in patients with haemochromatosis and are due to hepatic Fe accumulation. Cirrhosis does not usually develop until the hepatic Fe concentration reaches 4000–5000mcg/g of liver (normal 50–500mcg/g). Hepatocellular carcinoma is the cause of death in 20–30% but does not occur in the absence of cirrhosis which increases the risk over 200 ×. If venesection can be commenced prior to the development of cirrhosis and other complications of haemosiderosis the life expectancy is that of a normal individual. Reduction of Fe overload by venesection has only a small effect on symptomatology which has already developed: skin pigmentation diminishes, liver function may improve, cardiac abnormalities may resolve, diabetes and other endocrine abnormalities may improve slightly, arthropathy is unaffected.

### Further reading

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## Transfusion haemosiderosis

Fe overload occurs in patients with transfusion dependent anaemia, notably thalassaemia major, Diamond–Blackfan syndrome, aplastic anaemia, and acquired refractory anaemia. Also increasingly seen in transfused sickle patients. In many of these conditions Fe overload is aggravated by physiological mechanisms which promote ↑ dietary absorption of Fe in response to ineffective erythropoiesis. Each unit of blood contains 200–250mg Fe and average transfusion dependent adult receives 6–10g of Fe/year. Distribution of Fe is similar to haemochromatosis with primarily liver parenchymal cell accumulation followed by pancreas, heart, and other organs. Cardiac deposition occurs in patients who have received 100 units of blood (20g Fe) without chelation, and is followed by damage to the liver, pancreas, and endocrine glands. Pattern of distribution also determined by genetic factors.

Clinical features of Fe overload in children who require transfusion support for hereditary anaemia are listed. Similar problems excluding those related to growth and sexual maturation develop in patients who commence a transfusion programme for acquired refractory anaemia in later life.

### Features of transfusion haemosiderosis in hereditary anaemia

- Growth retardation in 2nd decade.
- Hypogonadism—delayed or absent sexual maturation.
- Skin pigmentation—slate grey or bronze discolouration.
- Hepatic dysfunction—hepatomegaly, chronic hepatitis, fibrosis, cirrhosis, hepatocellular carcinoma.
- Diabetes mellitus.
- Other endocrine dysfunction—rarely hypothyroidism, hypoparathyroidism, adrenal insufficiency.
- Cardiac dysfunction—cardiomyopathy, heart failure, dysrhythmias (main cause of death).
- Death from heart disease in adolescence.

### Management

- Fe chelation therapy using currently available chelation agents (desferrioxamine, deferiprone and deferasirox) is the mainstay of treatment for patients with transfusion haemosiderosis, who remain anaemic. Haemosiderosis due to previous transfusions in conditions where Hb now normal, e.g. treated AML, may be venesected to remove Fe.
- Regular treatment is required in transfusion dependent children if they are to avoid the consequences of Fe overload in the 2nd decade of life.
- SC administration of desferrioxamine by portable syringe pump over 9–12h on 5–7 nights/week is a common regimen. Ascorbic acid supplementation is given at the same time to aid excretion.



- Deferiprone (Ferriprox) is orally active but needs to be taken 3 times a day. Can also be given as combination therapy with desferrioxamine. Contraindicated for use in patients with DBA.
- Deferasirox (Exjade) is the newest licensed agent. Given as a suspension once daily. Avoid in renal impairment.
- Chelation therapy is started after a year of regular transfusions or when ferritin > 1000mcg/L. ↓ hepatic Fe and improves hepatic function, promotes growth and sexual development, and protects against heart disease and early death.
- Newer chelation therapies are under investigation.

### Natural history

The prognosis of the underlying haematological condition in transfusion-dependent elderly patients may eliminate the need for Fe chelation. In others with a longer life expectancy, a period of venesections (if good Hb) or iron chelation therapy should be considered.

### Other causes of haemosiderosis

Dietary Fe overload may also occur as a result of chronic over-ingestion of Fe-containing traditional home-brewed fermented maize beverages peculiar to sub-Saharan Africa, which overwhelms physiological controls on Fe absorption. Fe stores may >50g and Fe is initially deposited in both hepatocytes and Kupffer cells but when cirrhosis develops, accumulates in the pancreas, heart, and other organs. Over-ingestion of medicinal Fe may possibly have a similar though less dramatic effect but is certainly harmful to patients with Fe-loading disorders. The excessive Fe absorption seen in patients with chronic liver disease is associated with accumulation in Kupffer cells rather than hepatic parenchyma. Rare congenital defects associated with Fe overload have been reported.

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# White blood cell abnormalities

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## Neutrophilia

Neutrophils are derived from same precursor as monocytes. Cytoplasm contains granules; the nucleus has 3–4 segments. Functions include chemotaxis—neutrophils migrate to sites of inflammation by chemotactic factors, e.g. complement components (C5a and C3), and cytokines. Cytotoxic activity is via phagocytosis and destruction of particles/invading microorganisms (latter often antibody coated = *opsonized*). Granules contain cationic proteins → lyse Gram –ve bacteria, ‘defensins’, myeloperoxidase—interacts with  $H_2O_2$  and HCl → hypochlorous acid (HOCl); lysozyme (hydrolyses bacterial cell walls); superoxide ( $O_2^-$ ) and hydroxyl ( $OH^-$ ) radicals. Neutrophil lifespan is ~1–2d in tissues.

Normal neutrophil count  $2.0-7.5 \times 10^9/L$  (neonate differs from adult; ↻ Normal ranges, p.809).

Neutrophilia is defined as an absolute neutrophil count  $>7.5 \times 10^9/L$ .

### Mechanisms

- ↑ production.
- Accelerated/early release from marrow→blood.
- Demargination (marginal pool→circulating pool).

### Causes

- Infection (bacterial, viral, fungal, spirochaetal, rickettsial).
- Inflammation (trauma, infarction, vasculitis, rheumatoid disease, burns).
- Chemicals, e.g. drugs, hormones, toxins, haemopoietic growth factors, e.g. G-CSF, GM-CSF, adrenaline, corticosteroids, venoms.
- Physical agents, e.g. cold, heat, burns, labour, surgery, anaesthesia.
- Haematological, e.g. myeloproliferative disease, CML, PPP ( $1^{\circ}$  proliferative polycythaemia), myelofibrosis, chronic neutrophilic leukaemia.
- Other malignancies.
- Cigarette smoking.
- Post-splenectomy.
- Chronic bleeding.
- Idiopathic.

### Investigation

History and examination. Ask about cigarette smoking, symptoms suggesting occult malignancy.

### Other investigations

- ESR.
- CRP.

### Treatment

Usually treatment of underlying disorder is all that is required.

**Leukaemoid reaction**

May resemble leukaemia (hence name); see ↑ WBC (myeloblasts and promyelocytes prominent). Occurs in severe and/or chronic infection, metastatic malignancy.

## Neutropenia

Defined as absolute peripheral blood neutrophil count of  $<2.0 \times 10^9/L$ . Racial variation: black and Middle Eastern people may have neutrophil count of  $<1.5 \times 10^9/L$  normally.

### Congenital neutropenia syndromes

- *Kostmann syndrome*—↻ Paediatric haematology, p.597.
- *Chediak–Higashi*—↻ Paediatric haematology, p.603.
- *Shwachman–Diamond syndrome*—↻ Paediatric haematology, p.597.
- *Cyclical neutropenia*—3–4-week periodicity; often 21d cycle, lasts 3–6d.
- *Miscellaneous*—transcobalamin II deficiency, reticular dysgenesis, dyskeratosis congenita.

### Acquired neutropenia

**Table 3.1** Acquired neutropenia: commonest causes

Infection	Viral, e.g. influenza, HIV, hepatitis, overwhelming bacterial sepsis
Drugs	Anticonvulsants (e.g. phenytoin)
	Antithyroid (e.g. carbimazole)
	Phenothiazines (e.g. chlorpromazine)
	Antiinflammatory agents (e.g. phenylbutazone)
	Antibacterial agents (e.g. co-trimoxazole)
	Others (gold, penicillamine, tolbutamide, mianserin, imipramine, cytotoxics)
Immune mediated	Autoimmune (antineutrophil antibodies)
	SLE
	Felty's syndrome (rheumatoid arthritis + neutropenia + splenomegaly; no correlation between spleen size and degree of neutropenia)
As part of pancytopenia	
Bone marrow failure	Leukaemia, lymphoma, LGLL, haematitic deficiency, anorexia
Splenomegaly	Any cause

### Clinical features

When severe neutropenia: throat/mouth infection, oral ulceration, septicæmia. (See Table 3.1 for commonest causes.)

### Diagnosis

Examine peripheral blood film, check haematinics, autoimmune profile, antineutrophil antibodies, haematinics, bone marrow aspirate and trephine biopsy if indicated (e.g. severe or prolonged neutropenia, or features suggestive of infiltration of marrow failure syndrome).

### Treatment

Consists of prompt antibiotic therapy if infection, IVIg and corticosteroids may be helpful but effects unpredictable. In seriously ill patients consider use of G-CSF (need to exclude underlying leukaemia before starting therapy with growth factors). Consider prophylaxis with low-dose antibiotics (e.g. ciprofloxacin 250mg bd) and antifungal (e.g. fluconazole 100mg od) agents. Drug-induced neutropenia usually recovers on stopping suspected agent (may take 1–2 weeks).

### Further reading

Bux, J. *et al.* (1998). Diagnosis and clinical course of autoimmune neutropenia in infancy: analysis of 240 cases. *Blood*, **91**, 181–6.

Dale, D.C. *et al.* (2002). Cyclic neutropenia. *Semin Hematol*, **39**, 89–94.

## Lymphocytosis and lymphopenia

Lymphocytes are small cells with a high N:C ratio; some (e.g. natural killer cells) have prominent cytoplasmic granules. Two principal types: B and T lymphocyte. B-cells express monoclonal surface (not cytoplasmic) IgM and often IgD. B-cell stimulation through cross linkage of surface Ig molecules or via effector T cells causes their differentiation into plasma cells. Predominant role is humoral immunity via Ig secretion.

T cells are derived from stem cells that undergo maturation in thymus and express the T-cell receptor molecule (CD3) on the cell surface. Responsible for cell-mediated immunity, e.g. delayed hypersensitivity, graft rejection, contact allergy, and cytotoxic reactions against other cells.

### Lymphocytosis (peripheral blood lymphocytes $>4.5 \times 10^9/L$ )

- Leukaemias and lymphomas including: CLL, NHL, Hodgkin disease, acute lymphoblastic leukaemia, hairy cell leukaemia, Waldenström macroglobulinaemia, heavy chain disease, mycosis fungoides, Sézary syndrome, large granular lymphocyte leukaemia, ATLL.
- Infections, e.g. EBV, CMV, *Toxoplasma gondii*, rickettsial infection, *Bordetella pertussis*, mumps, varicella, coxsackievirus, rubella, hepatitis virus, adenovirus.
- 'Stress', e.g. myocardial infarction, sickle crisis.
- Trauma.
- Rheumatoid disease (occasionally).
- Adrenaline.
- Vigorous exercise.
- Post-splenectomy.
- $\beta$  thalassaemia intermedia.

### Lymphopenia (peripheral blood lymphocytes $<1.5 \times 10^9/L$ )

- Malignant disease, e.g. Hodgkin disease, some NHL, non-haematopoietic cancers, angioimmunoblastic lymphadenopathy.
- MDS.
- Collagen vascular disease, e.g. rheumatoid, SLE, GvHD.
- Infections, e.g. HIV.
- Chemotherapy.
- Surgery.
- Burns.
- Liver failure.
- Renal failure (acute and chronic).
- Anorexia nervosa.
- Fe deficiency (uncommon).
- Aplastic anaemia.
- Cushing's disease.
- Sarcoidosis.
- Congenital disorders (rare) such as SCID, reticular dysgenesis, agammaglobulinaemia (Swiss type), thymic aplasia (DiGeorge's syndrome), ataxia telangiectasia.



# Eosinophilia

## Differential diagnosis

### Common

- Drugs (huge list, e.g. gold, sulfonamides, penicillin); erythema multiforme (Stevens–Johnson syndrome).
- Parasitic infections: hookworm, *Ascaris*, tapeworms, filariasis, amoebiasis, schistosomiasis.
- Allergic syndromes—asthma, eczema, urticaria.

### Less common

- Pemphigus.
- Dermatitis herpetiformis (DH).
- Polyarteritis nodosa (PAN).
- Sarcoid.
- Tumours esp. Hodgkin.
- Irradiation.

### Rare

- Hypereosinophilic (Loeffler's) syndrome.
- Eosinophilic leukaemia.
- AML with eosinophilia esp. M4Eo (↻ see Table 4.1, p.127).

## Discriminating clinical features

- Drugs—history of exposure, time course of eosinophilia with resolution on cessation of drug.
- Allergic conditions—history of eczema, urticaria, or typical rashes. Symptoms and signs of asthma.
- Parasites—history of exposure from foreign travel, symptoms and signs of Fe deficiency anaemia (hookworm is commonest cause worldwide). Blood film may show filariasis. Stool microscopy and culture for ova, cysts, and parasites for amoebiasis, *Ascaris*, *Taenia*, schistosomiasis.
- Skin diseases—typical appearances confirmed by biopsy, e.g. dermatitis herpetiformis and pemphigus.
- PAN—renal failure, neuropathy, angiography and ANCA positivity.
- Sarcoid—multisystem features with non-caseating granulomata in biopsy of affected tissue or on BM biopsy; high serum ACE.
- Hodgkin—lymphadenopathy, hepatosplenomegaly—BM or node biopsy.
- Hypereosinophilic syndrome—history of allergy, cough, fever, and pulmonary infiltrates on CXR, may be cardiac involvement. Eosinophils on blood film have normal morphology and granulation. Diagnosis on exclusion of similar causes.
- Eosinophilic leukaemia—eosinophils on blood film have abnormal morphology with hyperlobular and hypergranular forms. BM heavily infiltrated with same abnormal cells. Other signs of myeloproliferative disease may be present.
- AML M4Eo—blasts with myelomonoblastic features on BM and blood film (↻ see Table 4.1, p.127).

## Basophilia and basopenia

Basophils are found in peripheral blood and marrow ( $\equiv$  mast cells in tissues). Short lifespan (1–2d), cannot replicate. Degranulation results in hypersensitivity reactions (IgE Fc receptors trigger), flushing, etc.

### Basophilia (peripheral blood basophils $>0.1 \times 10^9/L$ )

- Myeloproliferative disorders:
  - CGL.
  - Other chronic myeloid leukaemias.
  - PRV.
  - Myelofibrosis.
  - Essential thrombocythaemia.
  - Basophilic leukaemia.
- AML (rare).
- Hypothyroidism.
- IgE-mediated hypersensitivity reactions.
- Inflammatory disorders, e.g. rheumatoid disease, ulcerative colitis.
- Drugs, e.g. oestrogens.
- Infection, e.g. viral.
- Irradiation.
- Hyperlipidaemia.

### Basopenia (peripheral blood basophils $<0.1 \times 10^9/L$ )

- As part of generalized leucocytosis, e.g. infection, inflammation.
- Thyrotoxicosis.
- Haemorrhage.
- Cushing's syndrome.
- Allergic reaction.
- Drugs, e.g. progesterone.

## Monocytosis and monocytopenia

Bone marrow monocytes give rise to blood monocytes and tissue macrophages. Part of RES. Other components of RES: lung alveolar macrophages; pleural and peritoneal macrophages; Kupffer cells in liver; histiocytes; renal mesangial cells; macrophages in lymph node, spleen and marrow.

Contain 2 sets of granules: (1) lysosomal (acid phosphatase, arylsulphatase and peroxidase), and (2) function of second set unknown.

### Monocytosis (peripheral blood monocytes $>0.8 \times 10^9/L$ )

#### Common

- Malaria, trypanosomiasis, typhoid (commonest worldwide causes).
- Post-chemotherapy or stem cell transplant esp. if GM-CSF used.
- Tuberculosis.
- Myelodysplasia (MDS).

#### Less common

- Infective endocarditis.
- Brucellosis.
- Hodgkin lymphoma.
- AML (M4 or M5).

### Discriminating clinical features

- Malaria—identification of parasites on thick and thin blood films.
- Trypanosomiasis—parasites seen on blood film, lymph node biopsy, or blood cultures.
- Typhoid—blood culture, faecal and urine culture, and BM culture.
- Infective endocarditis—cardiac signs and blood cultures.
- Tuberculosis—AFB seen and cultured in sputum, EMU, blood, or BM, tuberculin positivity on intradermal challenge, caseating granulomata on biopsy of affected tissue or BM.
- Brucellosis—blood cultures and serology.
- Hodgkin—lymphadenopathy, hepatosplenomegaly, eosinophilia, biopsy of node or BM.
- MDS—typical dysplastic features on blood film or BM (see ➔ Myelodysplastic syndromes, p.228).
- AML (M4 or M5) —monoblasts on blood film and BM biopsy. Skin and gum infiltration common (see ➔ Acute myeloblastic leukaemia, p.124).

### Monocytopenia (peripheral blood monocytes $<0.2 \times 10^9/L$ )

- Autoimmune disorders, e.g. SLE.
- Hairy cell leukaemia.
- Drugs, e.g. glucocorticoids, chemotherapy.

## Mononucleosis syndromes

### Definition

Constitutional illness associated with atypical lymphocytes in the blood.

### Clinical features

Peak incidence in adolescence: may be subclinical or acute presentation consisting of fever, lethargy, sweats, anorexia, pharyngitis, lymphadenopathy (cervical > axillary > inguinal), tender splenomegaly ± hepatomegaly, palatal petechiae, maculopapular rash especially if given ampicillin. Rarely also pericarditis, myocarditis, encephalitis. Usually self-limiting illness but complications include lethargy persisting for months or years (chronic fatigue syndrome), depression, autoimmune haemolytic anaemia, thrombocytopenia, 2° infection, and splenic rupture.

### Causes

EBV, CMV, *Toxoplasma*, *Brucella*, Coxsackie and adenoviruses, HIV seroconversion illness.

### Pathophysiology

In EBV-related illness, EBV infection of B lymphocytes results in immortalization and generates a T-cell response (the *atypical lymphocytes*) which controls EBV proliferation. In severe immunodeficiency following prolonged use of ciclosporin, oligoclonal EBV-related lymphoma may develop which usually regresses with reduction of immunosuppressive therapy but may evolve to a monoclonal and aggressive lymphoma, e.g. after MUD stem cell transplant. In malarial Africa, EBV infection is associated with an aggressive lymphoma—Burkitt lymphoma (➔ Burkitt lymphoma, p.200).

### Diagnosis: haematological features

- Atypical lymphocytes on blood film (recognized by the dark blue cytoplasmic edge to cells and invagination (scalloping) around RBCs).
- Usually lymphocytosis with mild neutropenia.
- Occasionally anaemia due to cold antibody mediated haemolysis (anti-i)—identify with cold haemagglutinin titre.
- Paul–Bunnell/monospot test for presence of heterophile antibody +ve when cause is EBV but only in the first few weeks. False +ves can occur in lymphoma.
- ↑ bilirubin and abnormal LFTs.
- Serological testing should include EBV capsid Ag, CMV IgM, *Toxoplasma* titre, *Brucella* titre, HIV-1 and -2 Ag and Ab.
- Immunophenotype of peripheral blood B lymphocytes shows polyclonality (distinguishes from lymphoma and other lymphoproliferative disorders).

### Treatment

Rest and symptom relief are mandatory. No other specific treatment has been shown to influence outcome.

# Leukaemia

- Acute myeloblastic leukaemia (AML) 124
- Acute lymphoblastic leukaemia (ALL) 138
- Chronic myeloid leukaemia (CML) 148
- Chronic lymphocytic leukaemia (B-CLL) 158
- Prolymphocytic leukaemia (PLL) 168
- Hairy cell leukaemia and variant 170
- Large granular lymphocyte leukaemia (LGLL) 174
- Adult T-cell leukaemia-lymphoma (ATLL) 178

## Acute myeloblastic leukaemia (AML)

Malignant tumour of haemopoietic precursor cells of non-lymphoid lineage arising in the BM.

### Incidence

Most common acute leukaemia in adults; 3 per 100,000 annually. ↑ frequency with age (median 64 years; incidence 35/100,000 at age 90). Infrequent in children <15 years. 66% are >60 years of age.

### Aetiology

Unclear—association with pre-existing myelodysplasia, prior cytotoxic chemotherapy (particularly alkylating agents and epipodophyllotoxins), ionizing radiation, benzene exposure, constitutional chromosomal abnormalities (e.g. Down (older patients) and Fanconi syndromes) and smoking.

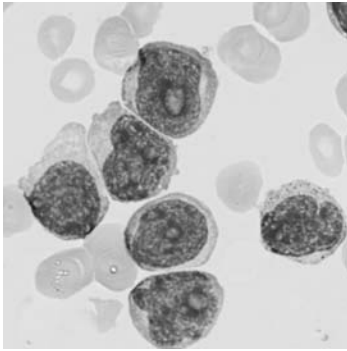
### Diagnosis

- FBC—usually shows leucocytosis, anaemia, and thrombocytopenia. Can show pancytopenia
- Blood film—usually contains blasts.
- BM aspirate—≥20% blasts (see Figs. 4.1–4.5).
- Trephine biopsy—to exclude fibrosis and multilineage dysplasia.
- Immunophenotyping to differentiate AML from ALL: CD3, CD7, CD13, CD14, CD33, CD34, CD64, CD117, cytoplasmic myeloperoxidase (MPO).
- Cytochemistry—MPO or Sudan Black (SB), combined esterase.
- Cytogenetic analysis—to identify prognostic group.
- Molecular analysis—RT-PCR and FISH in selected cases.

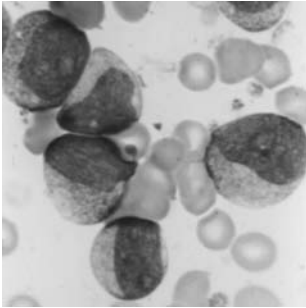
### Cytochemistry

*Formerly the mainstay of leukaemia diagnosis*—SB, MPO, and esterase (chloroacetate and non-specific esterase (NSE)) stains are +ve in AML and -ve in ALL (<3% blasts +ve).

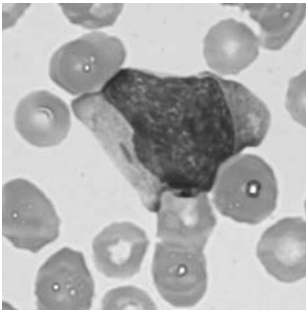
*Note:* M0 and M7 AML are MPO -ve. NSE is +ve in monocytic cells. Cytochemistry is not essential if 4-colour flow cytometry and estimation of cytoplasmic MPO is available.



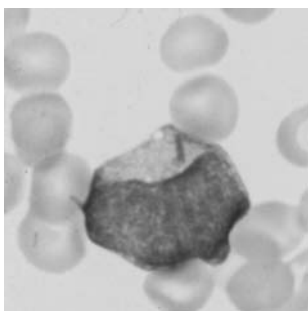
**Fig. 4.1** BM showing myeloblasts in AML (→ see colour plate section).



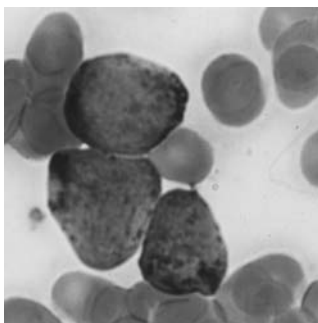
**Fig. 4.2** BM showing myeloblasts in AML (→ see colour plate section).



**Fig. 4.3** AML: myeloblast with large Auer rod (left) (→ see colour plate section).



**Fig. 4.4** AML: myeloblast with large Auer rod (top of cell) (↻ see colour plate section).



**Fig. 4.5** AML: Sudan Black stain (dark granules clearly seen in myeloblasts) (↻ see colour plate section).



### Morphological classification

The WHO classification (Table 4.2) is used and has superseded the French–American–British (FAB) system. The FAB classification, based on predominant differentiation pathway (Table 4.1), remains useful for preliminary classification of a newly diagnosed patient before the cytogenetics result is known. For this reason it remains included here.

**Table 4.1** FAB morphological classification of AML<sup>1,2,3</sup>

M0	AML with minimal differentiation (SB and MPO cytochemistry –ve but myeloid immunophenotyping; may also express CD4 and CD7); 3% of cases
M1	AML without maturation (<10% promyelocytes/myelocytes or monocytes; may have Auer rods) 20% of cases
M2	AML with maturation (≥10% promyelocytes/myelocytes; < 20% monocytes; may have Auer rods; t(8;21)) commonest subtype: 30% of cases
M3	Acute promyelocytic leukaemia (APL; >30% promyelocytes; multiple Auer rods (faggot cells); t(15;17)) 10% of cases
M3v	Microgranular variant of APL (high WBC count; minimal granulation; Auer rods rare; t(15;17))
M4	Acute myelomonocytic leukaemia (mixed myeloid (>20% blasts and promyelocytes) and monocytic (≥20%) maturation; monocytic cells are NSE +ve; may have Auer rods) 20% of cases
M4Eo	M4 variant with 5–30% eosinophils; associated with inv(16) chromosome abnormality. 5% of cases
M5a	Acute monoblastic leukaemia (poorly differentiated subtype with ≥80% monocytoid cells of which ≥80% are monoblasts; Auer rods unusual) 10–15% cases are M5a or M5b
M5b	Acute monocytic leukaemia (differentiated subtype with ≥80% monocytoid cells including NSE-+ve cells with typical monocytic appearance; Auer rods rare)
M6	Acute erythroleukaemia (myeloblasts sometimes with Auer rods plus ≥50% bizarre often multinucleated erythroblasts; erythroblasts often PAS-positive) 3–5% of cases but 10–20% of 2 <sup>o</sup> leukaemias
M7	Acute megakaryoblastic leukaemia (difficult to diagnose morphologically; often dry tap due to fibrosis; requires immunophenotyping with antiplatelet antibodies or electron microscope analysis of platelet peroxidase) rare

### WHO classification of acute myeloid leukaemia

Correlates morphological, genetic, and clinical features to categorize cases of AML<sup>4</sup> into unique clinical and biological subgroups. The WHO system is recommended for the definitive diagnosis and classification of AML.

- The blast threshold for the diagnosis of AML is reduced from 30% to 20% BM blast compared to older classification systems.
- Patients with clonal recurring abnormalities t(8;21)(q22;q22), inv(16)(q13q22), t(16;16)(p13;q22), or t(15;17)(q22;q12) should be considered to have AML regardless of the blast percentage.

**Table 4.2** 2008 WHO classification of acute myeloid leukaemia

#### Acute myeloid leukaemia with recurrent genetic abnormalities

AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*

AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*

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

AML with t(9;11)(p22;q23); *MLL3-MLL*

AML with t(6;9)(p23;q34); *DEK-NUP214*

AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*

AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1*

AML with mutated *NPM1*, provisional entry

AML with mutated *CEBPA*, provisional entry

#### Acute myeloid leukaemia with myelodysplasia-related changes

##### Therapy-related myeloid neoplasms

#### Acute myeloid leukaemia, not otherwise specified

AML with minimal differentiation (FAB M0)

AML without maturation (M1)

AML with maturation (M2)

Acute myelomonocytic leukaemia (M4)

Acute monoblastic/monocytic leukaemia (M5a/5b)

Acute erythroid leukaemia (pure erythroid leukaemia & erythroleukaemia, erythroid/myeloid) (M6)

Acute megakaryoblastic leukaemia (M7)

Acute basophilic leukaemia

Acute panmyelosis with myelofibrosis

#### Myeloid sarcoma

#### Myeloid proliferations related to Down syndrome

Transient abnormal myelopoiesis

Myeloid leukaemia associated with Down syndrome

#### Blastic plasmacytoid dendritic cell neoplasm

## Immunophenotyping

Monoclonal antibodies to cell surface antigens reliably differentiate AML from ALL and confirm the diagnosis of M0, M6, and M7 (see Table 4.3).

**Table 4.3** Immunophenotypic diagnosis of acute leukaemia<sup>5</sup>

Panel of monoclonal antibodies to differentiate AML and ALL	
Myeloid	Anti-MPO; CD13; CD33; CD45; CDw65; CD117
B lymphoid	CD19; cytoplasmic CD22; cytoplasmic CD79a; CD10
T lymphoid	Cytoplasmic CD3; CD2; CD7
Immunophenotypic patterns in AML subtypes	
Undifferentiated (M0)	Anti-MPO; CD13; CD33; CD34; CDw65; CD117; negative cytochemistry; lymphoid markers
Myelomonocytic (M1–M5)	anti MPO; CD13; CD33; CDw65; CD117
Monocytic (M4 & M5)	Stronger expression of CD11b & CD14
Erythroid (M6)	Anti-glycophorin A
Megakaryocytic (M7)	CD41; CD61

## Biphenotypic leukaemias

A minority of acute leukaemias have 2 distinct leukaemic cell populations on phenotyping and are characterized as biphenotypic leukaemias. Most commonly these cell populations express B-lymphoid and myeloid markers and are associated with a high frequency of t(9;22)(q34;q11), the Ph chromosome. These patients have variable response rates. Some may display 'lymphoid' features such as marked lymphadenopathy and high blast counts. Up to 50% of myeloid leukaemias may be positive for lymphoid antigens, most commonly CD2 (34%) and CD7 (42%) and this does not appear to have prognostic significance.

## Cytogenetic analysis

Should be performed in all cases of acute leukaemia. It detects translocations and deletions that provide independent prognostic information in AML.

Revised MRC prognostic classification based on multivariable analyses.

### Favourable risk cytogenetics

- t(15;17)(q22;q21): 15% adults <45 years, rare older; fusion gene *PML-RAR $\alpha$*  ('FAB M3').
- t(8;21)(q22;q22) Irrespective of additional cytogenetic abnormalities. 5–8% adults <55 years, rare older; fusion gene *AML1/ETO*. ('FAB M2').
- inv(16)(p13q22)/t(16;16)(p13;q22). 10% adults <45 years, rare older; fusion gene *CBFB/MYH11*. ('FAB M4Eo').

### Intermediate risk cytogenetics, 'standard risk'

Entities not classified as favourable or adverse.

**Adverse cytogenetics, 'poor risk'**

- abn(3q), excluding t(3;5)(q21~25;q31~35).
- inv(3)(q21q26)/t(3;3)(q21;q26).
- add(5q), del(5q), -5.
- -7, add(7q)/del(7q), excluding favourable karyotype cases.
- t(6;11)(q27;q23).
- t(10;11)(p11~13;q23).
- t(11q23), excluding t(9;11)(p21~22;q23) and t(11;19)(q23;p13).
- t(9;22)(q34;q11).
- -17/abn(17p).
- Complex ( $\geq 4$  unrelated abnormalities).

**Molecular analysis**

FISH and RT-PCR methods add sensitivity and precision to the detection of translocations, deletions, and aneuploidy in cases where conventional cytogenetics fails or gives normal results.

RT-PCR detects minimal residual disease overlooked by conventional methods and monitoring by quantification of PML-RARA transcript numbers in APL can identify patients at higher risk of relapse. This may also be useful in patients with t(8;21) or inv(16).

Presence of an activating mutation of the *FLT3* gene with internal tandem repeats (25–30% of all AML patients) is predictive for poor outcome in all cytogenetic subgroups.

Mutations of the nucleophosmin (NPM) gene which normally produces a protein with diverse functions including regulation of the p53 pathway, are found in many cases with a 'normal' karyotype and are associated with a favourable response to induction therapy. Abnormal localization of NPM in the cytoplasm can be detected by immunohistochemistry (NPMc+).<sup>6</sup>

**Clinical features**

- Acute presentation usual; often critically ill due to effects of BM failure.
- Symptoms of anaemia: weakness, lethargy, breathlessness, lightheadedness, and palpitations.
- Infection: particularly chest, mouth, perianal, skin (*Staphylococcus*, *Pseudomonas*, HSV, *Candida*).
- Fever, malaise, sweats.
- Haemorrhage (especially APL due to DIC): purpura, menorrhagia and epistaxis, bleeding gums, rectal, retina.
- Gum hypertrophy and skin infiltration (monocytic leukaemias (M4, M5)) (see Fig. 4.6).
- Signs of leucostasis, e.g. hypoxia, retinal haemorrhage, confusion, or diffuse pulmonary shadowing.
- Hepatomegaly occurs in 20%, splenomegaly in 24%; the latter should raise the question of transformed CML; lymphadenopathy is infrequent (17%).
- CNS involvement at presentation is rare in adults with AML.



**Fig. 4.6** Gum hypertrophy in AML (➔ see colour plate section).

### Investigations and diagnosis

- FBC and blood film.
- EBV, CMV and HIV serology.
- BM aspirate ± biopsy. BM infiltrated with blasts ( $\geq 20\%$ ).
- BM cytogenetics and molecular analysis.
- Immunophenotyping of blood or marrow aspirate.
- WBC usually  $\uparrow$  with blasts on film—but WBC may be low.
- Hb, neutrophils, and platelets usually  $\downarrow$ .

### Differential diagnosis

ALL, blast crisis of CML, infectious mononucleosis (in younger patients), Megaloblastic anaemia.

### Emergency treatment

- Seek expert help immediately and refer to AML treatment centre if appropriate.
- Intensive cardiovascular and respiratory resuscitation may be needed for septic shock or massive haemorrhage.
- Immediate empirical broad-spectrum antibiotic treatment for neutropenic sepsis.
- Leucapheresis if peripheral blast count high or signs of leucostasis (clinical, diffuse pulmonary shadowing on CXR, or hypoxia).  
*Note: not in APL.*
- Intensive hydration to prevent acute tumour lysis syndrome in patients with a high peripheral blast cell count
- Urgent chemotherapy; can usually be delayed until necessary clinical and laboratory assessments available. The exception is APL.

### Supportive treatment

- Explain diagnosis and offer counselling—the word 'leukaemia' and prospect of prolonged chemotherapy are distressing.
- RBC and platelet transfusion support will continue through treatment:
  - CMV –ve products? Most countries have switched to use of CMV unselected products due to leucodepletion at source.
  - Irradiated products for patients treated with purine analogues.
  - Platelet transfusion to maintain count  $>10 \times 10^9/L$ , unless septic, haemorrhagic or other haemostatic abnormality ( $>20 \times 10^9/L$ ); in bleeding APL  $>50 \times 10^9/L$ .
- Start neutropenic infection prophylaxis regimen. Prompt antibiotic treatment might be required if febrile/septic.
- Start hydration aiming for urine output  $>100\text{mL/h}$  throughout induction therapy.
- Start allopurinol or consider rasburicase (200mcg/kg for up to 7d) to prevent hyperuricaemia/tumour lysis syndrome.
- Insert tunnelled central venous catheter.
- Routine use of growth factors is not recommended.

### Chemotherapy

Where available patients should be entered into clinical trials.

Initial aim of chemotherapy<sup>7</sup> is to eliminate leukaemic cells and achieve complete haematological remission (CR), defined as normal BM cellularity (blast cells  $<5\%$  and normal representation of trilineage haematopoiesis), normalization of peripheral blood count with no blast cells, neutrophils  $\geq 1.5 \times 10^9/L$ , platelets  $\geq 100 \times 10^9/L$  and Hb  $>10\text{g/dL}$ . Leukaemia is undetectable by conventional morphology but may be demonstrated by more sensitive molecular techniques (when available). CR is not synonymous with cure. Standard treatment is with 4 cycles of intensive combination chemotherapy, each cycle followed by a 2–3-week period of profound myelosuppression, during which good supportive therapy is essential.

AML treatment consists of two phases:

1. *Remission induction* to achieve CR: usually 2 courses of anthracycline-containing combination chemotherapy; assess BM response after 3–4 weeks.
  - DA: daunorubicin  $35\text{--}60\text{mg/m}^2$  IV  $\times 3\text{d}$  plus cytarabine  $100\text{mg/m}^2/\text{d}$  as 12h IV infusion or divided dose bd bolus  $\times 8\text{--}10\text{d}$ .
2. *Consolidation therapy*: essential to reduce risk of relapse; optimum number unknown and part of ongoing trials. Standard 2 cycles, i.e. HD cytarabine.
  - Enter patient into national or international multicentre clinical trial if possible. Randomized studies are based on large patient numbers and compare incremental experimental therapy with best treatment arm from previous trials. Allows access to novel therapies.
  - Treatment protocols are generally age related; patients  $>60\text{--}70$  years will only tolerate less intensive treatments and rarely transplantation.

- Risk stratification using prognostic factors may determine consolidation therapy in some clinical trials or indeed for patients off-study.
- Patients who have resistant disease with >5% BM blasts after course 1 or adverse risk cytogenetic or molecular features should be considered for alternative treatment for high-risk AML preferably within study. This would include intensification of treatment with an aim to move to allo-SCT.
- Major complications are infective episodes which may be bacterial (Gram +ve and Gram -ve) or fungal (*Candida* and *Aspergillus*); less commonly viral (esp. HSV, HZV) and haemorrhage.
- No role for maintenance treatment.
- Effectiveness of therapy assessed by CR rate, relapse-free or disease-free survival, and overall survival.

### Stem cell transplantation (SCT)

- Allogeneic SCT<sup>7</sup> from a HLA-compatible donor should be offered to younger/fitter patients with poor-risk AML in 1st CR. Relapse risk falls to 20% from 50% with conventional chemotherapy but transplant-related mortality (TRM, 7–25%) erodes this advantage, although this is significantly lower with reduced intensity conditioning allo-SCT.
- Allo-SCT is an option for those with intermediate (standard) risk AML. Intensive consolidation is the preferred option for patients with favourable cytogenetics for whom SCT should be reserved as salvage treatment in the event of relapse.
- Some older patients with poor risk AML or beyond 1st CR may benefit from allogeneic SCT with reduced intensity conditioning.
- Autologous transplantation in AML has been abandoned.
- Haplo-identical SCT may be undertaken in younger patients with poor-risk AML or beyond 1st CR in the context of a clinical trial.

### Prognosis

- 70–80% of patients aged <60 years will achieve a CR; more intensive induction and consolidation regimens reduce the risk of relapse.
- Relapse risk at 5 years in patients <60 years with favourable risk cytogenetics is 29–42%; intermediate risk 39–60%; poor risk 68–90%.
- 50–60% of patients aged ≥60 years achieve CR with induction treatment (rate drops with each decade) but relapse occurs in 80–90%; a higher proportion have poor risk karyotype, previous myelodysplasia and co-morbidity; treatment-related morbidity and mortality is higher.

### Prognostic factors

The most important prognostic factors predicting for achievement of remission and for subsequent relapse are:

- Advancing patient age: <50 years favourable; >60 years unfavourable.
- Presence of specific cytogenetic abnormalities classifies 'favourable', 'intermediate', and 'poor risk' groups.
- Failure to achieve CR with 1st cycle of induction therapy predicts for refractoriness/relapse.

- Mutation of *FLT3* gene predicts for poor outcome in all cytogenetic subgroups.
- History of antecedent MDS or leukaemogenic therapy: unfavourable.
- Presenting blast count:  $<25 \times 10^9/L$  favourable;  $>100 \times 10^9/L$  unfavourable.

### Management of relapse

- 50% of patients in CR after conventional chemotherapy will relapse.<sup>7</sup>
- Most relapses occur in the first 2–3 years;  $<50\%$  achieve 2nd CR.
- Younger age and longer duration of 1st CR ( $> 6$  months) are good prognostic factors for achieving 2nd CR.
- $\sim 50\%$  of patients achieve 2nd CR with further therapy containing intermediate to high dose cytarabine (e.g. FLAG, FLAG-Ida, Vancouver DA);  $<10\%$  survive  $> 3$  years without a transplant.
- Allogeneic SCT is the treatment of choice for a younger/fitter patient who achieves 2nd CR after relapse. Patients who achieve 2nd CR with chemotherapy should proceed to allograft as soon as possible thereafter. Long-term DFS rates of 40–50% have been achieved. RIC allo-SCTs have  $\uparrow$  the patients eligible for transplant.
- In younger patients with relapsed AML refractory to 2 cycles of conventional chemotherapy, allogeneic SCT can achieve prolonged DFS in 20–30%.
- Donor lymphocyte infusion (DLI) may be used to treat recurrence after an allogeneic transplant.

### AML in the elderly

- Older patients have a significantly poorer prognosis, less frequent favourable cytogenetics, more frequent unfavourable cytogenetics, more frequent 2° leukaemia, more frequent MDR overexpression,  $\uparrow$  resistance to chemotherapy, and  $\uparrow$  treatment-related toxicity.
- Standard chemotherapy regimens may be appropriate in patients  $<70$  years with good performance status, aggressive salvage for relapse is rarely appropriate.
- Non-intensive chemotherapy in combination with good supportive care may control the WBC, minimize hospitalization, and provide optimum quality of life. The aim is palliative and to improve QoL.
- Schedules used:
  - Low-dose SC cytarabine.
  - Oral hydroxycarbamide or etoposide.
  - Azacitidine: licensed for use in AML with 20–30% blasts.
- In some, if not the majority of elderly patients, supportive care alone, including transfusion support, may be appropriate.

### AML in pregnancy

- Chemotherapy should be avoided in the 1st trimester if possible due to the high risk of fetal malformation. The option of termination should be discussed with the mother.
- Chemotherapy in the 2nd and 3rd trimesters is associated with  $\uparrow$  risk of abortion, premature delivery, fetal growth retardation, and neonatal pancytopenia. Early induction of labour between cycles should be discussed.
- Single-agent ATRA is the safest approach for APL in 2nd or 3rd trimesters; Avoid in 1st trimester as teratogenic.



### Extramedullary disease

- Ranges from gum and skin infiltration in monocytic (M4/M5) AML to tumour-like masses (chloroma, granulocytic sarcoma) at any site.
- Chloroma associated with t(8;21). Usually high presenting WBC but may occur without evidence of marrow or blood involvement by AML.
- Chloroma may be misdiagnosed as DLBCL no blasts in blood; immunohistochemistry will differentiate.
- Treat with AML chemotherapy.

### CNS disease

- Occurs in 0.5% at diagnosis; 5% of AML patients in CR relapse in CNS.
- Often followed by BM relapse if not already evident.
- IT prophylaxis shows no benefit in AML.
- Treat with IT cytarabine and systemic re-induction chemotherapy.
- Very poor prognosis.

### Acute promyelocytic leukaemia (APL/APML)

APL (FAB M3) requires a different therapeutic approach that relates to its biology. Risk of DIC prior to and during initial therapy due to release of thromboplastins from leukaemic cells is an indication for urgent treatment. Rapid confirmation of presence of PML-RARA fusion protein (due to t(15;17)) by PML immunofluorescence or FISH analysis predicts favourable response to all-*trans*-retinoic acid (ATRA) or arsenic trioxide (ATO). The variant PLZF-RARA fusion (t(11;17) 1%) confers resistance.

ATRA should be started as soon as APL is suspected. Dose: 45 mg/m<sup>2</sup> daily in 2 divided doses. ATRA induces differentiation of abnormal clone by overcoming molecular block resulting from t(15;17) translocation and reduces the risk of DIC. ATRA alone can induce remission, but this is not sustained. In combination with anthracycline chemotherapy, 70% of patients may be cured. Current 1st-line therapy is ATRA + anthracycline induction followed by consolidation.

**ATRA syndrome** is a complication that may follow ATRA therapy: marked neutrophilia, fever, pulmonary infiltrates, hypoxia, and fluid overload. Treat with dexamethasone 10mg bd IV. Discontinue ATRA in severe cases. Some centres treat preventatively but this is not supported in the literature.

Molecular monitoring is useful as persistence of the abnormal PML-RARA fusion product after therapy detected by RT-PCR predicts relapse.

Arsenic trioxide (ATO) is a useful agent to achieve 2nd CR in patients with PML-RARA-positive APL who relapse, but this should be consolidated by transplantation.

## References

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## Acute lymphoblastic leukaemia (ALL)

Malignant tumour of haemopoietic precursor cells of lymphoid lineage.

### Incidence

Most common malignancy in childhood with the majority of cases in the 2–10 years age group (median 3.5 years). Five times more frequent in childhood than AML. Rare leukaemia in adults, 0.7–1.8/100,000 annually. In adults, peaks at 15–24 years with further peak in older age (2.3/100,000 >80 years).

### Aetiology

Unknown. Predisposing factors are ionizing radiation (AML is more common) and congenital predisposition in Down syndrome (20-fold in childhood), Bloom, Klinefelter, and Fanconi syndromes. Chemicals, pollution, viruses, urban/rural population movements, father's radiation exposure, and radon levels have all been postulated.

### Immunophenotyping

A panel of monoclonal antibodies differentiates ALL from AML (➡ Table 4.3). A further panel of B- and T-lineage markers and lymphocyte maturation markers subclassify ALL.

### Immunological classification of ALL

#### B lineage ~85%

- *Pro B-ALL (early precursor B-ALL)*: HLA-DR+, TdT+, CD19+ (5% children; 11% adults).
- *Common ALL*: HLA-DR+, TdT+, CD19+, CD10+ (65% children; 51% adults).
- *Pre B-ALL (precursor B-ALL)*: HLA-DR+, TdT+, CD19+, CD10±, cytoplasmic IgM+. (15% children; 10% adults).
- *B-cell ALL*: HLA-DR+, TdT-, CD19+, CD10±, surface IgM+ (3% children; 4% adults)

#### T lineage ~15%

- *Pre-T ALL*: TdT+, cytoplasmic CD3+, CD7+ (1% children; 7% adults).
- *T-cell ALL*: TdT+, cytoplasmic CD3+, CD1a/2/3+, CD5+ (11% children; 17% adults).

### Classification

Historically ALL classification was by morphology (FAB classification). The FAB classification has since been superseded by the WHO classification, which includes cytogenetic, immunophenotypic and molecular information (Tables 4.4 and 4.5). Both classifications, however, are included for historical context.

**Table 4.4** WHO classification of acute lymphoblastic leukaemia/lymphoma<sup>a</sup>

## B-lymphoblastic leukaemia/lymphoma, NOS

B-lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities:

- ALL with t(9;22)(q34;q11.2); BCR-ABL (Philadelphia chromosome)
- ALL with t(v;11q23). MLL rearranged
- ALL with t(1;19)(q23;p13.3); TCF3-PBX1 (E2A-PBX1)
- ALL with t(12;21)(p13;q22); ETV6-RUNX1 (TEL-AML1)
- Hyperdiploid >50
- Hypodiploid
- ALL with t(5;14)(q31;q32); IL3-IGH

## T-lymphoblastic leukaemia/lymphoma

<sup>a</sup> 2008 classification.**Table 4.5** Historical morphological classification (French–American–British, FAB)

L1	Small monomorphic type—small homogeneous blasts, single inconspicuous nucleolus, regular nuclear outline; commonest subtype in children; <50% of adults.
L2	Large heterogeneous type—larger blasts, more pleomorphic and multinucleolate, irregular frequently clefted nuclei with conspicuous nucleoli; commonest in adults.
L3	Burkitt cell type—large homogeneous blasts, prominent nucleoli, abundant strongly basophilic cytoplasm with vacuoles; associated with B-cell phenotype.

**Cytogenetic analysis**

- Provides important prognostic information in both children and adults.
- Abnormalities detected in up to 85%. Each has an incidence in the order of 5–10% or less in adults.
- If no structural abnormalities present, abnormalities are classified by the modal chromosome number: <46 (hypodiploid); 46 with other structural abnormalities (pseudodiploid); 47–50 (hyperdiploid); >50 (hyper-hyperdiploid).
- t(9;22)(q34;q11), the Philadelphia (Ph) chromosome, found in 5% of children and 25% of adults with ALL; very strong adverse prognostic factor in both; resultant BCR-ABL hybrid product is same 210 kDa protein detected in CML in 33% but is smaller 180 kDa protein in 66%; can be used for MRD detection. Ph+ ALL rarely cured by chemotherapy alone.
- t(1;19) associated with precursor B-cell ALL.

- t(v;11q23), MLL rearranged. Occurs in 80% of infants with ALL and 6% of adults; fuses the *MLL* gene from 11q23; all these abnormalities associated with refractory disease and early relapse.
- t(8;14) is associated with B-cell ALL ('L3 morphology') and occurs in 5% of cases (dysregulates the *c-myc* proto-oncogene); variant *c-myc* translocations occur in t(2;8) & t(8;22).
- t(12;21)(p13;q22) is most common specific abnormality in childhood ALL (~25%); associated with *TEL-AML1* translocation and good prognosis; much less common in adults (~2%).
- Hyper-hyperdiploidy (>50 chromosomes) confers favourable prognosis (~25% children; ~5% adults); combined +4, +10 confers a favourable outcome in B-cell precursor ALL; patients with hypoploidy (<46 chromosomes) and pseudodiploidy have a poor prognosis.
- t(11;19) with *MLL-ENL* fusion and overexpression of *HOX11* confers good prognosis in T-cell ALL.

### Molecular analysis

FISH and RT-PCR methods add sensitivity and precision to the detection of prognostically significant translocations, deletions, and aneuploidy in cases where cytogenetics fails or gives normal results. Analysis of specific translocations, Ig, or T-cell receptor rearrangements allows detection of MRD permitting risk-stratified consolidation therapy.

### Clinical features

- Acute presentation usual; often critically ill due to BM failure:
  - Anaemia: weakness, lethargy, breathlessness, lightheadedness, and palpitations.
  - Infection: particularly chest, mouth, perianal, skin (*Staphylococcus*, *Pseudomonas*, HSV, *Candida*).
  - Fever, malaise, sweats.
  - Haemorrhage: purpura, menorrhagia, and epistaxis, bleeding gums, rectal, retina.
- Signs of leucostasis e.g. hypoxia, retinal haemorrhage, confusion, or diffuse pulmonary shadowing.
- Bone or joint pain is more common in children.
- Mediastinal involvement in 15%; may cause SVC obstruction especially T-ALL (see Fig 4.7).
- CNS involvement in 6% at presentation; may cause cranial nerve palsies especially facial nerve, sensory disturbances, and meningism.
- Signs include widespread lymphadenopathy in 55%, mild-to-moderate splenomegaly (49%), hepatomegaly (45%), and orchidomegaly.

### Differential diagnosis

- In adults: AML, aplastic anaemia, infectious mononucleosis, lymphoma with blood spill, transformed CML.
- In childhood: AML, aplastic anaemia, infectious mononucleosis, pertussis, neuroblastoma, rhabdomyosarcoma, Ewing sarcoma.

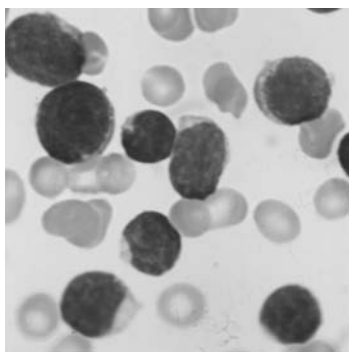
### Investigations and diagnosis

- FBC and blood film.
- BM aspirate ± biopsy.

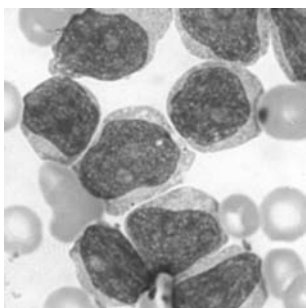
- BM cytogenetics and molecular analysis.
- Immunophenotyping of blood or marrow blasts.
- Total WBC usually high with blast cells on film but may be low ('aleukaemic leukaemia').
- Hb, neutrophils, and platelets often low; clotting may be deranged.
- BM heavily infiltrated with blasts ( $\geq 20\%$ ) (Figs 4.8 and 4.9).
- CXR and CT scan needed if ALL has B-cell or T-cell phenotype for abdominal or mediastinal lymphadenopathy respectively.
- Lumbar puncture mandatory to detect occult CNS involvement but (may be) postponed until treatment reduces high peripheral blast count to prevent seeding. Diagnostic LP combined with 1st intrathecal CNS directed therapy. *Note:* fundoscopy, CT scan of head, and platelet transfusion usually required.



**Fig. 4.7** Mediastinal mass in T-ALL.



**Fig. 4.8** BM: lymphoblasts in ALL L1 (↪ see colour plate section).



**Fig. 4.9** BM: T-cell ALL showing numerous lymphoblasts (➔ see colour plate section).

### Emergency treatment

Seek expert help immediately.

- Cardiovascular and respiratory resuscitation may be needed if septic shock or massive haemorrhage.
- Immediate empirical broad-spectrum antibiotic treatment for neutropenic sepsis.
- Leucapheresis may be needed if peripheral blast count high or signs of leucostasis (retinal haemorrhage, reduced conscious level, diffuse pulmonary shadowing on CXR or hypoxia).
- LP if meningism (note precautions discussed on ➔ Protocols and procedures, p.699).

### Supportive treatment

- Provide explanation and offer counselling—the word ‘leukaemia’ and prospect of prolonged chemotherapy are often distressing.
- RBC and platelet transfusion support will continue through treatment:
  - Irradiated products for patients treated with purine analogues.
  - Platelet transfusion to maintain count  $>10 \times 10^9/L$ , unless septic, on antibiotics, haemorrhagic or other haemostatic abnormality ( $>20 \times 10^9/L$ ).
- Start neutropenic regimen as prophylaxis against infections.
- Start hydration aiming for urine output  $>100\text{mL/h}$  throughout induction therapy.
- Tumour lysis syndrome: a particular problem in B-cell or T-cell ALL. Start IV fluids and allopurinol or rasburicase in high counts.
- Note: interaction with 6-mercaptopurine: discontinue allopurinol or reduce dose of 6-MP or give rasburicase instead.
- Insert tunnelled central venous catheter.



## Chemotherapy

Adult ALL regimens have evolved from successful treatments for childhood ALL. Initial aim of chemotherapy is to eliminate leukaemic cells and achieve complete haematological remission (CR), defined as normal BM cellularity (blast cells <5% and normal representation of trilineage haematopoiesis), normalization of peripheral blood count with no blast cells, neutrophils  $\geq 1.5 \times 10^9/L$ , platelets  $\geq 100 \times 10^9/L$  and Hb >10g/dL. Leukaemia is undetectable by conventional morphology but may be demonstrated by more sensitive molecular techniques and CR is not synonymous with cure.

- Major treatment related complications are infective episodes which may be bacterial (Gram +ve and Gram -ve), viral (esp. HSV, HZV), and fungal (*Candida* and *Aspergillus*).
- In the longer term, relapse is the main complication.

Enrol in high-quality multicentre trial if possible:

*Treatment for ALL consists of 4 contiguous phases:*<sup>1-3</sup>

### 1. Remission induction

- Vincristine, prednisolone (or dexamethasone), dauno/doxorubicin,  $\pm$  other cytostatics, and (peg-)asparaginase to achieve CR.
- More intensive induction: anthracycline improves leukaemia-free survival.

### 2. Consolidation therapy

- To further reduce or eliminate tumour burden and risk of relapse and development of drug-resistant cells; consists of alternating cycles of induction agents and other cytotoxics.
- Usually includes several '**intensification**' phases; combinations of methotrexate at high dose, cytarabine, etoposide, *m*-amsacrine, mitoxantrone (mitozantrone), and idarubicin.

### 3. CNS prophylaxis

- Can include craniospinal irradiation (18–24Gy in 12 fractions over 2 weeks) plus IT chemotherapy (methotrexate  $\pm$  cytarabine or prednisolone) early in the consolidation phase.
- Irradiation avoided in childhood ALL due to side effects and replaced by high-dose systemic methotrexate plus IT therapy.
- IT therapy continued in consolidation and maintenance phases.
- CNS prophylaxis reduces CNS relapse from 30% to 5%.
- Simultaneous administration of IV vincristine and IT methotrexate *must* be avoided as errors can be fatal.

### 4. Maintenance therapy

- Necessary for all patients who do not proceed to SCT.
- Daily 6-MP PO and weekly methotrexate PO with doses to limits of tolerance plus cyclical IV vincristine, oral prednisolone, and IT methotrexate for 2 years in girls and 3 years in boys.

### Allogeneic stem cell transplantation

- Ph+ ALL: allogeneic SCT transplant recommended in CR1 for all eligible patients.

- Consider allogeneic SCT in all adults with a compatible sibling or VUD (volunteer unrelated donor) in CR1, especially in MRD+ve patients and patients with poor risk cytogenetics.
- Leukaemia-free survival superior after 1st remission allograft in patients with high-risk disease (40% vs <10% for Ph/BCR-ABL+ ALL); RIC-allografts have significantly reduced treatment-related mortality.
- In low/good risk young/paediatric patients SCT can be reserved for second CR.

### Ph+/BCR-ABL+ ALL

- TKI (*tyrosine kinase inhibitor*): *imatinib mesylate* is included in regimen of patients with Ph+ ALL.<sup>4</sup> Improved CR, LFS, and OS. Also considered post-allogeneic transplant.
- Eligible patients should receive SCT early in 1st CR.

### Mature B-cell ALL/Burkitt cell leukaemia

- Old 'FAB L3' classification; Burkitt cell type: distinct treatment approach. No longer ALL in WHO classification: Burkitt lymphoma/Burkitt cell leukaemia.<sup>5</sup> Treatment as for Burkitt lymphoma.
- Improved prognosis; 75% CR; >65% LFS at 1 year: 40% DFS.

### T-cell ALL

- Higher incidence of CNS disease at diagnosis and relapse.<sup>6</sup>
- High cure rates when treated with cyclophosphamide-containing regimens yielding DFS of 50–70%.

### CNS leukaemia

- ↑ risk with high-risk genetics, T-cell ALL, and high blast count.
- Presents with headache, vomiting, lethargy, nuchal rigidity, or cranial or peripheral nerve dysfunction.
- Clinical examination may reveal papilloedema, meningism, neurological deficit, and LP shows blasts in CSF.
- Treat with intensified IT triple therapy plus craniospinal irradiation; IT methotrexate, cytarabine, and hydrocortisone 2–3 × times weekly over 3–4 weeks until 2 consecutive CSF samples are –ve; insertion of an Ommaya reservoir facilitates such frequent IT therapy.
- If relapse, will require systemic re-induction as BM relapse often present or follows.
- Poor prognosis.

### Minimal residual disease detection

Flow cytometry for clonal immunophenotypes or FISH or RT-PCR for fusion proteins or clonal Ig/TCR gene rearrangements identified at diagnosis can detect minimal residual disease (MRD) at a sensitivity of  $10^{-3}$ – $10^{-6}$ . Morphological and molecular CR can be distinguished.

MRD –ve status post-initial induction phase (d28–35) has very strong prognostic indications. MRD –ve patients carry a much better prognosis. MRD can be repeated at additional time-points depending on the regimen used. MRD +ve patients have a very high risk of relapse and are candidates for allogeneic SCT.

### Prognosis

- Overall ~75% of adults with ALL achieve a CR with a modern regimen and good supportive care; more intensive induction and consolidation reduces relapse risk but adds toxicity; results in patients >50 years are less good. Relapse rates remain high.
- Median remission duration of CR is around 15 months. 35–40% of adults with ALL treated with intensive chemotherapy survive 2 years.
- In contrast to high cure rate in childhood ALL, leukaemia-free survival (LFS) in adult ALL in general is <30% at 5 years (patients >50 years 10–20%). LFS after chemotherapy in patients without adverse risk factors is >50% but <10% for very high-risk Ph/BCR-ABL+ ALL; hence latter should have an allograft in 1st CR if possible.

### Prognostic factors

Prognosis in adult ALL is much poorer than childhood ALL (cure rate ~80%) due to higher frequency of poor prognostic features and treatment-related toxicity. The most important prognostic factors are listed. These are useful for risk stratification to identify patients who require more intensive therapy and SCT in 1st CR.

- Patient age (<50 years CR >80%, LFS >30%; ≥50 years CR <60%, LFS <20%).
- High leucocyte count ( $>30 \times 10^9/L$  in B precursor-ALL;  $>100 \times 10^9/L$  in T-ALL) poor risk.
- Immunophenotype: pro-B-ALL and pro-T-ALL have poorer outcomes; common pre-B-ALL still poor; mature B-cell ALL and T-cell ALL have better outcomes due to the use of more intensive regimens.
- Cytogenetics: Ph/BCR-ABL+ very poor prognosis: <10% LFS after chemotherapy;
- Long time to CR (>4 weeks): poor risk.
- High MRD level after induction ( $>10^{-3}$ ); persistent/increasing MRD during consolidation: poor risk.

### Management of relapse

- Relapse rate highest within first 2 years but may occur after 7 years.
- 20% occur outside BM, generally CNS; testis and other sites occur in 5%.
- Isolated extramedullary relapse often followed by haematological relapse; these patients require local treatment followed by systemic re-induction therapy.
- Best predictive factor for response is duration of 1st CR (better >18 months).
- 50–60% of patients achieve a short 2nd CR (generally <6 months).
- Prompt SCT offers only prospect of LFS and cure.
- Patients with BCR-ABL+ALL on maintenance imatinib at relapse have prospect (70%) of up to 6 months' haematological response to dasatinib.
- Clofarabine and nelarabine-containing regimens can be used.

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## Chronic myeloid leukaemia (CML)

Malignant tumour of pluripotent haemopoietic stem cell. The clonal marker is found in all 3 myeloid lineages and in some B and T lymphocytes demonstrating a primitive origin. Characterized by  $\uparrow\uparrow$  granulocytes with left shift and the presence of the Ph chromosome.

### Incidence

Rare disease. Frequency 1.25 per 100,000. Accounts for 15% adult leukaemias. Rare in children. Median age of onset 50 years. Slight  $\sigma^7$  excess.

### Aetiology

Unknown. Irradiation is only known epidemiological factor. BCR-ABL fusion proteins appear to play a role in the pathogenesis of CML and have been shown to transform haemopoietic progenitor cells *in vivo* and *in vitro*.

### Classification

Classified as a myeloproliferative disorder (WHO classification) with which it shares a number of clinical features but it also has unique biological features.

### Natural history

- Biphasic or triphasic disease: chronic phase (CP), accelerated phase (AP), and blast crisis (BC); 50% transform directly from CP to BC.
- >85% patients present in CP: defined by absence of features of AP or BC.
- AP characterized by blood counts and organomegaly becoming increasingly refractory to therapy; some have constitutional symptoms.
- BC resembles acute leukaemia with >20% blasts and promyelocytes in blood or marrow; rapidly fatal (Tables 4.6 and 4.7).

### Clinical symptoms and signs

- 30% asymptomatic at diagnosis; present after routine FBC.
- Fatigue, lethargy, weight loss, sweats.
- Splenomegaly in >75%; may cause (L) hypochondrial pain, (early) satiety, and sensation of abdominal fullness.
- Gout, bruising/bleeding, splenic infarction, and occasionally priapism.
- hepatomegaly (2%), lymphadenopathy unusual.
- Occasional signs of leucostasis at presentation.

### Diagnosis and investigations

- FBC and blood film show  $\uparrow$  WBC (generally  $>25 \times 10^9/L$ , often  $100\text{--}300 \times 10^9/L$ ): predominantly neutrophils and myelocytes; basophilia; sometimes eosinophilia (see Figs. 4.10 and 4.11).
- Anaemia common; platelets typically  $\leftrightarrow$  or  $\uparrow$ .
- Historical: low neutrophil alkaline phosphatase (NAP) score (no longer used).
- LDH and urate levels  $\uparrow$ .
- BM shows marked hypercellularity due to granulocytic hyperplasia (blasts <10% in chronic phase; >10% in AP; >20% blasts in BC); trephine biopsy useful to assess marrow fibrosis.
- Cytogenetic analysis of blood or marrow for t(9;22).

- Molecular analysis by real-time quantitative reverse transcription polymerase chain reaction (RQ-PCR) for *BCR-ABL* transcripts to obtain baseline for subsequent monitoring. FISH analysis of blood or marrow will confirm presence of *BCR-ABL*.

**Table 4.6** WHO criteria for accelerated phase

Blasts 10–19% of WBCs in peripheral blood and/or nucleated BM cells

Peripheral blood basophils  $\geq 20\%$

Persistent thrombocytopenia ( $<100 \times 10^9/L$ ) unrelated to therapy or persistent thrombocytosis ( $>1000 \times 10^9/L$ ) unresponsive to therapy

↑ spleen size and ↑ WBC count unresponsive to therapy

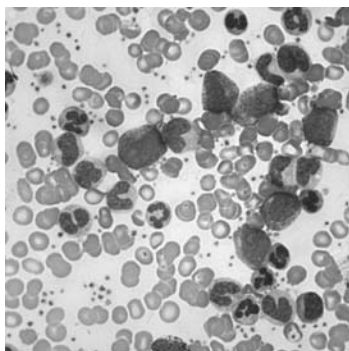
Cytogenetic evidence of clonal evolution

**Table 4.7** WHO criteria for blast crisis

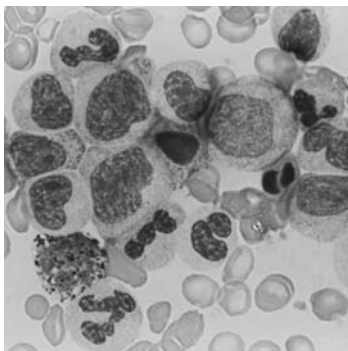
Blasts  $\geq 20\%$  of peripheral blood WBCs in or of nucleated BM cells

Extramedullary blast proliferation

Large foci or clusters of blasts in the BM biopsy



**Fig. 4.10** Peripheral blood film in CML: note large numbers of granulocytic cells at all stages of differentiation (low power) (↻ see colour plate section).



**Fig. 4.11** Peripheral blood film in CML (high power) (↻ see colour plate section).

### Cytogenetic and molecular analysis

- Characterized in >80% patients by the presence of the Ph chromosome. Reciprocal translocation between chromosomes 9 and 22, (t(9;22)(q34;q11)), involving 2 genes, *BCR* and *ABL* that form a fusion gene *BCR-ABL* on chromosome 22. This produces an aberrant 210 kDa protein (p210<sup>BCR-ABL</sup>) that is constitutively active and has greater tyrosine kinase activity than the normal *ABL* protein.
- 10% of patients have variant translocations involving chromosome 22 ± 9 and other chromosomes.
- A further 8% with typical clinical features lack the Ph chromosome, i.e. have Ph<sup>-ve</sup> CML; half of these have the hybrid *BCR-ABL* gene: Ph<sup>-ve</sup>, *BCR-ABL*<sup>+ve</sup> CML.
- Accelerated phase is marked by the acquisition of new cytogenetic abnormalities in 50–80% of patients.
- Myeloid blast crisis associated with additional copies of Ph chromosome, +8 and ↑ (17q).
- Lymphoid blast crisis associated with chromosome 7 anomalies.
- T315I: mutation predicting resistance to imatinib, nilotinib or dasatinib. Treatment with ponatinib indicated.

### Differential diagnosis

Differentiate CP from leukaemoid reaction due to infection, inflammation, or carcinoma and CMML (absolute monocytosis; trilineage myelodysplasia; absent Ph chromosome); 5% present with predominant thrombocytosis and must be differentiated from ET.



### Prognostic scores

- Sokal score based on age, spleen size, platelet count, and % blasts in blood at diagnosis used to identify good, moderate, and poor prognosis groups; based on patients treated with chemotherapy (see Table 4.8)

**Table 4.8** Prognostic scores

Score	= $\text{Exp}[0.0116 (\text{age}-43.4)]$ + 0.0345 (spleen size—7.51) + 0.188 ( $[\text{platelets}/700]^2-0.563$ ) + 0.0887 (blasts%—2.1)
Low risk	<0.8
Intermediate risk	= 0.8–1.2
High risk	>1.2

- Hasford calculation adds effect of basophilia and eosinophilia at diagnosis; based on patients treated with interferon- $\alpha$ . Now outdated.
- EUTOS score: predicts complete cytogenetic remission (CCgR) 18 months after the start of therapy, which is an important predictor for the course of disease. Patients without CCgR at this point of treatment are less likely to achieve one later on and are at a high risk of progressing to blastic and accelerated phase disease.  
*Formula:  $7 \times \text{basophils} (\%) + 4 \times \text{spleen size (cm under ribcage)}$*   
If the sum is >87, the patient is at high risk of not achieving a CCgR at 18 months, while a sum  $\leq 87$  indicates a low risk.
- Early cytogenetic response to imatinib is another important prognostic factor: PCyR at 6 months, 80% probability of CCyR at 2 years; minor or minimal CyR at 6 months, 50% probability; no CyR at 6 months, 15% probability.

### Treatment

- Commence allopurinol.
- Consider leucapheresis in patients with leucostasis or priapism.
- Hydroxycarbamide can be used for rapid reduction in white cell count.
- 1st-line treatment with allogeneic SCT in adults not recommended.
- TKI: continued indefinitely if response.

**Imatinib:** 400mg PO od 1st-line treatment of choice in newly diagnosed patients in CP; continue to intolerance, failure or suboptimal response.

- A small molecule signal transduction inhibitor that specifically targets BCR-ABL and some other tyrosine kinases.
- Achieves 96% complete haematological response (CHR), 87% major cytogenetic response (MCyR), and 76% complete cytogenetic response (CCyR);<sup>1</sup> 50% major molecular response (3 log  $\downarrow$ ).<sup>2</sup>

- 5-year follow-up of IRIS trial shows 69% CCyR at 12 months ↑ to 87% at 60 months; 7% progressed to AP or BC; 89% overall survival at 5 years.<sup>3</sup>
- Most patients achieve MCyR within first 6 months of therapy; patients with MCyR have lower risk of relapse; CCyR predicts for EFS.
- Commonest side effects: myelosuppression, oedema, nausea, rash, cramps, fatigue, diarrhoea, headache, arthralgia, and abnormal LFTs.
- Consider change to dasatinib, nilotinib, or clinical trial if not tolerated.

**Dasatinib:** 1st- or 2nd-line treatment:

- Orally active ABL kinase inhibitor that binds to both active and inactive conformations of ABL kinase domain.
- START-C trial of 70mg bd in 186 imatinib-resistant or intolerant CP patients: 90% CHR, 52% MCyR; 2% progressed or died after MCyR; 92% PFS after 8 months. Good results also in imatinib-resistant or intolerant patients in AP (major HR 64%; MCyR 33%) and BC (major HR ~33%; MCyR 31% in myeloid BC, 50% in lymphoid BC).
- The most frequently reported adverse effects are fluid retention, pleural effusion, diarrhoea, skin rash, headache, haemorrhage, fatigue, nausea, dyspnoea, and myelosuppression.
- 100mg od recommended for CP patients due to reduced toxicity and improved tolerability. 70mg bd in AP and BC.

**Nilotinib:** 1st or 2nd-line treatment:

- Orally active (400mg bd), highly selective inhibitor of BCR-ABL tyrosine kinase; 20–50 × more potent than imatinib in imatinib-resistant cell lines.
- High response rates in imatinib-resistant or intolerant CP (74% CHR; 40% CCyR) and in AP (24% CHR; 16% CCyR).
- Low-toxicity profile.

**Bosutinib:** 3rd-generation TKI. Licensed for use in patients refractory or resistant to imatinib, dasatinib, and/or nilotinib. Mature data is awaited. Mutation screening should be performed before starting second line treatment.

**Ponatinib:** active and licensed in patients with the T315I mutation. Not used in 1st-line treatment. Used once mutation screening is done. Mature data is awaited.

## Definitions of response to treatment

See Tables 4.9–4.11.

**Table 4.9** Haematological response

Complete (CHR):

Platelets  $<450 \times 10^9/L$

WBC  $<10 \times 10^9/L$

Differential: no immature granulocytes and  $<5\%$  basophils

Non-palpable spleen

Monitoring: check 2-weekly until CHR achieved and confirmed  $\times 2$ ; then every 3 months

**Table 4.10** Cytogenetic response (examination of  $\geq 20$  marrow metaphases)

Complete (CCyR)	Ph+ metaphases 0%
Partial (PCyR)	Ph+ 1–35%
Major (MCyR)	CCyR + PCyR
Minor	Ph+ 36–65%
Minimal	Ph+ 66–95%
Monitoring: check at least every 6 months until CCyR achieved and confirmed $\times 2$ ; then at least every 12 months.	

**Table 4.11** Molecular response (assessed on peripheral blood cells) and cytogenetic response (assessed on BM aspirate)

Complete	Transcripts not detectable
Major (MMoIR)	$\leq 0.1\%$ ( $\geq 3$ log reduction against International Scale)
Monitoring: check every 3 months; confirm $\times 2$ ; mutational analysis in case of failure, suboptimal response or transcript level increase	

### Disease monitoring

- Disease monitoring is critical to modern management of CML and selection of appropriate risk-adjusted therapy.
- Goals of therapy in order are CHR, CCyR, MMoIR and 'complete' molecular response.
- BM cytogenetics of value until CCyR; RQ-PCR for *BCR-ABL* transcripts more sensitive for patients in CCyR with imatinib ( $>75\%$ ).

#### 3-month evaluation

- CHR essential.
- *BCR-ABL*  $\leq 10\%$  and/or partial cytogenetic response or better: continue same dose of current TKI.
- Not in CHR and/or no minimal cytogenetic response: switch to alternate TKI; evaluate for SCT depending on response to 2nd-line treatment.

#### 6-month evaluation (including BM cytogenetics)

- *BCR-ABL*  $< 1\%$  and/or complete cytogenetic response: continue same dose of current TKI.
- *BCR-ABL*  $> 10\%$  and/or less than a partial cytogenetic response: switch to alternate TKI; evaluate for SCT depending on response to 2nd-line treatment.

#### 12-month evaluation (including BM cytogenetics)

- Major molecular response or better: continue same dose of current TKI.
- *BCR-ABL*  $> 1\%$  and/or less than complete cytogenetic response: change to alternate TKI, or continue same dose, or increase dose of imatinib as tolerated (max. 600–800mg).

**At any time**

- Major molecular response: continue current TKI.
- Loss of major molecular response, loss of complete cytogenetic response or loss of complete haematological response: switch to alternate TKI and evaluate for SCT depending on response to 2nd-line treatment.
- New development of clonal chromosomal abnormalities as per Table 4.12.

**Patients in CCyR**

- RQ-PCR for BCR-ABL transcript levels every 3 months (consider BM cytogenetics every 12–18 months for clonal cytogenetic abnormalities).
- MMolR after 12 months associated with a better EFS and PFS;<sup>4</sup> rise in level associated with mutations or loss of response.
- Repeat RQ-PCR in 1 month if rising transcript level (1 log ↑). If confirmed, monitor monthly; perform ABL kinase domain mutation analysis.
- Significance of mutations unclear; not consistently associated with relapse; several associated with resistance to imatinib.
- T315I mutation associated with resistance to imatinib, dasatinib, and nilotinib. Responsive to ponatinib.

**Table 4.12** Definitions of failure and optimal and suboptimal response (ELN 2013 update)

Time	Optimal response	Failure	Warnings
Diagnosis	N/A	N/A	High-risk score Major route CCA/Ph <sup>+</sup> <sup>a</sup>
3 months	BCR-ABL ≤ 10% and/or Ph+ ≤35% (PCyR)	No CHR and/or Ph+>95%	BCR-ABL >10% and/or Ph+ 36–95%
6 months	BCR-ABL <1% and/or Ph+ 0% (CCyR)	BCR-ABL >10% and/or Ph+>35%	BCR-ABL 1–10% and/or Ph+ 1–35%
12 months	MMolR (BCR-ABL ≤0.1%)	BCR-ABL >1% and/or Ph+>0%	BCR-ABL 0.1–1%
Then at any time	MMR or better	Loss of CHR Loss of CCyR Loss of MMolR <sup>b</sup> Mutations CCA/Ph+	CCA/Ph- (-7 or 7q-)

<sup>a</sup> Major route CCA/Ph+: major route clonal chromosomal abnormalities/Ph+: trisomy 8, 2nd Ph+ (+der(22)t(9;22)(q34;q11)), isochromosome 17 (i(17)(q10)), trisomy 19, and ider(22)(q10)t(9;22)(q34;q11).

<sup>b</sup> Confirmed in 2 consecutive tests.

### Actions

- *Intolerance to TKI*: consider SCT; interferon- $\alpha$   $\pm$  cytarabine or novel agent.
- *Failure*: change to alternate TKI and evaluate for SCT.
- *Suboptimal response*: re-assess and consider imatinib dose-escalation or treatment change now or in near future.
- *Warnings*: features suggesting possible incipient resistance to TKI and/or progression to advanced phase; needs closer monitoring and consideration of dose escalation, SCT, or other/investigational agents.
- *Failure*: mutation analysis should be done including T315I mutation.

### Other agents

- *Hydroxycarbamide, busulfan*: may be used to control leucocytosis or thrombocytosis and may 'normalize' FBC and reduce spleen size in CP in elderly patients TKI intolerant. No effect on cytogenetics or natural history.
- *Interferon alfa* (IFN- $\alpha$ ) at a dose of 3 million IU SC 3 times weekly corrects haematological abnormalities in 75% and produces 10–15% CCyR and 15–30% MCyR. Role in SCT-ineligible, TKI intolerant patients:
  - Treatment with IFN- $\alpha$  is associated with longer time to progression and longer survival (27–53% at 10 years) than hydroxycarbamide treatment, most significantly in patients with CCyR and MCyR.
  - IFN- $\alpha$  side effects (malaise, febrile reactions, anorexia and weight loss, depression) reduce quality of life and are not tolerable for many patients.

### Allogeneic SCT

- SCT remains a 1st-line treatment choice for paediatric patients.
- Best outcomes in patients aged <30 years in CP <1 year from diagnosis.
- Reduced intensity conditioning lowers treatment-related toxicity in older patients.
- Relapse after SCT has been successfully treated with imatinib, dasatinib, or nilotinib.
- DLIs: 60–80% response in molecular or cytogenetic relapse. GvHD is a side effect of DLI but is less frequent with incremental doses.

### Complications

- Modest  $\uparrow$  infection risk—sometimes atypical organisms.
- Progression to BC (75% myeloid, 25% lymphoid).
- Lymphoid BC: treat with TKI or modified ALL protocol (may survive >12 months). Follow with SCT where possible.
- Myeloid BC usually refractory to conventional chemotherapy, survival 2–5 months; may get brief response with TKI. SCT where possible.

### Treatment of advanced phase CML<sup>5,6</sup>

- Patients presenting in AP or BC not previously treated with a TKI should receive imatinib, nilotinib, or dasatinib.
- Patients who progress on imatinib may respond to other TKI. SCT should be offered where possible.

- SCT offers eligible patients with advanced phase CML the only prospect of prolonged survival and possible cure. Results are significantly less good than for SCT in CP (0–10% 5-year survival in BC) though achievement of 2nd CP pre-SCT improves results after BC.

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## Chronic lymphocytic leukaemia (B-CLL)

Progressive accumulation of mature-appearing, functionally incompetent, B lymphocytes in peripheral blood, BM, lymph nodes, spleen, liver, and sometimes other organs.

### Incidence

Most common leukaemia in Western adults (25–30% of all leukaemias). 2.5/100,000 per annum. 20–30 × more common in Western Caucasian and black populations than in India, China, and Japan. Predominantly disease of elderly (in over 70s, >20/100,000). Median age at diagnosis 65 years. ♂:♀ ratio ~2:1.

### Aetiology

Unknown. No causal relationship with radiation, chemicals, or viruses. Small proportion familial: ↑ lymphoid malignancies in 1st- and 2nd-degree relatives of patients with CLL.

### Clinical features and presentation

- 70–80% asymptomatic; lymphocytosis ( $>5.0 \times 10^9/L$ ) on routine FBC.
- With more advanced disease: lymphadenopathy: painless, often symmetrical, splenomegaly (66%), hepatomegaly and ultimately BM failure due to infiltration causing anaemia, neutropenia, and thrombocytopenia.
- Recurrent infection due to acquired hypogammaglobulinaemia: esp. herpes zoster.
- Patients with advanced disease: weight loss, night sweats, general malaise.
- Autoimmune phenomena occur; DAT +ve in 10–20% cases, warm antibody AIHA in <50% these cases. Autoimmune thrombocytopenia in 1–2%.

### Diagnosis

**FBC:** lymphocytosis  $>5.0 \times 10^9/L$ ; usually  $>20 \times 10^9/L$ , occasionally  $>400 \times 10^9/L$ ; anaemia, thrombocytopenia, and neutropenia absent in early stage CLL; autoimmune haemolysis ± thrombocytopenia may occur at any stage.

**Blood film:** lymphocytosis with ‘mature’ appearance; characteristic artefactual damage to cells in film preparation produces numerous ‘smear cells’ (note: absence of smear cells should prompt review of diagnosis); spherocytes, polychromasia and ↑ retics if AIHA; ↓ platelets if BM failure or ITP. In 15% of patients morphology is ‘atypical’ with >10% polymorphocytes (CLL/PLL) (see Figs. 4.12–4.14).

**Immunophenotyping:** differentiates CLL from other lymphocytoses  
First line panel: CD2; CD5; CD19; CD23; FMC7; Smlg ( $\kappa/\lambda$ ); CD22 or CD79b. CLL characteristically:

- CD2 and FMC7 –ve.
- CD5, CD19, and CD23 +ve.
- Smlg, CD22, CD79b weak;  $\kappa$  or  $\lambda$  light chain restricted.



**Immunoglobulins:** immunoparesis (hypogammaglobulinaemia) common; monoclonal paraprotein (usually IgM) <5%.

**BM:** >30% 'mature' lymphocytes.

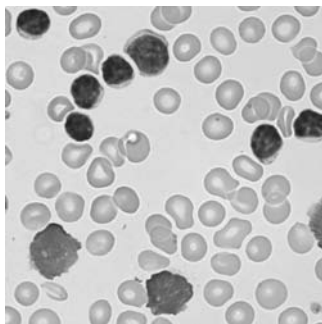
**Trephine biopsy:** provides prognostic information: infiltration may be nodular (favourable); interstitial; mixed; diffuse (unfavourable).

**Lymph node biopsy:** rarely required; appearances of lymphocytic lymphoma; may be useful if diagnosis uncertain or to exclude transformation to lymphoma in patients with bulky lymphadenopathy.

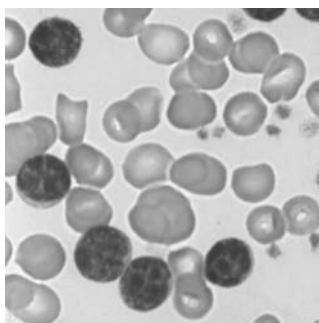
**Cytogenetics:** prognostic value; abnormalities in >80% using FISH. Clonal evolution over time. 11q- and 17q- associated with advanced disease. 11q-, 17q- very unfavourable; isolated 13q- favourable; isolated 6q- intermediate.

- 13q- (55%) if isolated (14–40%), favourable, median OS 133 months.
- 11q- (18%; ATM gene), median OS 79 months; fludarabine refractory.
- 12q+ (16%) if isolated, neutral prognostically.
- 17p- (7%; p53 mutation), median OS 32 months; fludarabine refractory.
- 6q- (7%) associated with plasmacytoid lymphoid cells.
- +12 associated with atypical morphology and disease progression.

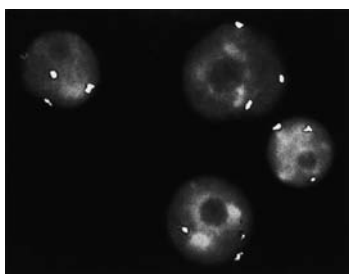
**Other tests:** direct antiglobulin test; reticulocyte count; U&E; urate; LFTs; LDH;  $\beta_2$ -microglobulin; imaging e.g. CXR, CT, or ultrasound as necessary for symptoms; newer prognostic indicators CD38 and/or Zap 70 expression by flow cytometry or immunochemistry.



**Fig. 4.12** Blood film in CLL showing smear cells (bottom of field) (→ see colour plate section).



**Fig. 4.13** Blood film in CLL.



**Fig. 4.14** FISH showing trisomy 12 (3 bright spots in each nucleus, each of which represents chromosome 12). Reproduced with permission from Souhami, R.L. et al (2001). *Oxford Textbook of Oncology*, 2e. Oxford University Press, Oxford.

### Differential diagnosis

Morphology and immunophenotyping will usually differentiate CLL from other chronic lymphoproliferative disorders.

### Scoring system in B-cell lymphoproliferative disorders

Devised to facilitate diagnosis based on the antigen profile of CLL using a panel of 5 monoclonal antibodies, the 'CLL-score'<sup>1</sup> (see Table 4.13).

**Table 4.13** Scoring system for CLL diagnosis

Marker		(Score)		(Score)
Smlg	weak	(1)	moderate/strong	(0)
CD5	positive	(1)	negative	(0)
CD23	positive	(1)	negative	(0)
FMC7	negative	(1)	positive	(0)
CD22/79b	weak	(1)	Strong	(0)

Total scores for CLL range from 3–5 and for non-CLL cases from 0–2.

**Poor prognostic factors<sup>2,3</sup>**

- ♂ sex.
- Advanced clinical stage.
- Initial lymphocytosis  $>50 \times 10^9/L$ .
- $>5\%$  prolymphocytes in blood film.
- Diffuse pattern of infiltrate on trephine.
- Blood lymphocyte doubling time  $<12$  months.
- Cytogenetic abnormalities 11q-, 17p-, or complex.
- p53 mutations (occurs in 10–15%)—correlates with refractory CLL.
- Unmutated IgVH genes ( $\leq 2\%$ )—predicts advanced/progressive disease.
- Cytoplasmic ZAP 70 expression ( $>20\%$ )—correlates with IgVH status.
- CD38 expression ( $>30\%$ )—independent of IgVH status.
- $\uparrow$  serum  $\beta_2$ -microglobulin—correlates with stage and poor response.
- $\uparrow$  serum LDH.
- Poor response to therapy.

**Atypical CLL**

Includes those with  $>10\%$  prolymphocytes 'CLL/PLL' which may show an aberrant phenotype (Smlg strong +ve, FMC7/CD79b +ve) is associated with trisomy 12 and p53 abnormalities and a more aggressive course.

**Monoclonal B-cell lymphocytosis (MBL)**

Lymphocyte count  $<5000/mm^3$  (but clonal population), no anaemia or thrombocytopenia: observe only.

**Clinical staging**

2 systems are used to classify patients as low, intermediate, or high risk—Rai modified staging (Table 4.14) and Binet clinical staging (Table 4.15).

**Table 4.14** Rai modified staging

Level of risk	Stage		Median survival (% of patients)
Low	0	Lymphocytosis alone ( $>15,000/\text{microL}$ and $>40\%$ in BM)	$>13$ years (30%)
Intermediate	I	Stage 0 + lymphadenopathy	8 years (25%)
	II	Stage 0–I with spleno-, or hepatomegaly or both	5 years (25%)
High	III	Stage 0–II with anaemia (Hb $<11.0\text{g/dL}$ ) <sup>a</sup>	2 years (10%)
	IV	Stage 0–III with thrombocytopenia ( $<100 \times 10^9/L$ ) <sup>a</sup>	1 year (10%)

<sup>a</sup> Not due to autoimmune anaemia or thrombocytopenia.

**Table 4.15** Binet clinical staging

Stage	Clinical features	Median survival (% of patients)
A	Hb $\geq$ 10g/dL and platelets $\geq$ 100,000 and <3 lymphoid regions enlarged	12 years (60%)
B	Hb $\geq$ 10g/dL and platelets $\geq$ 100,000 and $\geq$ 3 lymphoid regions enlarged	5 years (30%)
C	Hb $\leq$ 10g/dL and/or platelets <100,000 and any number of enlarged areas	2 years (10%)

### Clinical management

- Choice between observation, traditional alkylator-based palliation, or in fit patients, aiming for CR.
- If response duration was adequate, the same treatment could be used again.
- Management of a patient with CLL is based on:
  - Age, performance status, and co-morbidities.
  - Clinical stage, presence of symptoms, disease activity, and prognostic factors.

#### Early stage CLL

- Patients with asymptomatic lymphocytosis should be observed.
- Evidence that treatment improves outcome only in Rai stage III and IV or Binet stage B and C: monitor Rai stage 0–II or Binet stage A patients until treatment indicated for progressive or symptomatic disease.
  - Note: some patients have very indolent, 'smouldering CLL': Binet stage A, non-diffuse BM involvement; lymphocytes  $<30 \times 10^9/L$ , Hb  $>12g/dL$ , lymphocyte doubling time  $>12$  months; Binet stage A with somatic mutation of IgVH gene, median survival 25 years.

#### iwCLL indications for treatment<sup>4</sup>

- Constitutional symptoms due to CLL:  $\geq 10\%$  weight loss in 6 months; extreme fatigue; fevers  $>38^\circ C$  for  $\geq 2$  weeks without infection; night sweats without infection.
- Progressive marrow failure: development of or worsening anaemia and/or thrombocytopenia.
- Autoimmune haemolytic anaemia and/or thrombocytopenia unresponsive to steroids.
- Massive ( $>6cm$  below LCM) or progressive splenomegaly.
- Massive ( $>10cm$  nodes or clusters) or progressive lymphadenopathy.
- Progressive lymphocytosis with  $\uparrow >50\%$  over 2 months or anticipated doubling time of  $<6$  months.

## Treatments

Initial decision is to adopt a 'palliative' approach treating symptomatic disease with minimum toxicity or to aim for prolonged disease-free survival with hope of improved overall survival.

**Chlorambucil:** alkylating agent; traditional 'palliative' 1st-line therapy; dose 6–10mg/d (0.1–0.2mg/kg/d) PO for 7–14d in 28d cycles until disease stabilized (usually 6–12 cycles). Improves FBC and shrinks lymph nodes and spleen in 45–86%; CR 3–7%. Median response ~1 year. No effect on survival (~50% at 5 years). Prior responders to chlorambucil often respond on 1 or more further occasions. Further responses usually poorer and shorter. Resistance inevitably develops. Only 7% respond when fludarabine resistant.

- Side effect: myelosuppression. Prolonged use ↑ risk of myelodysplasia or 2° leukaemia.
- ± rituximab.
- Role in treatment of older, frail patients. Remains popular in Europe.

**R-FC: rituximab, fludarabine, and cyclophosphamide:** overall response (OR) 95%, with CR in 72%, nodular PR in 10%, PR due to cytopenia in 7%, and PR due to residual disease in 6%. Median follow-up of 6 years OS was 77% and PFS, 51%. The German CLL Study Group (GCLLSG) CLL8 trial showed a significant improvement in response rates and duration of response with R-FC compared with FC alone.

- Standard treatment for fit and younger patients.
- May be given in reduced doses.
- Purine analogues cause profound lymphodepletion with risk of opportunistic infection due to *P. jirovecii*, *M. tuberculosis*, herpes zoster, and other organisms. Patients should receive co-trimoxazole prophylaxis (480mg bd *tiw*) throughout therapy and for 3 months post therapy and irradiated cellular blood products indefinitely to prevent GvHD.

**Alemtuzumab:** achieves 39% OR rate in fludarabine-resistant patients and appears effective in some refractory patients with 17p–/p53 mutations. Not effective in bulky nodal disease (>5cm). Humanized anti-CD52 monoclonal antibody (30mg IV or SC *tiw* up to 18 weeks); preferentially eliminates CLL cells from blood, marrow, and spleen; 81% OR and 19% CR as initial therapy.

- Side effects: immunosuppression and virus reactivation (HZV and CMV—monitor weekly during and after treatment by CMV-PCR).
- Irradiate cellular blood products indefinitely to prevent GvHD.
- Co-trimoxazole prophylaxis of *P. jirovecii* for 6 months after treatment.
- Recommended for use for initial therapy for CLL with 17p– and fludarabine-refractory CLL.

**Bendamustine ± rituximab:** B 70mg/m<sup>2</sup> in cycle 1 ↑ to 90mg/m<sup>2</sup> if tolerated ± rituximab. High response rates and well tolerated.

**Rituximab monotherapy:** achieves only limited responses as a single agent in previously treated CLL.

**Ofatumumab:** also known as HuMax-CD20. Human monoclonal antibody (for the CD20 protein) which appears to inhibit early-stage B lymphocyte activation. Approved by the FDA for CLL refractory to fludarabine and alemtuzumab. Approved in Europe in refractory CLL.

**R-CHOP:** sometimes used in CLL.

**High-dose methylprednisolone:**  $1\text{g}/\text{m}^2/\text{d} \times 5\text{d}$  every 28d; achieves up to 77% OR rate; median duration 9 months. May be of particular use in patients refractory to fludarabine with bulky disease and/or p53 mutations. Infection frequent. Contraindicated in patients with active peptic ulceration. Caution with diabetes and CCF.

### Assessment of response

See Table 4.16

**Table 4.16** NCI-WG response criteria for CLL<sup>4</sup>

Criteria	Complete response	Partial response	Progressive disease
Symptoms	None		Richter's syndrome
Lymph nodes	None	≥50% decrease	≥50% increase or new nodes
Liver/spleen	Not palpable	≥50% decrease	≥50% increase or new
Lymphocytes	≤ $4.0 \times 10^9/\text{L}$	≥50% decrease	≥50% increase
Haemoglobin	>110g/L (untransfused)	>110g/L (untransfused) or 50% improvement*	
Neutrophils	≥ $1.5 \times 10^9/\text{L}$	≥ $1.5 \times 10^9/\text{L}$ <sup>a</sup>	
Platelets	> $100 \times 10^9/\text{L}$	> $100 \times 10^9/\text{L}$ <sup>a</sup>	
BM aspirate	<30% lymphocytes		
BM trephine	No interstitial or nodular infiltrate	May be residual lymphoid nodules	

<sup>a</sup> ≥1 of these features required for PR; features should be present for ≥2 months for CR or PR. Stable disease is defined as all other patients.

### Stem cell transplantation

Whether a SCT is indicated and at what point in a patient's treatment remains a big challenge. As most CLL patients are elderly this does often not cause any concerns.

The European Bone Marrow Transplant guidelines outline indications for SCT in CLL and the use of allogeneic SCT:

- Recommended in patients requiring treatment for CLL who have p53 abnormalities. Prognosis here is poor and they merit the risk of transplantation in 1st CR. These patients should be referred early to transplant centres.
- Allogeneic SCT is also recommended for some young(er) patients with CLL who fail to respond to 1st-line combination chemotherapy.
- Autologous SCT is not generally recommended.

### Treatment of complications

- Advise patients to report infection promptly as immunocompromised. Highest risk in elderly, advanced stage, and fludarabine treatment. Prophylactic antibiotics or monthly IV immunoglobulin replacement therapy may reduce recurrent infections in patients with hypogammaglobulinaemia but has no effect on survival.
- *Autoimmune cytopenias*: manage symptomatic AIHA or ITP with corticosteroids in the 1st instance; IVIg, chemotherapy, rituximab, or splenectomy may be required; test patients with pure red cell aplasia for parvovirus infection; treat with prednisolone, ciclosporin, or ATG.
- *Radiotherapy* helpful for persistent or bulky lymphadenopathy; splenic irradiation is sometimes helpful palliation in frail patients with symptomatic splenomegaly who are unfit for splenectomy.
- *Splenectomy* may sometimes be useful for massive splenomegaly, hypersplenism or refractory cytopenia (RR 50–88%).
- *Lymphomatous transformation* (Richter's syndrome) occurs in <10%; occurs in all stages; median interval from diagnosis 24 months; abrupt onset; progressive LN ↑; fever; weight loss; high LDH; usually diffuse large B-cell NHL but ~10% Hodgkin-like histology; chemoresistant; median survival 4 months with CHOP, better with ABVD for Hodgkin-like.

### Prognosis

CLL remains an incurable disease with current therapy apart from a few allografted patients. Most patients with early stage, asymptomatic CLL die of other unrelated causes. Infection is a major cause of morbidity and mortality in symptomatic patients. Advanced stage patients eventually develop refractory disease and BM failure.

### Cell markers in chronic lymphoproliferative disorders

See Tables 4.17 and 4.18.

**Table 4.17** Mature B-cell lymphoproliferative disorders

Marker	CLL	PLL	HCL	SMZL	FL	MCL
Surface Ig	Weak	++	++	++	++	+
CD5	++	-/+	-	-	-	++
CD10	-	-/+	-	-	+	-
CD11c	-/+	-	+	+/-	-	-
CD19	++	++	++	++	++	++
CD20	-/+	++	+	++	++	++
CD22	-/weak	++	++	++	+/-	+/-
CD23	++	+/-	-	-/+	-/+	-
CD25	+/-	-	++	-/+	-	-
CD79b	weak/-	++	+	++	++	++
FMC7	-/+	+	+	++	++	++
CD103	-	-	+	-/+	-	-
HC2	-	-	+	-/+	-	-
Cyclin D1	-	+	-/weak	-	-	++

CLL, chronic lymphocytic leukaemia; PLL, prolymphocytic leukaemia; HCL, hairy cell leukaemia; SMZL, splenic marginal zone lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma.



**Table 4.18** Mature T-cell lymphoproliferative disorders

Marker	T-LGLL	NK-LGLL	T-PLL	ATL	SS
TdT <sup>a</sup>	–	–	–	–	–
CD2	+	+	+	+	+
CD3	++	–	++	++	++
CD4	–	–	+/-	++	++
CD5	+	+	+	+	+
CD7	-/+	–	+++	–	-/+
CD8	++	–	-/+	–	–
CD16	+	+	–	–	–
CD25	–	–	-/+	++	–
CD56	-/+	+	–	–	–
Other		CD11b+		HTLV1+	
		CD57+			
Genotype	TCRR+	TCRR–	TCRR+	TCRR+	TCRR+

T-LGLL, T-cell large granular lymphocyte leukaemia; NK-LGLL, NK-cell large granular lymphocyte leukaemia; T-PLL, T cell prolymphocytic leukaemia; ATL, adult T cell leukaemia/lymphoma; SS, Sézary syndrome; TCRR, clonally re-arranged T-cell receptor.

<sup>a</sup>TdT: terminal deoxynucleotidyl transferase differentiates these cells from lymphoblasts of ALL.

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## Prolymphocytic leukaemia (PLL)

Uncommon aggressive clinicopathological variant of CLL with characteristic morphology and clinical features. B-cell and rare T-cell forms recognized.


### Epidemiology

Median age at presentation is 67 years; ♂:♀ ratio 2:1. Accounts for <2% cases of 'CLL'. B-PLL 75%; T-PLL 25%.

### Clinical features

- Symptoms of BM failure and constitutional symptoms—lethargy, weight loss, fatigue, etc.
- Massive splenomegaly, typically >10cm below costal margin may cause abdominal pain. Hepatomegaly common.
- Minimal lymphadenopathy in B-PLL, generalized lymphadenopathy more common in T-PLL.
- Skin lesions occur in 25% T-PLL as do serous effusions.

### Investigation and diagnosis

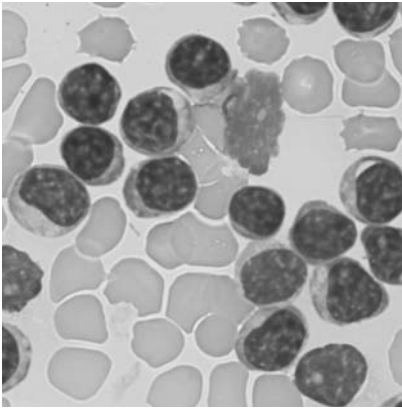
- FBC: high WBC (typically  $>100 \times 10^9/L$ ; commonly  $>200 \times 10^9/L$  in T-PLL); anaemia and thrombocytopenia usually present.
- Differential shows >55% (often >90%) prolymphocytes.
- Morphology: large lymphoid cells, abundant cytoplasm (B-PLL mainly), prominent single central nucleolus (see Fig. 4.15).
- BM diffusely infiltrated.
- Immunophenotype:  see Table 4.17.
- Cytogenetics—B-PLL: 14q+ in 60%; t(11;14)(q13;q32) in 20%; p53 gene abnormalities in 75%; 6q- and chromosome 1 abnormalities also described; T-PLL 14q11 abnormalities in >70%; loss of heterozygosity in 11q22–23 region affecting ATM gene expression in 67%; +8 in 50%.

### Differential diagnosis

B-PLL and CLL are not always easily distinguished and mixed 'CLL/PLL' is recognized (>10%, <55% prolymphocytes). Clinical features, morphology, and notably immunophenotyping are used to distinguish PLL from other lymphoproliferative disorders.

### Natural history

PLL is a relentlessly progressive disease and treatment is unsatisfactory. T-PLL carries poor prognosis with median survival 6–7 months. Median survival in B-PLL is 3 years. Only curative option is allogeneic SCT.



**Fig. 4.15** Blood film in PLL: cells are larger than those seen in CLL have large prominent nucleoli and moderate chromatin condensation (↻ see colour plate section).

### Management

- PLL is typically resistant to chlorambucil.
- *Combination chemotherapy*: RFC or RCHOP may achieve responses in about 33%.
- *Purine analogue therapy*: fludarabine, cladribine, or pentostatin (deoxycoformycin) may produce responses in some patients with B-PLL.
- *Alemtuzumab*: anti-CD52 monoclonal antibody produces responses in both B-PLL and T-PLL. In T-PLL CR rates of 40–60% have been achieved; may last several months and permit bridge to allogeneic SCT in eligible patients; some prolonged survival.
- *Rituximab*: anti-CD20 monoclonal antibody produces complete responses in B-PLL that may last several months and permit subsequent SCT in eligible patients.
- *SCT*: in view of the poor prognosis of both B- and T-PLL, younger patients who achieve a CR should be considered for allogeneic SCT where possible.
- *Splenectomy* may be symptomatically helpful, ‘debulking’, but must be followed with other therapy.
- *Splenic irradiation* offers symptomatic relief if unfit for splenectomy.

## Hairy cell leukaemia and variant

Uncommon low-grade B-cell lymphoproliferative disorder associated with splenomegaly, pancytopenia, and typical 'hairy cells' in blood and BM.

### Epidemiology

Accounts for 2% of leukaemias in adults, 8% of chronic lymphoproliferative disorders in the West. Rare in Japan. No known aetiological factors. Presents in middle age (>45 years) with ♂:♀ ratio of 4:1.

### Clinical features

- Typically non-specific symptoms: lethargy, malaise, fatigue, weight loss, and dyspnoea.
- 15% present with infections, often atypical organisms due to monocytopenia.
- 70% have recurrent infection; 30% bleeding or easy bruising.
- Splenomegaly in 80% (massive in 20–30%), hepatomegaly in 20%.
- Lymphadenopathy rare (<5%).
- Pancytopenia may be an incidental finding on a routine FBC.
- Vasculitic polyarthritis and visceral involvement similar to polyarteritis nodosa occurs in some patients with HCL.

### Investigation and diagnosis

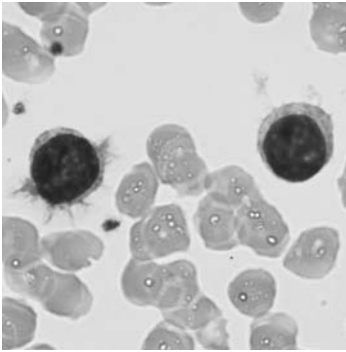
- FBC: moderate-to-severe pancytopenia; Hb <8.5g/dL in 35%.
- Blood film: low numbers of 'hairy cells' in 95%; florid leukaemic features unusual.
- Hairy cells: kidney-shaped nuclei, clear cytoplasm and irregular cytoplasmic projections (more notable on EM).
- WBC differential: neutropenia,  $<1.0 \times 10^9/L$  in 75%; *monocytopenia is a consistent feature*.
- Cytochemistry: +ve for tartrate-resistant acid phosphatase (TRAP) in 95%; Identified by flow cytometry.
- Immunophenotyping: typically CD11c, CD25, CD103, HC2+.
- BM: aspiration often unsuccessful—'dry tap' due to ↑ BM fibrosis; trephine shows diagnostic features with focal or diffuse infiltration of HCL where cells have a characteristic 'halo' of cytoplasm confirmed by immunocytochemistry with anti-CD20/DBA-44 and anti-TRAP.
- Staging CT neck, chest, abdomen, pelvis recommended. Intra-abdominal lymphadenopathy in 15–20%.

### Differential diagnosis

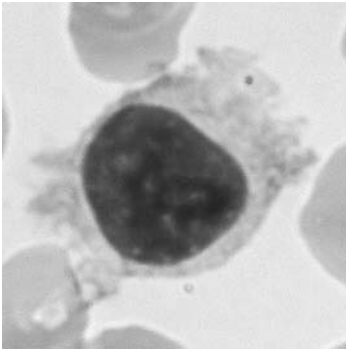
Confirmation of diagnosis may be difficult because of low numbers of circulating leukaemic cells and dry tap on marrow aspiration; trephine biopsy usually diagnostic; differential diagnosis includes myelofibrosis and other low grade lymphomas notably splenic marginal zone lymphoma.

### Prognostic factors

No established staging system. Response to therapy is probably the best prognostic indicator (see Figs. 4.16 and 4.17).



**Fig. 4.16** Peripheral blood film in HCL showing typical 'hairy' lymphocytes (medium power) (➡ see colour plate section).



**Fig. 4.17** Peripheral blood film in HCL showing typical 'hairy' lymphocytes (high power) (➡ see colour plate section).

## Management

Note: ↑ incidence of atypical mycobacterial infections in HCL. Prophylaxis is recommended.

- In <10% patients, often elderly with minimal or no splenomegaly and cytopenia, the disease remains relatively stable and may be observed.
- Therapy is required in patients with Hb <10g/dL, neutropenia  $<1.0 \times 10^9/L$ , thrombocytopenia  $<100 \times 10^9/L$ , symptomatic splenomegaly, recurrent infection, extranodal involvement, autoimmune complications, florid leukaemia, or progressive disease.
- Supportive management is important particularly in the early stages of therapy where cytopenias can worsen: treat infections promptly.

### 1st-line therapy

*Cladribine*: purine analogue; widely used as 1st-line therapy. 0.14 mg/kg SC injection once daily d1–5. 80% CR rate, remainder PR; temporary myelosuppression. Repeat at 6 months if no CR; 54% progression-free survival at 12 years; some patients likely cured.

*Pentostatin (deoxycoformycin)*: alternative purine analogue; 4 mg/m<sup>2</sup> IV bolus every 2 weeks to maximum response plus 2 cycles (generally 6–10); improvement expected after 2 cycles; maximum response generally 4–7 months; up to 90% OR; 75% CR; 15% continued CR at 8 years; some patients likely cured.

*G-CSF*: may be useful in patients with severe neutropenia.

*Splenectomy*: now rarely required; reserved for patients unresponsive to therapy. Avoid drug therapy for 6 months after splenectomy to assess response.

After purine analogue therapy CD4 lymphodepletion causes immunosuppression: requires *P. jirovecii* prophylaxis with co-trimoxazole for 6 months after treatment and irradiated blood products indefinitely.

Monitor response to therapy at 3 months by trephine biopsy.

### 2nd-line and subsequent therapy

Immediate retreatment is not always necessary when relapse is identified. If a remission of >5 years has been achieved then a further remission with the same agent is likely. The second remission is likely to be shorter. Early relapse or resistance may benefit from an alternative purine analogue, interferon alfa, or rituximab.

*Interferon alfa*: 3 million units SC 3×/week continued for 12–18 months may be useful for HCL refractory to purine analogues; initial therapy achieves PR in up to 80% but CR in <5%; lower responses later in disease; Side effects notably flu-like symptoms and fatigue cause intolerance in some patients.

*Rituximab*: 375mg/m<sup>2</sup>/week IV × 4–12; offers an alternative to patients resistant to purine analogues. 26–80% ORs and 10–55% CRs have been reported with median responses of 14–73 months.

### Natural history

Hairy cell leukaemia treated with a purine analogue is associated with prolonged survival (95% at 5 years, 80–87% at 10–12 years), with many patients achieving durable CRs; some patients may be cured. For others, careful application of available treatments at disease relapse or progression allows prolonged, good quality survival. Late relapses occur.

### Hairy cell variant

Describes a rare variant of HCL where the presenting WBC count is higher due to circulating leukaemic cells ( $40\text{--}60 \times 10^9/\text{L}$ ) and monocytopenia is absent. Cells are villous but have a central round nucleus and a distinct nucleolus-like PLL. Marrow is aspirated easily due to low reticulin but the trephine appearance is similar to HCL. There is often an associated neutropenia. Immunophenotype differs from typical HCL: CD11c+, CD25, and HC2 –ve, CD103 usually –ve. Response to pentostatin (deoxycoformycin) or interferon alfa is poor but chlorambucil appears active in this form, and the variant generally follows an indolent course.

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## Large granular lymphocyte leukaemia (LGLL)

Uncommon clonal lymphoproliferative disorders, characterized by an increase in LGLs in blood. Heterogeneous—may be either T-cell or NK-cell phenotype; each may follow an indolent or aggressive course.

### T-cell LGLL

Most frequent form of LGLL, 85% of LGL patients. Aetiology unclear.

#### Clinical features

- Median age at diagnosis 60 years; no gender preference.
- Usually asymptomatic; modest lymphocytosis on routine FBC with large granular lymphocytes  $>2 \times 10^9/L$  (NR  $0.2\text{--}0.4 \times 10^9/L$ ) (see Fig. 4.18).
- 80% develop neutropenia;  $>45\% <0.5 \times 10^9/L$ .
- Occasional presentation with chronic fatigue or recurrent bacterial infections—usually mucocutaneous.
- Arthralgia, itching, rash (25%), mouth ulcers; association with seropositive rheumatoid arthritis in 25–35% and Felty syndrome (neutropenia + splenomegaly + rheumatoid arthritis).
- Splenomegaly 20–50% of cases. Lymphadenopathy rare.

#### Laboratory findings

- Hb and platelets usually normal; chronic neutropenia (80%) and mild anaemia (48%) may be present. Thrombocytopenia 20%.
- Mild/moderate lymphocytosis (usually  $<10 \times 10^9/L$ ); medium-large cells; eccentric nucleus; abundant cytoplasm and distinct azurophilic granules.
- Phenotyping: most type as 'cytotoxic' T cells (CD3+ CD8+ CD16+ CD56– CD57+); variants co-express CD4 and CD8, CD4 alone or neither; weak expression of CD5 and CD7 distinguishes from reactive LGLs.
- Clonal rearrangement of T-cell receptor genes (TCR $\beta$  &  $\gamma$ ) confirms diagnosis by RFLP and Southern blotting (TCR $\beta$ ) and PCR (TCR $\gamma$ ).
- Polyclonal hypergammaglobulinaemia, rheumatoid factor, and antinuclear antibodies occur in 50% even without joint disease.
- BM involvement often subtle; may be diffuse or nodular usually non-paratrabeular; anti-CD3 immunohistochemistry helps.
- No characteristic cytogenetic pattern;  $<10\%$  abnormal.

#### Differential diagnosis

Reactive lymphocytosis (screen for infection especially EBV); other T-cell lymphoproliferative disorders (immunophenotype; ➔ see Table 4.16, p.164).



**Prognosis and management**

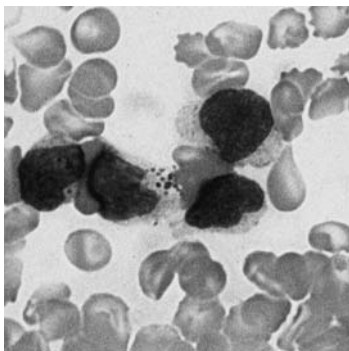
- Incurable but generally stable benign disease. Asymptomatic patients require observation only.
- Care is essentially supportive with prompt treatment of infection with appropriate broad-spectrum antibiotics.
- Treatment indicated by development of recurrent infections, severe neutropenia, symptomatic anaemia or thrombocytopenia or symptomatic splenomegaly or systemic symptoms.
- Immunosuppression with low-dose methotrexate (10mg/m<sup>2</sup> PO weekly), ciclosporin (2mg/kg PO bd) or cyclophosphamide (50–100mg PO od) effective in >50% of patients. Treat for at least 4 months to assess response; indefinite treatment may be necessary to sustain response.
- Symptoms may improve without improved neutrophil count; failure to eradicate clone does not prevent improvement in cytopenias.
- Corticosteroids in modest dosage may improve neutropenia but predispose to infection, including fungal infections; responses less durable. Prophylactic antibiotics can be helpful in severe neutropenia on steroid therapy.
- G-CSF may be of value in symptomatic chronic neutropenia.
- Alemtuzumab and purine analogue drugs are reported to be effective as single agents in refractory T-LGLL.

**Aggressive T-cell LGLL**

Rare; affects younger age-group: median age 41 years, range 9–64.

Present with rapidly progressive B symptoms (fever, night sweats, weight loss), hepatosplenomegaly, lymphadenopathy, lymphocytosis, anaemia, and/or thrombocytopenia.

- Poor prognosis; survival 2 months–2 years.
- Diagnosis by aggressive course plus FBC: LGLs  $>0.5 \times 10^9/L$ , many  $>10 \times 10^9/L$ .
- Immunophenotype: CD3+, CD8+, CD56+, TCR $\alpha\beta$ , or variants; clonally rearranged TCR  $\beta$  and  $\gamma$  genes.
- Differentiate from: indolent T-cell LGLL: indolent clinical course, neutropenia, autoimmune disease, and CD56-. Aggressive NK-LGLL: sCD3-, germline TCR genes, intracellular clonal episomal EBV; usually Asian patient.
- Optimal therapy undefined; treat with ALL-type regimen including CNS prophylaxis followed by SCT in 1st CR.



**Fig. 4.18** Blood film showing large granular lymphocytes in LGL leukaemia.

### Chronic NK-cell LGLL

- 5% of LGL patients; median age 58.
- Aetiology unclear. Possible role of viral infection.

#### *Clinical and laboratory features*

- Indolent disorder with favourable prognosis.
- Persistently elevated LGLs ( $>2 \times 10^9/L$ ).
- No fever, hepatosplenomegaly, or lymphadenopathy.
- Mild neutropenia and/or anaemia may occur; less severe and less frequent than in indolent T-cell LGLL.
- Immunophenotype: typically CD2+, CD3-, CD8-, CD16+, CD56+, CD57 variable.
- Demonstration of clonality and differential diagnosis from reactive NK-cell lymphocytosis can be difficult; panels vs NK-associated antigens can be helpful; reactive lymphocytosis associated with viral infection and connective tissue disorders and rarely last  $>6$  months; differentiate from indolent T-cell LGLL by immunophenotype and TCR clonal studies.

#### *Treatment*

As for indolent T-cell LGLL.

#### *Aggressive NK-cell LGLL*

10% of LGL patients; most prevalent in younger Asian patients. Median age 39 years; no gender preference. Association with EBV infection (?aetiological role).

#### *Clinical and laboratory features*

- Acute presentation with B symptoms, hepatosplenomegaly, and cytopenia. DIC and multiorgan failure may occur.
- BM: diffuse infiltration by NK cells.
- Diagnosis by immunophenotype CD2+, CD3-, CD56+, usually CD57-; germline TCR genes; clonal episomal EBV.
- Cytogenetics: del(6q21-q25) and -17p13 most common.

**Treatment**

CHOP-type therapy ineffective. Consider ALL-type regimen with CNS prophylaxis as initial therapy; consolidate responders with SCT.

**Further reading**

Alekshun, T.J. and Sokol, L. (2007). Diseases of large granular lymphocytes. *Cancer Control*, **14**, 141–50.

## Adult T-cell leukaemia-lymphoma (ATLL)

Aggressive neoplasm of CD4+ve T-lymphocytes caused by HTLV-I with a distinct geographical distribution.

### Incidence

Highest incidence among populations where HTLV-I infection endemic: Kyushi district of SW Japan, Caribbean, parts of Central and South America, Central and West Africa. Incidence 2/1000 ♂ and 0.5–1/1000 ♀ seropositive for HTLV-I (37% ♂ >40). HTLV-1 infection: ATLL risk 2.5% at 70 years. Non-endemic cases generally originate from these areas.

### Aetiology

HTLV-I involved in multistep pathogenesis. HTLV-I provirus in ATLL cells; all patients with ATLL are seropositive for prior HTLV-I infection. Transfer of live infected cells required for infection; 3 major routes: mother to infant (mainly by breast milk); parenteral transmission; sexual transmission. Not all infected patients develop ATLL. Often long latent period (40–60 years).

### Clinical subtypes

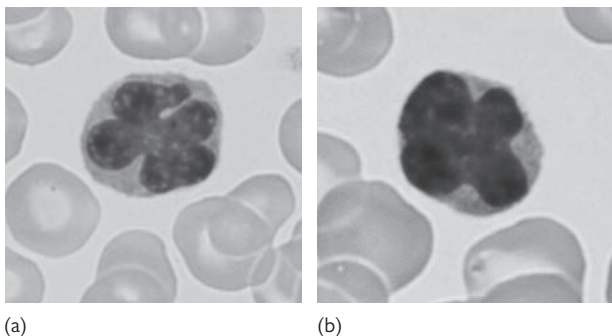
There are 4 subtypes: acute, chronic, smouldering, and lymphomatous forms. Acute subtype most common (66%), median survival 6 months despite therapy. Other forms have longer survival but often progress to the acute form after several months. Median survival of lymphomatous subtype 1 year and chronic subtype 2 years. The smouldering subtype is most indolent and is associated with survival >24 months.

### Clinical features and presentation

- Median age at diagnosis 58 years (range 20–90); ♂:♀ ratio 1:4.
- History of residence or origin in HTLV-I endemic area usual.
- Usually short history of rapidly increasing ill health.
- Opportunistic infection common.
- Acute subtype: abdominal pain, diarrhoea, pleural effusion, ascites, and respiratory symptoms (often due to leukaemic infiltration of lungs). Lymphadenopathy 60%; hepatomegaly 26%, splenomegaly 22%; skin lesions 39%.
- Lymphomatous subtype: prominent lymphadenopathy; few circulating cells.
- Chronic subtype: mild ↑ WBC; some skin lesions, enlarged LN, organomegaly.
- Smouldering subtype: few ATLL cells, skin lesions, and occasional pulmonary involvement.

### Diagnosis and investigation

- FBC: WBC usually markedly ↑ (up to  $500 \times 10^9/L$ ) in acute form but may be normal; anaemia and thrombocytopenia common.
- Blood film: large numbers of lymphoid cells with marked nuclear irregularity occasionally multilobulated with 'floral' or 'clover leaf' appearance (Fig. 4.19).
- Immunophenotyping: generally CD2+, CD3+, CD4+, CD5+, CD7-, CD8-, CD25+, HLA-DR+ T cells; rarely CD4-, CD8+ or CD4+, CD8+ (↻ see Table 4.18).
- Serology: +ve for HTLV-I.
- Cytogenetics: multiple abnormalities described; no consistent pattern.
- Serum chemistry: hypercalcaemia in 33–50% of patients at diagnosis; 70% during disease course due to ↑ osteoclasts.
- BM: diffuse infiltration by ATL cells.
- Monoclonal integration of HTLV-I provirus in lymphoid cells can be demonstrated by Southern blotting.



**Fig. 4.19** Peripheral blood films showing typical ATL cell with lobulated 'clover leaf nucleus' (↻ see colour plate section).

### Prognostic factors

Poor prognostic features:

- Raised LDH.
- Hypercalcaemia.
- Hyperbilirubinaemia.
- Raised WBC.

### Management and prognosis

- Treatment unsatisfactory; poor prognosis in acute and lymphomatous ATLL: median survivals 6 and 10 months respectively. Death is usually due to opportunistic infection.
- Patients with acute and lymphomatous ATLL require immediate treatment; patients with chronic or smouldering ATLL may be observed.
- The combination of AZT and IFN is highly effective and is standard 1st-line therapy in acute leukaemic ATLL, as well as smouldering and chronic ATLL. This combination has changed the natural history of the disease in patients with smouldering and chronic ATLL as well as a subset of patients with acute ATLL.
  - Use in leukaemic forms of the disease as 1st-line therapy and not after 1 or more cycles of chemotherapy.
  - Start with high doses of both agents.
- Lymphomatous ATLL: CHOP in addition to or followed by antiretroviral therapy with AZT/IFN. CHOP (with G-CSF) achieves 66% OR with 25% CR; median survival <1 year; OS at 3 years is 13%.
- Prophylaxis of opportunistic infections and supportive therapy should be given.
- Allogeneic SCT should be considered in suitable patients in 1st CR.
- GvL effect appears important. Reduced-intensity conditioning safer in older patients (most >50 years). Donors should be HTLV-I seronegative as donor cell transformation to ATL reported.

### Further reading

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Yasunaga, J. and Matsuoka, M. (2007). Human T-cell leukaemia virus type 1 induces adult T-cell leukaemia: from clinical aspects to molecular mechanisms. *Cancer Control*, **14**, 133–40.

# Lymphoma

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## Non-Hodgkin lymphoma (NHL)

NHL is a group of histologically and biologically heterogeneous clonal malignant diseases arising from the lymphoid system. The clinical presentation ranges from an indolent to an aggressive nature. Treatment decisions are preferably made in a multidisciplinary setting.

### Epidemiology

Lymphoma (both NHL and HL) is the most common haematological cancer (5% of all cancer cases). The annual incidence of 'lymphoma' in Western countries is 15–20 cases per 100,000. Incidence ↑ with age. ♂:♀ ratio 3:2.

The last decades have seen an increase; ↑ incidence is in part due to ↑ mean age of the population, improvements in diagnosis, the HIV pandemic, and immunosuppressive therapy and diagnosis (including HIV).

### Aetiology

The rearrangement and mutation of immunoglobulin genes that occur in B-cell differentiation and the response to antigen offers an opportunity for genetic accidents such as translocations or mutations involving immunoglobulin gene loci that have been characterized in many lymphomas. Most translocations involve genes associated with either proliferation (e.g. *c-MYC*) or apoptosis (e.g. *BCL-2*).

Other factors associated with NHL are:

- Congenital immunodeficiency: ataxia telangiectasia, Wiskott–Aldrich syndrome, X-linked combined immunodeficiency.
- Acquired immunodeficiency: immunosuppressive drugs, transplantation, HIV infection.
- Infection: HTLV-I (ATLL); EBV (Burkitt lymphoma, HL); *Helicobacter pylori* (gastric mucosa-associated lymphoid tissue (MALT) lymphomas).
- Environmental toxins: agricultural pesticides, herbicides, and fertilizers, solvents and hair dyes.
- Familial: risk ↑ 2–3-fold in close relatives (?environmental).

### Classification

The World Health Organization (WHO) classification of lymphoid neoplasms superseded the previous Revised European American Lymphoma (REAL) classification. The most recent 2008 WHO classification (☞ Table 5.1) lists over 80 different clinical-pathological lymphomas using morphology, immunophenotype, genotype, cell of origin, and clinical behaviour. 85% of lymphomas are B-cell type, the remaining lymphomas are of T/NK-cell origin.

The extensive list includes all entities and also includes provisional entities for which the WHO Working Group felt there was insufficient evidence to recognize as distinct diseases at this time.

The most common and least rare lymphomas are discussed in this chapter.

Clinical behaviour of lymphomas most often determines management strategies in clinical practice; treatment protocols remain largely based on classification systems that group diagnoses into indolent (low-grade) and aggressive (high-grade) NHL.



The more biologically relevant classification of lymphoma diagnosis using the WHO classification, together with emerging therapeutic options based on immunological and molecular characteristics will increase the diagnosis-specific nature of therapy in the future.

**Table 5.1** WHO classification of lymphoid neoplasms<sup>1,2</sup>

**Mature B-cell neoplasms**

Chronic lymphocytic leukaemia/small lymphocytic lymphoma
B-cell prolymphocytic leukaemia
Splenic B-cell marginal zone lymphoma
Hairy cell leukaemia
<i>Splenic B-cell lymphoma/leukaemia, unclassifiable</i>
<i>Splenic diffuse red pulp small B-cell lymphoma</i>
<i>Hairy cell leukaemia-variant</i>
Lymphoplasmacytic lymphoma
Waldenström macroglobulinaemia
Heavy chain diseases
Alpha heavy chain disease
Gamma heavy chain disease
Mu heavy chain disease
Plasma cell myeloma
Solitary plasmacytoma of bone
Extraneous plasmacytoma
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
Nodal marginal zone lymphoma
<i>Paediatric nodal marginal zone lymphoma</i>
Follicular lymphoma
<i>Paediatric follicular lymphoma</i>
Primary cutaneous follicle centre lymphoma
Mantle cell lymphoma
Diffuse large B-cell lymphoma (DLBCL), NOS
T-cell/histiocyte rich large B-cell lymphoma
Primary DLBCL of the CNS
Primary cutaneous DLBCL, leg type
<i>EBV-positive DLBCL of elderly</i>
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
ALK positive large B-cell lymphoma
Plasmablastic lymphoma
Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease
Primary effusion lymphoma
Burkitt lymphoma B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma

(Continued)

**Table 5.1** WHO classification of lymphoid neoplasms<sup>1,2</sup> (Continued)**Mature T-cell and NK-cell neoplasms**

T-cell prolymphocytic leukaemia  
 T-cell large granular lymphocytic leukaemia  
*Chronic lymphoproliferative disorder of NK-cells*  
 Aggressive NK cell leukaemia  
 Systemic EBV positive T-cell lymphoproliferative disease of childhood  
 Hydroa vacciniforme-like lymphoma  
 Adult T-cell leukaemia/lymphoma  
 Extranodal NG/T cell lymphoma, nasal type  
 Enteropathy-associated T-cell lymphoma  
 Hepatosplenic T-cell lymphoma  
 Subcutaneous panniculitis-like T-cell lymphoma  
 Mycosis fungoides  
 Sézary syndrome  
 Primary cutaneous CD30-positive T-cell lymphoproliferative disorders  
 Lymphomatoid papulosis  
 Primary cutaneous anaplastic large cell lymphoma  
 Primary cutaneous gamma-delta T-cell lymphoma  
*Primary cutaneous CD8 positive aggressive epidermotropic cytotoxic T-cell lymphoma*  
*Primary cutaneous CD4 positive small/medium T-cell lymphoma*  
 Peripheral T-cell lymphoma, NOS  
 Angioimmunoblastic T-cell lymphoma  
 Anaplastic large cell lymphoma, ALK positive  
 Anaplastic large cell lymphoma, ALK negative

**Hodgkin lymphoma**

Nodular lymphocyte predominant Hodgkin lymphoma  
 Classical Hodgkin lymphoma:  
 Nodular sclerosis Hodgkin lymphoma  
 Lymphocyte-rich classical Hodgkin lymphoma  
 Mixed cellularity Hodgkin lymphoma  
 Lymphocyte depleted Hodgkin lymphoma

*The italicized histological types are provisional entities, for which the WHO Working Group felt there was insufficient evidence to recognize as distinct diseases at this time.*

## Presentation


The features at presentation reflect a spectrum from 'low-grade' lymphoma (typically, widely disseminated at diagnosis but with indolent course) to 'high-grade' lymphoma (short history of localized rapidly enlarging lymphadenopathy  $\pm$  constitutional symptoms ('B-symptoms': drenching night sweats, >10% weight loss and/or fevers).

Almost 75% of adults present with nodal disease, usually superficial painless lymphadenopathy. 25% are extranodal (~50% in the Far East) and may present with oropharyngeal involvement (5–10%), GI involvement (15%), CNS involvement (5–10%), skin involvement (mainly T-cell lymphomas).

Patients with GI involvement have a higher frequency of oropharyngeal involvement (Waldeyer's ring) and vice versa. Hepatosplenomegaly is common in advanced disease.

## Diagnostic and staging investigations

- Histological diagnosis by expert haematopathologist: excision biopsy of lymph node or extranodal mass with immunohistochemistry  $\pm$  molecular analysis.
- NB Fine needle aspirate (FNA) is inadequate for reliable diagnosis and grading.
- Detailed history including B symptoms.
- Physical examination and performance status.
- FBC and blood film.
- LDH.
- CRP/ESR.
- Serum  $\beta_2$ -microglobulin.
- U&E, uric acid, LFTs.
- DAT screen.
- Serum protein electrophoresis.
- Plasma viscosity can be considered.
- BM aspirate and trephine biopsy.
- CXR.
- Imaging: whole-body CT neck, chest, abdomen, and pelvis or PET/CT scan; CT or MRI head/spine for patients with CNS symptoms; other imaging as indicated.
- LP also for high-grade disease with BM, testicular, paranasal sinus, parameningeal, peri-orbital or paravertebral involvement.
- HIV serology recommended in all patients.
- *Helicobacter pylori* serology (gastric MALT lymphoma).
- Echocardiogram pre-treatment with anthracyclin containing regimen.

Staging defines prognosis and helps in selecting therapy. The Ann Arbor staging system developed for Hodgkin lymphoma is also used in NHL (see  Table 5.9, p.217).

**(Possible) Laboratory features**


- Normochromic normocytic anaemia common.
- Leucoerythroblastic film if extensive BM infiltration  $\pm$  pancytopenia.
- Hypersplenism (occasionally).
- PB may show circulating lymphoma cells (MCL; cleaved 'buttock' cells in FL and blasts in high-grade disease)
- LFTs abnormal in hepatic infiltration.
- Serum LDH and  $\beta_2$ -microglobulin are useful prognostic factors. LDH often raised in high-grade lymphomas.
- 20% of patients with SLL or FL have a serum paraprotein, usually IgM and usually low level.

**Prognostic factors**

- Histological grade.
- Performance status.
- Constitutional ('B') symptoms unfavourable.
- Gene expression profile in DLBCL (GCB favourable; ABC poor).

**Unfavourable**

- Age >60 years.
- Disseminated disease (stage III–IV).
- Extranodal disease.
- Bulky disease (>10cm).
- Raised serum LDH.
- Raised serum  $\beta_2$ -microglobulin.
- High proliferation rate by Ki-67 immunochemistry.
- P53 mutations.
- T-cell phenotype.
- High-grade transformation from low-grade NHL.

Various international prognostic systems are used to predict prognosis and tailor treatment. The IPI, Revised IPI (R-IPI), FLIPI, and MIPI score are discussed on  pp.187–188.

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## International Prognostic Index

Validated in all clinical grades of NHL as a predictor of response to therapy, relapse, and survival (see Table 5.2). The IPI<sup>1</sup> was validated in the pre-rituximab era. 1 point is awarded for each of the following characteristics to identify 4 risk groups:

- Age >60 years.
- Stage III or IV.
- ≥2 extranodal sites of disease.
- Performance status (ECOG) ≥2.
- Serum LDH raised.

**Table 5.2** IPI<sup>1</sup>

Score	IPI risk group	% CR	5-year CR-DFS	5-year OS
0 or 1	Low	87%	70%	73%
2	Low/intermediate	67%	50%	51%
3	Intermediate/high	55%	49%	43%
4 or 5	High	44%	40%	26%

### Age-adjusted IPI<sup>4</sup>

Used for patients aged >60 years who may be eligible for more intensive therapy (see Table 5.3). 1 point is awarded for each of the following characteristics:

- Stage III or IV.
- Raised serum LDH.
- Performance status ≥2.

**Table 5.3** Age-adjusted IPI<sup>1</sup>

Score	IPI risk group (patients ≤60)	% CR	5-year CR-DFS	5-year OS
0	Low	92%	86%	83%
1	Low/intermediate	78%	66%	69%
2	Intermediate/high	57%	53%	46%
3	High	46%	58%	32%

*Revised International Prognostic Index (R-IPI)*<sup>2</sup> has been developed for patients with DLBCL treated with R-CHOP as the addition of rituximab has altered the survival of IPI prognostic groups (see Table 5.4). R-IPI uses the same risk factors as the IPI to identify 3 distinct groups.

**Table 5.4** R-IPI<sup>2</sup>

Score	R-IPI risk group	% patients	4-year PFS	4-year OS
0	Very good	10%	94%	94%
1 or 2	Good	45%	80%	79%
3, 4, or 5	Poor	45%	53%	55%

**Follicular Lymphoma Prognostic Index (FLIPI)<sup>3</sup>**

Provides better prognostic information in patients with follicular lymphoma as only ~11% are high risk on IPI (see Table 5.5). The FLIPI can be used to assess the need for early treatment and its likely outcome. 1 point is awarded for each of the following risk factors to identify 3 risk groups:

- Age >60 years.
- Stage III or IV.
- Haemoglobin <120g/L.
- Raised serum LDH.
- Number of nodal sites  $\geq 5$ .

**Table 5.5** FLIPI<sup>3</sup>

Score	FLIPI risk group	% patients	5-year OS	10-year OS
0 or 1	Low	36	91%	71%
2	Intermediate	37	78%	51%
$\geq 3$	High	27	52%	36%

**MCL International Prognostic Index (MIPI)**

Applies to advanced stage MCL (III-IV).

The full MIPI is calculated using the following formula:

$$[0.03535 \times \text{age (years)}] + 0.6978 \text{ (if ECOG >1)} \\ + [1.367 \times \log^{10}(\text{LDH/ULN})] + [0.9393 \times \log^{10}(\text{WBC count})]$$

A score <5.7 indicates low-risk disease, 5.7–6.2 indicates intermediate risk, and >6.2 high risk (see Tables 5.6 and 5.7).

**Table 5.6** Calculating the simplified MIPI<sup>4</sup>

Score	Age	PS	LDH/ULN	WCC
0	<50	0–1	<0.67	<6.7
1	50–59	–	0.67–0.99	6.7–9.9
2	60–69	2–4	1.0–1.49	10.0–14.9
3	$\geq 70$	–	$\geq 1.50$	$\geq 15.0$

LDH/ULN: LDH relative to the upper limit of normal

**Table 5.7** Simplified MIPI<sup>4</sup>

Score	Risk	% patients	Median OS (months)
0–3	Low	44	>90
4–5	Intermediate	35	58
6–11	High	21	37

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## Further reading

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## Indolent lymphoma

### Clinical features

Up to 40% of cases; typically slowly progressive disorders.

#### *Follicular lymphoma*

Most common in middle and old age (median age 55 years); can present with painless lymphadenopathy at  $\geq 1$  sites (most common), effects of BM infiltration or constitutional symptoms (15–20%). Median survival ~10 years; ~25% may transform to high-grade DLBCL. OS 5 years 72–77%. BM shows typical paratrabeular follicular infiltration. PB can show minor lymphocytosis.

#### *At diagnosis*

- 70% stage IV disease with BM involvement.
- 15–20% localized stage I or II disease.

#### *Grading*

FL is graded histologically based on the average number of centroblasts in 10 neoplastic follicles at high power field (HPF) examination ( $\times 40$ ).

- Grade 1: 0–5 centroblasts/HPF.
- Grade 2: 6–15 centroblasts/HPF.
- Grade 3a:  $>15$  centroblasts/HPF with many residual small cells.
- Grade 3b:  $>15$  centroblasts/HPF in monotonous sheets.

Note: cases of FL with a high proportion of centroblasts on histology follow a more aggressive clinical course and are treated as aggressive lymphomas.

Grade 3b FL is generally clinically treated as DLBCL.

#### *Small lymphocytic lymphoma (SLL)*

Nodal form of CLL and treated similarly; More common with age ( $>60$  years); disseminated peripheral lymphadenopathy and splenomegaly possible; lymphocyte count  $<4.5 \times 10^9/L$ ; BM involvement in 80%; constitutional symptoms  $<20\%$ ; serum paraprotein (usually IgM) in 30%; median survival 8–10 years; If the WCC is raised the diagnosis is CLL rather than SLL.

#### *Marginal zone lymphoma (MZL)*

- MALT lymphomas: local invasion at site of origin, e.g. stomach, small bowel, salivary gland, or lung. Gastric MALT lymphomas present with long history of dyspepsia, abdominal pain  $\pm$  GI bleeding; diagnosis by endoscopy and biopsy; localized in 80–90% and respond to antibiotic treatment for *H. pylori*; good prognosis ( $>80\%$  5-year survival).
- Nodal marginal zone lymphoma: rare; associated with Sjögren's syndrome and Hashimoto's thyroiditis; usually localized to ocular adnexae, thyroid, parotid, lung, or breast.

#### *Splenic marginal zone lymphoma (SMZL)*

- Median age at diagnosis ~65 years; rare  $<50$ . Rare ( $<1\%$  NHL).
- Formerly 'splenic lymphoma with villous lymphocytes' (SLVL).



- >50% survival at 5 years. Over 10% may require no treatment. Median survival >10 years from diagnosis. Transformation to diffuse large B-cell NHL occurs in ~10% in a median of 2–4 years.

#### *Clinical*

- Non-specific symptoms (fatigue, abdominal discomfort), moderate-to-massive splenomegaly, hepatomegaly in 50%, peripheral lymphadenopathy rare (splenic hilar LN frequently ↑).
- Auto-immune phenomena in 10%: AIHA, cold agglutinins, ITP, lupus anticoagulant, acquired von Willebrand's syndrome.
- Symptoms of cryoglobulinaemia in rare patients with concomitant hepatitis C infection.
- Differential diagnosis: MCL (especially 20% CD5+ SMZL) CLL, PLL, HCL, and hairy cell variant.

#### *Laboratory*

- Anaemia and thrombocytopenia in 25–30% usually due to hypersplenism.
- Total WBC ↑ due to lymphocytosis 95%; not grossly elevated. Intermediate size lymphocytes, larger than typical CLL cells, round/oval nuclei; clumped chromatin, indistinct nucleoli, usually with villous cytoplasmic projections at one/both poles; monocytopenia not a feature (cf. HCL).
- Monoclonal serum paraprotein in up to 30%; usually IgM.
- LDH usually normal; ↑ serum  $\beta_2$ -microglobulin.
- BM aspirate may show lymphocytosis with typical immunophenotype; biopsy may be normal (10%) but usually shows moderate patchy/nodular lymphoid infiltration; some interstitial or diffuse; intrasinusoidal CD20+ lymphoma cells characteristic of SMZL.
- Cytogenetic/molecular abnormalities in 80% but none specific.
- Spleen histology: characteristic with nodular infiltration involving the white pulp (cf. HCL).

### **Lymphoplasmacytic lymphoma (LPC-L)/Waldenström macroglobulinaemia**

Median age 60–65 years. Median survival 6.5 years.

Usually presents with isolated lymphadenopathy  $\pm$  serum paraprotein, usually IgM (Waldenström). Depending on level of paraprotein symptoms of hyperviscosity (if markedly ↑). BM infiltration common with small to medium-sized lymphocytes with lymphoplasmacytoid differentiation.

#### *International Prognostic Scoring System (IPSS) for Waldenström macroglobulinaemia (IPSSWM)*

Includes 5 (adverse) covariates: age >65 years, haemoglobin  $\leq 115$ g/L, platelet count  $\leq 100 \times 10^9$ /L,  $B_2$ -microglobulin >3mg/L, serum monoclonal protein concentration >70g/L.

The risk categories are of prognostic value (low:  $\leq 1$  adverse variable except age; intermediate: 2 adverse characteristics or age >65 years; high: >2 adverse characteristics) with 5-year OS 87%, 68%, and 36% respectively.

See ↻ Waldenström macroglobulinaemia, pp.368–377 for more details.

### Immunohistochemical and cytogenetic features

#### Follicular lymphomas

Pan-B markers; CD5–, CD10+, BCL-2+. t(14;18)(q32;q21) in 90% (cf. reactive lymphoid hyperplasia with normal follicles BCL-2–).

#### Small lymphocytic lymphoma

Pan-B markers (CD20+, CD79a+); CD5+, weak slg, CD23+, cyclin-D1– (cf. MCL).

#### Marginal zone lymphoma

Pan-B markers; CD5–, CD10–, BCL-2–. MALT t(11,18)(q21;q21) 50%; t(1;14)(p22;q32) rare, overexpress BCL-10 and poor response to *H. pylori* eradication; SMZL allelic loss at 7q21–32 in up to 40%.

#### Splenic marginal zone lymphoma

CD19+, CD20+, CD79b+, FMC7+, moderate-to-strong Smlg+; CD10–; most CD23–; usually CD5– CD25–; 20% CD25+ but CD11c– & CD103– differentiating from HCL; NB some DBA44+; 20% CD5+ but CD23– CD79b+ differentiating from CLL; cyclin D1– differentiates CD5+ SMZL from MCL.

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## Treatment of indolent lymphoma

Clinical trial entry where possible.

FL and SLL comprise the majority of patients. Both diseases are usually responsive to chemotherapy and radiotherapy but unless truly localized, incurable with inevitable recurrence. There is no firm evidence of a curative therapy for advanced disease. Many patients are asymptomatic and require no treatment initially. The decision to initiate treatment and the treatment chosen must take account of individual quality of life issues. At each stage, pros and cons of treatment options should be shared with the patient to reach an agreed treatment decision. Median OS with traditional treatment 8–10 years.

### Initial treatment of localized FL and SLL (stage I/II)

Involved field radiotherapy (35–40Gy) may be curative in the 15–30% of patients with truly localized disease. 5-year DFS >50% may be expected. Recurrence generally occurs outside radiation field. Late relapses occur. OS ~80% at 5 years, ~60% at 10 years. Addition of chemotherapy improves DFS but not OS.

### Initial management of advanced FL and SLL (stage III/IV)

Note histological grade 3b FL is usually treated like DLBCL.

Management of newly diagnosed patients involves a decision between ‘watchful waiting’ or conventional chemotherapy and/or radiotherapy. Symptomatic patients require treatment at diagnosis. ‘Watchful waiting’ is appropriate in asymptomatic patients, based on studies showing similar overall survival but better ‘quality of life’ in asymptomatic patients in whom therapy was deferred until indicated, compared to those in whom therapy was initiated at diagnosis.

#### ‘Watchful waiting’

Therapy may be deferred for many months/years after diagnosis in asymptomatic patients with low bulk disease until clinical symptoms or complications develop. Patients may have better QoL and avoid exposure to cytotoxic agents but must be monitored closely (every 3 months for 1 year then every 3–6 months) to identify progression or complications promptly. OS >80% at 5 years.

#### Indications for treatment (patient specific)

- B symptoms: unexplained weight loss, fever, or night sweats.
- Cytopenia.
- Bulky disease.
- Steady progression or threatened end-organ function.
- Evidence of transformation.
- Other patient-specific features such as local compression.

## Therapy

Established treatments:

- *Chemoimmunotherapy*: standard treatment includes a combination of rituximab (humanized monoclonal anti-CD20 antibody) with chemotherapy. ‘R-chemo’ regimens (R-CVP, R-CHOP, R-FC, R-MCP)

enhance OR and CR rates, DFS, and OS in newly diagnosed patients with indolent NHL compared to chemotherapy.

- **R-CVP:** addition of rituximab to CVP improves responses (OR 81% vs 57%; CR 41% vs 10%; OS 83% vs 77%).<sup>1</sup>
- **R-Chlorambucil (CBL):** possibly superseded as 1st choice in FL but still has a place in elderly patients; remains widely used in SLL in UK. Convenient oral regimen, well tolerated but prolonged or repeated use can cause myelosuppression.
- **Rituximab monotherapy:** Suitable for patients who cannot tolerate chemotherapy. 375mg/m<sup>2</sup> infusion weekly × 4 achieves 72% OR, 36% CR rates in untreated patients and median TTP 2 years.<sup>2</sup> Toxicity is mild. Risk of anaphylaxis with 1st infusion.
- **High-grade lymphoma regimens/R-CHOP:** high CR rates (~60%), but the continuous pattern of relapse and OS not convincingly improved in indolent FL (histological grades I and II). FL histological 'grade 3b' treated as 'high-grade' with R-CHOP; achieves responses equivalent to DLBCL but higher relapse rate. R-CHOP is widely used in USA as 1st-line therapy for FL.
- **Purine analogues combinations (R-FC, FMD):** fludarabine has OR rate of ~75% (~40% CRs) in untreated patients with FL. Combinations with rituximab and cyclophosphamide increases response rates (>90% CRs) and response duration. Combinations with mitoxantrone achieve similar response rates. In SLL fludarabine combinations achieve higher response rates and durations. Prophylaxis of PCP (*Pneumocystis jirovecii*) is required (e.g. co-trimoxazole) during and for 6 months after therapy as well as aciclovir prophylaxis. Cellular blood products must be irradiated. FCR is widely used for SLL in USA as well as the UK.
- **Maintenance rituximab:** a number of maintenance schedules demonstrate prolonged DFS after R-chemotherapy as induction. Median PFS 51.5 months vs 14.9 months with observation. R maintenance also improved OS: 85% at 3 years vs 77% with observation. Standard 375mg/m<sup>2</sup> every 2 months in first response; every 3 months in 2nd response for a duration of 2 years.
- **Bendamustine (R-bendamustine):** Generally well tolerated. Similar OS rated but prolonged DFS compared to other regimens. Fast increasing in 'popularity', though an older drug.
- **Radiotherapy** may be useful for treatment of local problems due to bulky disease, e.g. cord compression. Used with curative intent in truly localized FL/SLL.
- **Radioimmunotherapy:** tositumomab, <sup>131</sup>I-radiolabelled murine anti-CD20 monoclonal antibody; high response rates (95% OR and 75% CR) in untreated patients with median PFS >6 years;<sup>3</sup> up to 68% OR and 38% CR in treated patients. Current role only in early 'salvage' in chemo- or rituximab-resistant patients. Ibritumomab tiuxetan <sup>90</sup>Yt-labelled murine anti-CD20 monoclonal antibody; OR 74%, CR 15%. Role in relapsed FL refractory to rituximab.

### Treatment of relapsed FL/SLL

Relapsed asymptomatic disease is not necessarily an indication for further treatment. When indolent lymphoma progresses after partial or complete response to initial therapy, it is important to rule out transformation to DLBCL (esp. if LDH ↑, LN rapidly enlarging, constitutional symptoms, extranodal disease). Therapy should be chosen in the context of the patient's goals (palliative vs potentially curative), performance status and previous treatment(s) used, and their response(s). Treatment options are similar as for 1st-line treatments. Generally a chemotherapy treatment given before is not used again; exceptions might be where a very durable remission was achieved). Further responses are seen mainly in patients who achieved a previous durable response (>12 months). Resistance to treatment ultimately develops.

#### *Autologous SCT*

Considered in 2nd remission in young and otherwise fit patients. Auto-SCT achieves improved DFS at 5 years. Continuing risk of relapse. Risk of myelodysplasia and 2nd malignancy. Only recommended in 2nd (complete) remission; 1st CR auto-SCT remains investigational. Auto-SCT may offer younger patients with relapsed FL a survival advantage with a 4-year OS 71–77% vs 46%.<sup>4</sup> Not curative.

#### *Allogeneic SCT*

Potential cure though ↑ morbidity and mortality. Most evidence on the use of TBI-containing conditioning. CR rates >80% and relapse rates lower than after autograft (12% vs 55% at 5 years) and very few after 2 years. Some patients may indeed be cured. The benefit of better disease control is offset by a higher treatment-related mortality. Reduced-intensity conditioning ('mini'-allogeneic SCT) reduces toxicity, preserves the GvL effect, and widens the availability of this treatment. Allo-SCT should be considered in appropriate patients with poor prognosis disease, e.g. 1<sup>o</sup> refractory or early relapse after R-chemo. Allo-SCT using reduced intensity conditioning has achieved 73% OS and 65% PFS at 3 years.<sup>5</sup>

### High-grade transformation

~25% may transform to high-grade DLBCL. All low-grade NHLs can transform to high-grade lymphoma. Clinical suspicion if LDH ↑, LN rapidly enlarging, constitutional symptoms, or presence of extranodal disease.

~3.5 years median time to transformation in FL; 15% risk at 10 years, 22% risk at 15 years. ↑ risk with grade 3 histology and high-risk FLIPI score.<sup>6</sup>

Other risk factors are low albumin, high  $\beta_2$ -microglobulin, and absence of CR with initial therapy. Course is progressive, response to treatment poor, and survival short (median 1.2 year). Treatment is dependent on prior therapy. Generally high-grade NHL treatment schedules used  $\pm$  high-dose therapy and auto-SCT (~50% CR rate). Prognosis is generally poor and palliation may be appropriate.

## Treatment of MZL and SMZL

### Gastric MALT lymphoma (extranodal MZL)

- Generally stage I<sub>E</sub>; eradication of *H. pylori* (as per local guidelines. An often used regimen includes clarithromycin, amoxicillin, and omeprazole for 2 weeks): achieves CR in up to 80% of patients with 80% OS at 10 years. Regression of lesions can take 3–18 months so restaging should be done with this in mind. Re-staging at appropriate intervals (yearly once remission achieved). Regimen should be repeated if still *H. pylori* +ve and no progression. Late relapses can occur.
- Chlorambucil ± rituximab or local radiotherapy can achieve CR in non-responders (frequent in t(11;18) +ve), those who progress and *H. pylori* -ve patients.
- For more widespread disease with symptoms, non-responders, or recurrence post RT, treat as for stage III/IV indolent NHL.

### Non-gastric MALT lymphomas

Simple combination regimens, chlorambucil or local radiotherapy (20–30 Gy) generally achieve good responses; otherwise manage as for FL.

Nodal MZL: therapy similar to FL.

### Splenic MZL

- Asymptomatic patients with modest splenomegaly and no significant cytopenias can be observed.
- Splenectomy is recommended for bulky enlargement or hypersplenism (and/or to confirm diagnosis in some cases) and can correct cytopenias and associated symptoms for >5 years.
- Splenic irradiation is a safe and effective alternative to splenectomy in some cases and may be repeated.
- Progression after splenectomy may respond to chlorambucil (44% response rate; no CRs), fludarabine (very high response rates in small studies), and/or rituximab (very high response rates in small studies). Toxic effects with purine analogues may be marked in elderly patients; all patients should receive prophylactic co-trimoxazole and irradiated blood products.
- Treatment of transformation should follow that of DLBCL.

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## Aggressive lymphomas

~50% of cases of lymphoma; rapidly progressive if untreated. Patients require immediate treatment if appropriate. IPI can be used for prognostication.

### B-cell lymphomas

*Diffuse large B-cell lymphoma (DLBCL)*: most common lymphoma worldwide; ~30% of all lymphomas; occurs at all ages, generally >40 years; presents with localized stage I or II disease in 50% of patients but disseminated extranodal disease is not uncommon; constitutional symptoms in 33%; extranodal sites in 30–40%. CNS involvement more frequent in patients with testicular or BM involvement. Ascites and pleural effusions are common end-stage symptoms.

*T-cell rich B-cell lymphoma*: subtype of DLBCL; occurs in younger patients; more aggressive with early BM involvement.

*Mediastinal large B-cell lymphoma*: typically occurs in women <30 years; Anterior mediastinal mass sometimes causes superior vena cava obstruction; tendency to disseminate to other extranodal sites including CNS; cure rate with R-CHOP therapy similar to DLBCL.

*Mantle cell lymphoma (MCL)*: 4–8% of NHL; median 63 years; ♂:♀ ratio 4:1; B symptoms 50%; commonly advanced disease at presentation with generalized lymphadenopathy (57%), splenomegaly (47%), hepatomegaly (18%); extranodal involvement, particularly in GI tract is common (18%). Stage IV disease at diagnosis in 70%.

- Lymphocytosis in peripheral blood common (38%). Intermediate size lymphocytes; nucleus often has clefts and indentations; smear cells unusual.
- BM trephine biopsy demonstrates involvement in >70% with nodular, paratrabeular, interstitial, or diffuse patterns.
- Lymph node biopsy: mantle zone expansion or diffuse effacement of nodal architecture by uniform 'centrocytes'.
- Most patients have elevated LDH and serum  $\beta_2$ -microglobulin. Up to 30% have detectable paraprotein band, usually IgM.
- Poor therapeutic outcome: partial responses and eventual chemoresistance.
- Median survival 3–4 years.
- Improved remission rates and OS with schedules containing high-dose cytarabine and auto-SCT in 1st remission in younger patients.
- In some patients an indolent course is observed.

### T-cell lymphomas

*Peripheral T-cell lymphomas (PTCL)*: several subtypes. Mature T-cell lymphomas; median age 56; ♂:♀ ratio 2:1; variable clinical behaviour; nodal form more common in the West; more aggressive and less responsive to therapy than DLBCL. Heterogeneous group but poor prognosis overall; 80% stage III–IV at diagnosis; constitutional symptoms, BM and skin involvement common; 30–40% 5-year OS with combination therapy; IPI has predictive value, though more specific T-cell prognostic scoring systems exist. PTCL-unspecified most common form; extranodal



forms specified (common in Asia, particularly EBV+ nasal type with nasal obstruction, discharge, and facial swelling).

**Enteropathy type T-cell lymphoma:** PTCL subtype; occurs in gluten-sensitive enteropathy; abdominal pain, weight loss, small bowel obstruction and perforation; poor prognosis, median OS 7.5 months.

**Angio-immunoblastic T-cell lymphoma (AITL):** median 57–68 years; constitutional symptoms, generalized lymphadenopathy, hepatosplenomegaly, pruritic skin rash, arthritis, polyclonal hypergammaglobulinaemia, DAT+ve haemolytic anaemia, cryoglobulinaemia, cold agglutinins, and eosinophilia common; 33% progress to immunoblastic lymphoma; poor prognosis.

**Anaplastic large cell lymphoma (ALCL):** 3 subtypes. Systemic ALK+ ALCL: usually younger patients and children; typically lymphadenopathy at single site; (median age <30 years and chemosensitive); systemic ALK-ALCL: median age 50–60; unfavourable; 1° cutaneous ALK- ALCL: better prognosis, may spontaneously resolve.

**Mycosis fungoides:** mature T-cell lymphoma; presents as localized or generalized plaque or erythroderma; lymphadenopathy in 50%; median survival 10 years but prognosis poor with lymphadenopathy. Once PB/BM involvement: Sézary syndrome.

**Sézary syndrome:** leukaemic phase of a low-grade 1° cutaneous mature T-cell lymphoma (mycosis fungoides). See next section.

### Sézary syndrome

Occurs in up to 20% of cases of 1° cutaneous T-cell lymphoma (mycosis fungoides); median age 52 years; ♂:♀ ratio 2:1. Leukaemic phase of a low-grade 1° cutaneous mature T-cell lymphoma (mycosis fungoides).

#### Clinical

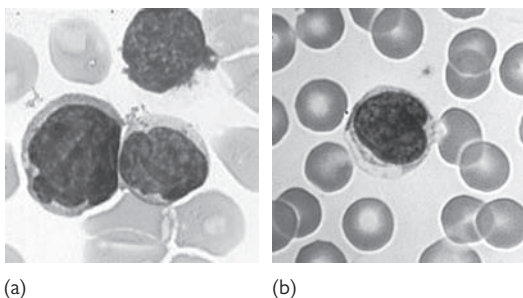
- Generally diagnosed as a result of FBC in a patient with exfoliative erythroderma; not necessarily end stage and may present *de novo*.
- MF classically progresses through eczematoid plaque stage, infiltrative plaque stage, and overt tumour stage and has characteristic histology on skin biopsy (epidermotropism and Pautrier microabscesses).

#### Investigations and diagnosis

- FBC: generally moderate leucocytosis (WBC rarely  $>20 \times 10^9/L$ ); Hb and platelets usually normal.
- Blood film: typically reveals large numbers of large lymphoid cells with characteristic 'cerebriform' folded nucleus (see Fig. 5.1).

#### Prognosis

Patients with Sézary syndrome have a poor prognosis. High Sézary cell count, loss of CD5 and CD7, and chromosomal abnormalities associated with poor outcome. Median survival 6–8 months.



**Fig. 5.1** Blood film in Sézary syndrome showing typical cerebriform nuclei. Image (b) is reproduced with permission from Souhami, R.L. et al. (2002). *Oxford Textbook of Oncology*, 2e. Oxford University Press, Oxford (see colour plate section).

### Very aggressive lymphomas

~5–10% cases of NHL; require immediate and aggressive treatment.

**Endemic Burkitt lymphoma:** presents in childhood/adolescence; mainly seen in Africa with large extranodal tumours in jaw or abdominal viscera; 90% associated with EBV infection; aggressive but curable disease.

**Sporadic Burkitt lymphoma:** median age 31 years; presents with rapidly growing lymphadenopathy, often intra-abdominal mass; BM, CNS, and blood involvement frequent; 30% associated with EBV infection; associated with HIV infection; aggressive but curable disease.

**Lymphoblastic lymphoma:** median age 15 years; >50% of childhood lymphomas; share features with ALL; T-cell LBL more frequent (85%) and usually associated with thymic mass; 33–50% present with BM involvement; CNS involvement common; commonly progresses to ALL; aggressive but potentially curable in children. Treatment the same as for ALL.

### HIV-associated lymphoma

Burkitt lymphoma, DLBCL and 1° CNS lymphoma are most common, and mostly seen in patients with very low CD4 counts. Incidence of NHL has ↓ as a result of highly active antiretroviral therapy (HAART). Hodgkin lymphoma and indolent lymphoma less frequent seen. Chemotherapy schedules used are identical to non-HIV patients. Administer HAART, prophylactic IT chemotherapy, and full-dose regimens with G-CSF as 1° prophylaxis.

### Post-transplantation LPD (PTLD)

Occurs in <2% of organ transplant recipients. Risk highest in heart–lung recipients. Monomorphic DLBCL most common; associated with immunosuppressive therapy (esp. ATG) and EBV (main risk EBV mismatched transplant). Tends to involve donor organ and multiple extranodal sites.

Treatment ranges from reduction of immunosuppression (early lesions, hyperplasia and polymorphic PTL), single agent rituximab (achieves OR ≤75%, CR ≤69%) to more intense immunochemotherapy schedules (monomorphic/HL subtypes) depending on subtype and morphology on biopsy.

**Different subtypes**

- Early lesions.
- Plasmacytic hyperplasia.
- Infectious mononucleosis—like PTLD.
- Polymorphic PTLD.
- Monomorphic PTLD (B and T/NK cell types).
- Classical HL-type PTLD.

**Immunohistochemical and cytogenetic features**

**Diffuse large B-cell lymphoma:**<sup>1</sup> pan-B markers (CD20+, CD79a+), generally slg+; Ki67 <90% favours DLBCL (cf. Burkitt >99%). 2 subgroups delineated by gene expression profiling using DNA microarrays: germinal centre-B-cell profile (GCB subtype: favourable; ~60% 5-year OS) and activated B-cell profile (ABC subtype: unfavourable; ~30% 5-year OS). Del(3)(q27) involving *BCL-6* gene mutations 35%; t(14;18) 25%; t(8;14) 15%.

**Mediastinal large B-cell lymphoma:** pan-B markers (CD20+, CD79a+), often CD30+; slg-; gene expression profile similar to classical HL.

**Mantle cell lymphoma:** pan-B markers; CD5+, strong slg, CD23-, cyclin-D1. t(11;14)(q13;q32) involves *IgH* gene on chromosome 14, juxtaposes and dysregulates *BCL-1* with overexpression of cyclin D1, a protein involved in cell cycle regulation, seen in 70%.

**Mature (peripheral) T-cell lymphomas:** pan-T markers (CD3+, CD2+); TdT- (cf. precursor T-lymphoblastic TdT+); Ki-67 has prognostic value.

**Angioimmunoblastic T-cell lymphoma:** characteristic PAS+ arborizing venules in LN; pan-T markers, CD4+, TdT-, CD10+, sometimes *BCL-6*+, *CXCL13* overexpression; B-cells are EBV+.

**Anaplastic large cell lymphoma:** pan-T markers but often CD3-; CD4+; TdT-, CD30+, ALK+, or ALK-. t(2;5)(p23;q35) in 60%. 1° cutaneous always ALK-.

**Mycosis fungoides and Sézary Syndrome**

Immunophenotyping: CD2+, CD3+, CD4+, CD5+, CD7-, CD8-, CD25- T cells. Cytogenetics: no typical pattern.

**Burkitt lymphoma**

Pan-B markers, CD10+, slg+, Ki67 >99% (cf. DLBCL <90%), TdT-. 80% t(8;14)(q24;q32), 15% t(2;8)(q11;q24), 5% t(8;22)(q24;q11) juxtapose *c-MYC* with *Ig* gene loci and cause *MYC* overexpression. Endemic cases have raised antibody titres to EBV antigens and multiple copies of EBV DNA in tumour (unusual in sporadic cases). Presence of t(14;18) associated with poor response to therapy.

**Precursor B-lymphoblastic:** pan-B markers, slg-, CD10+, TdT+; variable cytogenetics.

**Precursor T-lymphoblastic:** pan-T markers, TdT+; variable cytogenetics.

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## Initial treatment of aggressive lymphomas

Clinical trial entry where possible.

T-cell rich B-cell lymphoma and 1° mediastinal B-cell lymphoma treated as DLBCL.

### Localized DLBCL

Stage I and non-bulky (mass <10cm) stage II disease without adverse prognostic factors:

- 6 × R-CHOP (likely superior to CHOP, although stage I disease excluded from most trials) or R-CHOP × 3 plus involved field radiotherapy (IF-RT); PFS and OS >90%.<sup>2</sup>
- Patients with bulky disease and/or local extranodal disease: treated with 6 courses of R-CHOP plus IF-RT (30–40 Gy).<sup>1</sup>
- Patients with localized testicular DLBCL should have an orchidectomy, receive 6 courses of R-CHOP with IT CNS prophylaxis, followed by contralateral testicular RT. In the UK rituximab in stage I disease is not NICE approved.

### Advanced stage DLBCL

R-CHOP: Standard therapy if patient is able to tolerate. 6 cycles every 14d or 21d. Addition of rituximab has improved the CR rate (76% vs 63%), EFS (57% vs 38%), and OS (70% vs 57%) at 2 years with no added toxicity.<sup>2</sup> This was sustained on longer follow-up (5-year OS 58% vs 45%)<sup>3</sup> and confirmed in younger patients with DLBCL (3-year EFS 79% vs 59%).<sup>4</sup> Easy administration and generally well tolerated.

- Patients with testicular, sinus, BM, or epidural involvement should receive concomitant CNS directed IT chemotherapy prophylaxis (IT MTX × 3–6). Some groups use a high IPI as requiring CNS prophylaxis. All data on CNS relapse of DLBCL stems from the pre-rituximab era and views on CNS prophylaxis (agent, number of administrations, and in which patients) vary.
- Consider adjuvant RT to any site of bulk disease (>10cm) at diagnosis. Pros and cons of site of disease and age of patient need to be taken into consideration.
- Data do not support maintenance rituximab.
- Intensification of initial therapy remains under investigation (R-EPOCH).

### Response assessment

Evaluate response to initial therapy mid-treatment (unless response clinically apparent) by CT or PET/CT to identify non-response or progression despite therapy. Complete 6 courses if ≥50% PR has been achieved. Repeat all +ve interim scans on completion of therapy. If CR is not achieved further treatment should be considered.

### Follow-up

Patients who achieve CR should be reviewed every 3 months for 24 months then every 6 months for 36 months.<sup>1</sup>

## Mantle cell lymphoma

Incurable with conventional therapy.

*Not fit for intensive treatment:* chlorambucil, CVP, FCR, or CHOP + rituximab may be appropriate for patients not fit for more intensive therapy. CHOP: OR rate ~80% (CR ~50%) with PFS <18 months and OS 3 years. Addition of rituximab improves responses.

*Fit for intensive treatment*

- NORDIC schedule: alternating R-maxi-CHOP × 3 and HD cytarabine × 3 followed by BEAM autograft (maxi-CHOP: cyclophosphamide 1200mg/m<sup>2</sup>, doxorubicin 75mg/m<sup>2</sup>, vincristine 1.4mg/m<sup>2</sup> (max. 2mg), prednisolone 100mg, HD cytarabine: 3000mg/m<sup>2</sup> 4 doses per cycle (2000mg/m<sup>2</sup> if >60 years). *Standard treatment for fit patients.* Overall and complete response 96% and 54% respectively. 6-year overall, event-free, and progression-free survival 70%, 56%, and 66%, respectively, with no relapses occurring after 5 years.
- R-HyperCVAD alternating with MTX and cytarabine: very intensive with high toxicity: 89%CR; 3-year FFS 80% in patients <65 years.<sup>5</sup>
- R-EPOCH.<sup>6</sup>

*Maintenance rituximab:* improvement of RR and OS with addition of maintenance rituximab. Now generally recommended.

*Bortezomib:* proteasome inhibitor; single agent (1.3mg/m<sup>2</sup> SC d1, 4, 8, and 11 in 21d cycle × 8 cycles) achieves 31% OR in relapsed or refractory MCL; median duration 9 months.

*Thalidomide and lenalidomide:* show single-agent activity; in trials in combination with chemotherapy and rituximab. May be suitable for older patients.

*SCT:* high-dose treatment and autologous SCT recommended in 1st CR. Suitable patients with a sibling allogeneic SCT option should be considered for an allograft. Relapsed patients with MCL who have not received a SCT and respond to salvage therapy should be considered for SCT.

## T/NK cell lymphoma and MF/Sézary syndrome

No consensus on optimal therapy.<sup>7</sup> Both CHOP and gemcitabine-based regimens used. SCT should be considered in 1st CR.

- *ALK+ ALCL:* CHOP × 6. Very chemosensitive (5-year OS 60–90% following CHOP-type therapy). Consider additional IF-RT for stage I/II disease. SCT reserved for relapse in eligible patients.
- *ALK- ALCL:* CHOP × 6. Less chemosensitive (11–46% 5-year OS) and for stage III/IV consideration should be given to SCT consolidation in 1st CR or in salvage setting.
- *PTCLU:* inferior outcome to CHOP: 5-year OS <30%; SCT consolidation should be considered for stage III/IV in 1st CR.
- *AITL:* CHOP-type therapy can achieve 50–70% CR but 5-year OS only 10–30% due to early relapse or infective deaths. Consider SCT in 1st CR for stage III/IV.
- *Nasal NK/T lymphomas:* may respond to local RT plus chemotherapy and CNS prophylaxis.

- *MF/Sézary syndrome*: there is no evidence that single-agent or combination chemotherapy improves survival. High response rates achieved but short durations. Treatments used: phototherapy, bexarotene, vorinostat, liposomal doxorubicin, CHOP, gemcitabine. Allogeneic SCT should be considered in younger patients.

### Very aggressive lymphomas

These lymphomas display an exponential growth rate, a tendency to involve BM and meninges. There is a high risk of tumour lysis and pre-treatment hydration plus rasburicase are essential.

#### *Non-endemic Burkitt lymphoma (BL)*

High remission rates (90% EFS) and long-term DFS achieved with intensive short duration (3–6 months) multiagent chemotherapy regimens including high-dose MTX, high-dose cytarabine, etoposide, ifosfamide, and CNS-directed prophylaxis. Protocols designed for lymphoblastic lymphoma or ALL are inferior to specific BL protocols such as (R-) CODOX-M/IVAC. The role of high-dose therapy/SCT is uncertain but should be considered in relapsed setting. Patients who achieve CR should be reviewed every 3 months for 24 months then every 6 months for 36 months.

#### *Lymphoblastic lymphoma*

Based on ALL treatment. Management in adults follows more successful intensive regimens in childhood (including CNS prophylaxis and maintenance). High CR rates (~85%) and 5-year DFS up to 45% reported. High relapse rates in adults; SCT (autologous/allogeneic) should be considered in early management in eligible adult patients. Consider allograft in eligible relapsed patients.

*Adult T-leukaemia/lymphoma*: see ↻ p.178.

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## Salvage therapy in aggressive lymphomas

Patients who relapse or fail to achieve remission with initial therapy have a poor prognosis. Without effective 2nd-line (salvage) therapy, almost all die of progressive lymphoma in a median period of 3–4 months. A significant proportion of patients with DLBCL who relapse from 1st CR can be salvaged but prognosis for 1<sup>o</sup> refractory NHL is very poor.

### Conventional salvage chemotherapy

Several salvage schedules are available with similar response rates and none is clearly superior. There is therefore no 'standard' treatment and there will be geographical and institutional differences. Generally rituximab is added to salvage regimens although the evidence base in mainly the refractory setting for this is lacking.

Schedules used: R-DHAP, R-EPOCH, R-ESHAP, R-ICE, R-mini-BEAM.

Eligible patients (age <65 years) who respond to 2nd-line therapy (PR or CR) should receive high-dose therapy (e.g. BEAM or LEAM) followed by autologous SCT. ~50% of those achieving CR are long-term disease-free and may be cured.<sup>1</sup>

### High-dose therapy (HDT) and SCT

HDT followed by peripheral blood stem cell harvest (PBSCH) and autologous SCT is used to treat patients with relapsed or refractory aggressive or very aggressive NHL. In DLBCL the overall survival of 53%. This is clearly superior to conventional dosage salvage therapy (5-year EFS 12%; OS 32%).<sup>2</sup> Best results are obtained when lymphoma still responsive to conventional dosage therapy and SCT performed in a state of minimal residual disease. HDT is generally preceded by 2–3 cycles of salvage chemotherapy.

Syngeneic and allogeneic transplants have been used less frequently but are associated with cures. Patients who relapse after autologous SCT can sometimes be salvaged with an allogeneic SCT.

### New agents

Bortezomib, lenalidomide, mTOR inhibitors, other monoclonal antibodies, and targeted treatment are in ongoing clinical trials in either combination or as single agents.

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## Response criteria for lymphoma

See Table 5.8 for the response criteria.

**Table 5.8** Response criteria for lymphoma (including PET)<sup>1</sup>

Response	Definition	Nodal masses	Spleen, liver	BM
Complete response (CR)	Disappearance of all evidence of disease	(a) FDG-avid or PET +ve prior to therapy: mass of any size permitted if PET -ve; (b) Variably FDG-avid or PET -ve: regression to ↔ size on CT	Not palpable, nodules disappeared	Infiltrate cleared on repeat biopsy; if indeterminate by morphology, immunohistochemistry should be -ve
Partial response (PR)	Regression of measurable disease and no new sites	50% decrease in SPD of up to 6 largest dominant masses; no increase in size of other nodes (a) FDG-avid or PET +ve prior to therapy: ≥1 +ve at previously involved site (b) Variably FDG-avid or PET -ve: regression on CT	50% decrease in SPD of nodules (for single nodule in greatest transverse diameter); no increase in size of liver or spleen	Irrelevant if +ve prior to therapy; cell type should be specified.
Stable disease (SD)	Failure to achieve CR/PR or PD	(a) FDG-avid or PET +ve prior to therapy; PET +ve at prior sites of disease and no new sites on CT or PET (b) Variably FDG-avid or PET -ve; no change in size of previous lesions on CT		
Relapsed disease or progressive disease (PD)	Any new lesion or increase 50% of previously involved sites from nadir	Appearance of a new lesion(s) >1.5cm in any axis, 50% increase in SPD of >1 node, or 50% increase in longest diameter of a previously identified node >1cm in short axis. Lesions PET +ve if FDG-avid lymphoma or PET +ve prior to therapy	>50% increase from nadir in the SPD of any previous lesions	New or recurrent involvement

### Reference

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## Role of FDG-PET scanning in NHL diagnosis and treatment<sup>1</sup>

CT is a reliable technique for pre-treatment staging but is inadequate for assessment of patients with apparent incomplete resolution of, usually previously bulky, nodal sites after therapy. Pre-treatment PET/CT is not currently standard but does facilitate interpretation of equivocal post-therapy scans especially DLBCL. At restaging a +ve PET scan is predictive of relapse in DLBCL (~85%) and the -ve predictive value is strong (85%).

PET is more accurate than CT for response assessment. Different NHL subtypes have different/variable FDG uptake. DLBCL, BL & MCL are generally FDG-avid; indolent lymphomas are generally much less avid. T-NHL has variable FDG-avidity. PET/CT might have a role in diagnosing/excluding high-grade transformation seen in low-grade NHL.

False +ve avidity occurs due to inflammation, infection, granulomatous disease, brown fat, thymic hyperplasia, marrow and splenic hyperplasia after G-CSF, previous radiotherapy. A gap of at least 3 weeks after chemotherapy and 8–12 weeks after radiotherapy is recommended.

False -ve findings occur due to lesions below scanner resolution (5–10mm), too brief an interval between injection and scan (2h recommended), masking of tumour by physiological uptake by brain, heart, renal or GI tract, and in case of hyperglycaemia reducing uptake by tumour.

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## CNS lymphoma

### 1° CNS lymphoma (PCNSL)<sup>1-3</sup>

2–3% of NHL cases; 3% of all brain tumours.

- Incidence ↑ 3 × 1990s and early 2000 partly due to the HIV pandemic (occurs mainly when CD4s < 50/μL; Incidence is now falling due to early intervention and success of HAART.
- Non-HIV related PCNSL occurs in ages 55–70 years; Usually classified as a DLBCL, occasionally BL-like.
- Commonly involving the frontal lobes, corpus callosum, or deep periventricular structures; may involve eyes, meninges, or spinal cord; Simultaneous systemic lymphoma uncommon.
- Commonly presents with focal neurological deficit, cognitive or personality change, symptoms of raised intracranial pressure (headache and vomiting); 10% seizures; cauda equina syndrome; spinal cord disease causes neck or back pain or myelopathy.
- LP (if safe, exclude raised ICP first) for CSF cytology gives variable results: flow cytometry and PCR analysis for Ig gene re-arrangement may help.
- Imaging: MRI or CT. No role for PET/CT.
- Diagnosis by stereotactic needle biopsy. Note: diagnostic accuracy is improved by avoiding routine use of steroids for cerebral oedema; steroids should therefore only be used in emergency.
- 50% relapse risk; most relapses in CNS, others mainly leptomeningeal and ocular, <10% systemic.

### Treatment

Systemic treatment has largely superseded IT treatment due to adequate penetration of blood–brain barrier with high doses of chemotherapy.

*Unfit for systemic chemotherapy:* steroids and whole-brain radiotherapy (WBRT); palliative approach.

*Fit for systemic chemotherapy:*

- Optimal schedule remains unclear but in patients fit for intensive chemotherapy, the schedule should include methotrexate in adequate dose (>3g/m<sup>2</sup>) with folinic acid rescue.
- Addition of HD Cytarabine (2000 mg/m<sup>2</sup> for 4 doses) is recommended. Improves RR but no documented OS as yet.
- <60yr addition of WBRT is recommended
- >60yrs no WBRT or attenuated dose WBRT recommended. Delayed neurotoxicity post-WBRT is common especially in older patients (5-year cumulative incidence 24%): progressive subcortical dementia, psychomotor slowing, memory dysfunction, behavioural changes, ataxia, urinary dysfunction, and incontinence.
- Consolidation of response to high-dose MTX-based therapy (without WBRT) with thiotepa-based high-dose treatment and auto-SCT has produced prolonged responses in patients with poor prognostic features and can be considered.
- Patients who relapse after chemotherapy/SCT may respond again to WBRT if not previously administered.

### Ocular lymphoma

- Intraocular lymphoma presents as ocular floaters and blurred vision; It can progress to involve both eyes; 50% develop brain involvement; 15% of PCNSL develop concurrent or later eye involvement;
- Standard therapy is fractionated radiotherapy (35–40 Gy over 5 weeks) to globe and contralateral globe with consideration of systemic chemotherapy (including high-dose MTX) to prevent CNS relapse. See ↻ Primary CNS lymphoma (PCNSL), ↻ p.211.

### 2° CNS lymphoma

- Systemic NHL with CNS involvement or CNS relapse post-treatment for systemic NHL.
- Usually meningeal involvement; occurs in up to 10% of cases of NHL.
- Poor prognosis; median survival <3 months.
- In fit patients a treatment schedule similar to that used in PCNSL can be considered.

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## Hodgkin lymphoma (HL)

First described by Thomas Hodgkin in 1832. Clinical presentation varies but often asymptomatic. Treatment decisions are preferably made in a multidisciplinary setting.

### Incidence

- 1% cancer registrations per annum. Annual incidence ~3 per 100,000 in Europe and USA (less common in China and Japan).
- Bimodal age incidence—major peak between 20 and 29 years and 2nd smaller peak at 60 years; median age 35; >90% of cases age 16–65.
- Overall higher incidence in ♂. Nodular sclerosing (NS) histology is most common subtype in young adults (>75% of NS cases are <40 years) and has a ♀ preponderance.

### Risk factors

- Familial: 99 × ↑ risk in identical twins; 7 × risk for siblings of young adults (no ↑ for sibs of older adults); ?genetic or environmental effect.
- EBV: 4 × ↑ risk of HL in individuals with history of infectious mononucleosis with median interval of 4.1 years;<sup>1</sup> EBV encoded nuclear RNAs detected in Reed–Sternberg (RS) cells; 26–50% cases +ve for EBV by molecular analysis (esp. mixed cellularity (MC) HL).
- HIV: ~8 × ↑ risk of HL; usually when good CD4 counts (usually patients with known diagnosis and on HAART); usually EBV+; associated with advanced stage at presentation, unusual sites, and subsequent poorer outcome.

### Pathophysiology

Cell of origin historically controversial. Single-cell PCR for Ig gene amplification demonstrated that the neoplastic cells in HL are clonal germinal centre (GC) or post-GC B-lymphocytes. HL therefore considered B-cell lymphomas.

- RS cells: seen in classical HL: appear to be transformed post-germinal centre B-cells with re-arranged but non-expressed Ig genes (due to crippling mutations) destined for apoptosis that survive through several mechanisms including latent EBV infection, NF-κB expression and a permissive inflammatory cell infiltrate.
- Lymphocytic and histiocytic (L&H) ‘popcorn’ cells in nodular lymphocyte-predominant HL are neoplastic germinal centre B cells that express BCL-6; frequently have translocations involving bcl-6; usually EBV –ve.

### Histology and classification

The WHO classification puts HL into 2 distinct entities (↔ Table 5.1, pp.183 and 184): classical HL and nodular lymphocyte-predominant HL. Both have a different immunophenotype, natural history, and response to therapy.

#### Classical HL

Most common; large mononuclear (Hodgkin cells) or binucleate/multinuclear RS cells make up only 1–2% of the cellularity of the lymph node.



These cells are CD30+ and typically CD3-, CD15+, CD20-, CD45-, CD75-, CD79a-. 4 histological subtypes are identified.

*Differential diagnosis:* anaplastic large cell lymphoma; peripheral T-cell NHL; DLBCL; T-cell rich B-cell lymphoma; mediastinal large B-cell lymphoma.

- Nodular sclerosing HL (NSHL): ~80%; prominent bands of fibrosis and nodular growth pattern; lacunar Hodgkin cells; variable numbers of RS cells.
- Mixed cellularity HL (MCHL): ~17%; mixed infiltrate of lymphocytes, eosinophils, and histiocytes with classical RS cells.
- Lymphocyte depleted HL (LDHL): rare; diffuse hypocellular infiltrate with necrosis, fibrosis, and sheets of RS cells.
- Lymphocyte rich classical HL (LRCHL): uncommon; diffuse predominantly lymphoid infiltrate with scanty RS cells of 'classical' phenotype.

#### *Nodular lymphocyte-predominant HL (NLPHL)*

3-8%; contains large atypical B cells L&H 'popcorn' cells. These are CD30-, CD15-, CD3-, CD20+, CD45+, CD75+, CD79a+ and BCL-6 protein+, EMA-. Typical RS cells absent. L&H cells associated with CD4+/CD57+ T-cell rosettes. Nodules contain CD21+/CD35+ follicular dendritic cell meshwork. No evidence of EBV. Differential diagnosis: T-cell rich B-cell lymphoma; lymphocyte-rich classical HL.

### **Clinical features**

*Often presents with asymptomatic lymphadenopathy (often cervical);* Initial mode of spread occurs predictably to contiguous nodal chains. Waldeyer's ring involvement is rare and suggests a diagnosis of NHL. Lymphadenopathy in HL may wax and wane during observation.

- Spleen involved in ~30%; hepatomegaly 5%. Abdominal lymphadenopathy is unusual without splenic involvement.
- Bulky mediastinal and hilar lymphadenopathy may produce local symptoms (e.g. bronchial or SVC compression) or direct extension (e.g. to lung, pericardium, pleura, or rib). Pleural effusions in 20%.
- Extranodal spread and BM involvement rare. Presence of disseminated extranodal disease is generally accompanied by generalized lymphadenopathy and splenic involvement and usually a late event.
- 33% patients have  $\geq 1$  associated 'B' symptom at presentation; 'B' symptoms correlate with disease extent, bulk, and prognosis.
- Sometimes: generalized pruritus and alcohol-induced lymph node pain.
- A defect in cellular immunity has been documented in patients with HL:  $\uparrow$  risk of infections (TB, fungal, protozoal, and viral infections).

*NSHL:* typically in young adults (median age 26 years); usually localized involving cervical, supraclavicular and mediastinal nodes; good prognosis if stage I/II.

*MCHL:* median age 30 years but more prevalent in paediatric and older patients; associated with advanced stage and intermediate prognosis.

*LDHL:* more common in older adults; often extensive disease relatively poor prognosis. Most frequently seen in HIV patients.

*LRCHL*: ♂ > ♀; tendency to localized disease; favourable prognosis.

*NLPHL*: more frequent in ♂ (2–3 ×); median age 35 years; typically localized at presentation; usually cervical or inguinal; infrequent ‘B’ symptoms; indolent course; late relapses occur; favourable prognosis; 10-year OS 80–90%.

### Investigation, diagnosis, and staging

- Clinical history: B symptoms, alcohol intolerance, pruritus, fatigue, and performance status.
- Lymph node excision biopsy; FNA inadequate. Multiple biopsies of suspicious nodes may be necessary as hyperplastic nodes may occur.
- FBC: may show normochromic normocytic anemia, reactive leucocytosis, eosinophilia, and/or a reactive mild thrombocytosis.
- ESR.
- U&E and LFTs.
- Urate and LDH.
- HIV serology recommended in all patients.
- CXR.
- PET/CT whole body or CT neck, chest, abdomen, and pelvis.
- BM trephine biopsy: in patients with stage IB/IIB or III/IV disease (not essential in stage IA/IIA disease).
- Fertility counselling; egg harvesting or semen cryopreservation prior to chemotherapy or pelvic radiotherapy if appropriate.
- Cardiac ejection fraction and pulmonary function tests if ABVD or BEACOPP planned.

### Ann Arbor staging

Accurate staging is critical to selection of the most appropriate therapy. The Ann Arbor staging classification has strong prognostic value and is determined by the number of lymph node regions (not sites) involved and the presence or absence of ‘B’ symptoms (➡ Table 5.9). The Cotswolds modification reflects the use of modern imaging techniques and recognizes and clarifies differences in disease distribution and bulk.

**Table 5.9** Ann Arbor staging classification (Cotswolds modification)

Stage I	Involvement of a single lymph node (LN) region/structure (e.g. spleen, thymus, Waldeyer's ring) or single extranodal site (I <sub>E</sub> )
Stage II	Involvement of 2 or more LN regions on the same side of the diaphragm; localized involvement of 1 extranodal organ/site and LN region on same side of diaphragm (II <sub>E</sub> ); number of anatomical sites can be indicated by a subscript, e.g. II <sub>3</sub>
Stage III	Involvement of LN regions/structures on both sides of the diaphragm, which may also be accompanied by localized involvement of an extranodal organ/site (III <sub>E</sub> ), involvement of spleen (III <sub>S</sub> ) or both (III <sub>SE</sub> )
Stage IV	Diffuse involvement of one or more extranodal sites (e.g. BM, liver, or other extranodal sites not contiguous with LN—cf. 'E' below).
A	Absence of constitutional symptoms (B symptoms)
B	Fevers >38°C, weight loss >10% in 6 months or drenching night sweats
Additional subscripts at any disease stage:	
X	Bulky disease (widening of mediastinum by >33% or mass ≥10cm)
E	Involvement of a single extranodal site contiguous or proximal to known nodal site.

### Clinical imaging criteria

- Lymph node involvement: >1cm on CT scan is considered abnormal.
- Spleen involvement: splenomegaly may be 'reactive'; filling defects on CT or USS confirm involvement. PET/CT useful.
- Liver involvement: hepatomegaly insufficient; filling defect on imaging and abnormal LFTs confirm involvement. PET/CT useful.
- Bulky disease: ≥10cm in largest dimension or mediastinal mass >1/3 the maximal intrathoracic diameter.

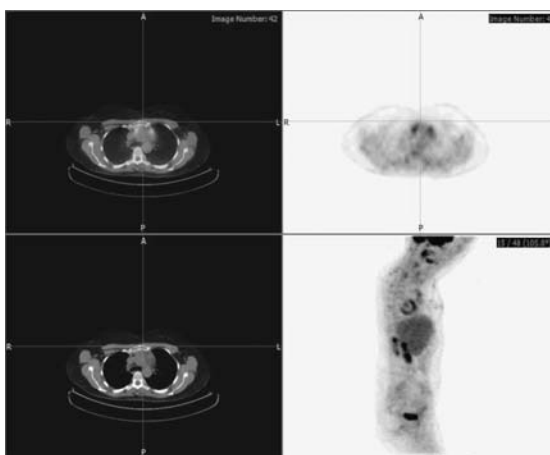
### FDG-PET scanning in HL

- PET/CT scanning has become routine practice: higher sensitivity (>80%) and specificity (~90%); The stage is altered in 15–20% and treatment altered in 5–10%.<sup>2</sup> (See Fig. 5.2).
- PET scanning has a clear role in restaging after therapy completed, particularly if CT shows residual mass(es). High -ve predictive value (NPV; ~85%). Lower +ve predictive value (PPV; ~65%).
- PET scan after 2 cycles of chemotherapy ('early PET scan') may be predictive of DFS and OS with PPV 69% and NPV 95% in one study.<sup>2</sup> Another study of PET after 2 cycles found 2-year PFS of 95% for PET-ve patients vs 12.8% for PET+.<sup>3</sup>

- A role for CT definitely remains, though inferior in assessment of patients with apparent incomplete resolution of, usually previously bulky, nodal sites after therapy. PET is more accurate than CT for response assessment (~80% vs 50%).

*False +ves:* due to inflammation, infection, granulomatous disease, brown fat, thymic hyperplasia in HL, marrow and splenic hyperplasia after G-CSF, previous radiotherapy. Delay of at least 3 weeks after chemotherapy and 8–12 weeks after radiotherapy recommended.

*False –ves:* due to lesions below scanner resolution (5–10mm), too brief an interval between injection and scan (2h recommended), masking of tumour by physiological uptake by brain, heart, or GI tract, hyperglycaemia reducing uptake by tumour.



**Fig. 5.2** PET and CT scans in patient with nodular sclerosing Hodgkin lymphoma demonstrating active disease (moderate FDG uptake on PET) in residual anterior mediastinal mass on CT after ABVD chemotherapy (see ↻ colour plate section).

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## Treatment of HL

### Clinical trial entry where possible

- Treatment results for HL have improved dramatically over the last 4 decades and a patient aged <60 years now has a >80% prospect of cure.
- The aim of 'risk-adapted therapy' is to provide each patient with the best probability of cure while minimizing early and late treatment-related morbidity.
- Treatment is determined by stage, symptoms, disease bulk, and patient characteristics such as age and comorbidities.
- Clinical trials remain essential to evaluate therapeutic regimens in order to achieve this objective for all patients without over-treatment.
- Patients with classical HL are generally divided into 3 treatment groups: early favourable, early unfavourable (intermediate), and advanced stage. Therapy of stage I/II NLPHL reflects its indolent nature.

### Prognostic factors

Prognostic factors for patients with stage I/II HL that identify favourable and unfavourable groups have been identified by French and German study groups and define treatment groups and these are now widely applied in Europe to inform treatment choice. There are small differences in the risk factors identified and treatment groups defined (GHSG adds IIB with large mediastinal mass or extranodal disease to the advanced stage group<sup>1</sup>).<sup>1</sup> In the USA, a similar approach is adopted using B symptoms and bulky disease to identify patients who require more therapy: NCCN guidelines recommend that non-bulky stage I/II B HL are treated like stage III/IV HL.<sup>2</sup> (See Table 5.10.)

**Table 5.10** European study group risk factors and treatment groups in Hodgkin lymphoma

	EORTC/GELA	GHSG
Risk factors (RF)	A. Large mediastinal mass <sup>a</sup> B. Age ≥50 years C. Elevated ESR <sup>b</sup> D. ≥4 involved regions	A. Large mediastinal mass <sup>a</sup> B. Extranodal disease C. Elevated ESR <sup>b</sup> D. ≥3 involved regions
Treatment groups		
NLPHL	Supradiaphragmatic CS I/II; no RFs	CS I/II; no RFs
Early stage favourable	Supradiaphragmatic CS I/II; no RFs	CS I/II; no RFs
Early stage unfavourable (Intermediate)	Supradiaphragmatic CS I/II; with ≥1 RF	CS I, CSIIA with ≥1 RF; CS IIB with RF C/D but not RF A/B
Advanced stage	CS III/IV	CS IIB with RF A/B; CS III/IV

EORTC, European Organisation for Research and Treatment of Cancer; GELA, Groupe d'Etude des Lymphomes de l'Adulte; GHSG, German Hodgkins Study Group.

<sup>a</sup> Mediastinal mass ratio >0.35 (mass ≥10cm widely applied); <sup>b</sup> ESR >50mm/h or >30 in presence of 'B' symptoms.

**International Prognostic Score (IPS), Hasenclever score**

For advanced HL (stage III/IV) has been developed to distinguish patients who may be cured by conventional therapy from those who require more intensive therapy.<sup>3</sup> 7 unfavourable factors are combined into a simple prognostic score predictive of 5-year FFP and OS (Table 5.11):

- Serum albumin <40g/L
- Hb <105g/L
- Male gender
- Stage IV disease
- Age  $\geq 45$  years
- WBC  $\geq 15 \times 10^9/L$
- Lymphopenia  $<0.6 \times 10^9/L$  or  $<8\%$  of differential.

Patients with no adverse factors have an 84% 5-year FFP. Each additional factor reduced FFP by ~7% until patients with 4–7 factors have ~47% 5-year FFP.

**Table 5.11** International Prognostic Score (IPS) for advanced HL

Number of factors	% of patients	5-year FFP (%)	5-year OS (%)
0–1	29	79	90
2	29	67	81
3	23	60	78
4–7	19	47	59

**Classical HL, favourable stage I/II: no risk factors**

About 30% of patients have early stage favourable HL (stage IA/IIA) at presentation. The aim of treatment is cure with minimal side effects. Expected outcome: >90% FFS and >95% OS at 5 years.

**Treatment of choice**

ABVD 2–4 cycles  $\pm$  IFRT. Historically combined modality treatment with abbreviated chemotherapy, usually ABVD  $\times$  2–4 cycles and IFRT (20–30Gy) to sites of disease was used. This strategy is still followed by many centres. However, due to high cure rates with chemotherapy alone the risk of long-term complications has to be minimized and the role of radiotherapy in younger patients is more and more uncertain. Studies have showed no significant difference in 5-year FFP and OS between ABVD  $\times$  6 and combined modality treatment.<sup>4</sup> In young patients 6 cycles of ABVD without IFRT can be considered. In patients PET–ve post cycle 2, a total of 4 cycles can be considered.

Alternative treatments used: Stanford V  $\times$  8 weeks.

Randomized trials continue to evaluate further reductions in treatment intensity seeking reduced toxicity by reduction of numbers of cycles, omission of bleomycin and dacarbazine, IFRT, or use of ABVD alone. The role of PET scans at 2 cycles to tailor further therapy is also being examined (including escalation of treatment if early PET positive).

ABVD often poorly tolerated by elderly patients due to cumulative doses of doxorubicin and bleomycin; G-CSF support may be necessary; pulmonary toxicity reported in 30% of patients, fatal in ~2%.<sup>5</sup>

### *Restaging*

Early PET scanning is being adopted by many centres but the evidence regarding this is not conclusive. Trials continue to look at the value of early scanning (after 2 cycles). Interim scanning is currently recommended after cycle 4 to assess that patient is responding; earlier if clinical concern. Patients who fail to achieve complete metabolic remission (CMR) on completion of therapy or have progressive disease should proceed to salvage therapy.

### **Classical HL unfavourable stage I/II**

Aim of treatment is cure with acceptable side effects. Expected outcome:

- ~85% DFS and ~90% OS at 5 years.
- ~15% will relapse within 5 years and ~5% have 1<sup>o</sup> refractory disease.

### *Treatment of choice*

ABVD × 4–6 cycles + IFRT (bulky disease 30–36Gy; non-bulky disease 20–30Gy) is widely used. However, due to high cure rates with chemotherapy alone the risk of long-term complications has to be minimized and the role of radiotherapy is more and more uncertain. IFRT is (generally) recommended for bulky disease.

Alternative treatments used: Stanford V × 12 weeks; BEACOPP/ABVD.

Clinical trials are continuing to evaluate the role of PET/CT scan in adjusting treatment according to response, as well as more intensified chemotherapy.

### *Restaging*

Early PET scanning is being adopted by many centres but the evidence regarding this is not conclusive. Trials continue to look at the value of early scanning (after 2 cycles). Interim scanning is currently recommended after cycle 4 to assess that patient is responding; earlier if clinical concern. Patients who fail to achieve CMR on completion of therapy or have progressive disease should proceed to salvage therapy.

### **Classical HL advanced stage (III/IV)**

About 30% of patients have stage III/IV HL at presentation. Advanced stage HL has been curable with combination chemotherapy for >3 decades. Standard treatment is with ABVD.<sup>6</sup>

### *Treatment of choice*

ABVD × 6–8 cycles. IFRT is (generally) recommended for bulky disease. Alternative treatments Stanford V, escalated BEACOPP.

### *Restaging*

Interim scanning is currently recommended after cycle 4 to assess that patient is responding; earlier if clinical concern. Patients who fail to achieve CMR on completion of therapy or have progressive disease should proceed to salvage therapy.



### Autologous SCT consolidation in first remission

Not recommended. No advantage over standard chemotherapy proven.<sup>7</sup>

### Nodular lymphocyte predominant HL (NLPHL)

#### Early stage non-bulky NLPHL

- 90% OS at 10 years.
- Can be treated with IFRT alone; at median follow up >7 years, more die of treatment-related toxicity than recurrent HL.
- Observation after excision biopsy and rigorous staging may be appropriate in some patients.
- In some patients treatment as for classical HL is indicated.

#### Stage III/IV NLPHL

- 96% CR and 74% FFS and 89% OS at 8 years.
- Treatment identical to classical HL indicated.<sup>8</sup>
- Symptomatic patients not candidates for chemotherapy may benefit from RT or single-agent rituximab.

### Evaluation of response

- Restaging as outlined on ↻ p.222. PET/CT is extremely useful.
- The revised response criteria for lymphoma (↻ Table 5.8, p.209) are also used in HL with the added *Cotswolds response criteria*:
  - CR: complete resolution of all radiological and laboratory evidence of active HL.
  - CRu: 'uncertain CR', identifies presence of a residual mass that remains stable or regresses on follow-up.
- CMR is now used to interpret PET results.
- The recommendations remain to restage after 4 courses of ABVD chemotherapy to ensure adequate response. The role of early-PET scanning after cycle 2 is as yet unclear but this strategy has made its way into many a clinical practice already.
- If progressive disease at any restaging or <CRu or no CMR at the end of treatment, proceed to salvage treatment.
- Residual masses sometimes persist on CT at completion of therapy, notably in mediastinum: may be residual fibrotic tissue with no viable tumour. PET scan is the best method to exclude active disease; if -ve it obviates the need for invasive biopsy.

### Salvage therapy

Durable FFP and prolonged survival not generally achieved by conventional chemotherapy for patients refractory to or relapsing after treatment. Salvage chemotherapy followed by high dose therapy (HDT) and autologous SCT are indicated.

#### Primary refractory HL (5–10%)

- Low Karnofsky score, age >50 years and failure to achieve temporary remission to 1st line are adverse factors for OS.<sup>9</sup>
- A MSKCC study showed that response to 2nd-line standard CT is predictive of response to HDT ( $\geq 25\%$  PR 10-year EFS 60% vs 17% <25% PR).<sup>10</sup>

*Relapsed patients*

- 10–30% will relapse, 90% within 2 years. Patients should undergo re-biopsy.<sup>2</sup>
- Better EFS and PFS after HDT compared to salvage chemotherapy alone: up to 80% CR and >50% EFS after 3 years irrespective of duration of remission (BNLI<sup>11</sup> and GHSG<sup>11</sup>).

*Salvage chemotherapy and HDT*

- Many schedules used; no consensus on best schedule, no regimen clearly superior. Most important is to achieve CR and for patient to go on to HDT and autologous SCT. Usually 2 cycles of chemotherapy prior to PBSCH. HDT most effective after CR achieved or with minimal residual disease.
- Schedules used: CHVPP, DHAP, ESHAP, ICE, mini-BEAM, IGEV, (escalated) BEACOPP.
- HDT and autologous transplant: most commonly used schedules are BEAM or LEAM. Low morbidity and mortality (<5%) in selected patients.
- Patients who relapse after HDT have a poor prognosis and responses to further treatment are generally brief though median survival is 2 years.

*Patients ineligible for HDT:* older age or co-morbidity: require individualized therapy: IFRT for localized relapse; palliative chemotherapy salvage. remission <12 months, B symptoms, and advanced stage are adverse factors for OS.

*Allogeneic SCT with standard conditioning:* associated with higher early mortality; reduced intensity conditioning lowers risk and DLI can be effective, showing possible GVL effect.<sup>12</sup> Allogeneic transplant remains investigational.

*Brentuximab vedotin (SGN-35)*

Chimeric monoclonal antibody (targets the cell-membrane protein CD30) linked to the antimetabolic agent monomethyl auristatin E.

34% CR and 40% PR in refractory HL. Remissions often not sustained but can be used as bridge to transplant. Accelerated FDA approval in 2011 and included on the UK national CDF list in 2013.

**Follow-up and monitoring for late complications<sup>2</sup>**

- Clinical review and check FBC, ESR and chemistry every 3 months for 1–2 years, then every 6 months for 3–5 years.
- TSH annually if previous RT to neck.
- Imaging not routinely indicated.
- Annual influenza immunization especially after chest RT or bleomycin.
- Early breast cancer screening: starting 5–8 years post-therapy or age 40 years if earlier.

### Late complications of chemotherapy (both NHL and HL)

10–15 years after treatment the risk of late complications of therapy exceeds the risk of relapse.

**Sterility:** ABVD produces significantly less infertility than other schedules used historically.

**Paramediastinal fibrosis post RT:** persistent effort dyspnoea.

**Second malignancy:** note that the data included here is from the era when RT was used (much more) routinely; this information has since led to change in practice. Included partly for historical purposes.

- **Solid tumours:** commonly breast, lung, melanoma, soft tissue sarcoma, stomach, and thyroid; comprise >50% of 2nd malignancies. Risk ~13% at 15 years and ~22% at 25 years; associated with RT, dose, and volume and young age at time of treatment; 75% occur within radiation fields; breast cancer occurs within 30 years of treatment in 30% of women treated with thoracic RT at age <30 years; lung cancer risk ↑ 20 × in heavy smokers after thoracic RT for HL.
- **Acute myeloblastic leukemia** risk ~3% at 10 years after treatment; peak incidence between 5 and 9 years; risk ↓ 10 years after therapy; particularly associated with older (and no longer used) MOPP chemotherapy (5% risk within 5 years), age >40 years and RT dose >30Gy to mediastinum; risk 10 years after ABVD alone <1%.
- **NHL** risk 7%; rises after 10 years and declines after 15 years; no clear association with type of therapy.

**Cardiac toxicity:** mainly related to RT and chemotherapy agents (anthracycline); higher risk in patients <40 years at treatment; risk of coronary artery disease up to 6% at 10 years and 10–20% at 20 years.

**Pulmonary toxicity:** generally mild, usually asymptomatic changes in pulmonary function; associated with mediastinal irradiation and bleomycin containing chemotherapy (ABVD).

**Thyroid toxicity:** 50% risk at 20 years after radiation to the neck and upper mediastinum; highest risk ages 15–25 years; usually hypothyroidism.

**Patients treated with HDT and autologous SCT:** ↑ incidence of myelodysplasia and AML; azoospermia in males and pre-mature ovarian failure in females is usual.

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
### Further reading

NCCN Clinical Practice Guidelines in Oncology: Hodgkin Lymphoma.  [www.nccn.org/professionals/physician\\_gls/PDF/hodgkins.pdf](http://www.nccn.org/professionals/physician_gls/PDF/hodgkins.pdf)

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# Myelodysplasia

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- 

## Myelodysplastic syndromes (MDS)

The myelodysplastic syndromes (MDS) are a group of biologically and clinically heterogeneous clonal disorders characterized by ineffective haematopoiesis and subsequent peripheral cytopenia. There is a variable tendency to evolve to BM failure or acute myeloblastic leukaemia, depending on the subtype.

### Incidence

Predominantly affects the elderly but may occur at any age; median age 69; annual incidence 4/100,000 in general population; rising from 0.5/100,000 aged <50 years to 89/100,000 aged ≥80 years.

### Risk factors

- Age.
- Prior cancer therapy: notably with radiotherapy, alkylating agents (chlorambucil, cyclophosphamide, melphalan; peak 4–10 years after therapy) or epipodophyllotoxins (etoposide, teniposide; peak within 5 years).
- Note: prolonged alkylator therapy also used in other specialties (rheumatology).
- Prior autologous SCT (up to 20% of patients with NHL).
- Environmental toxins: notably benzene and other organic solvents; related to intensity and duration of exposure; also smoking, petroleum products, fertilizers, semi-metal, stone dusts, and cereal dusts.
- Genetic: rare familial syndromes; MDS ↑ in children with congenital BM failure syndromes, Schwachman–Diamond syndrome, Fanconi anaemia, and neurofibromatosis type 1.

### Pathophysiology

- Clonal haematopoietic stem cell disorder characterized by genetic progression possibly due to a combination of genetic predisposition and environmental exposures.
- Early mutations cause differentiation arrest and dysplasia with later mutations leading to proliferation, clonal expansion, and AML (variable).
- Recurring cytogenetic abnormalities well recognized but molecular pathogenesis and basis for progression remain largely undefined.
- The *MLL* gene at 11q23 is up-regulated (with *HOXA9* activation) in significant % of MDS and AML patients including normal karyotypes.<sup>1</sup>
- Abnormalities in the marrow microenvironment described: e.g. aberrant cytokine production (↑ inhibitory pro-apoptotic cytokines including TNF- $\alpha$ , IL-6, TGF- $\beta$ , IFN- $\alpha$ , and Fas ligand) and altered stem cell adhesion.
- MDS marrow stem cells display lowered apoptotic threshold to TNF- $\alpha$ , IFN- $\alpha$ , and anti-Fas antibodies and less response to haemopoietic growth factors.
- Early indolent pro-apoptotic MDS transforms to aggressive proliferative MDS as genetic lesions accumulate (*ras*, *FLT3*, *FMS*, and *p53* mutations associated with disease progression).

- Symptoms relate not only to the degree of cytopenia but to impaired function of granulocytes and platelets and may occur at near normal levels.

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## Classification of MDS

The historical FAB classification has largely been superseded by the revised 2008 WHO classification of MDS.<sup>1</sup> The NCCN guidelines<sup>2</sup> include the FAB classification for historical context, the same is done here.

### World Health Organization (WHO) system<sup>1</sup>

(See Table 6.1.)

- Classification of MDS based on morphology, karyotype, and clinical features that defines subgroups and provides valuable prognostic information useful for clinical decision-making. Initially proposed in 2001. Revised in 2008.<sup>1,3–6</sup>
- Diagnosis of AML if  $\geq 20\%$  blasts in PB or BM (as opposed to the FAB classification which has a 'RAEB-t' category, which is acknowledged as a footnote to the WHO classification).
- Defines 2 subtypes of RAEB: RAEB-1 (5–9% BM blasts) and RAEB-2 (10–19% BM blasts) reflecting worse clinical outcomes with  $\geq 10\%$  blasts. A footnote to the classification acknowledges the RAEB-t category from the old FAB classification. WHO defines this as AML.
- Recognizes the '5q– syndrome' as a distinct narrowly defined entity.
- Removed CMML to a newly created disease group: MDS/MPD.

The 2008 revision included a new subtype, refractory cytopenia with unilineage dysplasia (RCUD). Unilineage dysplasia is accepted for diagnosis provided it persists  $\geq 6$  months and other causes of dysplasia are excluded. Further subtyped in refractory anaemia (RA), refractory neutropenia (RN), and refractory thrombocytopenia (RT).

### French–American–British (FAB) system

- Its use has been superseded by WHO system.
- Morphology-based classification used since 1982.<sup>7</sup>
- Requires dysplastic changes in  $\geq 2$  lineages.
- Useful for predicting prognosis and risk of evolution to acute leukaemia.

#### FAB classification

- Refractory anaemia (RA): cytopenia of 1 peripheral blood (PB) lineage; normo- or hypercellular marrow with dysplasia  $\geq 2$  lineages;  $< 1\%$  PB blasts;  $< 5\%$  BM blasts. 25% of patients.
- Refractory anaemia with ringed sideroblasts (RARS): defined as for RA plus ringed sideroblasts constituting  $> 15\%$  nucleated erythroid cells; 15% of patients.
- Refractory anaemia with excess blasts (RAEB): cytopenia of  $\geq 2$  PB lineages; dysplasia of all 3 BM lineages;  $< 5\%$  PB blasts; 5–20% BM blasts; 35% of patients.
- Refractory anaemia with excess blasts in transformation (RAEB-t): cytopenia of  $\geq 2$  PB lineages; dysplasia of all 3 BM lineages;  $\geq 5\%$  PB blasts; 21–30% BM blasts or Auer rods in blasts; 15% of patients.
- Chronic myelomonocytic leukaemia (CMML): PB monocytosis ( $> 1 \times 10^9/L$ );  $< 5\%$  PB blasts;  $\leq 20\%$  BM blasts; 10% of patients.



**Table 6.1** WHO classification system<sup>1</sup>

Condition	PB finding	BM findings
<i>Refractory cytopenia with unilineage dysplasia (RCUD)</i>	No or rare blasts 3 subtypes: ● Refractory anaemia (RA) ● Refractory neutropenia (RN) ● Refractory thrombocytopena (RT)	Dyserythropoiesis, or Dysgranulopoiesis, or Dysmegakaryocytopoiesis only  <5% blasts <15% ringed sideroblasts
<i>Refractory anaemia with ringed sideroblasts (RARS)</i>	Anaemia No blasts	≥15% ringed sideroblasts erythroid dysplasia only <5% blasts
<i>Refractory cytopenia with multilineage dysplasia (RCMD)</i>	Cytopenias (bi- or pan-) No or rare blasts No Auer rods <1 × 10 <sup>9</sup> /L monocytes	Dysplasia in ≥10% cells in ≥2 myeloid cell lines <5% blasts No Auer rods <15% ringed sideroblasts
<i>RCMD-RS</i>	As RCMD	As RCMD but with ≥15% ringed sideroblasts
<i>Refractory anaemia with excess blasts (RAEB-1)</i>	Cytopenias <5% blasts No Auer rods <1 × 10 <sup>9</sup> /L monocytes	Unilineage or multilineage dysplasia 5–9% blasts No Auer rods
<i>Refractory anaemia with excess blasts (RAEB-2)</i>	Cytopenias 5–19% blasts Auer rods ± <1 × 10 <sup>9</sup> /L monocytes	Unilineage or multilineage dysplasia 10–19% blasts Auer rods ±
<i>MDS with isolated del(5q)</i>	Anaemia <5% blasts Platelets ↔ or ↑	↔ to ↑ megakaryocytes with hypolobated nuclei <5% blasts No Auer rods Isolated del(5q)
<i>MDS—unclassified (MDS-U)</i>	Cytopenias	Unilineage dysplasia or no dysplasia Characteristic MDS cytogenetics Blasts <5%

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## Clinical features of MDS

- Patient may be asymptomatic. Incidence ↑ with age.
- Difficult diagnosis in patient with multiple comorbidities where anaemia of chronic disease is also in the differential diagnosis.
- Presentation blood results range from mild anaemia to profound pancytopenia.
  - Macrocytic or normocytic anaemia usual (60–80%).
  - ± neutropenia (50–60%).
  - ± thrombocytopenia (40–60%).
- Isolated thrombocytopenia is an unusual presentation for MDS.
- Symptoms of underlying cytopenias and cellular dysfunction may develop:
  - Anaemia—fatigue, shortness of breath, exacerbation of cardiac symptoms.
  - Neutropenia and dysfunctional granulocytes—recurrent infection.
  - Thrombocytopenia and dysfunctional platelets—spontaneous bruising, purpura, bleeding gums.
- Constitutional symptoms including anorexia, weight loss, fevers, and sweats usually features of more ‘advanced’ subgroups.
- Splenomegaly/organomegaly is not seen in MDS.

### Investigation and diagnosis

*History:* prior exposure to chemotherapy/radiation; FH of MDS/AML; symptoms of anaemia, recurrent infection, bleeding or bruising; timing, severity, and speed of onset of cytopenias; previous transfusion; medication history and co-morbidities.

*Examination:* pallor; infection; bruising.

*FBC:* macrocytic/normocytic anaemia ± neutropenia ± thrombocytopenia ± thrombocytosis:

- Hb <110g/L and/or
- Neutrophils <1.5 × 10<sup>9</sup>/L and/or
- Platelets <100 × 10<sup>9</sup>/L.

*Blood film:* essential. Morphological dysplastic features: macrocytosis; dimorphic red cells; basophilic stippling in RBCs; dysplastic granulocytes: pseudo-Pelger forms (Fig. 6.1), hypersegmented neutrophils, hypogranular neutrophils, dysmorphic monocytes ± blasts; platelets can be large or hypogranular.

*Reticulocyte count:* not ↑.

*U&E, LFTs, ECG, and CXR:* no typical abnormalities; to assess co-morbidity.

*Serum ferritin, vitamin B<sub>12</sub>, and RBC folate:* usually ↔ levels; ferritin may be elevated in RARS/RCMD-RS.

*Serum EPO level:* (prior to transfusion); indicates potential for response to EPO.

*BM aspirate:* assess cellularity, M:E ratio and blast %; demonstrates >10% dysplastic cells in ≥1 lineage; megaloblastoid erythropoiesis, nuclear-

cytoplasmic asynchrony in myeloid/erythroid precursors, dysmorphic megakaryocytes or micro-megakaryocytes; Perl's stain: normal/ ↑ storage Fe; ≥15% ringed sideroblasts (RARS/RCMD-RS).

**BM trephine biopsy:** assess cellularity, usually ↑/↔ but note hypocellular variant (hypoplastic MDS); may show multifocal accumulation of immature (CD34+) progenitors (formerly termed 'abnormal localization of immature myeloid precursors' (ALIPs) centrally in interstitium),<sup>1</sup> megakaryocyte dysplasia, ↑ mast cells; ↑ reticulin or fibrosis; ↑ storage Fe; excludes occult neoplasia or alternative haematological diagnosis.

**Immunohistochemistry:** minimum of CD34, CD31, CD42, or CD61 (megakaryocytes) recommended in most cases; CD3/15/20/25/117 in selected cases.<sup>1</sup>

**BM cytogenetic analysis:** may demonstrate clonal chromosome abnormality(s) confirming diagnosis and provide prognostic information; karyotype evolution is associated with progression; diagnosis of MDS associated with isolated del(5q).

- FISH useful if low metaphase yield or difficult case.
- To exclude Fanconi and dyskeratosis congenita in familial cytopenia.

**HLA-typing and CMV serology:** for patients eligible for allogeneic SCT.

**HIV serology:** in selected cases.

**Flow cytometry:** in selected cases; to exclude PNH (hypoplastic marrow).

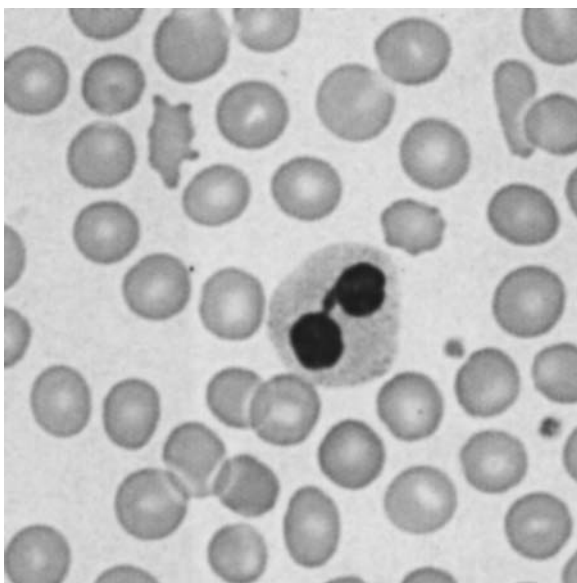
**HLA-DR15 screening:** in younger patients with hypoplastic marrow and normal cytogenetics (possible response to immunosuppression).

**Clonality studies:** used in some centres to establish diagnosis in difficult cases; flow cytometry for clonal BM blasts expressing phenotypic deviations.

**Consider HIV testing in all patients:** diagnostic criteria are summarized in ➔ Table 6.1, p.231 according to the WHO classification (2008). Cytopenias are fluctuant and their trend needs to be observed. A diagnosis of 'idiopathic cytopenia of uncertain (undetermined) significance' (ICUS) was introduced to describe cases with cytopenia in ≥1 lineage for ≥6 months but that do not meet the minimal criteria for MDS and cannot be explained by another diagnosis. This entity is different to the MDS-U category. A repeat BM if MDS is continued to be suspected might be indicated at a later time point.

The definitive diagnosis of early MDS (e.g. isolated cytopenia) may be difficult; regular review with repeat blood count and film assessment is recommended; in all cases a measure of the speed of onset and evolution of the disease over several months is of prognostic value.

Labelling a patient as having MDS should only be done once the diagnosis is clear and confirmed. If features are mild or diagnostic criteria are not met the patient should be monitored. Repeat investigations might be indicated at a later stage.



**Fig. 6.1** Blood film in MDS showing bilobed pseudo-Pelger neutrophil (➔ see colour plate section).

### Cytogenetic analysis

- Cytogenetic abnormalities are found in 40–70% *de novo* MDS and 80–90% 2° MDS.
- Cytogenetic analysis yields 3 patterns:
  - Normal karyotype.
  - Single abnormalities.
  - Complex karyotypes ( $\geq 3$  abnormalities): 30% *de novo* MDS and 50% 2° MDS.
- More complex abnormalities are associated with more aggressive subtypes and higher % blasts and with 2° MDS. They are part of the prognostic scoring systems. Single or complex abnormalities at diagnosis may evolve during course of disease.
- Most frequent abnormalities involve chromosomes 5, 7, 8, 11, 12, 13, 17, and 20; most typical are 8+, 7– or 7q–, 5– or 5q–.

### Prognostic value

- Specific abnormalities hold prognostic value. See ➔ Tables 6.2 to 6.7 later in this chapter.
- Excluded: t(8;21), t(15;17), inv(16). These findings constitute AML (irrespective of blast count).

## Differential diagnosis

Exclude:

- Other causes of anaemia—haematinic deficiency, haemolysis, blood loss, renal failure.
- Other causes of neutropenia—drugs, viral infection.
- Other causes of thrombocytopenia—drugs, ITP.
- Other causes of bi-/pancytopenia—drugs, infection, aplastic anaemia.
- Reactive causes of BM dysplasia—megaloblastic anaemia, HIV infection, alcoholism, recent cytotoxic therapy, severe intercurrent illness.
- Other causes of marrow hypoplasia in hypoplastic MDS—aplastic anaemia, PNH.
- Anaemia of chronic disease.

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## Prognostic factors in MDS

### International Prognostic Scoring System (IPSS) and revised IPSS (IPSS-R)

- (The initial IPSS from 1997/1998<sup>1</sup> has since been revised in 2012 in the Revised IPSS (IPSS-R).<sup>2</sup> Both scoring systems are included here (Tables 6.2 to 6.5).)
- Uses % BM blasts, BM cytogenetics, and number of cytopenias to calculate a risk score and stratify patients into 4 distinct groups.
- Improved prognostic power for both survival and evolution into AML compared with earlier systems.
- IPSS score should be calculated during a stable clinical state and not, for example, during florid infection at initial presentation.
- IPSS score is the 'gold standard' for risk assessment in patients with *de novo* DS and may be used to assist management decisions.

**Table 6.2** Calculation of IPSS Score

Prognostic variable	Score value				
	0	0.5	1.0	1.5	2.0
BM blast %	<5	5–10	–	11–20	21–30
Karyotype	Good	Intermediate	Poor		
Cytopenias	0/1		2/3		

Karyotype: Good: normal, –Y alone, del(5q) alone, del(20q) alone. Poor: complex ( $\geq 3$  abnormalities) or chromosome 7 anomalies. Intermediate: other abnormalities.

Cytopenias: Hb  $<100\text{g/L}$ ; neutrophils  $<1.8 \times 10^9/\text{L}$ ; platelets  $<100 \times 10^9/\text{L}$ .

**Table 6.3** Prognosis by IPSS Score<sup>1</sup>

IPSS risk group (% patients)	Combined score	Median survival	25% AML evolution
Low (33)	0	5.7 years	9.4 years
Intermediate–1 (38)	0.5–1.0	3.5 years	3.3 years
Intermediate–2 (22)	1.5–2.0	1.2 years	1.1 years
High (7)	>2.5	0.4 years	0.2 years



**Table 6.4** Calculation of IPSS-R Score

Prognostic variable	Score value						
	0	0.5	1.0	1.5	2.0	3	4
BM blast %	≤2		2–<5		5–10	>10	–
Cytogenetics	Very good		Good		Int	Poor	Very poor
Haemoglobin	≥100		80–<100	<80			
Platelets	>100	50–<100	<50				
ANC	≥0.8	<0.8					

Karyotype: Very good: -Y, del(11q); Good: normal, del(5q), del(12p), del(20q), double including del(5q). Intermediate: del(7q), +8, +19, i(17q), any other single or double independent clones. Poor: -7, inv(3)/t(3q)/del(3q), double including -7/del(7q), complex (3 abnormalities). Very poor: complex (>3 abnormalities).

**Table 6.5** Prognosis by IPSS-R Score

IPSS-R risk group (% patients)	Overall score	Median survival	25% AML evolution
Very low (19)	≤1.5	8.8 years	Not reached
Low (38)	1.5–3.0	5.3 years	10.8 years
Intermediate (20)	>3–4.5	3 years	3.2 years
High (13)	>4.5–6	1.6 years	1.4 years
Very high (10)	>6	0.8 years	0.7 years

**WHO-based Prognostic Scoring System (WPSS)**

The WHO classification-based system takes into account the transfusion requirement of patients. It is shown that this has a negative prognostic impact. Initially transfusion requirement was part of the scoring system, but due to its relative subjectivity, the scoring system was refined to include 'severe anaemia'.<sup>3</sup> (Tables 6.6 and 6.7.)

**Table 6.6** Calculation of WPSS Score

Prognostic variable	Score value			
	0	1	2	3
WHO category	RCUD RARS 5q-	RCMD	RAEB-1	RAEB-2
Karyotype	Good	Intermediate	Poor	–
Severe anaemia ( $<90\text{g/L}$ ♂; $<80\text{g/L}$ ♀)	Absent	Present	–	–

Karyotype: Good: normal, -Y alone, del(5q) alone, del(20q) alone. Poor: complex ( $\geq 3$  abnormalities) or chromosome 7 anomalies. Intermediate: other abnormalities.

**Table 6.7** Prognosis by WPSS Score

IPSS risk group (% patients)	Combined score
Very low	0
Low	1
Intermediate	2
High	3–4
Very High	5–6

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## Clinical variants of MDS

These variants though partly discussed on p.231 have specific diagnostic relevance and distinct clinical characteristics. It remains important to identify several of these because of new therapeutic options.

### 5q-syndrome = MDS with isolated del(5q)

- Separately defined in WHO classification.
- ♀ preponderance.
- More indolent clinical course.
- Lower rate of evolution to AML (10%).
- Severe anaemia; pronounced macrocytosis; ↔ or moderate ↓ WBC; ↔ or moderate ↑ platelets; dysplastic megakaryocytes; no blast excess.
- High response rates to lenalidomide.

### RARS (See Fig. 6.2.)

- Good survival: 77% OS at 3 years.
- Very low risk of AML transformation.
- High risk of transfusion siderosis.
- Good response to EPO ± G-CSF.

### 2° MDS

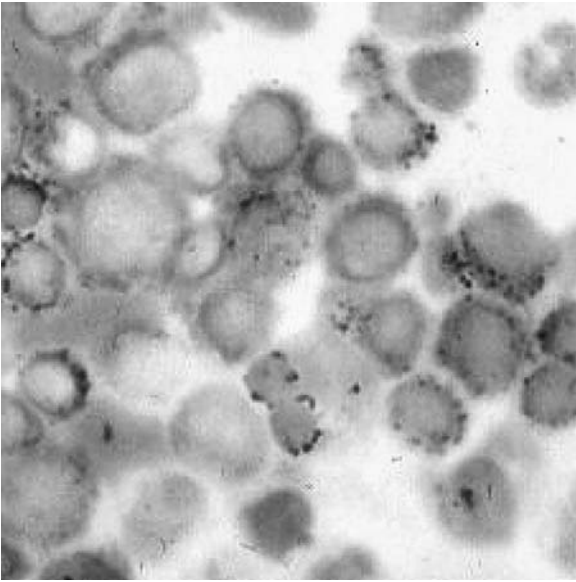
- Incidence ↑ due to successful chemotherapy and ↑ pollution.
- Multiple chromosomal abnormalities in almost all patients.
- Poorer prognosis than *de novo* MDS.
- Prior cancer therapy: notably with radiotherapy, alkylating agents (chlorambucil, cyclophosphamide, melphalan; peak 4–10 years after therapy) or epipodophyllotoxins (etoposide, teniposide; peak within 5 years).

### Hypoplastic MDS

- <15% of cases of MDS have hypocellular BM on biopsy (<30% cellularity age <60 years; <20% aged ≥60 years); dysplastic megakaryocytes ± myeloid cells or excess blasts may be difficult to see.
- May be difficult to distinguish from aplastic anaemia in which pancytopenia usually more severe.
- Cytogenetic findings typical of MDS may be necessary for diagnosis; ↔ or ↑ %CD34+ cells suggests MDS.
- Not age associated.
- May respond to immunosuppressive therapy.

### MDS with myelofibrosis (MDS-MF)

- <15% of patients with MDS on BM have marked fibrosis.
- More common in 2° MDS.
- BM hypercellular with MF, diffuse coarse reticulin fibrosis and dysplasia in at least 2 cell lineages.
- PB shows pancytopenia and dysplastic features and sometimes leucoerythroblastic picture; organomegaly unusual.
- Rapid deterioration usual; must distinguish from acute megakaryocytic leukaemia, acute panmyelosis with fibrosis and chronic myeloproliferative disorders, metastatic cancer, lymphoma and HCL.



**Fig. 6.2** BM in RARS stained for Fe: note Fe granules round the nucleus of the erythroblast (➔ see colour plate section).

## Management of MDS

WHO classification and IPSS(-R) risk category are used in deciding therapy. The speed of onset of symptoms/findings over several weeks/months is an important parameter. Most patients are elderly and performance status and individual patient preferences should be taken into consideration.

Guidelines recommend stratifying patients into 2 major risk groups:

- Lower risk: haematological improvement is the therapeutic aim.
- Higher risk: alteration of the natural history is the priority.<sup>1</sup>

For patients with *lower risk MDS*, a 'watch and wait' approach can be adopted prior to the introduction of therapy. Supportive care may be the only treatment indicated. However, development of transfusion-dependence significantly worsens OS<sup>2,3</sup> and more intensive treatment can be appropriate here.

Intensive therapy is indicated for patients with *higher risk MDS* if appropriate. Palliative or investigational therapy is indicated for patients with higher risk MDS ineligible for intensive therapy. Apart from potential cure with allo-HSCT, treatments for MDS have a palliative intent.

### Supportive care

Supportive care has the aim of reducing morbidity and maintaining QoL. For many patients this will be the mainstay of management. It involves monitoring, psychosocial support, and QoL assessment.

### Red cell transfusion

Should be administered only for symptomatic anaemia; symptomatology rather than a 'trigger' Hb level should initiate red cell support.

#### Platelet transfusion

Should be administered for patients with haemorrhagic problems with the aim of maintaining a platelet count  $>10 \times 10^9/L$ . If no bleeding symptoms lower platelet counts can be accepted.

#### Anti-infective therapy

Empirical broad-spectrum antibiotics and/or antifungals should be administered promptly for neutropenic sepsis; no evidence to support routine use of prophylactic anti-infectives; may be useful in neutropenic patients with recurrent infection.

#### G-CSF

May be useful for patients with neutropenia and recurrent or antibiotic resistant infections; not recommended simply for chronic prophylaxis.

### Iron chelation therapy

Should be considered once a patient has received 20–25 units of RBCs if long-term transfusion is required (RA, RARS, 5q- syndrome), in those with serum ferritin  $>2500\text{mg/L}$ , and those with concurrent cardiac or hepatic dysfunction. Iron overload is a significant cause of morbidity and mortality in low-risk patients with isolated erythroid dysplasia.<sup>2</sup>

**Desferrioxamine:** inconvenient administration (SC infusion) and toxicity; dose 20–40mg/kg by 12h SC infusion 5–7 nights/week; aim for serum ferritin <1000mg/L; monitor renal function; audiometric and ophthalmological assessments prior to therapy and then annually.

**Deferasirox:** 20–30mg/kg PO od; comparable efficacy to desferrioxamine.<sup>4</sup> Monitor renal and hepatic function monthly and eyes and ears pre-therapy and annually.

**Deferiprone** is another approved orally active chelator; dose 25mg/kg tds; agranulocytosis has been reported.

## Treatments

Treatment strategies on  p.244.

Consider clinical trials where appropriate.

### Immunosuppression

Notable responses in hypoplastic MDS; some responses seen in other lower risk patients.

Younger age, shorter duration of transfusion dependence, HLA-DR15, hypoplastic BM, and presence of a PNH clone associated with response to immunosuppression:

- ATG 40mg/kg/d × 4d can achieve transfusion independence in ~33% patients (median response >2 years); erythroid improvement in up to 66%, and sustained neutrophil and platelet responses in up to 50%; response associated with significant survival benefit.<sup>5,6</sup>
- Cyclosporin has achieved transfusion independence in a high proportion of patients with RA in small clinical trials.<sup>7</sup>
- ATG+cyclosporin may be used in combination as in aplastic anaemia.<sup>8</sup>

### Lenalidomide

Achieves major clinical responses and cytogenetic responses in most patients with 5q- syndrome.<sup>9–11</sup> Mode of action appears to be direct cytotoxic effect suppressing 5q- clone. Long-term follow-up suggests it may alter natural history of MDS with durable responses (median 2.2 years), improved OS (10-year estimate of 78% for cytogenetic responders vs 4% in non-responders) and reduced AML transformation (15% at 10 years in responders vs 67%) at 10 years.<sup>12</sup> median duration of major responses >2 years. Similar but less durable responses in MDS with 5q- plus additional abnormalities where it also normalized % BM blasts in 75%.

### Epigenetic therapy: hypo-methylating agents azacitidine and decitabine

Inhibit DNA methyl transferase, reduce DNA methylation, and reactivate abnormally suppressed gene expression. In a subset of MDS patients they ameliorate anaemia, ↓ the risk of leukaemic transformation, and improve OS.

#### Azacitidine

- Achieves haematological responses, delays AML transformation, and improves QoL (47% OR, 10% CR; 36% haematological improvement (HI) with a trend to ↑ OS.<sup>13</sup>

- Usual dose 75mg/m<sup>2</sup>/d SC 7d or 100mg/m<sup>2</sup>/d SC 5d monthly. Most responses observed between courses 4 and 6.
- Response often only after several months; minimum 3 cycles required before evaluating response.


#### *Decitabine*

- IV administration; requires hospitalization.
- Up to 39% CR described but no phase III survival benefit data.

#### *Non-intensive chemotherapy*

Classical low-dose chemotherapy (hydroxycarbamide, cytarabine, etoposide) may be used and tolerated by elderly patients with transformed or 'transforming' MDS. However may lead to ↑ cytopenia and transfusion dependence. Its role has markedly reduced with the introduction of epigenetic therapy.

#### *Intensive chemotherapy*

AML induction chemotherapy should be considered in patients <60 years with higher-risk MDS and good performance status. See treatment strategies on  p.132.

- Lower responses (40–50%) and higher treatment-related morbidity and mortality than in *de novo* AML.
- Prolonged DFS rare. Without SCT option, benefit marginal and survival not prolonged in most patients.<sup>14</sup>
- Consolidate with HSCT where possible.

#### *HSCT*

(Mini-) Allogeneic SCT offers the best prospect of prolonged survival and curative therapy (35–40% 3-year DFS); few patients are eligible but treatment of choice for younger patients with a higher MDS risk score.

- IPSS int-2/high-risk patients 60 years should proceed to HLA-matched sibling SCT at diagnosis for best OS but SCT should be delayed in low/int-1 MDS patients until progression.<sup>15</sup>
- Need for remission induction chemotherapy pre-transplant unclear.
- High treatment-related mortality (~40%).
- Relapse rate up to 40%; relapse risk relates to IPSS score—low risk <5%, high risk >25%.
- 5-year DFS relates to IPSS: low/int-1 60%, int-2 36% and high risk 28%; favourable outcome associated with younger age, shorter disease duration, compatible graft, 1<sup>o</sup> MDS, <10% blasts, and good risk cytogenetics.
- RIC regimens have reduced toxicity, TRM, and increase eligible age range for SCT.<sup>16</sup>

### **Lower risk group treatment strategy (IPSS low/int-1; IPSS-R and WPSS very low/low/int)**

Consider clinical trials where appropriate.

#### *Symptomatic anaemia and serum EPO ≤500 mU/mL (and no 5q-)*

- Erythropoietin ±G-CSF; 20–30% respond to EPO alone, 40–60% to EPO+G-CSF; synergistic effect. Responses generally occur within 6–8 weeks; median response 1–2 years, longer responses associated with lower % BM blasts, low/int-1 IPSS.



- rHu EPO 40–60,000U SC 1–3 ×/week or darbepoetin alfa 150–300 mcg/week SC for 6 weeks; in non-responders consider adding daily G-CSF (1–2mcg/kg/d or 1–3 ×/week) SC or double dose EPO or both for further 6 weeks.
- In responders, reduce G-CSF to 3-weekly and EPO in steps to lowest dose retaining response.
- No response after 2–3 months = treatment failure. If good probability to respond to immune suppressive therapy (IST): consider IST with antithymocyte globulin (ATG) or ciclosporin A (CsA).

#### *Symptomatic anaemia and serum EPO >500 mU/mL (and no del5q–)*

- Good probability to respond to IST: consider ATG or CsA.
- Poor probability to respond to IST: consider azacitidine/decitabine; consider lenalidomide; consider allo-HSCT in rare/selected cases if above fails.

#### *Clinically relevant thrombocytopenia or neutropenia or increased blasts*

- Consider azacitidine/decitabine.
- Consider IST. Pre-transplant cytoreductive chemotherapy not necessarily recommended.
- Best supportive care.

#### *MDS with isolated del(5q)*

- Lenalidomide.
- Dose: 10mg/d for 21d every 4 weeks.
- Monitor patients with renal dysfunction carefully.

### **Higher risk group treatment strategy (IPSS int-2/high; IPSS-R int, high, very high; WPSS high, very high)**

Consider clinical trials where appropriate.

#### *High-intensity therapy candidate*

- Donor available: allo-HSCT.
- No donor available:
  - Azacitidine (1st choice, phase III trial data showed survival benefit).
  - Decitabine.
  - High-intensity chemotherapy (AML like chemotherapy).

#### *Not high-intensity candidate*

- Azacitidine (1st choice) or decitabine.
- Best supportive care.

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## Response criteria

An International Working Group proposed<sup>1</sup> and subsequently modified<sup>2</sup> response criteria to standardize assessment of clinical trials of therapy, to identify risk-based treatment goals and to identify clinically meaningful responses. 4 aspects of response were defined:

- Altering the natural history of MDS— see definitions in Table 6.9.
- Cytogenetic response—requires examination of 20 metaphases.
- Haematological improvement (Table 6.8 and 6.9)—pre-therapy baseline is average of  $\geq 2$  measurements over at least 1 week, not affected by transfusion.
- QoL—correlation with Hb level.<sup>3</sup> QoL assessment should be an integral part of MDS management.

**Table 6.8** Modified IWG response criteria for haematological improvement<sup>2</sup>

Haematological improvement (HI)	Response criteria (must last $\geq 8$ weeks)
Erythroid response (pre-treatment $< 110 \text{g/L}$ ) HI-E	Hb $\uparrow$ by $\geq 15 \text{g/L}$ or $\downarrow$ of RBC transfusions (for Hb $\leq 90 \text{g/L}$ ) by $\geq 4 \text{U}$ / 8 weeks compared to previous 8 weeks
Platelet response (pre-treatment $< 100 \times 10^9/\text{L}$ ) HI-P	$\uparrow$ of $\geq 30 \times 10^9/\text{L}$ for patients starting $> 20 \times 10^9/\text{L}$ ; $\uparrow$ from $< 20$ to $> 20 \times 10^9/\text{L}$ and by at least 100%
Neutrophil response (pre-treatment $< 1.0 \times 10^9/\text{L}$ ) HI-N	At least 100% $\uparrow$ and absolute $\uparrow > 0.5 \times 10^9/\text{L}$
Progression or relapse after HI	At least 1 of: <ul style="list-style-type: none"> <li>• <math>\geq 50\%</math> <math>\downarrow</math> from max. response in neutrophils or platelets</li> <li>• <math>\downarrow</math> Hb by <math>\geq 15 \text{g/L}</math></li> <li>• Transfusion dependence</li> </ul>

**Table 6.9** Modified IWG response criteria: altering MDS natural history<sup>2</sup>

Category	Response criteria (must last $\geq 4$ weeks)
Complete remission (CR)	<ul style="list-style-type: none"> <li>● BM <math>\leq 5\%</math> myeloblasts</li> <li>● <math>\pm</math> persistent dysplasia</li> <li>● FBC: Hb <math>\geq 110\text{g/L}</math>; platelets <math>\geq 100 \times 10^9/\text{L}</math>; neutrophils <math>\geq 1.0 \times 10^9/\text{L}</math>; blasts 0%</li> </ul>
Partial remission (PR)	All CR criteria if abnormal pre-treatment except BM blasts $\downarrow \geq 50\%$ but still $>5\%$
Marrow CR	<ul style="list-style-type: none"> <li>● BM <math>\leq 5\%</math> blasts and <math>\downarrow \geq 50\%</math></li> <li>● FBC: if HI responses, to be noted in addition</li> </ul>
Stable disease	Failure to achieve $\geq$ PR but no evidence of progression for $>8$ weeks
Failure	Death during treatment or worsening cytopenia, $\uparrow$ % BM blasts or progression to more advanced subtype
Relapse after CR/PR	At least 1 of: <ul style="list-style-type: none"> <li>● Return to pre-therapy % BM blasts</li> <li>● <math>\downarrow \geq 50\%</math> from max. neutrophil/platelet counts</li> <li>● Hb <math>\downarrow \geq 15\text{g/L}</math> or transfusion dependence</li> </ul>
Cytogenetic response	<ul style="list-style-type: none"> <li>● Complete: disappearance of abnormality without new one</li> <li>● Partial: <math>\geq 50\%</math> reduction of abnormality</li> </ul>
Disease progression	If: <ul style="list-style-type: none"> <li>● <math>&lt;5\%</math> blasts: <math>\geq 50\%</math> <math>\uparrow</math> to <math>&gt;5\%</math></li> <li>● 5–10% blasts: <math>\geq 50\%</math> <math>\uparrow</math> to <math>&gt;10\%</math></li> <li>● 10–20% blasts: <math>\geq 50\%</math> <math>\uparrow</math> to <math>&gt;20\%</math></li> <li>● 20–30% blasts: <math>\geq 50\%</math> <math>\uparrow</math> to <math>&gt;30\%</math></li> </ul> Or any of: <ul style="list-style-type: none"> <li>● <math>\geq 50\%</math> <math>\downarrow</math> max. neutrophils/platelets</li> <li>● <math>\downarrow</math> Hb <math>\geq 20\text{g/L}</math></li> <li>● Transfusion dependence.</li> </ul>
Survival	<ul style="list-style-type: none"> <li>● Endpoints: OS death from any cause</li> <li>● EFS: failure/death from any cause</li> <li>● PFS: disease progression/death from MDS</li> <li>● DFS: time to relapse</li> <li>● Cause-specific death: death related to MDS</li> </ul>

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## Myelodysplastic/myeloproliferative diseases (MDS/MPD)

This category was created in the WHO classification of myeloid neoplasms for a group of disorders that have both dysplastic and proliferative features at diagnosis and are difficult to assign to either myelodysplastic or myeloproliferative groups.<sup>1</sup>

### WHO classification of MDS/MPD diseases

- Chronic myelomonocytic leukaemia (CMML).
- Atypical chronic myeloid leukaemia (aCML).
- Juvenile myelomonocytic leukaemia (JMML).
- MDS/MPD unclassifiable (MDS/MPD-U).

### CMML

Many patients who present with low WBC count and minimal splenomegaly, however ultimately progress to meet 'proliferative' criteria.

#### Clinical features

- Predominantly presents in >60 years age group, median 65–75 years; ♂:♀ ratio ~2:1.
- Often asymptomatic and found on routine FBC.
- Weight loss, fatigue, night sweats may occur.
- Skin and gum infiltration may occur.
- Splenomegaly (50%) and hepatomegaly (up to 20%) usually only in cases with leucocytosis and constitutional symptoms.
- Serous effusions (pericardial, pleural, ascitic, and synovial) associated with high PB monocytosis.

#### Investigations and diagnosis

- Investigations as for MDS.
- Variable leucocytosis; marked in 50%; neutrophilia in some patients.
- Monocyte count  $>1.0 \times 10^9/L$  is diagnostic minimum.
- Variable anaemia; platelets usually ↔ or ↓.
- Marrow typically hypercellular; blasts and promyelocytes <20%.
- Immunophenotype of PB and BM: CD13+, CD33+, CD14±, CD64±, CD68±; ↑ % CD34 may be associated with early AML transformation.
- Karyotypic abnormalities associated with MDS found in 20–40% (8+, 7-, 7q-, abnormal 12p); none specific for CMML apart from rarity of 5q-; Exclude 5q31–33 translocation and/or PDGFRβ gene re-arrangement; occurs in <1–2% of CMMLs, usually with marked eosinophilia; may be a unique entity; these patients may respond to imatinib.<sup>2</sup>
- Hypokalaemia may be present.
- Reactive causes of monocytosis must be excluded.

#### WHO diagnostic criteria for CMML

- Persistent peripheral blood monocytosis  $>1.0 \times 10^9/L$ .
- No Ph chromosome or BCR-ABL fusion gene.
- No rearrangement of PDGFRA or PDGFRB.
- <20% myeloblasts, monoblasts, and promyelocytes in PB or BM.

- Dysplasia in  $\geq 1$  myeloid lineages. If myelodysplasia absent or minimal, CMML may still be diagnosed if above criteria met, and:
  - An acquired clonal cytogenetic abnormality is present in BM cells, or
  - Monocytosis has been persistent for  $\geq 3$  months, and
  - All other causes of monocytosis have been excluded.

### 3 subcategories have been defined:

- **CMML-1:**  $<5\%$  PB blasts and  $<10\%$  BM blasts.
- **CMML-2:**  $5\text{--}19\%$  PB blasts or  $10\text{--}19\%$  BM blasts or when Auer rods present with  $<20\%$  BM or PB blasts.
- **CMML-1 or CMML-2 with eosinophilia:** above criteria met plus PB eosinophil count  $>1.5 \times 10^9/L$ ; associated with complications related to eosinophil degranulation: fever, fatigue, cough, angioedema, muscle pains, pruritus, diarrhoea, endomyocardial fibrosis, pulmonary infiltrates.

### Prognostic factors

BM blasts: median survival for CMML with  $<5\%$  BM blasts 53 months vs 16 months for those with  $5\text{--}20\%$ .

### Management

- Asymptomatic cases with near  $\leftrightarrow$  haematology apart from a monocytosis of  $>1.0 \times 10^9/L$  require no intervention and should simply be monitored. Therapy otherwise supportive.
- Patients with symptoms, organomegaly, and/or  $\uparrow\uparrow$  WBC may respond to oral chemotherapy; hydroxycarbamide performed better than oral etoposide in a randomized study.<sup>3</sup>
- Azacitidine: ORR 39%; CR rate 11%; PR rate 3%; haematological improvement (HI) 25%. The median overall survival 12 months. Overall survival advantage in responders compared with non-responders: 15.5 months versus 9 months, respectively.
- Rarely: younger patients may be treated with high-dose therapy and allogeneic SCT which offers only curative option. EBMT reported 5-year estimated OS 21% and DFS 18% and 52% TRM.<sup>4</sup>

### Natural history

Prognosis for asymptomatic patients is favourable (several years). For those requiring therapy, median survival is 6–12 months. Median survival overall is 20–40 months. AML (usually FAB M4/M5) develops in  $\sim 20\%$  after median of 1.5–2 years; poorly responsive to intensive chemotherapy.

### aCML<sup>5</sup>

Ph chromosome and *BCR-ABL* fusion gene –ve CML. Rare; 1–2 cases per 100 cases of CML.

- Occurs in elderly; median age in 7th or 8th decade.
- Short median survival (11–18 months).
- Most have symptoms related to anaemia.
- Splenomegaly frequent.
- FBC: WCC  $>13$ ;  $\uparrow$  WBC due to dysplastic immature ( $>10\text{--}20\%$ ) and mature neutrophils; generally  $35\text{--}96 \times 10^9/L$  though sometimes  $>300 \times 10^9/L$ ; PB monocytes rarely  $>10\%$ ; PB blasts usually  $<5\%$ ,

always <20%; anaemia and thrombocytopenia common; no or minimal basophilia (<2%) (cf. CML).

- BM: hypercellular due to ↑ and dysplastic myeloid series; M:E ratio usually >10; often multilineage dysplasia; blasts <20%.
- Cytogenetic abnormalities frequent: 8+, 13+, 20q-, i(17q), 12p-.
- No rearrangement of PDGFRA/B.

#### Management:

Hydroxycarbamide or etoposide may control the leucocytosis and ↓ splenomegaly. Evolution to AML in 20–40%; patients die of BM failure.

### JMML<sup>6</sup>

Clonal disorder arising in pluripotent stem cell causing selective hypersensitivity to GM-CSF due to dysregulated signal transduction through RAS/MAPK pathway.

Affects infants and young children (75% <3 years); ♂:♀ ratio ~2:1; 10% of cases occur in children with neurofibromatosis type 1.

- Most present with malaise, pallor, fever infection, or bleeding.
- Maculopapular rash in 40–50%.
- Hepatosplenomegaly almost universal; due to leukaemic infiltration.
- FBC: ↑ WBC (usually 25–35 × 10<sup>9</sup>/L; >100 × 10<sup>9</sup>/L in 5–10%) due to neutrophilia (with promyelocytes and myelocytes) and monocytosis (>1.0); eosinophilia and basophilia in a minority; anaemia and thrombocytopenia usual; nucleated RBCs frequent.
- Polyclonal hypergammaglobulinaemia and ↑ LDH in majority.
- BM: hypercellular with myeloid proliferation; monocytes usually 5–10%.
- Abnormal karyotype in 30–40%; none specific. 33% carry *PTPN11* mutations, 15–20% without evidence of neurofibromatosis *NF1* mutations and 15–20% *RAS* mutations.

#### Diagnostic criteria

- PB monocytosis >1 × 10<sup>9</sup>/L.
- Blasts (including promonocytes) <20% in PB and BM.
- No Ph chromosome or *BCR-ABL* fusion gene.
- Plus ≥2 of the following:
  - Haemoglobin F ↑ for age.
  - Immature granulocytes in PB.
  - WBC >10 × 10<sup>9</sup>/L
  - Clonal chromosomal abnormality (e.g. 7-).
  - GM-CSF hypersensitivity of myeloid progenitors *in vitro*.

#### Management

No consistently effective therapy. Allogeneic SCT is the target-treatment of choice (up to 55% relapse). 5-year OS ~50%.<sup>7</sup> Untreated, 30% progress rapidly and die within 1 year of diagnosis.

### MDS/MPD-U<sup>8</sup>

Applied to patients with both myelodysplastic and myeloproliferative features but who do not meet the criteria for the 3 conditions on ↻ p.252.



### Diagnostic criteria

- Clinical, laboratory, and morphological features of one of the categories of MDS with <20% blasts in PB & BM; and
- Prominent myeloproliferative features e.g. platelets  $\geq 600 \times 10^9/L$  or WBC  $\geq 13 \times 10^9/L \pm$  prominent splenomegaly; and
- Has no prior history of MPD or MPS, cytotoxic, or growth factor therapy and no BCR-ABL fusion gene, 5q-, t(3;3)(q21;q26) or inv3(q21;q26); or
- Patient has mixed features of MPD and MDS and cannot be assigned to any other category of MDS, MPD, or MDS/MPD.
- JAK2<sup>V617F</sup> mutation found in 25%.<sup>9</sup>

### Management

Manage as in patients with MDS or atypical MPD.

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# Myeloproliferative neoplasms

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## Myeloproliferative neoplasms (MPNs)

The term 'myeloproliferative neoplasm' (MPN) replaces the term 'chronic myeloproliferative disorder' (MPD) in the 2008 revision of the WHO classification.<sup>1</sup> This revision reflects information on molecular pathogenesis not previously available in 2001.<sup>2</sup> The MPNs are a heterogeneous group of clonal haemopoietic stem cell neoplasms with excessive proliferation of 1 or more of the erythroid, megakaryocytic, or myeloid lineages and relatively normal maturation resulting in ↑ numbers of red cells, platelets, and/or granulocytes in the peripheral blood. Constitutive tyrosine kinase activation appears to be a common pathogenetic mechanism. Phenotypic diversity is a result of different mutations affecting protein kinases or related molecules causing different signal transduction abnormalities.<sup>3</sup> MPNs are characterized by effective haematopoiesis devoid of dyserythropoiesis, granulocytic dysplasia, or monocytosis. Splenomegaly and hepatomegaly are common. The MPNs share a potential to progress to myelofibrosis, ineffective haematopoiesis or blastic transformation. The MDS/MPN over-lap syndromes are discussed in ↻ Chapter 6 (MDS). The myeloid/lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA/B or FGFR1 are discussed here.

### 2008 WHO classification<sup>1,4</sup>

#### MPNs

- Chronic myelogenous leukaemia, CML (discussed on ↻ p.148).
- Polycythaemia vera (PV).
- Essential thrombocythaemia (ET).
- 1° myelofibrosis (MF).
- Chronic neutrophilic leukaemia.
- Chronic eosinophilic leukaemia (CEL), not otherwise categorized.
- Hypereosinophilic syndrome (HES).
- Mastocytosis: cutaneous, extracutaneous, and systemic mastocytosis, mast cell leukaemia, and mast cell sarcoma.
- MPNs, unclassifiable.

#### *Myelodysplastic/myeloproliferative neoplasms:*

↻ See Chapter 6.

This category was created in the WHO classification of myeloid neoplasms for a group of disorders that have both dysplastic and proliferative features at diagnosis and are difficult to assign to either myelodysplastic or myeloproliferative groups:

- Chronic eosinophilic leukaemia (CEL), not otherwise categorized.
- CMML.
- Atypical CML, BCR-ABL1 negative.
- Juvenile monomonocytic leukaemia.
- Myelodysplastic/myeloproliferative neoplasm, unclassifiable.


#### *Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PGFRA, PDGFRB, or FGFR1*

Discussed later in this chapter (↻ pp.307–308).

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## Pathogenesis of the MPNs

Although included in the WHO classification of MPNs, CML is widely regarded as a separate entity characterized by the presence of *BCR-ABL* which is a consequence of t(9;22) and has a clear role in pathogenesis (see  Chronic myeloid leukaemia (CML), p.148).

Mutations of a number of protein kinases have now been described in the *BCR-ABL* negative MPNs.

The *JAK2-V617F* mutation was detected using different methods.<sup>1,2</sup> This G >T transversion in exon 14 is found in up to 95% of patients with PV.<sup>3</sup> Several different activating mutations have been found in *JAK2* exon 12 in *JAK2-V617F* negative patients with PV with isolated erythropoiesis without leucocytosis or thrombocytosis.<sup>4</sup> Thus 98% of patients with PV have a *JAK2* mutation.

This point mutation in the -ve regulatory pseudokinase domain of *JAK2* leads to constitutive activation of the JAK-STAT (signal transducers and activators of transcription) signalling pathway involved in regulation of cell growth and differentiation. Cytokines and growth factors (e.g. EPO) activate this pathway by binding to cell surface receptors. JAKs bound to the cytoplasmic domain of the receptor are activated by binding of ligand. These activate STAT proteins which dimerize, translocate to the nucleus and activate transcription of target genes. *JAK2* is the sole JAK kinase involved in EPO-receptor signalling.<sup>1,2</sup> The mutation has been found in different lineages including B and T lymphocytes. The name 'Janus' is taken from the 2-faced Roman god of beginnings and endings, because these kinases have 2 near-identical phosphate-transferring domains. One domain exhibits the kinase activity, while the other negatively regulates the kinase activity of the first.

Expression of *JAK2-V617F* (or an exon 12 mutant) confers cytokine hypersensitivity and cytokine-independent growth to haemopoietic cells<sup>1,2</sup> and in a mouse model results in erythrocytosis and marrow fibrosis but not thrombocytosis.<sup>5</sup> Constitutive activation of the JAK-STAT pathway causes excessive erythropoiesis in PV though its relationship to thrombotic complications and progression to myelofibrosis remains unclear.<sup>1</sup> However leucocytosis (mainly neutrophilia) appears to be a risk factor for thrombosis in PV and ET and *JAK2-V617F*-mediated granulocyte activation may contribute to thrombosis.<sup>6,7</sup>

The finding of *JAK2-V617F* -ve but clonal AML transformation in PV suggests that a prior abnormality may be present as appears to occur in familial clonal MPN.<sup>1,8</sup> Rare families have an ↑ incidence of MPN with clonal haematopoiesis and most affected members carry a somatic mutation of *JAK2*. It is suggested that this low penetrance, late onset familial clonal MPN is due to an inherited predisposition to somatic mutations.<sup>1,8</sup>

The *JAK2-V617F* mutation has also been found in 58% of patients with PMF, 50% with ET<sup>3</sup> and 50% with refractory anaemia with ringed sideroblasts associated with thrombocytosis (RARS-T),<sup>9</sup> a provisional 'MDS/

MPD unclassifiable' entity, and much less commonly in 5q- MDS (5%) and other myeloid neoplasms.

The reason why this mutation is associated with 3 different MPN clinical phenotypes remains unclear. Additional genetic factors clearly contribute to the clinical phenotype of *JAK2-V617F* +ve MPN. Homozygosity for *JAK2-V617F* resulting from mitotic recombination and duplication of the mutant allele (acquired uniparental disomy (UPD)) is common in PV (30%), rare in ET, and occurs occasionally in PMF (13%).<sup>10</sup> Duplication may be important to pathogenesis of PV. Other differences are: (i) the proportion of chromosomes expressing the mutation (gene dosage) is higher in PV and PMF than in ET; (ii) the allelic frequency of *JAK2-V617F* remained stable over time in ET but ↑ slightly in PV; and (iii) in PV the allelic frequency is identical in neutrophils and platelets, in ET it is higher in platelets.<sup>11</sup>

It has been suggested that *JAK2-V617F* +ve ET is a forme-fruste of PV and may have a different clinical course from *JAK2-V617F* -ve ET.<sup>12</sup> Evidence has been contradictory (reviewed in references 1 and 8).

Presence of *JAK2-V617F* may correlate with poorer survival in PMF.<sup>13</sup>

Clonal haematopoiesis is found in *JAK2-V617F* -ve MPN suggesting that other alleles cause myeloproliferation in these patients.<sup>14</sup>

Somatic mutations of codon 515 at the transmembrane–juxtamembrane junction of the thrombopoietin receptor gene (*MPL*), *MPL-W515L* or *MPL-W515K* have been detected in ~10% of patients with *JAK2-V617F* -ve PMF and a smaller proportion of patients with *JAK2-V617F* -ve ET.<sup>15</sup> Expression of *MPL-W515L* transforms haematopoietic cells to factor-independent growth and activates STAT, MAPK, and P13K-Act signalling pathways in a manner similar to *JAK2-V617F* but *in vivo* results in thrombocytosis and myelofibrosis.<sup>1</sup>

A *KIT-D816F* allele has been detected in systemic mastocytosis (SM)<sup>16</sup> and an interstitial deletion producing a fusion protein *FIP1L1-PDGFR*A involving platelet-derived growth factor- $\alpha$  has been detected in patients with a diagnosis of chronic eosinophilic leukaemia (CEL) or SM.<sup>17</sup>

*TET2* mutations occur in both *JAK2-V617F* positive and negative MPNs and are more frequent in MPN-U patients.

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## Polycythaemia vera (PV)

Erythrocytosis is defined as an increase in total red cell mass (RCM). The term 'polycythaemia' is widely used synonymously but lacks precision and can lead to confusion. It is suspected by finding a raised haematocrit (Hct) or packed cell volume (PCV).

Persistent elevation of Hct  $>0.48$  in adult ♀ and  $>0.51$  in adult ♂ is abnormal. An elevated Hct can occur with a normal RCM (absence of erythrocytosis) if the plasma volume is reduced: 'relative' erythrocytosis (RE). 2° causes of polycythaemia must be excluded.

PV is a neoplastic clonal disorder of the BM stem cell causing excessive proliferation of the erythroid, myeloid, and megakaryocyte lineages and carries a risk of thrombotic complications. PV was traditionally diagnosed using the criteria of the Polycythaemia Vera Study Group by the presence of an ↑ red cell mass. The detection of *JAK2* mutations in most patients with PV<sup>1</sup> has revolutionized diagnostic procedures.

### Incidence

PV is the most common MPN with an incidence of up to 3 per 100,000 per annum. It occurs in all races but is 10 × less frequent in Japan. Median age at diagnosis is 60 years. PV occurs at all ages but is very rare below 30 years. It occurs extremely rarely in childhood. The ♂:♀ ratio is 1.2:1.

### Aetiology

As described on p.260, mutation of the cytoplasmic tyrosine kinase *JAK2* gene (*JAK2*-V617F and *JAK2* exon 12 mutations) has a clear pathogenetic role. Incidence of PV is ↑ in populations exposed to radiation (including Japan) and petroleum refinery and chemical workers.

### Differential diagnosis of erythrocytosis

See Table 7.1.

**Table 7.1** Differential diagnosis of erythrocytosis

1° erythrocytosis	<i>Congenital</i>	1° familial and congenital polycythaemia (PFCP): gain-of-function mutation of <i>EPO</i> receptor gene → truncated receptor; 10 different alleles described; autosomal dominant; high penetrance; early age of onset; ↑ RCM, low sEPO, polyclonal haematopoiesis, ↔ WBC and platelet counts.
	<i>Acquired</i>	Familial clonal MPN: low penetrance, late onset, often <i>JAK2</i> -V617F +ve PV (previously: polycythaemia rubra vera (PRV)); 1° proliferative polycythaemia (PPP).
2° erythrocytosis (SE) due to 1° endogenous EPO production	<i>Congenital</i>	High O <sub>2</sub> -affinity haemoglobinopathy

(continued)

**Table 7.1** Differential diagnosis of erythrocytosis (*continued*)

(2° erythrocytosis (SE) due to 1° endogenous EPO production)	Congenital 2,3-DPG deficiency Autonomous high EPO production; autosomal recessive mutation of von Hippel–Lindau ( <i>VHL</i> ) gene; Chuvash polycythaemia ( <i>VHL-598C &gt;T</i> ) resulting in ↑ levels of hypoxia-induced transcription factor ( <i>HIF1</i> ) and ↑ expression of <i>EPO</i> gene; other alleles described.
<i>Acquired</i>	
<i>Hypoxaemia</i>	Chronic lung disease Cyanotic congenital heart disease with right→left shunt Living at high altitude Chronic alveolar hypoventilation e.g. gross obesity Sleep apnoea syndromes
<i>Other causes of impaired tissue O<sub>2</sub> delivery</i>	Smoking (↑ COHb)
<i>Renal disease (EPO mediated)</i>	Polycystic kidneys Renal tumours Renal artery stenosis Post-renal transplantation
<i>Tumours causing pathological EPO production</i>	Hepatoma Cerebellar haemangioblastoma Uterine leiomyoma Bronchial carcinoma Adrenal tumours Parathyroid carcinoma
<i>Liver disease</i>	Cirrhosis Hepatitis
<i>Drugs</i>	EPO abuse/doping Androgens
Idiopathic erythrocytosis (IE)	Persistent ↑ RCM, no cause found but no evidence of MPN or clear cause of 2° erythrocytosis.
Relative erythrocytosis (RE). <i>Syn.</i> apparent polycythaemia, spurious erythrocytosis, pseudo-polycythaemia	Normal RCM and reduced plasma volume; Diuretic therapy or dehydration; Gaisbock's syndrome; smoking, alcohol, hypertension, obesity.

### Clinical evaluation of a patient with suspected erythrocytosis

A patient with erythrocytosis may be asymptomatic or may present with thrombosis (up to 40% with PV), haemorrhage (up to 20% with PV), or vague symptoms (~40% with PV) of headache, dizziness, weakness, excessive sweating, tinnitus, visual upset, or gout.

Take a detailed history with attention to smoking habits, alcohol consumption, diuretic therapy, dyspepsia, and thrombosis. A history of pruritus (especially after bathing) suggests PV (occurs in ~50%). A burning sensation in fingers and toes with erythema, pallor, or cyanosis (erythromelalgia) is typical of PV. Patients presenting with abdominal vein (portal vein) thrombosis should be screened for a possible underlying MPN.

Physical examination may identify facial plethora or abnormalities associated with 2° erythrocytosis such as gross obesity, hypertension, evidence of obstructive airways disease or cyanotic cardiac conditions. At diagnosis splenomegaly and hepatomegaly occur in 67% and 40%, respectively.

### Investigations

- **FBC and film:** ↑↑ RCC and ↓ or ↔ MCV and MCH (may be evidence of Fe deficiency) in PV; neutrophils and platelets frequently ↑ in PV (rare in other causes of erythrocytosis).
- **JAK2 mutation analysis:** screen for JAK2-V617F mutation; found in 95% of patients with PV; differentiates PV from SE and RE but not from PMF or ET (58% and 50% JAK2-V617F +ve);<sup>1</sup> several JAK2 exon 12 mutations described in patients with JAK2-V617F -ve PV and should be screened for if JAK2-V617F negative;<sup>2</sup> False negatives due to low mutant allele burden possible.<sup>3</sup>
- **Serum erythropoietin (sEPO):** included in initial work-up; usually ↓ but can be ↔ in PV, extremely unlikely to be ↑; ↑ sEPO suggests 2° erythrocytosis; may be ↓ in RE and idiopathic erythrocytosis.
- **BM examination:** not always indicated. See diagnostic algorithm on p.267. Trepine may be diagnostic in PV; typically hypercellular for age due to trilineage hyperplasia (mainly erythroid and megakaryocytic); megakaryocytes conspicuous, pleomorphic (small to giant) and clustered round sinusoids or near trabeculae with deeply lobulated nuclei; usually ↓ Fe stores (95%); ↑ reticulin fibrosis present in 30%; ↔ BM histology does not exclude PV but is more usual in SE; late in the disease collagen fibrosis may supervene with clusters of dysmorphic megakaryocytes.
- **Serum ferritin:** ↓ (esp. PV) or ↔; occasionally overt Fe deficiency.
- **Serum chemistry:** U&E (to exclude renal disease); uric acid (often ↑ in MPNs); LFTs (to exclude liver disease).
- **Urinalysis:** haematuria or proteinuria should prompt further renal investigations.
- **Arterial oxygen saturation:** pulse oximetry most convenient to detect chronic hypoxia. SaO<sub>2</sub> <92% suggests causal relationship with absolute erythrocytosis.
- **ABG:** arterial blood gas for pO<sub>2</sub> assessment.
- **CXR:** to exclude pulmonary disease and congenital cardiac abnormalities.

**Additional investigations useful in selected patients**

- *BM examination: as above.*
- Abdominal USS: for hepatosplenomegaly, renal or pelvic abnormalities.
- BM cytogenetics: not routine; up to 30% have abnormalities, typically 20q-, +8, +9, 7-, and 10-.
- BFU-E culture: not widely available and thus not routinely done; PV progenitors show ↑ sensitivity to growth factors and develop 'endogenous erythroid colonies' (EEC) without added EPO.
- Haematinic assays: serum vitamin B<sub>12</sub>: levels commonly ↑ in PV due to ↑ transcobalamin release reflecting associated granulocytosis. Folate deficiency may occur.
- Sleep studies may be indicated if history of snoring, waking unrefreshed, and somnolence.
- Pulmonary function tests are indicated if lung disease is suspected.
- O<sub>2</sub> dissociation curve: in patients with erythrocytosis and suspected high-affinity haemoglobin.
- *EPO-R* or *VHL* gene analysis if congenital erythrocytosis suspected.
- RCM and plasma volume: former mainstay of diagnostic process; no longer routinely done; patient red cells labelled with <sup>51</sup>Cr and re-injected; simultaneous plasma volume measurement using <sup>131</sup>I-labelled albumin. RCM ↑ >25% above mean predicted value is diagnostic of absolute erythrocytosis. Plasma volume also ↑ (if marked splenomegaly present (see Table 7.2).

**Table 7.2** Red cell mass and plasma volume studies in erythrocytosis

	PV	SE	RE
RCM	↑	↑	↑ within NR or ↔
Plasma volume	N or ↑	N	↓ within NR or ↔

PV, polycythaemia vera; SE, 2° erythrocytosis; RE, relative erythrocytosis.

**Diagnostic algorithm for suspected PV**

In a patient with suspected PV, the following diagnostic algorithm based on the 2008 WHO criteria is recommended (Table 7.3).<sup>3</sup>

**Screen blood for JAK2-V617F mutation and measure serum EPO.**

- V617F +ve and sEPO ↓: PV highly likely → BM biopsy encouraged but not essential.
- V617F +ve but sEPO ↔ or ↑: PV likely → BM biopsy recommended for confirmation.
- V617F -ve but sEPO ↓: PV possible → BM biopsy and JAK2 exon 12 mutation screening → if results still not consistent with PV, consider congenital polycythaemia with EPO receptor mutation.
- V617F -ve and sEPO ↔ or ↑: PV unlikely → consider SE including congenital polycythaemia with VHL mutation.

**Table 7.3** 2008 WHO diagnostic criteria for PV<sup>3</sup>

Major criteria	1. Hgb >185g/L (♂), > 165g/L (♀) or Hgb or Hct >99 <sup>th</sup> percentile of reference range for age, sex, or altitude of residence or Hgb >170g/L (♂), >150g/L (♀) if associated with sustained increase $\geq$ 20g/L from baseline that cannot be attributed to correction of Fe deficiency or Elevated RCM >25% above mean $\leftrightarrow$ predicted value 2. Presence of JAK2-V617F or functionally similar mutation (such as exon 12 mutation)
Minor criteria	1. BM: hypercellular for age with trilineage growth (panmyelosis) with prominent trilineage proliferation 2. Subnormal serum EPO level 3. EEC growth <i>in vitro</i>

Diagnosis of PV requires that the patient meets both major criteria plus 1 minor criterion or the presence of the first major criterion and 2 minor criteria.

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## Natural history of PV

Untreated PV carries a significant risk of early thrombotic complications and a further long-term risk of transformation into myelofibrosis or less commonly AML. There is also an ↑ risk of bleeding notably from peptic ulcers. The aim of treatment is to reduce the risk of thrombotic complications and to prevent progression to myelofibrosis (MF) or leukaemia. Current treatments improve median survival to >15 years. Some myelo-suppressive treatments (notably chlorambucil and <sup>32</sup>P) have been associated with an ↑ risk of AML and are no longer routinely used.

Cardiovascular complications, notably MI, stroke, and VTE are the most common causes of death (41%). The rate of non-fatal thrombosis is 3.8 per 100 patients per annum. Major and fatal haemorrhage is rare (0.8 and 0.15 per 100 patients per annum).<sup>1</sup> Age and thrombotic history are the most important risk factors for thrombosis.

PV characteristically presents during a proliferative phase when control of erythrocytosis and prevention of thrombotic complications is often an urgent priority. This is often followed by a stable phase of variable duration. In some patients near ↔ counts are maintained without therapy as a result of ↓ proliferative capacity due to early myelofibrosis. Subsequently an advanced phase can develop, which is due to extensive myelofibrosis and associated with progressive hepatosplenomegaly, pancytopenia, and systemic symptoms (fever and weight loss). Incidence of this transformation is 10–15% after 10 years rising to >30% at 20 years; after transformation median survival <18 months. The incidence of AML is estimated at 2%.

### Risk stratification

Age and thrombotic history are predictive for vascular events in PV (and ET) and are used for risk stratification to inform treatment decisions (Table 7.4). Other (possible) risk factors include:

- Platelet count  $>1500 \times 10^9/L$  is a risk factor for bleeding but not for thrombosis.<sup>2</sup>
- ↑ leucocyte count ( $>20\text{--}25 \times 10^9/L$ ).<sup>3</sup>
- Quantitation of *JAK2* mutant allele (*JAK2*-V617F/*JAK2* wild type ratio  $>75\%$ ).<sup>4</sup>
- Age  $>60$  years.
- Symptomatic splenomegaly.<sup>5</sup>

**Table 7.4** Risk stratification in PV<sup>2</sup>

Risk category	Age $>60$ years or history of thrombosis	Cardiovascular risk factors <sup>a</sup>
Low	No	No
Intermediate	No	Yes
High	Yes	Not applicable

<sup>a</sup> Hypertension, hypercholesterolaemia, diabetes, smoking.



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## Management of PV

### Modifiable factors and lifestyle

In all PV patients, identify and treat any additional risk factors (e.g. hypertension) and encourage the patient to adopt a healthy lifestyle (e.g. stop smoking, take exercise).

### Patients with a low risk of thrombosis

#### Venesection

↓ RCM to ↔ as rapidly as possible to prevent complications (target Hct <0.45). No firm data exist to support these targets.<sup>1,2,3</sup> Removal of RBCs by venesection is the quickest way of reducing RCM. 450mL blood (± isovolaemic replacement with 0.9% saline) removed safely from younger adults every 2–3d (↓ volume to 200–300mL and frequency to twice weekly in elderly or patients with cardiovascular disease). If Hct very high (>0.60) venesection may be technically difficult due to extreme viscosity.

#### Maintenance therapy

Venesection alone can be used to maintain the Hct at 0.40–0.45. Once target achieved monitor 4–8-weekly to establish the requirement for further venesection. Individual requirements are variable (e.g. 2 procedures per year to monthly venesection). Fe supplements should not be given. Early studies showed ↑ risk of thrombosis in first 3 years after treatment with venesection alone thus additional cytoreductive treatment is required in patients with a higher risk of thrombosis.

#### Aspirin

75–100mg daily is recommended in all PV patients without a history of major bleeding or gastric intolerance. The ECLAP study randomized 518 mainly asymptomatic low-risk PV patients to aspirin 100mg/d or placebo.<sup>2</sup> Aspirin significantly reduced the risk of the combined endpoint of non-fatal MI, non-fatal stroke, pulmonary embolism, major venous thrombosis, or death from cardiovascular causes (RR 0.40; CI 0.18–0.91;  $p=0.03$ ). The incidence of major bleeding was not significantly ↑ (RR 1.62; CI 0.27–9.71). It has been argued that the risks and benefits of aspirin should be carefully considered in very low-risk patients, e.g. <55 years with no additional risk factors as benefits are proportional to vascular risk and below a certain level bleeding events due to aspirin may exceed vascular events prevented by it.<sup>4</sup>

### Patients with an intermediate risk of thrombosis

Treatment of these patients must be individualized. Most may be managed as for low-risk patients but cytoreductive therapy may be required in some. Poor compliance to venesection or progressive myeloproliferation (splenomegaly, constitutional symptoms, leucocytosis, and thrombocytosis) can be an indication for cytoreductive therapy.<sup>5</sup>

## Additional treatment for patients with a high risk of thrombosis

### *Hydroxycarbamide (HC)*

HC (formerly hydroxyurea) an antimetabolite (ribonucleotide reductase inhibitor), is cytoreductive treatment of choice. Onset of myelosuppression with HC is rapid, and overdosage is quickly corrected by temporary withdrawal. Start at 15–20mg/kg/day until Hct <0.45, then adjust to a maintenance dose to preserve response without WCC <3 × 10<sup>9</sup>/L. Review every 2 weeks initially and in steady-state monitor FBC every 3 months. Randomized trials in ET<sup>6,7</sup> have demonstrated the antithrombotic effect of HC but similar trials have not been reported in PV. However HC has been shown to be effective and safe in PV and is the most widely used agent.<sup>5</sup> Continuous treatment is required and some patients find the need for follow-up difficult. Longstanding concerns that HC may still carry a risk are not supported by a large observational study<sup>8</sup> and >10 years use in sickle cell disease. Nonetheless use HC cautiously in younger patients. Some patients experience GI upset, skin pigmentation, and leg ulcers. Leg ulcers occur in up to 10% and do not heal until HC is discontinued. The antithrombotic effect of HC may be due to more than myelosuppression including qualitative changes in leucocytes, ↓ expression of endothelial adhesion molecules, and ↑ NO generation.<sup>1</sup>

### *Interferon alfa*

IFN-α can control erythrocytosis and ↓ leucocytosis, thrombocytosis, and modest splenomegaly. IFN-α directly inhibits fibroblast progenitors and antagonizes PDGF, TGF-β, and other cytokines that may be involved in myelofibrosis. Commence 3 million units SC daily and when Hct <0.45 achieved reduce to lowest dose that maintains response.<sup>1</sup> Monitor patients frequently initially. Side effects (fever, flu-like symptoms, weakness, myalgia, depression) can reduce compliance. ~33% discontinue it due to toxicity.<sup>9</sup> Long-term follow-up in 55 patients (median 13 years) shows ↓ thrombotic and haemorrhagic events and confirms the absence of leukaemogenesis.<sup>10</sup> Pegylated-IFN may be better tolerated; begin at 0.5mcg/kg/week, double dose if response not achieved after 12 weeks then reduce to maintenance dose. IFN is treatment of choice in pregnant ♀, and is preferred by some in patients <40 years.<sup>1,11</sup>

### *Anagrelide*

Oral imidazoquinazoline with anticyclic AMP phosphodiesterase activity and profound effect on megakaryocyte maturation resulting in ↓ platelet production. Useful for control of thrombocytosis in PV patients who do not tolerate or fail to respond to HC or IFN. More useful in ET as no effect on progression of PV: erythrocytosis or splenomegaly. Commence at 500mcg bd, adjusting according to response with weekly increments of 500mcg/d; usual therapeutic dose 2–3mg/d. Therapeutic effect usually within 14–21 days. No evidence of mutagenic activity. Side effects may reduce long-term tolerance: headache (50%), forceful heartbeat, fluid retention, dizziness, arrhythmia (<10%), CCF (2%), and diarrhoea. Use cautiously in patients with known or suspected cardiac disease. May be used in combination with HC or IFN to achieve disease control with reduced side effects.

### Radioactive phosphorus ( $^{32}\text{P}$ ) and busulfan

These are historical early treatments for PV. Produce  $\downarrow$  in RCM 6–12 weeks after administration ( $^{32}\text{P}$  2.3 mCi/m<sup>2</sup> by IV injection every 12 weeks as necessary; busulfan by single oral dose 0.5–1mg/kg). Either agent may be repeated after 3–6 months if further myelosuppression is required. Both individually  $\downarrow$  thrombosis risk and myelofibrosis but markedly  $\uparrow$  the risk of AML. The incidence of AML after  $^{32}\text{P}$  alone is 2.5–15%.<sup>11</sup> The use of  $^{32}\text{P}$  with either busulfan or hydroxycarbamide in an individual carries a very high risk of AML. Neither is therefore recommended for patients  $\leq 75$  years who should receive hydroxycarbamide. However, in patients  $>75$  in whom compliance or regular monitoring of hydroxycarbamide dose is a problem, busulfan is likely the best choice of treatment.<sup>11,12</sup>

### Supportive treatment

- Maintain adequate fluid intake and avoid dehydration.
- Give allopurinol (300mg/d) in the early phase of treatment to minimize risks of hyperuricaemia.
- Acute gout is managed by standard therapies.

### New treatments for PV

JAK2 inhibitor: ruxolitinib. Oral agent approved for use in MF. 97% of patients achieve a haematocrit  $<45\%$ . 80% patients achieve  $>50\%$  spleen reduction. Leucocytosis normalization in 73%, thrombocytosis normalization in 69%. CR in 50%.<sup>13</sup> Trials are ongoing comparing ruxolitinib to best available therapy.

### Continued care and follow-up

Patients with PV and idiopathic erythrocytosis should have long-term haematological follow-up. Measure Hct at least 3-monthly. For patients on cytotoxic therapy with hydroxycarbamide the FBC should be checked every 8–12 weeks.

### Treatment of advanced phase PV

Symptomatic management should be prioritized. Patients often require blood product support. Splenectomy is often considered due to discomfort, recurrent infarction, or hypersplenism but often followed by massive hepatomegaly due to extramedullary haematopoiesis.

### Complications of PV

**Pruritus:** typically aquagenic, a troublesome complication for some patients. Unfortunately there is no satisfactory treatment. Sometimes abates when excess myeloproliferation controlled and Hct reduced but may persist despite adequate control. Antihistamines, H<sub>2</sub>-antagonists (cimetidine 400mg bd), IFN- $\alpha$  (3 million units 3  $\times$  weekly), the selective serotonin re-uptake inhibitor (SSRI) paroxetine (20mg/d), or phototherapy with psoralen and UV light have been used.

**Erythromelalgia:** due to microvascular disturbance. Aspirin (75 or 100mg/d maintenance) is usually effective.

**Thrombosis:** manage venous thromboembolic events according to current guidelines. Follow LMWH with warfarin (INR 2.0–3.0) for 3–6

months. Low-dose aspirin (75–100mg/d) is recommended following ischaemic stroke, TIA, peripheral arterial occlusion, MI, unstable angina, or in patients with evidence of coronary artery disease.<sup>1</sup> Role of clopidogrel unclear. Cytoreduction recommended in all patients with acute vascular events.

**Haemorrhage:** major haemorrhage uncommon. Most frequent from skin, mucous membranes and GI tract. May be precipitated by antithrombotic therapy: avoid in patients with history of haemorrhagic events, gastric ulcers, or varices. Associated with platelet count  $>1500 \times 10^9/L$ . May be related to acquired vWD due to loss of large vWF multimers resulting in a functional defect. Normalization of the platelet count corrects the coagulopathy.<sup>14</sup> Treat by withdrawal of any antithrombotic therapy and correction of thrombocytosis with HC. Consider antifibrinolytic therapy and/or vWF containing concentrates.<sup>15</sup>

### Surgery in PV patients

Relatively contraindicated in uncorrected PV. Defer surgery until Hct and platelets normalized for  $\geq 2$  months due to risk of thrombotic and haemorrhagic complications; if emergency surgery necessary perform venesection.

### PV in pregnancy

Rare. Assess risk of thrombosis and complications of pregnancy. Pre-plan conception when possible with cessation of any teratogenic agents and control of Hct. Administer low-dose aspirin throughout pregnancy and prophylactic LMWH for up to 6 weeks post-partum. When cytoreduction is necessary for uncontrolled Hct or progressive myeloproliferation, IFN- $\alpha$  is recommended.<sup>1,11,16</sup>

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## Secondary erythrocytosis

See causes of 2° erythrocytosis in  Table 7.1, p.264.

### Effects of the ↑ red cell mass

- ↑ peripheral vascular resistance.
- ↓ cardiac output.
- ↓ systemic O<sub>2</sub> transport resulting in, e.g. ↓ cerebral blood flow and O<sub>2</sub> and glucose delivery to the brain.
- Thromboembolic complications also occur. Thus ‘compensatory’ erythrocytosis is a pathological rather than physiological condition.

### Symptoms

Non-specific and those of the underlying cause (particularly if cardiac or pulmonary) may predominate. Pruritus, splenomegaly, or the presence of leucocytosis or thrombocytosis suggest the alternative diagnosis of PV.

### Investigations

As listed under PV, notably JAK2 mutation analysis, serum EPO, arterial O<sub>2</sub> saturation, renal and hepatic function, urinalysis, renal ultrasound and, if necessary, abdominal CT.

### Treatment

The aim of therapy must be correction of the underlying cause where possible and reduction of the RCM. Venesection is the treatment of choice and if possible should be continued until a target Hct is achieved. The indications for venesection and recommended target Hct vary with the underlying cause:<sup>1</sup>

- High O<sub>2</sub> affinity haemoglobins:
  - Indications—dizziness, dyspnoea or angina, thrombosis, comparable family member with thrombosis; target Hct <0.60 or if thrombosis or symptoms with Hct <0.60, target <0.52.
- Hypoxic pulmonary disease:
  - Indications—symptoms of hyperviscosity or Hct >0.56; target Hct 0.50–0.52.
- Cyanotic congenital heart disease:
  - Indications—chest and abdominal pain, myalgia and weakness, fatigue, headache, blurred vision, amaurosis fugax, paraesthesiae, slow mentation and ↑ viscosity; target individualized; isovolaemic venesection for symptoms. Avoid Fe deficiency which may compromise O<sub>2</sub> delivery.
- Post-renal transplant erythrocytosis:
  - Indication—failure to respond to ACE inhibitor or angiotensin II receptor antagonist; target Hct <0.45.

In patients with cyanotic congenital heart disease, pulmonary disease or high O<sub>2</sub> affinity haemoglobin, the benefit of venesection can be determined by symptomatic response or by using the serum EPO level as a measure of tissue hypoxia.

*Myelosuppressive therapy is not indicated in any of these disorders.*

### Reference

1. McMullin, M.F. et al. (2005). Guidelines for the diagnosis, investigation and management of polycythemia/erythrocytosis. *Brit J Haematol*, **130**, 174–95.



## Relative erythrocytosis

Elevation of Hb and Hct with  $\leftrightarrow$  or minimally  $\uparrow$  RBC mass and  $\leftrightarrow$  or  $\downarrow$  plasma volume defines RE. Apparent polycythaemia, spurious polycythaemia, pseudopolycythaemia, stress erythrocytosis and Gaisbock's syndrome (RE associated with hypertension and nephropathy) are synonymous terms for this disorder. However the existence of chronic plasma contraction is contentious.

### Aetiology

Unclear. Some cases may represent extreme ends of  $\leftrightarrow$  ranges for red cell and plasma volumes, but in most obesity, cigarette smoking, and hypertension are present singly or in combination. Haemoconcentration from dehydration or diuretic therapy should be excluded.

### Investigations

- FBC generally shows only modest  $\uparrow$  Hct. Confirm persistence with at least 2 measurements over a 3-month period.
- Serum EPO usually  $\leftrightarrow$ ; may be  $\downarrow$ .
- *JAK2-V617F* mutation  $-ve$ .
- RCM studies now rarely performed as PV excluded by sEPO and *JAK2* analysis, however can be useful here. Demonstrate  $\leftrightarrow$  RCM and  $\downarrow$  plasma volume in  $\sim 33\%$ ; most have high  $\leftrightarrow$  RCM and low  $\leftrightarrow$  plasma volume.
- Important to exclude renal disease and arterial hypoxaemia.
- BM biopsy not usually necessary but normal when carried out.

### Management

- Involves dealing with reversible associated features, i.e. weight reduction, cessation of smoking, control of  $\uparrow$  BP (without thiazide diuretic), reduction of alcohol intake, and reduction in stress (where possible). Correction of these factors may result in spontaneous improvement.
- Venesection is not standard management; however, it is suggested that patients with recent thrombosis or Hct levels chronically  $>0.54$  should be considered for venesection.<sup>1</sup>
- Monitor untreated patients to exclude further  $\uparrow$  in Hct.

### Natural history and treatment

- Not clear. Retrospective analysis appears to suggest an  $\uparrow$  incidence of vaso-occlusive episodes that may relate to associated risk factors in the lifestyle of the patients rather than the  $\uparrow$  PCV.
- Low-dose aspirin (75mg/d) advisable for patients with overt thrombotic risks and no GI contraindication.
- No role for myelosuppressive therapy.
- Many patients improve with the simple measures specified.
- Follow-up can be done in primary care.

### Reference

1. McMullin, M.F. *et al.* (2005). Guidelines for the diagnosis, investigation and management of polycythemia/erythrocytosis. *Brit J Haematol*, **130**, 174–95.

## Idiopathic erythrocytosis

Heterogeneous group with absolute erythrocytosis ( $\uparrow$  RCM) but no clear cause for 1° or 2° erythrocytosis. Some patients formerly given this diagnosis have been shown to have JAK2 exon 12 mutations.

- May be physiological variant: extreme 'high-normal' values.
- 5–10% showed definite features of PV after several years of follow-up.
- Others develop clear evidence of SE, e.g. sleep apnoea.
- Long-term follow-up is required in these patients.
- Venesection to a Hct  $<0.45$  has been recommended because of an incidence of fatal thromboembolic and haemorrhagic events similar to PV.<sup>1</sup>
- Myelosuppressive therapy is contraindicated.

### Reference

1. McMullin, M.F. et al. (2005). Guidelines for the diagnosis, investigation and management of polycythemia/erythrocytosis. *Brit J Haematol*, **130**, 174–95.

## Essential thrombocythaemia

ET (*syn.* 1° or idiopathic thrombocythaemia) is characterized by persistent thrombocytosis that is neither reactive (i.e. 2° to another condition) nor due to another myeloproliferative neoplasm or a myelodysplastic disorder. It is a diagnosis of exclusion and is biologically heterogeneous.

### Incidence

True incidence unknown but probably up to 3 per 100,000 annually;<sup>1</sup> slight excess in ♀; median age at diagnosis 60 years; frequently occurs <40 years; very rare <20 years.

### Pathogenesis

Aetiology unknown. No association with radiation, drugs, chemicals, or viral infection. Heterogeneous pathogenesis. The *JAK2-V617F* mutation is found in ~50% of patients with ET,<sup>2</sup> though it is not clear how this mutation produces 3 different clinical phenotypes of MPN. The mutation load in ET is lower than in PV and IMF and very rarely shows homozygous *JAK2* mutation.<sup>3,4</sup> Further differences are lower gene dosage in ET, generally stable frequency of the *JAK2-V617F* mutant allele over time, and higher expression in platelets than in neutrophils.<sup>5</sup>

There is evidence of clonality in some patients with *JAK2-V617F* -ve ET including the presence of a mutation of the thrombopoietin (TPO) receptor (MPL). Somatic mutations of codon 515 at the transmembrane–juxtamembrane junction of MPL (*MPL-W515L* or *MPL-W515K*) have been identified in about 10% of *JAK2-V617F* -ve PMF patients and 1% with *JAK2-V617F* -ve ET.<sup>6</sup> Hereditary thrombocytosis has been described due to mutations causing hypersensitivity of MPL to TPO<sup>7</sup> or ↑ translation of TPO from mRNA.<sup>8</sup>

ET patients with the *JAK2-V617F* mutation may have a higher frequency of thrombotic complications and it has been suggested that they have a clinical phenotype similar to PV and a different clinical course from ET patients without the mutation.<sup>9</sup>

Platelets in ET are often functionally abnormal showing impaired aggregation *in vitro*. High platelet counts ( $>1000 \times 10^9/L$ ) are associated with an acquired von Willebrand syndrome.<sup>10</sup> The mechanism may be ↑ catabolism of large-molecular-weight multimers; reduction in the platelet count corrects the abnormality and reduces haemorrhagic episodes.<sup>10,11</sup>

### Clinical features

- Diagnosis often follows detection of thrombocytosis on a routine FBC; up to 50% patients are asymptomatic. Others may present with 'vasomotor', thrombotic, and/or haemorrhagic symptoms.
- Vasomotor symptoms occur in 40%: headache, light headedness, syncope, atypical chest pain, visual upset, paraesthesiae, livedo reticularis, and erythromelalgia (erythema and burning discomfort in hands or feet due to digital microvascular occlusion).

- Haemorrhagic symptoms occur in 25% (major <5%): easy bruising, mucosal or GI bleeding, or unexplained or prolonged bleeding after trauma or surgery.
- Thrombosis occurs in ~20% (major <10%): arterial > venous, e.g. MI, CVA, splenic, or hepatic vein thrombosis.
- Splenomegaly found in <40% (less common and less marked than in other myeloproliferative disorders).
- Splenic atrophy may occur from repeated microvascular infarction.
- Recurrent abortions and fetal growth retardation due to multiple placental infarctions may occur in young women with ET.
- Patients presenting with abdominal vein (portal vein) thrombosis should be screened for a possible underlying MPN.

### Differential diagnosis

Most cases of isolated thrombocytosis are reactive and <10% are due to a 1° haematological disorder. The overlapping clinical phenotypes of ET, PV, PMF, and CML can make diagnosis difficult. Before a diagnosis of ET is made, causes of reactive thrombocytosis (RT) must be excluded (discussed on [p.290](#)) by detailed history, examination and investigation, as must PV, CML, PMF, and MDS with a predominant thrombocytosis which can all mimic ET.

### Investigations

- FBC:
  - Platelets persistently  $>450 \times 10^9/L$  (may be as high as  $5000 \times 10^9/L$ ); the degree of thrombocytosis is not a criterion for differentiating ET from RT.
  - Hb usually  $\leftrightarrow$ ; may be  $\downarrow$  with  $\downarrow$  MCV due to chronic blood loss; WBC usually  $\leftrightarrow$ .
  - Mean platelet volume (MPV) usually  $\leftrightarrow$  but can be raised; raised platelet distribution width (PDW).
  - Automated FBC may give erroneous data in severe cases as giant platelets may be counted as RBCs.
- Blood film:
  - Thrombocytosis, variable shapes and sizes (platelet aniso-poikilocytosis), giant platelets, and platelet clumps; megakaryocyte fragments; basophilia may be present; variable degree of RBC abnormality: may be hypochromic and microcytic if Fe deficient; may be changes of hyposplenism, e.g. Howell–Jolly bodies.
- Peripheral blood mutation screening for JAK2-V617F mutation:
  - Detected in ~50% of patients with ET; presence excludes reactive thrombocytosis; absence does not exclude ET.
  - Bear in mind false +ve results due to highly sensitive allele-specific assays and false -ve due to low mutant allele burden especially in ET.<sup>12</sup>
- BM examination:
  - Required to help differentiate ET from RT in JAK2-V617F -ve patients and to differentiate ET from other MPNs, notably pre-fibrotic PMF, and from MDS.
  - Aspirate: may show  $\uparrow$  platelet clumps,  $\uparrow$  large megakaryocytes with 'hypermaturation' appearances,  $\uparrow$  nuclear lobation, and large cytoplasm; little or no granulocyte or erythroid hyperplasia. Fe stores present in 40–70%.

- Trepine biopsy: ↑ megakaryocytes, dispersed throughout the marrow or with loose clustering; large, 'hypermaturation' with deeply lobated and hyperlobated nuclei; bizarre atypical forms seen in PMF not usually seen in ET; little or no granulocyte or erythroid hyperplasia. Reticulin normal or minimal ↑ (up to 25%); no fibrosis. No dysplastic features.
- Cytogenetics: abnormal in 5%; no specific diagnostic abnormalities; Always Ph-ve.
- Other tests:
  - Uric acid: ↑ in 25%.
  - Pseudohyperkalaemia: in 25%.
  - ESR and acute phase proteins (CRP and fibrinogen) usually ↔ in ET, often raised in RT but not discriminatory as inflammation can also occur in patients with ET.
  - Serum ferritin: if low Fe replacement therapy should fail to ↑ Hb to the PV range.

### 2008 WHO diagnostic criteria for ET<sup>12</sup>

Availability of the JAK2-V617F clonal marker and an ↑ use of BM histology has resulted in a recommended lower platelet count threshold for the diagnosis of ET than the historically used 600 to currently used  $450 \times 10^9/L$ . Diagnosis of ET requires that all 4 of these criteria are met (Table 7.5).

**Table 7.5** WHO diagnostic criteria for ET (2008)<sup>12</sup>

1	Persistent elevation of the platelet count $\geq 450 \times 10^9/L$
2	Megakaryocyte proliferation with large and mature morphology; no or little granulocyte or erythroid proliferation
3	Not meeting WHO criteria for CML, PV, PMF, MDS, or other myeloid neoplasm
4	Demonstration of JAK2-V617F or other clonal marker or in the absence of a clonal marker, no evidence of reactive thrombocytosis

### Diagnostic algorithm for suspected ET

Once RT has been excluded (no reactive cause) by history and examination, the diagnostic algorithm recommended by Tefferi and Vardiman<sup>12</sup> and based on the 2008 WHO criteria and 2013 BCSH guidelines may be used in a patient with suspected ET.

#### Peripheral blood mutation screening for JAK2-V617F

- V617F +ve: ET, PV, or PMF highly likely. Requires BM biopsy and cytogenetics.
- V617F -ve: ET and PMF still possible and CML should also be considered. Requires BM biopsy and cytogenetics.

### Risk factors in ET

- Thrombosis risk associated with previous thrombosis and age >60 years; no clear relationship between marked thrombocytosis and thrombotic risk; presence of leucocytosis is an additional risk factor,<sup>13</sup> as is JAK2-V617F mutation positivity and homozygosity.<sup>7</sup>

- Haemorrhage associated with extreme thrombocytosis ( $>1500 \times 10^9/L$ ) and anti-platelet therapy.

### Risk stratification in ET<sup>14</sup>

#### Low risk

- Age  $<40$  years, and
- No history of thrombosis, and
- No high-risk features/cardiovascular risk factors.

#### Intermediate risk

- Neither low risk nor high risk.

#### High risk

- Age  $\geq 60$  years, or
- Previous history of thrombosis.
- Platelets  $>1500 \times 10^9/L$  (though in younger patients higher levels could be accepted).

### Natural history

ET is usually associated with a more favourable prognosis than the other MPNs. It generally follows an indolent course and life expectancy is near normal. The risk of life-threatening complications or of leukaemic transformation is very low. However, the risk of AML has in the past been  $\uparrow$  by cytotoxic therapy (busulfan and  $^{32}P$ ) though the risk is much less with hydroxycarbamide. Need for therapy must be individualized, balancing the risk of therapy against risk of thrombosis or haemorrhage, FBC results, co-morbidity, and age. Risk of transformation is low for a patient with ET in the absence of busulfan or  $^{32}P$  therapy: 2% leukaemic transformation and 4% evolution to myelofibrosis at 15 years.<sup>15</sup> Death due to thrombosis is reported in 11–25% with a cumulative rate of cardiovascular events of 2–3% per patient year with arterial thrombosis accounting for 60–70% of events.<sup>16</sup>

### Management

Aim is to alleviate symptoms of microvascular disturbance (e.g. headache, lightheadedness, atypical chest pain, or erythromelalgia) and reduce the risks and incidence of thrombotic and haemorrhagic complications by use of aspirin and normalization of platelet count (target  $<450 \times 10^9/L$ ); balance these risks against the short and long-term risks of therapy (see Table 7.6).

- All patients with ET should be advised to make lifestyle changes (smoking, exercise, obesity) to reduce their risk of thrombosis and atherosclerosis and reversible cardiovascular risk factors should be treated (hypertension, hypercholesterolaemia).
- NSAIDs should be avoided due to risk of haemorrhage.
- Note: acquired von Willebrand syndrome (AvWS) occurs in some patients with ET with  $>1000 \times 10^9/L$  platelets; use aspirin cautiously and consider screening for AvWS and cytoreduction prior to aspirin therapy; in symptomatic patients cytoreductive therapy usually corrects the clinical symptoms and laboratory abnormality; cytoreduction is advised in asymptomatic patients with AvWS only if RiCoF  $<30\%$ ; low-dose aspirin may be used if the RiCoF remains  $>50\%$ .<sup>10</sup>

### Low-risk patients

- These patients have no ↑ risk of thrombosis (<2/100 patient years) or haemorrhage (~1/100 patient years) compared to age- and sex-matched controls;<sup>17</sup> thrombotic deaths are rare; a 'watch and wait' policy is recommended.<sup>14</sup>
- No added risk with surgery.
- Cytoreductive therapy should be avoided unless complications occur.
- Observation ± aspirin 75mg/d (if no contraindication).
- Although the antithrombotic benefit of low-dose aspirin was demonstrated in the ECLAP study in PV<sup>18</sup> there is no evidence to support 1° prophylaxis in asymptomatic low-risk ET; the Italian guidelines do not recommend it<sup>19</sup> though others do in the absence of a contraindication.<sup>20,21</sup> The risks and benefits of low-dose aspirin should be carefully balanced in the individual patient.<sup>22</sup>
- Microvascular symptoms (e.g. erythromelalgia) are an indication for low-dose aspirin and usually respond promptly (75–100mg/day).<sup>23</sup>

### Intermediate-risk patients

- Many patients in this group may simply be treated with low-dose aspirin (if no contraindication: previous GI haemorrhage, symptomatic AvWS, or RiCoF >50%) and observation.
- Smokers should be encouraged to stop smoking and obese patients to lose weight to reduce their risks of thrombosis.
- Cytoreductive therapy: individualize treatment. Might be indicated for patients with marked thrombocytosis ( $>1500 \times 10^9/L$ ) associated with a bleeding diathesis, or who are at ↑ risk of haemorrhage, or have cardiovascular risk factors.
- The ongoing PT-1 study randomizes between aspirin ± hydroxycarbamide.
- If treated: aim for platelet count  $<450 \times 10^9/L$ .<sup>24</sup>

### High-risk patients

- **Aspirin:** 75–100mg/d recommended for all high-risk patients; quickly relieves erythromelalgia (2–4d); extreme caution in patients with marked thrombocytosis, haemorrhagic complications or history of peptic ulceration; H<sub>2</sub>-antagonist or PPIs may be needed; markedly ↑ bleeding risk with higher aspirin doses. *Dipyridamole* is an alternative for patients unable to tolerate aspirin. No data on the use of clopidogrel.
- **Hydroxycarbamide (HC):** established as standard treatment of choice in combination with aspirin (when not contraindicated) for high-risk patients, definitely those >60 years. Dose 0.5–1.5g/d maintenance after higher initial doses aiming to bring platelets  $<450 \times 10^9/L$ .
  - Control of thrombocytosis with HC reduces risk of thrombosis in high-risk patients (3.6% vs 24% after 27 months in randomized study vs observation)<sup>25</sup>;
  - HC + aspirin superior to anagrelide + aspirin in the UK MRC PT-1 randomized trial in 809 patients with high-risk ET: despite similar ↓ in platelets, ↓ rates of arterial thrombosis, major haemorrhage, transformation to myelofibrosis (3 ×) and treatment withdrawal though ↑ rate of venous thrombosis.<sup>26</sup>

- Can be used in patients <60 years, though many physicians/centres continue to prefer anagrelide or IFN in this group of patients. Some patients require HC in combination with anagrelide or IFN- $\alpha$  to achieve a normal platelet count.
- Side effects: myelosuppression, oral ulceration, rash; contraindicated in pregnancy and breastfeeding; risk of leukaemia not  $\uparrow$  by HC.<sup>10,26,27</sup>
- Up to 10% of patients do not achieve the desired reduction in platelets; others develop unacceptable side effects, e.g. leg ulcers, cytopenias, or fever; a working group has developed criteria to define clinical resistance and intolerance to HC in ET to facilitate analysis of trials and management of individual patients.<sup>28</sup>
  - Platelets  $>600 \times 10^9/L$  after 3 months of at least 2g/day HC (2.5g/d if weight  $>80\text{kg}$ ); or
  - Platelets  $>400 \times 10^9/L$  and WBC count  $<2.5 \times 10^9/L$  at any dose of HC; or
  - Platelets  $>400 \times 10^9/L$  and Hb  $<10\text{g/dL}$  at any dose of HC; or
  - Presence of leg ulcers or other unacceptable mucocutaneous manifestations at any dose of HC; or
  - HC-related fever.
- *Interferon*: (start at 3 million units, 3  $\times$  weekly; usual maintenance 1–5 million units, 3  $\times$  weekly) can control thrombocytosis due to ET; useful in some younger patients and patients intolerant to HC or anagrelide; no risk of leukaemogenesis; 85% response rate with 54% achieving normal platelet counts; reduction in splenomegaly in 66% with 17% normalization of spleen size;<sup>19</sup> rarely used due to inconvenience of SC administration and poor tolerance; drug of choice in pregnancy; pegylated IFN has equal efficacy and a more convenient once weekly dose schedule.
- *Anagrelide*: commence 500mcg bd, adjust according to response with weekly increments of 500mcg/d to usual therapeutic dose of 2–3mg/d. Has been preferred in younger patients, though HC often remains the standard treatment. Interferes with megakaryocyte differentiation; therapeutic effect usually seen within 14–21d; side effects: headache ( $>50\%$ ), palpitations ( $>50\%$ ), fluid retention, diarrhoea (15–20%); contraindicated in pregnancy and patients with CCF or known cardiac disease.
- *Busulfan*: alkylating agent; produces prolonged control of platelet count;  $\uparrow$  risk of AML; restricted to patients  $>75$  years unable to comply with regular HC therapy; administer intermittently at 4–6mg/d for 2–6 weeks until response or as single dose of 0.5–1mg/kg repeated if necessary after 2–3 months.
- *Radioactive phosphorus ( $^{32}\text{P}$ )*: may be considered in elderly patients ( $>75$  years) unable to comply with regular HC therapy; side effects: myelosuppression, long-term risk of AML (10%); 2.3mCi/m<sup>2</sup> IV; may be repeated after 3–6 months.
- *JAK2-inhibitors*: may prove useful for accelerated-phase disease. Given excellent prognosis of ET, the role of JAK2 inhibitors in the majority of ET patients is unclear.



**Table 7.6** Treatment for ET<sup>20</sup>

Risk group	Age <60 years	Age >60 years	♀ of childbearing age
Low risk	?Low-dose aspirin <sup>a</sup>	Not applicable	?Low-dose aspirin <sup>a</sup>
Intermediate risk <sup>b</sup>	Low-dose aspirin <sup>a</sup>	Not applicable	Low-dose aspirin <sup>a</sup>
High risk	Hydroxycarbamide/ IFN- $\alpha$ /anagrelide <sup>c</sup> + low-dose aspirin	Hydroxycarbamide + low-dose aspirin	IFN- $\alpha$ + low-dose aspirin

<sup>a</sup> In the absence of a contraindication, including a RiCoF <50%.

<sup>b</sup> Decision to use cytoreductive agents in intermediate risk ET should be made on individual patient basis, but not standard.

<sup>c</sup> Choice dependent on guidelines used and physician/centre preference.

### ET in pregnancy

- Close monitoring is recommended in all ET patients (including low-risk patients). A multidisciplinary approach with close maternal and fetal monitoring is recommended. Additional thromboprophylaxis may be indicated.
- 1st trimester spontaneous abortion occurs frequently in young women with ET (25–40%) with late pregnancy loss in 10%; abruptio placentae occurs in 3.6% and intrauterine growth retardation (IUGR) in 4–5%.<sup>29</sup>
- Maternal thrombosis and haemorrhage are uncommon.
- High risk in ♀ with a history of severe complications in a previous pregnancy.
- Patients with the JAK2-V617F mutation have a higher risk of developing complications in pregnancy; aspirin did not prevent these and may worsen the outcome in mutation –ve mothers.<sup>30</sup>
- If possible, stop potentially teratogenic drugs (hydroxycarbamide, anagrelide) at least 3 months before conception.
- Low-dose aspirin is recommended for pregnant ♀ with ET with a history of microvascular symptoms or a previous adverse pregnancy event; generally low-dose aspirin is given throughout pregnancy and for at least 6 weeks after delivery for all ♀ with ET and PV without contraindications.<sup>31</sup> If possible, aspirin should be commenced before conception.
- LMWH is recommended for prophylaxis and treatment of DVT in selected high-risk pregnant women with ET and a history of late fetal loss, pre-term delivery, VTE and IUGR (enoxaparin 40mg od, then bid from 16 weeks then od for 6 weeks post-partum).<sup>29</sup>
- Interferon alfa is cytoreductive treatment of choice when indicated: previous history of major thrombosis or haemorrhage; platelet count >1000–1500 × 10<sup>9</sup>/L; familial thrombophilia or cardiovascular risk factors.<sup>29</sup>

### Life-threatening haemorrhage in ET

- Main sites: skin, mucous membranes, and GIT.
- Most frequent in patients with platelet counts >1000–1500 × 10<sup>9</sup>/L.
- Stop antiplatelet agents.
- Identify site of bleeding.

- Hydroxycarbamide 2–4g/d  $\times$  3–5d to  $\downarrow$  platelets (takes 3–5d for effect).
- Antifibrinolytic agents, e.g. tranexamic acid may be used.
- Desmopressin/factor VIII concentrate of limited value if evidence of AvWS.<sup>10</sup>
- Platelet transfusion may be helpful if no evidence of AvWS.

### Arterial thrombosis in ET

- Commence aspirin 75mg/d (rapid response in TIA and erythromelalgia) if no major contraindication.
- Cytoreductive therapy to normalize platelet count.

### Venous thrombosis in ET

- Commence heparin followed by oral anticoagulant therapy (target INR 2.0–3.0) according to standard protocols.
- Commence concomitant cytoreductive therapy to reduce risk of recurrence.
- Monitor platelet count and INR closely because of greater bleeding risk.

### Surgery in ET

- $\uparrow$  morbidity and mortality risk if surgery undertaken with poor control.
- Withhold aspirin for at least 5 days before elective surgery if surgically required.
- Commence/adjust hydroxycarbamide/cytoreductive therapy to achieve platelet count  $<450 \times 10^9/L$ ; especially if splenectomy planned.
- Resume oral cytoreductive therapy as soon as possible post-operatively; resume aspirin 24h after surgery if no excessive bleeding.
- Consider LWH prophylaxis as per standard protocols.
- Surgical procedures may require specific antithrombotic strategies, e.g. heparin. Monitor patient with ET carefully for haemorrhage or thrombosis.
- Thrombotic risks reduced if platelet count normal.

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### Further reading

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## Reactive thrombocytosis

Platelet counts of  $>450 \times 10^9/L$  occur as a reactive phenomenon and may be seen in:

- Infection.
- Following surgery.
- Post splenectomy or functional asplenia.
- Malignancy, e.g. occult carcinoma.
- Trauma.
- Chronic inflammatory states, e.g. collagen disorders.
- Blood loss.
- Fe deficiency.
- Haemolytic anaemia.
- Rebound in response to haematinics and/or chemotherapy.
- Any severely ill patient on ITU.

Raised platelet count in clonal haematological disorders occurs in CML, ET, PV, PMF, and also in MDS (esp. 5q- syndrome).

In RT platelets are usually  $<1000 \times 10^9/L$  but levels of  $1500 \times 10^9/L$  may occur. Platelet morphology usually normal but differentiation from ET relies on full clinical evaluation. BM examination if done reveals  $\uparrow$  megakaryocytes with  $\leftrightarrow$  morphology.

RT is generally not associated with an  $\uparrow$  risk of thrombosis or haemorrhage and no specific treatment is required. However, short-term anti-coagulant or antiplatelet therapy is advised for marked thrombocytosis occurring in the immediate post-splenectomy period as an  $\uparrow$  incidence of thrombosis has been described in this situation.

Improvement or resolution of the underlying cause usually results in normalization of the platelet count in RT.



## Primary myelofibrosis

1° myelofibrosis (syns. idiopathic myelofibrosis (IMF), myelofibrosis with myeloid metaplasia) is a MPN characterized by marrow fibrosis, splenomegaly, extramedullary haematopoiesis, and teardrop poikilocytes and leucoerythroblastosis in the peripheral blood film. Cases of myelofibrosis (MF) that evolve from PV (10–15% evolve into MF) or ET (~2% evolve to MF) are designated post-PV myelofibrosis and post-ET myelofibrosis respectively. The term PMF is reserved for *de novo* cases.

### Incidence

Rare disorder; ~5 cases per million per annum; predominantly elderly patients (median 65 years); affects ♂ and ♀ equally.

### Pathogenesis

- PMF is a clonal neoplastic proliferation arising from an early haematopoietic stem cell that leads to marked hyperplasia of morphologically abnormal megakaryocytes and clonal monocytes that stimulate reactive fibrosis of the BM.
- The *JAK2-V617F* mutation has been found in up to 58% of patients with PMF;<sup>1</sup> it is homozygous in 13% of patients with PMF compared to 30% with PV and rare in ET.<sup>2,3</sup>
- Presence of *JAK2-V617F* may correlate with poorer survival in PMF;<sup>4</sup> homozygosity is associated with more frequent unfavourable cytogenetic abnormalities.<sup>5</sup>
- The *MPL-W515L* or *MPL-W515K* mutation of the transmembrane domain of the thrombopoietin receptor (cMPL) has been detected in 9% of patients with *JAK2-V617F* -ve PMF; these mutations can co-exist in the same patient; 30% of patients with a cMPL mutation also have the *JAK2-V617F* mutation;<sup>6</sup> the burden of each mutation appears to remain constant over time.<sup>7</sup>
- Patients with *MPL-W515L/K* mutations may be older, have more severe anaemia, and be more likely to require transfusion support.<sup>8</sup>
- ~50% of patients with clinically similar PMF have no detectable mutation of *JAK2* or cMPL, so these mutations cannot be the only cause of PMF and other genetic events clearly contribute to the development of PMF; gains of genetic material occur in >50% of patients, most commonly 9p, 2q, 3p, 4, 12q, and 13q.<sup>9</sup>
- PMF fibroblasts are non-clonal; fibrosis is a reactive process in response to cytokines released by clonal megakaryocytes and monocytes, notably TGF- $\beta$ ,<sup>10</sup> PDGF, IL-1, EGF, calmodulin, and bFGF may also play a role.
- Exaggeration of normal BM reticulin pattern progresses to intense collagen fibrosis that disrupts and finally obliterates normal marrow architecture; ultimately osteosclerosis may develop.
- High levels of endothelial progenitors appear in PB in the prefibrotic phase and haematopoietic progenitors (CD34 cells) increase as PMF advances which facilitates seeding of extramedullary haematopoiesis.<sup>11</sup>

- Extramedullary haematopoiesis develops in spleen and/or liver, and occasionally other sites, e.g. lymph nodes, skin, and serosal surfaces.
- Marrow fibrosis can also occur 2° to other disorders: a minority may follow chemotherapy or radiotherapy; can develop in response to chronic myeloproliferative disorders, myelodysplasia, and 2° to carcinomatous involvement.

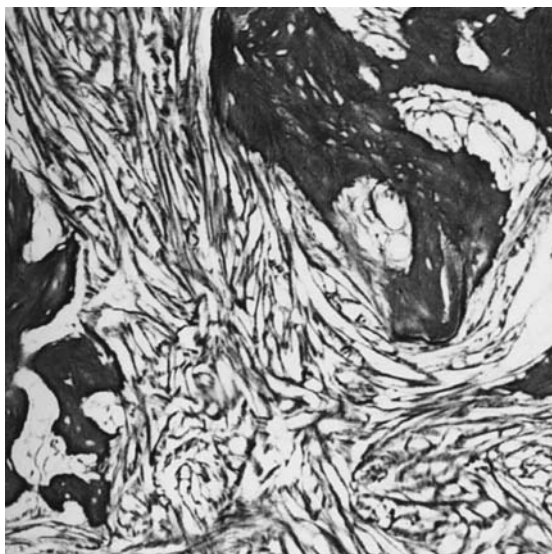
### Clinical features and presentation

- ~20% may be asymptomatic at diagnosis: mild abnormalities identified on routine FBC or splenomegaly on clinical examination.
- 70–80% are diagnosed in the fibrotic stage; most present with symptoms of progressive anaemia and hepatosplenomegaly associated with hypercatabolic features of fatigue, weight loss, night sweats, and low-grade fever.
- Abdominal discomfort (heavy sensation in left upper quadrant), early satiety and/or dyspepsia from pressure effects of splenic enlargement may prompt presentation.
- Symptoms and signs of marrow failure: lethargy, infections, bleeding.
- Splenomegaly is almost universal (>90%): moderate-to-massive (35%) enlargement; variable hepatomegaly (up to 70%); lymphadenopathy is uncommon (<10%).
- Gout in ~5%; portal hypertension, pleural effusion, and ascites (due to portal hypertension or peritoneal seeding) also occur.
- 20–30% of patients with PMF are diagnosed in pre-fibrotic stage which may clinically mimic ET; it evolves from hyperproliferation of megakaryocytes with moderate-to-marked thrombocytosis (88%  $\geq 500 \times 10^9/L$ ), occasionally with thrombosis or bleeding, mild anaemia, mild-to-moderate leucocytosis, and no or modest splenomegaly (15%) to classical fibrotic PMF.<sup>12</sup>

### Investigations and diagnosis

- FBC: Hb usually ↓ or ↔ (<10g/dL in 60%); normochromic normocytic indices; WBC may be ↓, ↔ or ↑ (~30%; rarely  $>100 \times 10^9/L$ ); basophilia and eosinophilia in 10–30%; platelets usually ↓ or ↔; occasionally ↑.
- Blood film:
  - Prefibrotic stage: thrombocytosis and mild leucocytosis but no or minimal red cell poikilocytosis, teardrops, or leucoerythroblastosis.
  - Fibrotic stage: leucoerythroblastic anaemia (nucleated red cells, myelocytes) with teardrop poikilocytes (96%) and polychromasia; giant platelets and megakaryocyte fragments.
- BM aspirate: usually unsuccessful ('dry tap').
- BM trephine biopsy: essential for diagnosis:
  - Characteristically shows patchy haemopoietic cellularity (often focally hypercellular); ↑ numbers of small-to-large irregular megakaryocytes with aberrant nuclear–cytoplasmic ratio and hyperchromatic, bulbous, or irregularly folded nuclei and dense clustering; bare megakaryocyte nuclei common; usually reticulin fibrosis (often coarse and branching) and/or collagen fibrosis; distended marrow sinusoids with intravascular haematopoiesis (Fig. 7.1).

- Prefibrotic stage: minimal or absent reticulin fibrosis,  $\uparrow$  cellularity due to granulocyte proliferation and often  $\downarrow$  erythropoiesis; differentiate from ET by  $\uparrow$  atypical megakaryocytes.
- JAK2 mutation analysis: +ve in 58%.
- Coagulation screen: features of (chronic) DIC in 15%; usually occult but causes problems at surgery (e.g. splenectomy); defective platelet aggregation common.
- Cytogenetics: abnormalities in up to 50%: 13q-, 20q-, +8, +9, t(1;7), der(6), t(1;6), (q21~23;p21.3), 12p-, 7-, 7q-, and 1q+ most frequent; exclude Ph chromosome by FISH on PB if dry tap on BM.
- Serum chemistry: bilirubin  $\uparrow$  in 40%; alkaline phosphatase and ALT  $\uparrow$  in 50%; urate  $\uparrow$  in 60%.
- LDH  $\uparrow$  due to ineffective erythropoiesis and haemolysis;  $\uparrow$  in 20% of prefibrotic MF.
- Serum ferritin: to assess Fe stores;  $\downarrow$  by occult GI blood loss in subclinical PV.
- Serum EPO: patients with level  $<125\text{U/L}$  respond more readily to EPO therapy.
- Vitamin B<sub>12</sub> often raised.
- Skeletal radiology: to assess osteomyelosclerosis.
- MRI: readily distinguishes fibrotic BM from cellular BM.



**Fig. 7.1** BM trephine in myelofibrosis: note streaming effect caused by intense fibrosis (➔ see colour plate section).



### Differential diagnosis

- Exclude other myeloproliferative neoplasms (CML, PV, ET), AML M7 ('acute MF'), myelodysplasia, lymphoproliferative disorders (particularly HCL), metastatic cancer (esp. breast, lung, prostate, gastric), tuberculosis, histoplasmosis and SLE. See Table 7.7.
- PMF is -ve for Ph chromosome and BCR-ABL fusion gene.
- Metastatic cancer in marrow, especially breast, prostate, and thyroid, can give similar FBC features but without splenomegaly; metastatic carcinoma cells are usually apparent on marrow biopsy and/or aspirate.
- Prefibrotic stage may be difficult to distinguish from PV/ET; prominent neutrophil proliferation, reduced erythroid precursors and particularly the markedly abnormal megakaryocytes assist in correct diagnosis.

**Table 7.7** Conditions associated with BM fibrosis<sup>13</sup>

Haematological disorders	Non-haematological disorders
● PMF	● Metastatic cancer (breast, lung, prostate, gastric)
● PV	● Autoimmune myelofibrosis
● CML	● Systemic lupus erythematosus
● ET	● Kala-azar (leishmaniasis)
● AML (AML-M7)	● Tuberculosis
● Myelodysplastic syndromes	● Histoplasmosis
● CMML	● Paget disease
● ALL	● HIV infection
● HCL	● Vitamin D-resistant rickets
● Hodgkin lymphoma	● Renal osteodystrophy
● Non-Hodgkin lymphoma	● Hyperparathyroidism
● MM	● Grey platelet syndrome
● CEL	● Familial infantile myelofibrosis
● Mast cell disease	● Radiation exposure
● Malignant histiocytosis	● Osteopetrosis

### Diagnostic criteria

The 2008 revised WHO diagnostic criteria for PMF define 3 major and 4 minor criteria and require all 3 major criteria plus 2 minor criteria for the diagnosis of PMF (Table 7.8).<sup>14</sup>

### Prognostic factors

Adverse prognosis is associated with:

- Marked anaemia. Hb < 100 g/L is significant prognostic marker.
- Older age.
- WBC count.
- Number of blasts in peripheral blood.
- Constitutional symptoms.
- Abnormal karyotype (other than 13q- or 20q-); strongest predictor of poor survival in post-PV MF and post-ET MF.<sup>15</sup>
- High CD34+ cell count.
- Presence of JAK2-V617F mutation.

**Table 7.8** 2008 WHO diagnostic criteria for PMF<sup>14</sup>

Major criteria	1. Megakaryocyte proliferation and atypia <sup>a</sup> accompanied by either retuculin and/or collagen fibrosis,
	<i>or</i> In the absence of reticulin fibrosis, the megakaryocyte changes must be accompanied by ↑ marrow cellularity, granulocytic proliferation, and often ↑ erythropoiesis (i.e. pre-fibrotic PMF)
	2. Not meeting WHO criteria for CML, PV, MDS, or other myeloid neoplasm
Minor criteria	3. Demonstration of <i>JAK2-V617F</i> or other clonal marker (e.g. <i>MPL-W515K/L</i> )
	<i>or</i> No evidence of reactive marrow fibrosis
	1. Leucoerythroblastosis
	2. ↑ serum LDH
	3. Anaemia
	4. Palpable splenomegaly

<sup>a</sup> Small-to-large megakaryocytes with an aberrant nuclear/cytoplasmic ratio and hyperchromatic and irregularly folded nuclei and dense clustering.

### Risk assessment

Risk assessment has become important to identify patients for whom the risks of SCT are appropriate. A number of systems have been developed but none is clearly superior.

The simple Lille scoring system (Table 7.9) is based on 2 adverse prognostic factors has been widely used in both PMF and post-PV/ET MF.<sup>15</sup>

### Prognostic scoring systems

#### Lille prognostic scoring system

Two factors:

- Hb <100 g/L.
- WBC <4 × 10<sup>9</sup>/L or >30 × 10<sup>9</sup>/L.

**Table 7.9** Lille prognostic scoring system for MF

Number of factors	Risk group	Cases (%)	Median survival (months)
0	Low	47	93
1	Intermediate	45	26
2	High	8	13

#### International Prognostic Scoring System (IPSS)<sup>16</sup>

- 5 risk factors for estimating survival from time of diagnosis:
- Age >65 years.
- Hemoglobin level <100 g/L.
- Leucocyte count >25 × 10<sup>9</sup>/L.
- Circulating blasts ≥1%.
- Presence of constitutional symptoms.

See Table 7.10.

**Table 7.10** IPSS for PMF<sup>16</sup>

Number of factors	Risk group	Expected median survival (years)
0	Low	11.3
1	Intermediate 1	7.9
2	Intermediate 2	4.0
≥3	High	2.3

- Dynamic IPSS-plus (DIPSS-plus)—modified IPSS for use at any time during the disease course.<sup>17,18</sup>

See Table 7.11.

**Table 7.11** DIPSS-plus prognostic scoring system<sup>17,18</sup>

Prognostic variable	0	1
Age (years)	≤65	>65
WBC	≤25	>25
Hb (g/L)	≥100	<100
PB blasts (%)	<1	≥1
Constitutional symptoms	No	Yes
RC transfusion need	No	Yes
Platelet count <100	≥100	<100
Unfavourable karyotype <sup>a</sup>	No	Yes

<sup>a</sup> Complex karyotype or sole or 2 abnormalities that include +8, -7/7q-, i(17q), inv(3), -5/5q-, 12p-, or 11q23 rearrangement.

- The 8 DIPSS-plus risk factors are used to define low (no risk factors), intermediate 1 (1 risk factor), intermediate 2 (2 or 3 risk factors), and high (≥ 4 risk factors) -risk groups with respective median survivals of 15.4, 6.5, 2.9, and 1.3 years.

## Management

- PMF is incurable except by allogeneic SCT but few patients are eligible; no other treatment is currently proven to alter disease course, prevent leukaemic transformation, or prolong survival. However, results from ongoing trials with novel agents such as JAK2-inhibitors (ruxolitinib) are awaited.
- Treatment is largely palliative and aims to improve anaemia, alleviate symptomatic organomegaly, and hypercatabolic symptoms.
- Asymptomatic cases with minimal FBC abnormalities and splenic enlargement should simply be observed with regular follow-up.
- Allopurinol should be commenced to treat or prevent hyperuricaemia.

### Treatment of anaemia

- Treatment for anaemia should be considered at Hb <10g/dL, in symptomatic patients and those with reduced functional capacity.<sup>19</sup>
- *Red cell transfusion* for symptomatic anaemia; transfuse on basis of symptoms not at a specific Hb level; Fe chelation therapy should be initiated when long-term transfusion therapy is commenced.
- *Androgen therapy*: semi-synthetic androgen danazol commenced at a dose of 200mg tds, tapering to minimum effective dose in responders after 6 months improves Hb in ~40%;<sup>20</sup> median time to response 5 months; most respond within 2–3 months, some later (6–9 months); response usually transient; fewer side effects than other androgens, e.g. oxymetholone; frequent slight ↑ in LFTs and androgenic effects in ♀; monitor LFTs; may have synergistic effect with EPO.<sup>21</sup> Continues to be used in treatment of anaemia in PMF.
- *Corticosteroids*: prednisolone (1mg/kg/d) primarily indicated for PMF patients with DAT+ haemolysis.<sup>19</sup>
- *EPO*: rHuEPO at initial dose of 10,000 units 3 × weekly achieves responses in 40–50% of patients which may be maintained >12 months in half;<sup>21,22</sup> Darbepoetin alfa at initial dose 150mcg/week rising to 300 mcg/week if no response after 4–8 weeks achieves similar response rate;<sup>23</sup> serum EPO level <125 U/L increases the probability of response; EPO recommended in treatment of PMF-associated anaemia in patients unresponsive to danazol or in whom it is inappropriate;<sup>19</sup> alternatively if serum EPO inadequate give trial of rHuEPO/darbepoetin for 3 months and continue in responders.

### Cytoreductive therapy

- Controls proliferative features (leucocytosis, thrombocytosis, splenomegaly, and constitutional symptoms); no effect on disease course; can worsen anaemia.
- *Hydroxycarbamide (HC)*: cytoreductive therapy of choice;<sup>19</sup> usually promptly reduces leucocytosis, thrombocytosis, and hypercatabolic symptoms; splenomegaly response may take several months; commence at 15–20mg/kg/d and titrate to maintain normal counts; generally well tolerated apart from worsening anaemia.
- *Cytotoxic alternatives to HC*: low-dose *melphalan* (2.5mg tiw) and *busulfan* (2mg/d for 1–2 months at 3–6-month intervals) can normalize counts and reduce splenomegaly but are associated with higher rates of leukaemic transformation up to 30%. Only used in elderly patients and palliative situations.
- *Cladribine (2-CDA)*: recommended in symptomatic patients refractory to other therapies;<sup>19</sup> also useful for management of thrombocytosis, leucocytosis, and progressive hepatomegaly after splenectomy;<sup>24</sup> dose 0.05–0.1mg/kg for 7d per month for up to 5 cycles.
- *Interferon alfa*: 5 million units tiw controls hyperproliferative features but poor tolerance limits its utility.<sup>25</sup> Has been recommended for younger patients.
- *Anagrelide*: may control thrombocytosis in patients intolerant of other cytoreductive therapy but no impact on myelofibrosis.

### Anti-angiogenic agents

- *Thalidomide*: at low dose (50mg/d) in combination with prednisolone (0.5mg/kg/d) can improve anaemia (in up to 70%), thrombocytopenia (in up to 75%), and reduce spleen size (in 19%);<sup>26</sup> higher doses of thalidomide are poorly tolerated and benefit has been difficult to demonstrate; low-dose thalidomide + prednisolone is recommended in non-responders to danazol and EPO.<sup>19</sup>
- *Lenalidomide*: (10mg/d for 3–4 months) can improve anaemia (22%), thrombocytopenia (50%), and splenomegaly (33%)<sup>27</sup> and reduce cells bearing marker chromosomes and JAK2-V617F.<sup>28</sup>

### Splenectomy

- Palliative measure indicated for massive or symptomatic splenomegaly, excessive transfusion requirements, refractory thrombocytopenia, hypercatabolic symptoms unresponsive to cytoreduction, uncontrollable haemolysis, and portal hypertension.
- Evaluate coagulation system preoperatively; contraindicated in patients with thrombocytosis—control preoperatively.
- A review of 314 patients treated from 1976–2004 reports 7% operative mortality, 28% perioperative morbidity (infection, thrombosis, bleeding), 76% symptomatic improvement, 50% improvement in anaemia, and 19-month median overall survival; survival was reduced in patients with preoperative thrombocytopenia  $<100 \times 10^9/L$ ; post-splenectomy thrombocytosis occurred in 29% and accelerated hepatomegaly in 10%; perioperative thrombohaemorrhagic complications were ↓ by prompt use of cytoreductive agents.<sup>29</sup>
- No evidence that splenectomy is followed by an ↑ risk of leukaemic transformation.

### Radiotherapy

- *Splenic irradiation* may be used to reduce spleen size and discomfort in patients unfit for splenectomy (3–6-month benefit);<sup>30</sup> due to transient benefit and the risk of severe pancytopenia (~45%), not recommended for most patients; irradiation pre-splenectomy increases risk of post-op bleeding.
- Hepatic irradiation contraindicated due to same problems.
- Low-dose involved field irradiation is useful treatment for extramedullary haemopoietic (EMH) infiltrates at other sites, e.g. paraspinal, pericardium, pleural, or peritoneal cavities (causing effusion or ascites) and whole lung irradiation is effective when pulmonary EMH causes pulmonary hypertension.<sup>13,25</sup>

### Allogeneic SCT

- Only therapy for PMF with curative potential; consider in all patients ≤65 years with high-risk features at diagnosis or later in the disease course.
- Myeloablative conditioning regimens have been associated with high transplant-related mortality (25–48%).<sup>31</sup>
- Advanced age is an important prognostic factor in myeloablative SCT: 14% patients ≥45 years survived at 5 years vs 62% younger patients.<sup>32</sup>

- RIC conditioning with cyclophosphamide + busulfan appears to give better outcomes than TBI-based conditioning regimens due to lower toxicity.
- Myeloablative SCT may be expected to achieve 33–53% CR rate, 27–48% TRM at 1 year and 41–64% OS between 2.8–5 years (reviewed in reference 31).
- Reduced-intensity conditioning (RIC) reduces toxicity in older patients including those with unrelated donors; the 2 largest studies each with 21 patients with median age ~54 years, reported CR in ~75%, 10–16% TRM at 1 year, and ~85% OS between 2.2–2.7 years.
- The optimum RIC regimen has not been established.
- The place of splenectomy before SCT is unclear though it may be associated with more rapid haematological reconstitution.
- Responses to donor lymphocyte infusions (DLI) in patients who have relapsed after allo-SCT have been reported.<sup>25</sup>
- Myeloablative or RIC SCT is indicated in patients with high-risk PMF aged <45 years; RIC SCT should be considered in patients suitable for intensive therapy with high-risk PMF aged 45–65 years; RIC SCT should be considered in patients with intermediate risk PMF on an individual basis.

#### *JAK2-inhibitors*<sup>33,34</sup>

- Ruxolitinib is approved for use in PMF in the USA and UK (via the national Cancer Drug Fund). Oral agent; well tolerated. Impressive clinical/symptomatic response though no change in disease course documented as yet. Trials ongoing.

#### *Novel therapies*<sup>31,33,34</sup>

- Other JAK2 inhibitors: partial or complete resolution of JAK splenomegaly and reduction in constitutional symptoms.
- Bortezomib, the proteasome inhibitor that inhibits NFκB remains in early phase trials in PMF.
- Azacitidine and decitabine are hypomethylating agents in early phase studies as single agents or in combination with a histone deacetylase inhibitor.
- Inhibitors of vascular endothelial growth factor (VEGF) remain in early phase trials.
- Pomalidomide remains investigational.

### **Prognosis**

- Worst prognosis of the MPNs: median survival 3.5–5 years (range 1–30 years).
- Morbidity and mortality commonly due to leukaemic transformation, portal hypertension, infection, haemorrhage, and thrombosis.
- Hypersplenism often develops as the spleen enlarges.
- Progressive cachexia occurs due to hypercatabolic state in advanced PMF.
- Death in symptomatic cases usually due to infection or haemorrhage.
- Up to 20% transform to AML refractory to intensive chemotherapy.

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## Chronic neutrophilic leukaemia

- Characterized by peripheral blood mature neutrophilia ( $\geq 25 \times 10^9/L$ ) without significant left shift, monocytosis, eosinophilia, or basophilia.<sup>1</sup>
- Very rare; historically a minority fulfil criteria for 'true' CNL and this diagnosis has encompassed a number of distinct pathological entities.<sup>2</sup>
- Median age ~65 years, range 15–86; ♂:♀ ratio 2:1.<sup>2</sup>
- Usually asymptomatic at diagnosis; fatigue most common symptom.<sup>3</sup>
- Blood tests: usually no anaemia or thrombocytopenia at presentation.
- ↑ serum B<sub>12</sub> and ↑ uric acid (and history of gout) common.

### Diagnostic criteria (WHO 2008)

- Peripheral blood leucocytosis  $\geq 25 \times 10^9/L$  with >80% neutrophils and bandforms and blasts <1%.
- Hypercellular BM with ↑ granulocytes with normal maturation. Blasts <5%.
- Hepatosplenomegaly.
- No identifiable cause for physiological neutrophilia, or demonstration of clonality.
- No Philadelphia chromosome or *BCR-ABL1* fusion gene.
- No rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1*.
- No evidence for PB, ET or PMF.
- No evidence of MDS or MDS/MPN.

### Prognosis

Variable prognosis described for patients with CNL with survival up to 20 years.<sup>1</sup> 'True' CNL may have median OS of 30 months with only 28% 5-year OS.<sup>2,3</sup>

- Disease acceleration: progressive treatment-refractory neutrophilia, marked splenomegaly, anaemia, thrombocytopenia, and the appearance of immature myeloid progenitors including blasts in the peripheral blood.<sup>3</sup>
- A significant proportion of patients with CNL will progress to AML.
- Death due to blast transformation, progression without transformation, bleeding complications and infection.
- No standard treatment recommendation; optimum therapy undefined; Treatment may be unnecessary if asymptomatic.

### Treatment

- Hydroxycarbamide (HC) may control progressive neutrophilia and splenomegaly and maintain a prolonged stable phase; recommended 1st-line therapy with median response of 12 months.<sup>3</sup>
- Busulfan can achieve the same effect.
- Interferon alfa is option as 2nd-line therapy<sup>3</sup> though responses to 2nd-line therapy often short-lived and poorly tolerated.<sup>4</sup>
- AML-type induction therapy is ineffective.<sup>4</sup>
- Splenic irradiation and splenectomy can reduce tumour bulk and relieve abdominal discomfort; splenectomy may aggravate leucocytosis.<sup>5</sup>
- Allogeneic SCT is potentially curative<sup>5,6</sup> and in view of the poor prognosis of patients with true CNL, should be considered in eligible patients.

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## Eosinophilic syndromes and neoplasms

Persistent eosinophilia in the peripheral blood and tissues may occur in a heterogeneous group of conditions and requires investigation. First step is to exclude a 2° eosinophilia. If reactive causes have been excluded a clonal diagnostic entity can be expected, or if none found, idiopathic hypereosinophilic syndrome. The diagnostic entities historically were classified as myeloproliferative disorders, though distinct categories now exist.<sup>1</sup>

### Diagnostic groups/entities (discussed here)<sup>1,2</sup>

- Reactive or 2° eosinophilia.
- Myeloid and lymphoid neoplasms with abnormalities of *PDGFRA*, *PDGFRB*, or *FGFR1*.
- Chronic eosinophilic leukaemia, not otherwise specified (CEL, NOS).
- Lymphocyte-variant hypereosinophilia.
- Idiopathic hypereosinophilic syndrome (HES), which is a diagnosis of exclusion.

### Investigation of a patient with eosinophilia

All patients with eosinophilia should be assessed with a detailed history including drugs and travel, a full physical examination, blood count and film, routine chemistry, serum IgE, serum B<sub>12</sub>, ECG, echocardiogram, CXR, pulmonary function tests, and CT chest and abdomen. Further investigations may be indicated by the findings, e.g. parasite screen, HIV serology.

If eosinophilia remains unexplained, the diagnostic algorithm recommended by Tefferi and Vardiman<sup>1</sup> and based on the 2008 WHO criteria should be used:

- Perform BM biopsy, tryptase stain, T-cell clonality studies (immunophenotyping and TCR gene rearrangement studies), cytogenetic studies, and FISH or RT-PCR for *FIP1L1-PDGFR*A:
  - *FIP1L1-PDGFR*A +ve → *PDGFRA* rearranged myeloid neoplasm with eosinophilia.
  - 5q33 translocations → *PDGFRB* rearranged myeloid neoplasm with eosinophilia.
  - 8p11 translocations → *FGFR1* rearranged myeloid neoplasm with eosinophilia.
  - BM histology shows abnormalities other than eosinophilia → use histology to make specific diagnosis
  - BM unremarkable other than eosinophilia and no clonal T cells
    - → PB blasts >2% or BM blasts >5% or abnormal cytogenetics: CEL-NOC/HES.
    - → PB blasts <2% and BM blasts <5% and no abnormal cytogenetics: idiopathic HES.

### Conditions associated with 2° or reactive eosinophilia

- Allergic: asthma, allergic bronchopulmonary aspergillosis, drug reactions.
- Infectious and parasitic: helminths, ectoparasites (scabies), protozoa, fungi (coccidiomycosis), mycobacteria, and viruses (HIV, HSV).
- Inflammatory: Loeffler syndrome, collagen vascular disorders (Wegener granulomatosis, PAN, SLE), sarcoid.
- Neoplastic: CML, AML with inv(16), ALL, angioimmunoblastic lymphoma, chronic MPNs, Hodgkin and non-Hodgkin lymphoma, adenocarcinoma.
- Immune dysregulation: Wiskott–Aldrich syndrome, phenotypically aberrant T lymphocytes with abnormal release of cytokines.
- Metabolic: hypoadrenalism.

### Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB, or FGFR1

The 2008 WHO classification<sup>1</sup> has redefined myeloid and lymphoid neoplasms with molecularly characterized clonal eosinophilia, previously classified under CEL/HES, as a new category of their own and removed them from the MPN category.

#### *Myeloid and lymphoid neoplasms associated with PDGFRA rearrangement (platelet derived growth factor)*

- Rare disorder. Karyotype usually normal.
- Activating mutation of the gene for PDGFRA due to interstitial deletion at 4q12 producing a FIP1L1-PDGFRA fusion gene.<sup>3</sup> Associated with HES, CEL, and systemic mastocytosis (SM).<sup>3–5</sup>
- t(4;22)(q12;q11) producing BCR-PDGFRA fusion gene also described associated with atypical CML.<sup>6</sup>
- FIP1L1-PDGFRA detected by FISH in 14% of patients with 'primary eosinophilia'; also detectable by RT-PCR; almost all classified histologically as SM due to mast cell infiltrates in the BM.<sup>5</sup> SM-CEL has been suggested as nomenclature.<sup>7</sup>
- Occurs almost exclusively in ♂; associated with eosinophil-related tissue damage, elevated serum tryptase, splenomegaly, BM hypercellularity with reticulin fibrosis, and atypical mast cells.
- Extremely sensitive to imatinib therapy: 100mg/d achieved 100% durable clinical and haematological CR in 8 patients;<sup>8</sup> if clinical or molecular relapse occurs, the dose should be ↑ to 400mg/d;<sup>9</sup> concomitant prednisolone therapy (1mg/kg/d) is recommended for initial 1–2 weeks to reduce risk of drug-induced cardiogenic shock.<sup>7</sup>
- Acute phase disease may be myeloid or T-lymphoblastic.

#### *Myeloid neoplasms associated with PDGFRB rearrangement (platelet derived growth factor)*

- Rare disorder.
- Activating mutation of PDGFRB gene at 5q33 associated with eosinophilia sometimes accompanied by monocytosis.<sup>10</sup>
- Usually involves translocation e.g. t(5;12)(q33;p13) and karyotype usually abnormal.
- Detected in patients previously diagnosed with CEL or CMML.<sup>11</sup>
- Rarely affects ♀; also responds to imatinib.

*Myeloid and lymphoid neoplasms associated with FGFR1 rearrangement*

- Rare aggressive disorder also known as 8p11 myeloproliferative syndrome. Stem cell leukaemia-lymphoma syndrome causing eosinophilia associated with myeloproliferative and myelodysplastic features. Acute phase of disease can be T-cell ALL or progression to AML.<sup>12</sup>
- Translocations involving 8p11 and multiple partners result in constitutive activation of a fusion tyrosine kinase involving *FGFR1*.<sup>12</sup>
- Unresponsive to imatinib.
- Only allogeneic SCT is effective at suppressing the malignant clone.<sup>12</sup>

**CEL, not otherwise categorized (CEL-NOC)**

Rare condition. Clonal proliferative disorder of eosinophil precursors causing persistent ↑ in mature and immature eosinophils in PB ( $\geq 1.5 \times 10^9/L$ ) and in BM.

Hyper-eosinophilic syndrome (HES) is a synonym though its terminology not recommended due to the confusion with *idiopathic* HES which is a different diagnostic entity.

*WHO defined diagnostic criteria (2008 classification)*

- Persistent peripheral blood eosinophilia  $\geq 1.5 \times 10^9/L$ .
- No Ph chromosome or *BCR-ABL1* fusion gene or other MPN (PV, ET, PMF) or MDS/MPN (CMML or aCML).
- No t(5,12) or other rearrangement of *PDGFRB*.
- No *FIP1L1-PDGFR* fusion gene or other rearrangement of *PDGFRA*.
- No rearrangement of *FGFR1*.
- Blasts PB and BM <20% and no inv(16), t(16,16) or other feature diagnostic of AML.
- No clonal cytogenetic or molecular genetic abnormality; blast cells >2% in PB or >5% in BM.

Marked ♂ predominance; usually age 20–50 years; incidental finding or short history of fever, malaise, fatigue, sweats, weight loss, skin rash, angioedema, muscle pains, pruritus, diarrhoea, and ↑ susceptibility to infection; splenomegaly in 30–50%.

PB eosinophils mainly mature with few eosinophilic myelocytes or promyelocytes; some show abnormalities: sparse granulation, vacuolation, hypersegmentation, hyposegmentation, or large forms; neutrophilia with left shift frequent; platelet count variable.

BM hypercellular; usually 10–30% eosinophils; often >5% blasts; no specific cytogenetic or molecular abnormality; no dysplastic features, Ph chromosome or *BCR-ABL* fusion gene.

Associated with tissue infiltration by immature eosinophils, anaemia, and thrombocytopenia; end-organ damage may occur due to leukaemic infiltration or release of eosinophil cytokines or enzymes: scarring of mitral/tricuspid valves, peripheral neuropathy, CNS dysfunction, pulmonary symptoms, and arthritis.

### Treatment

Eosinophilia may be controlled initially by steroids followed by hydroxycarbamide (up to 2g/d); does not generally respond to imatinib. Other antimetabolites or cytostatic drugs have been used. Alemtuzumab has been used with variable results. Some patients respond to Imatinib (even though *FIP1L1-PDGFR* -ve). In younger patients consider sibling allogeneic SCT.

### Prognosis of CEL-NOC/HES

Variable survival; up to 80% at 5 years decreasing to 42% at 15 years. Poor prognostic factors include presence of a concurrent myeloproliferative disorder, lack of response to steroids, cardiac disease, ♂ gender, and degree of eosinophilia.<sup>13</sup> The WHO identified marked splenomegaly, PB blasts, BM blasts, cytogenetic abnormalities and myeloid lineage dysplasia as unfavourable features.<sup>14</sup>

## Idiopathic HES

### Diagnosis

Diagnosis of exclusion. Characterized by PB eosinophilia  $\geq 1.5 \times 10^9/L$  for >6 months for which no underlying cause is found and by symptoms relating to end-organ damage. It requires the same first 5 criteria listed for CEL-NOC in the WHO classification,<sup>1,14</sup> but in addition:

- No evidence of a clonal cytogenetic abnormality, and
- $\leq 2\%$  PB blasts, and
- $\leq 5\%$  BM blasts.

Often incidental finding or symptoms as per CEL-NOC; ♂:♀ ratio ~9:1. Peak incidence in 4th decade.

Note: 20–50% of patients previously diagnosed with HES had *FIP1L1-PDGFR* abnormality which occurs almost exclusively in ♂.<sup>15</sup>

PB eosinophils indistinguishable from CEL-NOC; neutrophilia with left shift frequent; platelets variable; polyclonal increase in immunoglobulins including IgE common; splenomegaly 40%, anaemia 50%; BM hypercellular with 25–75% eosinophils with left shift.

An episodic form associated with angioedema, urticaria, weight gain, fever, and a polyclonal gammopathy including elevated levels of IgE that follows a relatively benign course and is steroid responsive has been described (Gleich syndrome).<sup>16</sup>

Endomyocardial fibrosis progressing to restrictive cardiomyopathy, skin lesions (angioedema, urticaria), thromboembolic disease, pulmonary lesions, arthritis, and CNS dysfunction occur commonly due to infiltration and release of enzymes.

### Treatment

- Eosinophilia may be rapidly controlled by prednisolone (1mg/kg/d) tailing slowly to a maintenance dose (70% respond).
- HC (starting dose 500mg bd, titrating up to 2g/d as necessary) may be added in patients with tissue damage.<sup>7</sup>
- Interferon alfa (starting dose 1 million units tiw) remains an option for treatment<sup>7,17</sup> and may maintain prolonged remissions associated with improved clinical symptoms and organ disease.<sup>17,18</sup>

- Interferon alfa + HC allows dose reduction and better control than with either agent alone.<sup>13</sup>
- Vincristine, cyclophosphamide, 6-thioguanine, and cytarabine have been used with variable results.
- Alemtuzumab (anti-CD52) has been used with variable results.
- A trial of imatinib may be effective in some *FIP1L1-PDGFR* –ve patients.<sup>17</sup>

### Eosinophilia associated with aberrant T lymphocytes

- Eosinophilia may result from the presence of immunophenotypically aberrant, often demonstratively clonal, IL-5-secreting T lymphocytes.<sup>19</sup>
- A study of 60 patients, recruited mainly from dermatology clinics reported 16 patients with circulating T-cells with aberrant phenotype of whom 8 had clonal T-cell receptor (TCR) rearrangement; 4 patients subsequently developed T-cell lymphoma.<sup>15</sup>
- Aberrant phenotype includes absent, reduced, or ↑ expression of several antigens (e.g. CD2, CD3, CD4, CD5, CD6, CD7, or CD95), usually CD3–, CD4+, CD8–, or expression of activation markers (CD25 or HLA-DR).
- Elevated serum IgE may be further evidence of Th2 activation.
- Equal frequency in ♂ and ♀; dermatological involvement in majority; GI symptoms and obstructive lung disease also common but endomyocardial fibrosis and myelofibrosis rare; CD3–, CD4+, CD8– and del(6q) associated with ↑ risk of progression to T-cell lymphoma.<sup>18</sup>
- In a patient with skin abnormalities, lymphocytosis, or abnormal lymphocytes, immunophenotype and TCR gene analysis are recommended.<sup>19</sup>
- Prednisolone as a single agent or in combination with interferon alfa is usually effective; HC has been used with variable to good results.

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## Mastocytosis (mast cell disease)

Mastocytosis is a heterogeneous group of diseases characterized by abnormal proliferation of mast cells in 1 or more organ systems including skin, BM, liver, spleen, and lymph nodes. They range from skin lesions that may spontaneously regress to aggressive multisystem disease with a poor prognosis (Table 7.12).

**Table 7.12** WHO classification of mastocytosis<sup>1</sup>

Variant	Subvariants
Cutaneous mastocytosis (CM)	<ul style="list-style-type: none"> <li>• Maculopapular CM (MPCM)/urticaria pigmentosa (UP)</li> <li>• Diffuse CM (DCM)</li> <li>• Mastocytoma of skin</li> </ul>
Indolent systemic mastocytosis (ISM)	<ul style="list-style-type: none"> <li>• Smouldering SM (SSM)</li> <li>• Isolated bone marrow mastocytosis (BMM)</li> </ul>
Systemic mastocytosis with an associated clonal haematological non-mast cell lineage disease (SM-AHNMD) <sup>a</sup>	<ul style="list-style-type: none"> <li>• SM-AML</li> <li>• SM-MDS</li> <li>• SM-MPN</li> <li>• SM-CMML</li> <li>• SM-NHL</li> <li>• SM-CEL</li> <li>• SM-HES</li> </ul>
Aggressive systemic mastocytosis (ASM)	<ul style="list-style-type: none"> <li>• Lymphadenopathic SM with eosinophilia<sup>b</sup></li> </ul>
Mast cell leukaemia (MCL)	<ul style="list-style-type: none"> <li>• Typical MCL</li> <li>• Aleukaemic MCL<sup>c</sup></li> </ul>
(Extracutaneous) mast cell sarcoma (MCS)	
Extracutaneous mastocytoma	

<sup>a</sup> The non-mast cell lineage disease must also be defined by WHO criteria.

<sup>b</sup> In a subgroup of these patients the *FIP1L1/PDGFR*A-fusion gene is detectable.

<sup>c</sup> Circulating mast cells <10%.

### Epidemiology

Occurs at any age; median age of SM 50–60 years; range 5–88; CM most common in children: median age 2.5 months, may be present at birth, usually evident by 6 months;<sup>2</sup> after age 10 years median age of CM 26 years.

### Pathogenesis

Mast cells are derived from pluripotential haemopoietic cells and are the effector cells of the immediate allergic reaction via high affinity receptors for IgE. Most variants of SM are clonal and a somatic mutation of *c-KIT* (usually D816V on exon 17), the proto-oncogene, located at 4q12, that encodes the transmembrane tyrosine kinase receptor for stem cell factor (SCF), is present in >80%.<sup>3–5</sup> Other mutations described in ~25% of cases lacking the D816V mutation but each individually rare.<sup>5–7</sup> These

mutations lead to constitutive activation of KIT protein independent of SCF binding, causing mast cell proliferation and suppressing apoptosis. In paediatric mastocytosis and CM *KIT*-activating mutations are rare.

Clinical symptoms in mastocytosis are due: (i) to the release of mast cell granules containing pro-inflammatory and vasoactive mediators (including histamine, tryptase, heparin, TNF- $\alpha$ , PGD<sub>2</sub>, cytokines, and chemokines) that have both local and systemic effects, and (ii) to organ infiltration.

In cutaneous mastocytosis (CM) mast cell proliferation is confined to skin; involvement of at least one extracutaneous organ defines systemic mastocytosis (SM), whether there is evidence of skin involvement or not.<sup>2</sup> Both are discussed here.

### Cutaneous mastocytosis

#### *Clinical features and presentation*

- Most cases of CM are seen in infants and children; involvement is generally limited to the skin; most common form is *maculopapular CM/urticaria pigmentosa (MPCM/UP)* with widespread cutaneous involvement with a few or many small lightly pigmented red-brown macules and papules. Intensifies on rubbing (Darier's sign). Usually transient; begins in 1st year and disappears at or shortly after puberty; progression to SM is unusual.<sup>6,8</sup>
- *Diffuse CM* is less frequent, almost exclusive to children and may cause smooth, red, or greatly thickened skin.
- *Solitary mastocytoma of the skin* occurs as a tumour; rare; generally occurs in infants; benign course.
- *Adult MPCM/UP* is associated with small heavily pigmented macular lesions; onset in young adults; lesions remain stable or increase with time though rarely decrease spontaneously;<sup>6,8</sup> often progressive with systemic organ involvement usually BM (46%), lymph nodes (~25% at diagnosis), spleen (~50% at diagnosis), liver, and GIT.
- *Familial mastocytosis* causes cutaneous disease in infancy, persists into adult life, and may progress to systemic involvement; rare.

#### *Investigation and diagnosis*

- Diagnosis of CM (usually in children) requires demonstration of typical clinical findings with histological evidence of skin infiltration by mast cells and no evidence of systemic involvement.
- Adults with rash frequently misdiagnosed as CM, but SM criteria present in most cases. Undertake SM investigations in all adult patients.
- Assessment:
  - Inspection of lesional skin (plus photography).
  - Skin biopsy with tryptase immunohistochemistry (IHC). Monomorphic MC infiltrate, either large aggregates of tryptase +ve cells (>15 per cluster) or scattered MC.
  - *KIT* mutation analysis.
- Most patients with CM have a normal serum tryptase (median 5ng/mL); almost all patients with SM have a level >20 ng/mL.<sup>6,9</sup>

- In children with serum tryptase >100ng/mL or symptoms/signs of SM and in all adults undertake BM examination to exclude SM.
- In children with serum tryptase <20ng/mL diagnosis of CM may be made without marrow examination unless other signs of SM present.
- In children with serum tryptase 20–100ng/mL, without other signs of SM, provisional diagnosis CM; monitor until puberty, if persist examine BM.

### Grading

A clinical grading system has been proposed<sup>6</sup> based on the severity of symptoms: pruritus, flushing, blistering and bullae formation (Table 7.13).

**Table 7.13** Grading of skin-specific symptoms<sup>a</sup> in patients with mastocytosis<sup>6</sup>

Grade	Definition
0 = no symptoms	Prophylaxis <sup>b</sup> , but requires no other therapy <sup>c</sup>
1 = mild, infrequent	Prophylaxis ± therapy as required
2 = mild/moderate, frequent	Requires and can be controlled by daily therapy <sup>d</sup>
3 = severe, frequent	Suboptimal or unsatisfactory control with daily and combination therapy <sup>d</sup>
4 = severe adverse event <sup>e</sup>	Requires immediate therapy and hospitalization

<sup>a</sup> Pruritus, flushing, blistering, bullae formation.

<sup>b</sup> All patients with mastocytosis are advised to avoid precipitating factors and events—for most, prophylactic antihistamines (H<sub>1</sub> and H<sub>2</sub> receptor antagonists) are recommended.

<sup>c</sup> Unless patient is focused on cosmetic consequences of the disease or suffers from systemic mediator-related symptoms.

<sup>d</sup> Despite avoidance of precipitating factors.

<sup>e</sup> The frequency of severe adverse events should be reported: 4A: <1/year; 4B: >1/year and <1/month; 4C: >1/month.

### Management

- Patients should avoid agents and situations that provoke a reaction.
- Grade 0 patients are usually not treated unless they suffer from significant non-organic symptoms or systemic mediator-related symptoms; grade 4 patients may be hospitalized and require antimediator drugs, stabilizing agents and topical therapy. Therapeutic approach is shown in Table 7.14.
- Response to therapy is scored as complete regression, major regression (>50% reduction in affected skin), partial regression (10–50%), and no regression (<10%).<sup>6</sup>
- Serum tryptase can be used for monitoring patients with CM if elevated at baseline; a significant increase can be regarded as evidence of an increase in MC burden and an indication for histological re-assessment of the BM to exclude indolent SM.<sup>9</sup>

**Table 7.14** Therapeutic options for cutaneous symptoms in patients with mastocytosis<sup>6</sup>

Symptom (variant)	1st-line, grade 1–2	1st-line, grade 3 and 2nd-line grade 2
Pruritus (all)	H <sub>1</sub> antihistamines Topical sodium cromoglicate	UV irradiation, PUVA, H <sub>1</sub> and H <sub>2</sub> antihistamines, leukotriene antagonists, glucocorticoids
Flushing (all)	H <sub>1</sub> antihistamines Leukotriene antagonists	H <sub>1</sub> and H <sub>2</sub> antihistamines UV irradiation, PUVA
Blistering (all)	Local therapy H <sub>1</sub> antihistamines	H <sub>1</sub> and H <sub>2</sub> antihistamines Systemic glucocorticoids Topical sodium cromoglicate
Bullae (usually diffuse CM and mastocytomas)	Local care, dressing	H <sub>1</sub> and H <sub>2</sub> antihistamines Systemic glucocorticoids Topical sodium cromoglicate
Mastocytoma lesion with symptoms or increasing size	Local immunosuppressants, local UV irradiation and psoralen bath or cream	Excision

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## Systemic mastocytosis

### Clinical features and presentation

- Patients typically present with symptoms of mediator release which vary in severity (mild to life-threatening) and may be chronic and recurrent, or acute, lasting minutes or hours:
  - Urticaria, flushing, dermatographism, pruritus, angioedema, anaphylaxis.
  - Paroxysmal hyper- or hypotension.
  - GI symptoms (80%): abdominal cramps, dyspepsia, nausea, diarrhoea, malabsorption, multiple peptic ulcers, haemorrhage; consider aggressive SM if malabsorption or weight loss.
  - Wheezing, dyspnoea, rhinorrhoea.
  - Neuropsychiatric symptoms (headache, fatigue, irritability, cognitive disorganization, nightmares).
  - Musculoskeletal pain (25%).
- Less often, patients present with an abnormal blood count (~20%), organomegaly, lymphadenopathy, or pathological fracture.
- May present without skin involvement; frequent in aggressive SM (ASM) and mast cell leukaemia though may have isolated BM involvement in subvariant of indolent SM (ISM).
- SM may follow either an indolent or an aggressive course; organomegaly due to mast cell infiltration may occur in patients with an indolent course;<sup>1</sup> aggressive SM is characterized by progressive mast cell infiltration of organs causing impaired function: BM failure, hepatocellular failure with ascites, severe diarrhoea and malabsorption, pathological fractures, organomegaly, and pancytopenia.
- Clinical features of SM have been classified as 'B-findings' and 'C-findings' and assist in staging, identification of clinical variants, selection of therapy, and assessment of response:<sup>2-4</sup>
  - *B-findings*: define spread of disease; indication of high burden of mast cells, and expansion of the disease without impairment of organ function; B = borderline benign:<sup>2,5</sup>
    - BM biopsy showing >30% infiltration by mast cells (focal, dense aggregates) and/or serum tryptase level >200ng/mL.
    - Signs of dysplasia or myeloproliferation, in non-mast cell lineage, but insufficient for a diagnosis of AM-AHNMD, with normal or slightly abnormal blood counts.
    - Hepatomegaly without impaired liver function, and/or palpable splenomegaly without hyperplenism and/or lymphadenopathy on palpation or imaging.
  - *C-findings*: define aggressiveness of the infiltrate; indication of impaired organ function due to mast cell infiltration:<sup>2,6</sup>
    - BM dysfunction:  $\geq 1$  cytopenia(s): ANC  $< 1.0 \times 10^9/L$ , Hb  $< 10g/dl$ , Plt  $< 100 \times 10^9/L$ .
    - Palpable hepatomegaly with impairment of liver function, ascites and/or portal hypertension.
    - Skeletal involvement with large osteolytic lesions and/or pathological fractures.

- Palpable splenomegaly with hypersplenism.
- Malabsorption with weight loss due to GI mast cell infiltrates.

### Investigations and diagnosis

When SM is suspected a biopsy of the affected organ should be done if possible and a BM (aspirate and trephine) examined with tryptase stain, flow cytometry for phenotypically abnormal mast cells (CD25+), and, if available, mutation screening for *KIT*-D816V.<sup>7</sup>

#### BM biopsy/tissue biopsy

- Diagnostic criteria as per Table 7.15.
- Usually sharply demarcated mast cell aggregates in perivascular or peritrabecular locations or randomly distributed; often comprise varying proportions of mast cells, lymphocytes, eosinophils, and fibroblasts; frequently significant fibrosis (90%) and thickening of adjacent bone; in advanced SM diffuse infiltration with marked reticulin or collagen fibrosis.<sup>2</sup>
- Exclude a coexisting haematopoietic neoplasm (SM-AHNMD), hypercellularity or dysmyelopoiesis (prognostic value); exclude reactive non-clonal mast cell hyperplasia that may accompany several haematological disorders where mast cells lack atypia and are sprinkled throughout the marrow rather than in aggregates.<sup>2</sup>
- Immunohistochemistry for tryptase, CD117 (*KIT*) and CD25 to demonstrate infiltrate of tryptase +ve spindle-shaped mast cells.
- If isolated infiltrate only or multifocal small aggregates (<15 MCs) consider minor diagnostic criteria (Table 7.15).
- If subdiagnostic ( $n < 3$ ), repeat biopsy; if remains subdiagnostic in patient with high clinical suspicion monitor closely. *KIT*-mutation analysis might help.

**Table 7.15** WHO diagnostic criteria for SM<sup>2-4</sup>

Major criterion <sup>a</sup>	Multifocal, dense infiltrates of mast cells ( $\geq 15$ mast cells per aggregate) in BM and/or other extracutaneous organ(s) confirmed by tryptase immunohistochemistry
Minor criteria <sup>a</sup>	<ol style="list-style-type: none"> <li>1. In biopsy sections of BM or other extracutaneous organs &gt;25% of mast cells show an abnormal spindle-shaped morphology or &gt;25% of mast cells in BM aspirate smears are immature or atypical</li> <li>2. Detection of <i>c-KIT</i> mutation D816V or other activating mutation at codon 816 in extracutaneous organ(s); marrow is recommended for screening</li> <li>3. <i>KIT</i>+ MCs in BM or other extracutaneous organs express CD2 or/and CD25</li> <li>4. Serum tryptase &gt;20ng/mL (does not count in patients who have AHNMD-type disease)</li> </ol>

<sup>a</sup> If at least 1 major and 1 minor criterion or at least 3 minor criteria are fulfilled, the diagnosis of SM can be established.

**BM aspirate**

- $\geq 5\%$  mast cells suggests an unfavourable prognosis;  $\geq 20\%$  defines mast cell leukaemia; mild myelodysplasia is found in most patients with SM.<sup>3</sup>
- Mast cells in SM show aberrant morphological features.<sup>3</sup>
- Non-metachromatic myeloblasts suggest AHNMD and dysplasia;  $>5\%$  SM-MDS and  $>20\%$  SM-AML.
- Aspirate can be negative whilst trephine is diagnostic.

**Flow cytometry**

- Neoplastic SM mast cells usually aberrantly express CD25  $\pm$  CD2.
- BM cells should be analysed; minimum recommended panel includes CD2, CD25, CD45, and CD117; CD34 may be added when CD117 expression low (frequent in *KIT* mutation<sup>8</sup>) to define MC as CD34-/CD117+/CD45+.<sup>3</sup>

***KIT*-D816V and other *KIT* mutation analysis**

- Neoplastic mast cells in  $>80\%$  adults with SM have activating *KIT* mutation at codon 816; D816V most common (SM 70–90%; CM 10–30%), all others rare.<sup>3</sup>
- RT-PCR and RFLP analysis of unfractionated BM cells after RBC-lysis or marrow mononuclear cells; PB is not an acceptable alternative as often –ve in ISM.<sup>3</sup>
- If –ve result with small infiltrate, interpret with caution; if large infiltrate, confirm –ve result in reference laboratory as wt-*KIT* and certain other mutants confer imatinib-sensitivity.

**Serum tryptase**

- Almost all patients with SM have serum tryptase level  $>20\text{ng/mL}$ .
- In severe allergic reactions, tryptase may be markedly elevated so wait 2d after clinical resolution before measuring; if elevated, consider SM.<sup>3</sup>
- Also elevated in myeloid non-mast cell disorders, AML, CML, MPN, and MDS; therefore a minor criterion only in absence of AHNMD.
- Tryptase level higher with high mast cell burden; in ISM level remains reasonably constant over years; in ASM or mast cell leukaemia levels often increase as disease progresses.
- In ASM or mast cell leukaemia tryptase levels often decrease with successful cytoreduction.<sup>3</sup>

**FBC and film**

- No characteristic features; circulating mast cells very rare (2%) unless very advanced disease or mast cell leukaemia.
- Mild-to-moderate anaemia  $\sim 45\%$ ; eosinophilia up to 25%; thrombocytopenia  $\sim 20\%$ ; monocytosis  $\sim 15\%$ ; pancytopenia may develop due to BM infiltration or hypersplenism.

**Other investigations**

- Bone scan/skeletal survey shows bone lesions in 60%; generalized osteosclerosis, focal sclerosis, osteopenia, osteoporosis, or osteolysis.
- Bone densitometry (DEXA scan) in all patients with SM.
- Ultrasound abdomen.
- Serum biochemistry.



- Coagulation screen.
- Endoscopy in patients with GI symptoms.
- EEG: if clinically indicated.

### Variants of SM

- **Indolent SM (ISM):** most common form of SM (~66%); no 'B' or 'C' findings; mast cell burden low; usually involves skin and BM; associated with maculopapular skin lesions (90%); flushing and abdominal cramps frequently reported; slow involvement of organs; prolonged course and good prognosis in almost all with survival >20 years; poor quality of life if inadequate control of mediator symptoms; small number transform into ASM or SM-AHNMD.<sup>2,5,6</sup> Subvariants identified:<sup>4</sup>
  - *BM mastocytosis (BMM):* rare subvariant of ISM with isolated marrow involvement and no skin lesions or multiorgan involvement, low MC burden and good prognosis.<sup>2</sup>
  - *Smouldering SM (SSM):* as ISM with 2 or more 'B' findings but no 'C' finding; some have prolonged stable course others develop ASM or AHNMD.<sup>2,3,5,6</sup>
- **Aggressive SM (ASM):** ~5% of all SM patients; ≥1 'C' finding; may present with hepatosplenomegaly and/or generalized lymphadenopathy without typical skin lesions; characterized by impaired organ function due to progressive infiltration of BM, liver, spleen, GI tract, or skeletal system; predisposition to severe mediator release attacks with haemorrhagic complications; median survival 3–5 years.<sup>2,6</sup>
  - *Lymphadenopathic mastocytosis with eosinophilia:* rare subvariant of ASM; progressive lymphadenopathy with PB eosinophilia often with extensive bone involvement and hepatosplenomegaly but usually without skin lesions.<sup>2,6</sup>
- **SM with associated haematological non-mast cell disease (SM-AHNMD):** 20–30% of SM patients; <50% have skin lesions; 80–90% myeloid, most commonly CMML; 10–20% lymphoid, most commonly myeloma; classify using WHO criteria; prognosis poor, largely determined by accompanying disorder and poorer survival than patients without SM.<sup>2,6</sup>
- **Mast cell leukaemia:** rare; ≥20% MC in BM aspirate and ≥10% in PB (aleukaemic variant <10%); diffuse infiltration on trephine biopsy; no skin lesions, severe peptic ulcer disease, hepatosplenomegaly, anaemia, multiorgan failure; poor response to chemotherapy and short survival (most <1 year).<sup>2,5,6</sup>
- **Mast cell sarcoma (MCS):** extremely rare; unifocal tumour consisting of atypical mast cells; high grade cytology; locally destructive growth; no skin or systemic involvement at diagnosis; prognosis grave; rapid progression with terminal phase resembling Mast cell leukaemia.<sup>2,6</sup>
- **Extracutaneous mastocytoma:** extremely rare; most reported cases involve lung; unifocal tumour; low-grade cytology; non-destructive growth pattern; no skin or systemic involvement.<sup>2,6</sup>

### Differential diagnosis

Diagnosis often delayed due to non-specific symptoms and presentation. Exclude reactive mast cell hyperplasia, mast cell activation syndromes, MPNs with ↑ mast cells, carcinoid syndrome, pheochromocytoma, liver disease, CEL/HES, and lymphoma.

### Management

- No curative therapy; management consists of prevention of mediator effects, an attempt to control organ infiltration by mast cells by cytoreductive therapy where appropriate, and treatment of accompanying haematological disease where present.
- Selection of intensive therapy should be based on signs and symptoms of aggressive disease (C-findings).
- B-findings: borderline benign; wait and watch.
- C-findings: consider cytoreduction with chemotherapy or with targeted drugs.
- Patients should avoid factors triggering acute mediator release: extremes of temperature, pressure, friction, aspirin, NSAIDs, opiates, alcohol, specific allergies.
- In SM-AHNMD, treat the associated haematological disorder as for cases without SM and treat SM component as required.<sup>3</sup> Generally patients achieve a short partial remission at best; splenectomy may help pancytopenic patients. SCT should be considered in appropriate patients.
- SM-CEL with *FIP1L1-PDGFR*A fusion gene will respond to imatinib.

#### Treatment of acute mast cell mediator release

- Anaphylaxis: epinephrine (adrenaline) adult dose every 10–15min as needed.
- Refractory hypotension and shock: fluid resuscitation and epinephrine (adrenaline) IV bolus plus infusion; add inotropes if unresponsive.
- Patients at risk of severe hypotension should be advised to carry 2 or more epinephrine (adrenaline) self-injectors.<sup>3</sup>
- Commence H<sub>1</sub> plus H<sub>2</sub>-receptor antagonists and steroids.

#### Treatment of chronic mast cell mediator release

- H<sub>1</sub> plus H<sub>2</sub> receptor antagonists: H<sub>1</sub> antihistamines: diphenhydramine (25–50mg PO 4–6-hourly; 10–50mg IM/IV), hydroxyzine (25mg PO tid or qid; 25–100 mg IM/IV) or loratadine (non-sedating; 10mg PO od); H<sub>2</sub> antihistamines: ranitidine (150mg PO bd; 50mg IV) or cimetidine (400–1600mg/day PO in divided doses; 300mg IV). Titrate doses for individual patient requirements.
- GI symptoms unresponsive to H<sub>1</sub> and H<sub>2</sub> antagonists may respond to sodium cromoglicate or short-term corticosteroids; prednisolone 40–60 mg/d PO for malabsorption, tailing dose.
- Bisphosphonates for osteoporosis; analgesia for bone pain, radiotherapy in severe cases; osteolysis is a sign of advanced disease and an indication for cytoreductive therapy/IFN.<sup>3</sup>
- PUVA for urticaria pigmentosa.
- A small number of patients gain symptomatic relief from interferon alfa or ciclosporin for refractory symptoms.

**Cytoreductive treatment<sup>3,5</sup>**

- First establish diagnosis of ASM, Mast cell leukaemia, or MCS and *KIT* mutation status.
- ISM or SSM: cytoreductive treatment generally not required, but in SSM with rapidly progressive B-findings:
  - Consider interferon alfa (3 million units SC tiw) ± corticosteroids (50–75mg/d, commence before interferon alfa and monitor carefully during initial days of treatment<sup>5</sup>).
  - Cladribine: CR but prolonged remission rare.
- *KIT*-D816V –ve (wild type or other mutation) ASM or mast cell leukaemia: consider imatinib; N.B. *KIT*-D816V+ SM is imatinib resistant.
- ASM with *slow progression*: interferon alfa (15–20% major clinical responses in ASM/MCL<sup>6</sup>) ± corticosteroids; if interferon alfa intolerant, or no response, consider cladribine.
- ASM with *rapid progression*: polychemotherapy, e.g. cytarabine fludarabine and/or daunorubicin; consider SCT in selected cases who respond to polychemotherapy; Alternatives: IFN, cladribine, splenectomy for hypersplenism, hydroxycarbamide as palliation.
- Mast cell leukaemia: AML-type polychemotherapy ± cladribine ± interferon alfa; consider SCT; splenectomy for hypersplenism; hydroxycarbamide as palliation.

**Novel therapies:** nilotinib, dasatinib, midostaurin, NF-κB inhibitors, HSP90 inhibitors, geldanamycin, alemtuzumab, and gemtuzumab are all being studied.<sup>9,10</sup> Case reports have shown thalidomide to achieve durable major responses.

**Response assessment**

Response criteria for patients receiving cytoreductive drugs have been agreed and relate to C-findings.<sup>3,9</sup>

- **Major response:** complete resolution of ≥1 C-finding *and* no progression in other C-findings:
  - Complete remission: disappearance of mast cell infiltrates in affected organs *and* decrease of serum tryptase to <20ng/mL *and* disappearance of SM-associated organomegaly.
  - Incomplete remission: decrease in mast cell infiltrates in affected organs *and/or* substantial decrease of serum tryptase level *and/or* visible regression of splenomegaly.
  - Pure clinical response: without decrease in mast cell infiltrates, without decrease in tryptase levels *and* without regression of splenomegaly.
- **Partial response:** incomplete regression of ≥1 C-finding without complete regression *and* without progression:
  - Good partial response: >50% regression; no progression in other C-findings.
  - Minor response; <50% regression; no progression in other C-findings.
- **No response:** C-finding(s) persistent or progressive.
  - Stable disease: C-finding parameters show constant range.
  - Progressive disease: ≥1 C-finding shows progression.

### Prognosis

Most patients with SM have only slowly progressive disease and many survive several decades. The life expectancy of patients with pure CM, ISM and BMM is similar to that of individuals without mastocytosis.<sup>8</sup>

~33% of patients with SM evolve into a haematological malignancy, frequently leukaemia. Mast cell leukaemia is resistant to intensive chemotherapy and has a survival of only a few months.

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### Further reading

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## MPN—unclassifiable

This is a group of MPN-like disorders that do not meet the diagnostic criteria for either the classic or non-classic MPNs and may account for 10–20% of MPNs.<sup>1,2</sup> Most cases fall into 2 groups:

- Initial stages of PV, PMF, or ET presenting before characteristic features have developed.
- Advanced MPN where marked myelofibrosis, osteosclerosis, or transformation obscures the underlying disorder.

The former should be screened for MPN-associated mutations and monitored until a clear diagnosis can be determined. The latter have advanced disease and should be monitored and treated appropriately.

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# Paraproteinaemias

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## Paraproteinaemias

A heterogeneous group of disorders characterized by deranged proliferation of a single clone of plasma cells or B lymphocytes and usually associated with detectable monoclonal immunoglobulin (paraprotein or M-protein) in serum and/or urine.

### Conditions associated with paraprotein production

#### *Stable production*

- Monoclonal gammopathy of undetermined/uncertain significance (MGUS).
- Asymptomatic myeloma ('smouldering' myeloma).
- Cryoglobulinaemia.

#### *Progressive production*

- Multiple myeloma (MM):
  - Complete immunoglobulins: IgG, IgA, or IgD; very rarely IgM or IgE.
  - Free light chains (LCs):  $\kappa$  or  $\lambda$  (Bence Jones proteinuria).
  - Non-secretory.
- Plasma cell leukaemia (PCL).
- Solitary plasmacytoma of bone (SPB).
- Solitary extramedullary plasmacytoma (SEP).
- POEMS syndrome.
- Waldenström macroglobulinaemia (WM).
- B-LPD (including CLL).
- NHL (mainly low grade).
- Heavy chain disease (HCD).
- AL amyloidosis (AL).





## Monoclonal gammopathy of undetermined significance (MGUS)

MGUS describes an asymptomatic premalignant disorder characterized by limited monoclonal plasma cell proliferation and the presence of a stable monoclonal paraprotein in serum or less commonly in urine in the absence of clinicopathological evidence of MM, WM, AL, or another lymphoproliferative disorder (LPD).

### Epidemiology

Prevalence 3.2%  $\geq 50$  years in large population-based study. Slight  $\sigma$  excess (1.3  $\times$ ). Prevalence rises with age: 1.7% age 50–59; 3% 60–69; 4.6% 70–79; 6.6%  $\geq 85$  years. In USA,  $>2\times$  more frequent in African Americans compared to Caucasians. Median age 72 years; 2%  $<40$ ; 59%  $\geq 70$  years. MGUS diagnosed  $\sim 4\times$  as frequently as MM.

### Pathophysiology

MGUS appears to arise from a pre-germinal centre cell whose progeny pass through the germinal centre and undergo mutation. Antigenic stimulation related to autoimmune, infectious, or inflammatory disorders (but not allergies) may be an initiating event.

There is genomic instability on molecular analysis and 90% of patients have abnormalities. Progression to MM may be due to outgrowth of a single clone but the precise trigger is unclear. There is a continuing rate of progression to MM, WM, AL, or other LPDs. This constant rate suggests a random 2nd hit. FISH demonstrates the same MM cytogenetic abnormalities in MGUS often acquired over time, e.g.  $-13$  (in 50% MGUS). Expression microarrays show MGUS much closer to MM than to normal plasma cells.

### Natural history

- $>50\%$  die of unrelated causes after  $>25$ -year follow-up.
- 1% per annum progress to MM, WM, AL, or other LPD or CLL.
- Actuarial rate of progression 17% at 10 years; 39% at 25 years but true probability is lower when other causes of death are taken into account.
- 69% who progress develop MM, 12% AL, 11% WM, and 8% LPD.
- Some may show an  $\uparrow$  in paraprotein  $\geq 30\text{g/L}$  or BM PC  $>10\%$  without developing symptomatic MM or WM or requiring therapy.

### Clinical features

- Typically asymptomatic and an incidental finding on investigation of  $\uparrow$  ESR/PV or  $\uparrow$  globulin on routine LFTs.
- No abnormal physical findings (end-organ damage) except due to unrelated pathology.
- Lack of progression and absence of additional evidence of progressive plasma cell or B-cell lymphoproliferative malignancy.

## Investigations and diagnosis

(See Table 8.1.)

- Check FBC, renal biochemistry, serum calcium, serum immunoglobulins, serum protein electrophoresis and paraprotein quantitation, serum free light chains (SFLC) (and/or urine for Bence Jones proteinuria), and skeletal survey.
- BM aspirate and trephine biopsy indicated if paraprotein  $\geq 15\text{g/L}$  or non-IgG paraprotein present *and* abnormal FBC, serum creatinine, serum calcium, SFLC ratio, or skeletal survey.
- FBC: no anaemia or other cytopenia except due to unrelated causes.
- Serum chemistry: no hypercalcaemia or otherwise unexplained renal impairment.
- Serum protein electrophoresis with immunofixation and densitometry to detect, characterize, and quantitate paraprotein levels:
- SFLCs: ~30% have abnormal  $\kappa:\lambda$  ratio.
- Urine electrophoresis: identifies low levels of Bence Jones proteinuria in up to 30%; ~60%  $\kappa$ , ~40%  $\lambda$ ; generally  $<1\text{g}/24\text{h}$ , ~15%  $>150\text{mg}/24\text{h}$ .
- Skeletal radiology: no evidence of lytic lesions or pathological fracture; osteoporosis may co-exist from other causes e.g. postmenopausal ♀.
- Serum  $\beta_2$ -microglobulin levels  $\leftrightarrow$  (unless renal impairment).
- BM aspirate:  $<10\%$  plasma cells in BM; median ~5%.
- BM cytogenetics:  $\leftrightarrow$  by conventional techniques but all abnormalities in MM described in MGUS by FISH;  $\text{del}(13)$ ,  $\text{t}(4;14)$ ,  $\text{ras}$  mutations,  $p16$  and  $p53$  inactivation less common.
- BM trephine biopsy: no evidence of diffuse plasma cell infiltration or osteoclast erosion of trabeculae.
- Note: patients with MGUS should have stable paraprotein and other parameters on prolonged observation.

## Differential diagnosis

- Exclude conditions with associated paraprotein, notably MM, WM, and AL amyloidosis.
- Bone pain/damage, unexplained anaemia, or impaired renal function suggests MM.
- Lymphadenopathy or splenomegaly with an IgM paraprotein suggests WM.
- The presence of neuropathy attributable to the monoclonal gammopathy would be termed 'monoclonal gammopathy associated with neuropathy'.

## Risk factors for progression

No specific features at initial presentation predict the patients who will progress but risk of progression  $\uparrow$  by:

- Paraprotein level: progression rate at 20 years:  $10\text{g/L} = 16\%$ ;  $15\text{g/L} = 25\%$ ;  $20\text{g/L} = 41\%$ ;  $25\text{g/L} = 49\%$ .
- Paraprotein type:  $\text{IgM} > \text{IgA} > \text{IgG}$ .
- Abnormal SFLC ratio ( $<0.26$  or  $>1.65$ ).
- BM plasma cells  $>5\%$  ( $2 \times$  risk of  $<5\%$ ).
- Circulating plasma cells by immunofluorescence.

**Risk stratification**

A scoring system has been developed using 3 parameters (Table 8.2):

- Quantity of paraprotein (<15g/L vs ≥15g/L).
- Isotype of paraprotein (IgG vs any other subtype).
- SFLC ratio (↔ vs abnormal).

**Management**

- No treatment; observation with long-term follow-up and review of clinical and laboratory features required due to risk of progression.
- Clinical and laboratory (FBC, renal function, serum Ca<sup>2+</sup>, serum Igs, paraprotein quantitation, SFLC, and urine electrophoresis) re-evaluation at 6 months then annually.
- Less frequent follow-up (e.g. every 2 years) may be appropriate in low-risk patients.
- Where diagnosis in doubt review over 3–6 months usually differentiates MGUS from MM.
- Advise patients to seek early assessment if relevant or unexplained symptoms develop.

**Table 8.1** Diagnostic criteria for MGUS

- 1 Serum monoclonal protein <30g/L
- 2 Bone marrow plasma cells <10% (IgM MGUS—lymphoplasmacytic cells <10%)
- 3 No evidence of other B-cell lymphoproliferative disorder
- 4 No myeloma-related end-organ or tissue impairment, e.g. lytic bone lesions, anemia, hypercalcaemia, or renal failure (IgM MGUS—no anemia, constitutional symptoms, hyperviscosity, lymphadenopathy or hepatosplenomegaly)

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**Table 8.2** Risk stratification system for MGUS<sup>1</sup>

Risk group	Number of patients	Relative risk	Absolute risk of progression at 20 years (%)	20-year progression risk with death as competing risk (%)
<i>Low-risk:</i> M-band <15g/L; IgG isotype; normal SFLC ratio (0.26–1.65)	449	1	5	2
<i>Low-intermediate risk</i> (any 1 factor: M-band ≥15g/L; non-IgG-isotype, or abnormal SFLC ratio)	420	5.4	21	10
<i>High-intermediate risk</i> (any 2 factors)	226	10.1	37	18
<i>High risk</i> (all 3 factors)	53	20.8	58	27

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## Asymptomatic multiple myeloma ('smouldering' myeloma)

Asymptomatic or smouldering MM (SMM) identifies patients with a paraprotein  $>30\text{g/dL}$  and/or  $>10\%$  plasma cells in the BM but in whom the natural history is that of MGUS rather than MM, i.e. no clinical evidence of complications associated with MM or progression.

### Incidence

Account for  $\sim 15\%$  of all new cases of myeloma.

### Pathogenesis

Almost all have either translocation involving IgH (50%) or hyperdiploidy ( $\sim 40\%$ ).

### Prognosis

- Clinical stability may persist for months or years but most patients progress to symptomatic MM.
- Risk of progression is higher than MGUS and time dependent: 10% per annum for first 5 years,  $\sim 3\%$  per annum for next 5 years, and 1% per annum for next 10 years. Cumulative probability of progression 73% at 15 years.
- Median time to progression  $\sim 5$  years.
- Some evidence suggests early treatment for patients with 'high-risk' smouldering myeloma delays progression to active disease and increases overall survival.<sup>1</sup>
- Survival is the same as for newly diagnosed myeloma from the time chemotherapy is started.

### Clinical and laboratory features

- Absence of symptoms or physical signs attributable to myeloma.
- Perform investigations listed for MM.
- FBC: haemoglobin  $>10\text{g/dL}$ .
- Serum chemistry: post-rehydration creatinine  $<130$  micromol/L;  $\leftrightarrow$  serum  $\text{Ca}^{2+}$ .
- $\beta_2$ -microglobulin:  $\leftrightarrow$  or minimally raised.
- BM aspirate: plasmacytosis  $>10\%$  but normally  $<25\%$ .
- BM cytogenetics: FISH identifies the abnormalities associated with MM; some patients have detectable chromosomal abnormalities by standard karyotype analysis.
- Skeletal radiology normal
- Stable paraprotein and other parameters on prolonged observation.

### Differential diagnosis

Exclude MGUS, MM, WM, and AL amyloidosis (see Table 8.3).

**Table 8.3** Diagnostic criteria for asymptomatic multiple myeloma<sup>2</sup>

- |   |   |
|---|---|
| 1 | Serum monoclonal protein $\geq 30\text{g/L}$ and/or bone marrow plasma cells $\geq 10\%$                          |
| 2 | No evidence of other B-cell LPD   |
| 3 | No myeloma-related end-organ or tissue impairment (lytic bone lesions, anemia, hypercalcaemia, or renal failure). |

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Same criteria in updated 2008 WHO classification of tumours of the haematopoietic and lymphoid tissues.

### Risk factors for early progression of asymptomatic MM

Shorter time to progression associated with:

- Serum paraprotein  $>30\text{g/L}$ .
- IgA protein type.
- Urinary Bence Jones proteinuria  $>50\text{mg/d}$ .
- BM plasmacytosis  $>25\%$ .
- Suppression of uninvolved Igs.
- SFLC ratio  $<0.125$  or  $>8$ .

These risk factors have no impact on survival after progression. Other possible risk factors for progression are circulating plasma cells,  $\beta_2$ -microglobulin  $>2.5\text{mg/L}$ , and karyotype abnormalities on conventional cytogenetics.

### Risk stratification

- Table 8.4 shows a scoring system using 2 parameters:
  - Quantity of paraprotein ( $<30\text{g/L}$  vs  $>30\text{g/L}$ ).
  - Isotype of paraprotein (IgG vs IgA).
- Table 8.5 shows a more recent scoring system including SFLC ratio. 3 parameters, with 1 point awarded for each:
  - BM plasmacytosis  $\geq 10\%$ .
  - Serum M protein  $\geq 30\text{g/L}$ .
  - SFLC ratio  $<0.125$  or  $>8$ .

### Management

- Chemotherapy is not indicated for these patients until there is evidence of clinical progression; no benefit for therapy before symptomatic disease develops.
- Review clinical and laboratory features as for MGUS but more frequently: every 3–4 months recommended.
- Median survival following chemotherapy 3–5 years, identical to that of *de novo* symptomatic myeloma.

**Table 8.4** A risk stratification scoring system in SMM (see p.333)<sup>3</sup>

Risk group	Factors	Proportion of patients	Median time to progression
Low-risk	M-band $\leq 30\text{g/L}$ <b>and</b> IgG isotype	~45%	>4 years
Intermediate-risk	M-band $>30\text{g/L}$ <b>or</b> IgA isotype	~45%	~2 years
High-risk	M-band $>30\text{g/L}$ <b>and</b> IgA isotype	<10%	9 months

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**Table 8.5** A risk stratification scoring system in SMM (see p.333)<sup>4</sup>

Risk group	Number of factors	Number of patients (%)	Relative risk (95%CI)	Absolute risk at 5 years	Absolute risk at 10 years
Low-risk	1	81 (28%)	1	25%	50%
Intermediate-risk	2	114 (42%)	1.9 (1.2–2.9)	51%	65%
High-risk	3	78 (30%)	4.0 (2.6–6.1)	76%	84%

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## Multiple myeloma

MM is a clonal B-cell malignancy characterized by proliferation of plasma cells that accumulate mainly within BM and usually secrete monoclonal Ig or Ig LCs (paraprotein or M-protein ('M-band')). MM may be associated with BM failure, hypercalcaemia, renal impairment, lytic bone lesions, diffuse osteoporosis, or pathological fracture. Normal Ig production is usually impaired (immunoparesis; hypogammaglobulinaemia). Uncommon cases are 'non-secretory' with no detectable serum or urine paraprotein. (See Fig. 8.1.)

### Epidemiology

MM accounts for 1% of all malignancies; 10% of haematological malignancies. Incidence ~4 per 100,000 per annum. Median age 66 years; <3% <40 years. ♂:♀ ratio 1.5. Incidence in Afro-Caribbeans >2 × Caucasians; lowest in Asians. Most present *de novo* but some documented as arising from MGUS (most cases of MM may follow undetected MGUS). Familial clusters have been reported, suggesting a possible genetic element. The apparent ↑ incidence of MM is due to improved diagnosis and ageing populations.<sup>1</sup>

### Pathophysiology<sup>2,3</sup>

MM arises from a post-germinal centre B-cell that has undergone antigen selection, VDJ recombination, somatic hypermutation of V regions, and switch-recombination of IgH genes.

#### 2 pathogenetic pathways have been postulated:

*Hyperdiploid* (48–75 chromosomes): ~ 50%; typically with multiple trisomies involving chromosomes 3, 5, 7, 9, 11, 15, 19, and 21; rarely with a recurrent IgH translocation (usually t(4;14)).

*Non-hyperdiploid* (<48 or >75 chromosomes): aberrant class-switch recombination or somatic hypermutation may contribute to neoplastic transformation; IgH translocations at 14q32, usually within or near switch regions, detected by FISH in 55–70% of MM; usually associated with dysregulation of a gene in 1 of 7 recurrent partner chromosomes grouped by gene affected:

- *Cyclin D*: 11q13 (cyclin D1), 20%; 12p13 (cyclin D2), <1%; 6p21 (cyclin D3), 2%.
- *MAF*: 16q23 (c-*MAF*), 5%; 20q11 (*MAFB*), 2%; 8q24.3 (*MAFA*), <1%.
- *MMSET/FGFR3*: 4p16.3 (*MMSET* and usually *FGFR3*), 15%.

#### 2 other early events have been described:

- Loss of chromosome 13 or 13q14 deletion: occurs in ~50% MM and 40–50% MGUS; concurrent 13/13q- occurs in both pathogenetic pathways; much more prevalent (80–90%) in t(4;14) or t(14;16) than others (30–40%); low incidence in hyperdiploid MM.
- Dysregulation of a cyclin D gene: ↑ levels of cyclin D1 (less commonly cyclin D2) are expressed by almost all MM tumours: 25% due to IgH translocation.

Plasmablasts generated from post-GC B cells, migrate to the BM where they differentiate into long-lived plasma cells. The BM microenvironment is also critical to MM clonal expansion. Adhesion to stromal cells enhances MM cell growth and survival and confers protection against drug-induced apoptosis. MM cells activate the stroma, triggering paracrine and autocrine secretion of a range of cytokines and growth factors including IL-6, IGF-1, VEGF, TNF- $\alpha$ , bFGF, MIP-1 $\alpha$ , SCF, HGF, and IL-1 $\beta$ . IL-6 is the major growth and survival factor in MM and confers resistance to dexamethasone. VEGF induces MM-cell growth, migration, and survival as does IGF-1 which also confers drug resistance. Angiogenesis is altered (VEGF is main angiogenic factor) and  $\uparrow$  BM microvascular density correlates with disease progression and poor prognosis.

Bone disease in MM is due to osteoclast (OC) activation and osteoblast (OB) inhibition causing the characteristic 'punched-out' lytic lesions and hypercalcaemia associated with a normal alkaline phosphatase. OC proliferation and activation is triggered by a variety of OC-activating factors (OAFs) produced by MM-cells and stroma (including MIP-1 $\alpha$ , RANK-ligand, VEGF, TNF- $\alpha$ , IL-1 $\beta$ , PTHrP, HGF, and IL-6). OC activation in turn modulates MM-cell growth and survival. In addition there are  $\downarrow$  numbers of OBs and  $\downarrow$  bone formation due to dysregulation of Runx2/Cbfa1, Wnt, and IL-3.

BM infiltration by the neoplastic clone causes anaemia. Immunoparesis of normal Ig production predisposes to infection. The physico-chemical properties of the paraprotein determine whether amyloid deposition, renal damage, or hyperviscosity (IgM > IgA > IgG) occur.

### Clinical features and presentation

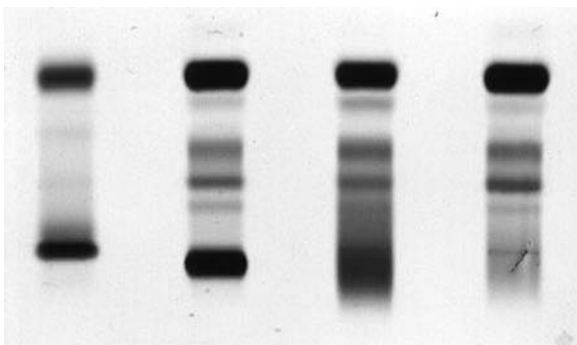
- Spectrum from asymptomatic paraproteinaemia detected on routine testing (~20%) to a rapidly progressive illness with extensive, destructive bone disease. IgG and IgA myeloma are most common; IgM myeloma is rare (0.5%).
- Most patients present with bone (usually back) pain (~75%) or pathological fracture; kyphosis and loss of height may occur from vertebral compression fractures.
- Weakness and fatigue (>50%), recurrent infection (10%) and thirst, polyuria, nocturia or oedema due to renal impairment (~10%) are also common presenting symptoms.
- Acute hypercalcaemia, symptomatic hyperviscosity (mental slowing, visual upset, purpura, haemorrhage), neuropathy, spinal cord compression, amyloidosis, and coagulopathy are less frequent at presentation.
- 'CRAB': hyper-calcaemia, renal impairment, anaemia, and bone problems.

### Differential diagnosis

Exclude MGUS, asymptomatic myeloma, and other conditions associated with a paraprotein, notably solitary plasmacytoma, systemic AL amyloidosis and LPDs.

### Investigations and diagnosis

A full history and physical examination should be undertaken in all patients with suspected MM. The investigations detailed in Table 8.6 should be performed to confirm or exclude the diagnosis and if confirmed, to establish tumour burden and prognosis. (See Figs. 8.2–8.4). Further investigations that are not routine may be necessary in individual patients (e.g. MRI) (➔ see colour plate section, plates 1–4). Diagnostic criteria are listed in Table 8.7.



**Fig. 8.1** Electrophoresis: from L to R: urine with BJP and generalized proteinuria (albumin band at top of strip, BJP near foot); serum M band in myeloma; polyclonal gammopathy; normal sample, showing albumin, A, B, and  $\gamma$  globulins (➔ see colour plate section).

**Table 8.6** Investigations of patients with suspected myeloma<sup>4-7</sup>**Screening tests**

FBC and film	Normochromic normocytic anaemia in 60%; film may show rouleaux
ESR or PV	↑ in 90% ; not in LC or non-secretory MM (NS MM)
Serum urea, creatinine, and electrolytes	May identify renal impairment (~25%)
Uric acid	May be ↑
Serum albumin, calcium, phosphate, alk. phos.	May reveal low albumin or hypercalcaemia (~20%) with normal alk. phos.
Serum protein electrophoresis with immunofixation	To detect serum paraprotein (80%)
Serum immunoglobulins	To detect immunoparesis
SFLC	To detect LC MM (22%); useful response parameter in LC MM, amyloidosis and 75% of non-secretory MM
Routine urinalysis	To detect proteinuria (~70%)
Urine electrophoresis with immunofixation:	To detect Bence Jones proteinuria: (22% have BJP only and no serum M-band: LC MM)
24h urine collection	For creatinine clearance and 24h proteinuria —to assess renal damage
X-ray sites of bone pain	May reveal pathological fracture(s) or lytic lesion(s)

**Diagnostic tests**

BM aspirate	Demonstrates plasma cell infiltration—may be only way to diagnose NS MM
Radiological skeletal survey	Identifies lytic lesions, fractures, and osteoporosis (80%; 5–10% osteoporosis only)
Paraprotein immunofixation and densitometry	Characterizes and quantifies paraprotein; IgG 50%; IgA 20%; LC 20%; IgD/E/M/biclonal <10%. Note: serum and urine EPS -ve in NS ~1% but SFLC abnormal in 75% of NS MM

**Tests to establish tumour burden and prognosis**

Serum $\beta_2$ -microglobulin	Measure of tumour burden
Serum C-reactive protein	Surrogate measure of IL-6 which correlates with tumour aggression
Serum LDH	Measure of tumour aggression; ↑ in plasmablastic MM
Serum albumin	Hypoalbuminaemia correlates with poor prognosis
BM cytogenetics and FISH	Clear prognostic value, esp. t(4;14) and del(17p) (see Table 8.8)
BM trephine biopsy with immunohistochemistry	Shows LC restriction, extent of infiltration and haematopoietic reserve

(continued)

**Table 8.6** Investigation of patients with suspected myeloma<sup>4-7</sup> (continued)**Tests which may be useful in some patients**

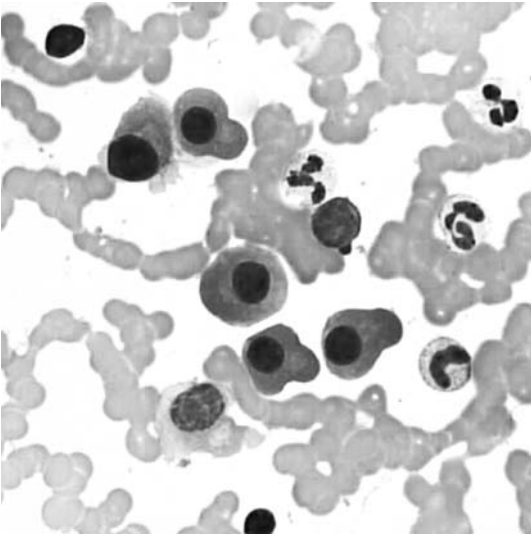
MRI	Urgent in patients with suspected cord compression; MRI whole spine/pelvis important in staging solitary plasmacytoma of bone to exclude occult disease; useful to clarify ambiguous CT findings; abnormal in ~25% of MM patients with ↔ skeletal survey
CT	For detailed evaluation of localized sites of disease, e.g. extraosseous plasmacytoma, including CT-guided biopsy; urgent CT indicated in suspected cord compression where MRI contraindicated or unavailable; useful to clarify ambiguous radiographic findings; may identify lesions that are -ve on radiography
FDG-PET scan	Not recommended as routine but may clarify extent of extramedullary disease if other imaging techniques do not do so; identifies focal recurrent disease and focal extramedullary disease; abnormal in ~25% with ↔ skeletal survey; persistent +ve post-therapy may predict early relapse
PET/CT	May be useful in solitary plasmacytoma of bone
BM flow cytometry	Confirms monoclonal PC infiltration and aberrant phenotype; useful when BM PC <10%
Tissue biopsy	To diagnose solitary plasmacytoma of bone or extraosseous plasmacytoma
Bone densitometry	Provides baseline in patients with osteoporosis but not recommended as routine

**Table 8.7** Diagnostic criteria for MM<sup>a</sup> 8,9

- Monoclonal protein in serum and/or urine (Note: no minimum level).
- Clonal BM plasma cells (Note: no minimum level; 5% have <10% plasma cells) or plasmacytoma.
- Myeloma-related organ or tissue impairment (acronym 'ROTI')
  - Elevated Ca<sup>2+</sup> levels: serum Ca<sup>2+</sup> >0.25mmol/L (>1mg/dL) above upper limit of ↔ or corrected serum Ca<sup>2+</sup> >2.75mmol/L (>11mg/dL).
  - Renal insufficiency: (creatinine >173 micromol/L or >2mg/dL).
  - Anemia: Hb 2g/dL below ↔ range or Hb <10g/dL.
  - Bone lesions: lytic lesions or osteoporosis with compression fractures recognized by conventional radiology.
  - Others: symptoms of hyperviscosity; amyloidosis; recurrent bacterial infection.

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<sup>a</sup> Criteria identical in WHO classification (2008).



**Fig. 8.2** BM aspirate in myeloma showing numerous plasma cells (→ see colour plate section).

### Cytogenetics<sup>2,9,10</sup>

- Conventional techniques demonstrate abnormal karyotypes in only 30–50% due to the low proliferative rate of MM cells and low proportion within samples for cytogenetic analysis; heterogeneous pattern and complex abnormalities common.
- High-density comparative genetic hybridization arrays show abnormalities in almost 100%.
- Interphase FISH on sorted or labelled MM-cells demonstrates aneuploidy in nearly all patients:
  - Hyperdiploidy in ~50%; median 54 chromosomes.
  - Abnormalities involving 14q32 (IgH locus) in ~60% (esp. non-hyperdiploid karyotypes): t(11;14)(q13;q32) ~20%, t(4;14)(p16;q32) ~15%, t(14;16)(q32;q23); ~5% and non-recurrent abnormalities.
  - del(17p;13.1): ~10%; loss of p53 tumour suppressor gene.
- Prognostic value of cytogenetic findings: see Tables 8.8 and 8.9.

**Table 8.8** Cytogenetic prognostic groups in MM<sup>9</sup>

Unfavourable risk	Deletion 13 or aneuploidy by metaphase analysis t(4;14) or t(14;16) or t(14;20) by FISH Deletion 17p13 by FISH Hypodiploidy
Favourable risk	Absence of unfavourable risk cytogenetics and presence of hyperdiploidy, t(11;14) or t(6;14) by FISH

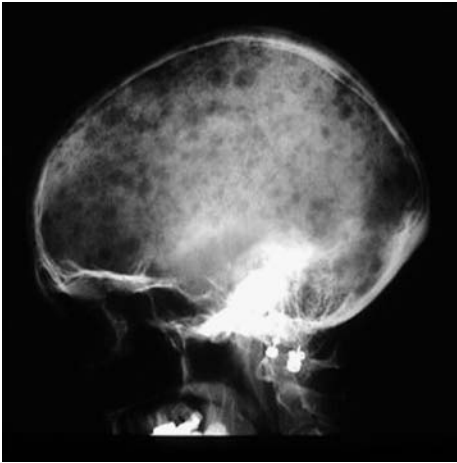
**Table 8.9** Mayo stratification of myeloma and risk-adapted therapy<sup>11</sup>

High risk	Standard risk
~25% of patients	~75% of patients
Presence of any of the following:	All other FISH or cytogenetic abnormalities, including:
<ul style="list-style-type: none"> <li>● FISH del(17p)</li> <li>● FISH t(4;14)</li> <li>● FISH t(14;16)</li> <li>● Cytogenetic del(13q)</li> <li>● Cytogenetic hypodiploidy</li> <li>● PC labelling index <math>\geq 3\%</math></li> </ul>	<ul style="list-style-type: none"> <li>● Hyperdiploidy</li> <li>● FISH t(11;14)</li> <li>● FISH t(6;14)</li> </ul>

**Prognostic factors: adverse factors at diagnosis**

- Age >65 years.
- Performance status 3 or 4.
- High paraprotein levels (IgG >70g/L; IgA >50g/L; BJP >12g/24h).
- Low haemoglobin (<10g/dL).
- Hypercalcaemia.
- Advanced lytic bone lesions.
- Abnormal renal function (creatinine >180 micromol/L).
- Low serum albumin (<30g/L).
- High  $\beta_2$ -microglobulin ( $\beta_2$ -M) ( $\geq 6$ mg/mL).
- High C-reactive protein ( $\geq 6$ mg/mL).
- High serum LDH.
- High % BM plasma cells (>33%).
- Plasmablast morphology.
- Adverse cytogenetics.
- Circulating plasma cells in PB (plasma cell leukaemia).
- High serum IL-6 (not routinely done; measured in only a few centres).





**Fig. 8.3** Skull x-ray in a patient with myeloma: multiple lytic lesions.



**Fig. 8.4** Humerus in a patient with myeloma: marked osteoporosis, lytic lesions, and healing pathological fracture.

### Staging systems

The *Durie–Salmon staging system* (Table 8.10) has been widely used since 1975.<sup>12</sup> It attempts to assess tumour bulk but may not provide as good prognostic discrimination as more recent systems; Largely superseded by the ISS staging, but still included here.

**Table 8.10** Durie–Salmon staging system.<sup>12</sup> Patients staged as I, II, or III and A or B; stage represents tumour burden

	Stage I	Stage II	Stage III
Tumour cell mass	<i>Low</i> All of the following:	<i>Medium</i> Not fitting stage I or III	<i>High</i> One or more of the following:
Monoclonal IgG (g/L)	<50		>70
Monoclonal IgA (g/L)	<30		>50
BJP excretion (g/24h)	<4		>12
Hb (g/dL)	>10		<8.5
Serum Ca <sup>2+</sup> (mmol/L)	≤2.6 (≤12mg/dL)		>2.6 (>12mg/dL)
Lytic lesions	None or one		Advanced
Stage A: serum creatinine	<175 micromol/L (<2.0mg/dL)		
Stage B: serum creatinine	≥175 micromol/L (≥2.0mg/dL)		

The *International Staging System (ISS)*<sup>13</sup> is another widely used staging system and was devised using data from 10,750 untreated patients from America, Europe, and Asia. It uses serum  $\beta_2$ -M and serum albumin to define 3 different prognostic groups. Patients with stage I (serum  $\beta_2$ -M <3.5mg/L and serum albumin ≥35g/L) have a median survival of 62 months, patients with stage II (serum  $\beta_2$ -M <3.5mg/L and serum albumin <35g/L or serum  $\beta_2$ -M 3.5–<5.5mg/L) have a median survival of 44 months, and patients with stage III (serum  $\beta_2$ -M ≥5.5mg/L) have a median survival of 29 months.

*Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART)*<sup>12</sup> defines a high-risk group based on cytogenetic and proliferation data (including using conventional cytogenetics to correlate with a high MM cell proliferative rate) to identify patients who should be managed differently from standard-risk patients (Table 8.9)

### Response assessment

2 systems are in current use. The system initially developed by the European Group for Blood and Marrow Transplant (EBMT)<sup>14</sup> for patients treated with SCT has been widely used (Table 8.11) but may be superseded by the more recently published system developed by the International Myeloma Working Group<sup>14</sup> (Table 8.12). Although primarily designed for clinical trials, these criteria are useful in clinical practice.

**Table 8.11** EBMT response criteria for MM<sup>15</sup>

Response category	Definition
Complete response (CR)	<p>All of the following:</p> <ul style="list-style-type: none"> <li>● Absence of paraprotein in serum and urine by immunofixation, maintained for <math>\geq 6</math> weeks</li> <li>● <math>&lt; 5\%</math> plasma cells (PC) in BM and trephine biopsy, if performed; if absent paraprotein sustained 6 weeks, no need to repeat BM</li> <li>● No increase in size or number of lytic bone lesions (development of a compression fracture does not exclude CR)</li> <li>● Disappearance of soft tissue plasmacytomas</li> </ul>
Partial response (PR)	<p>All of the following:</p> <ul style="list-style-type: none"> <li>● <math>\geq 50\%</math> <math>\downarrow</math> in serum paraprotein, maintained for <math>\geq 6</math> weeks</li> <li>● <math>\downarrow</math> in 24h urinary LC excretion by either <math>\geq 90\%</math> or to <math>&lt; 200\text{mg}</math>, maintained for <math>\geq 6</math> weeks</li> <li>● For patients with NS myeloma only, <math>\geq 50\%</math> <math>\downarrow</math> in PC in BM aspirate and trephine biopsy, if performed, maintained <math>\geq 6</math> weeks</li> <li>● <math>\geq 50\%</math> <math>\downarrow</math> in size of soft tissue plasmacytomas (by radiology or clinical examination)</li> <li>● No <math>\uparrow</math> in size or number of lytic bone lesions (development of a compression fracture does not exclude PR)</li> </ul>
Minimal response (MR)	<p>All of the following:</p> <ul style="list-style-type: none"> <li>● 25–49% <math>\downarrow</math> in serum paraprotein level maintained for <math>\geq 6</math> weeks</li> <li>● 50–89% <math>\downarrow</math> in 24h urinary LC excretion, which still exceeds 200mg/24h, maintained for <math>\geq 6</math> weeks</li> <li>● For patients with NS myeloma only, 25–49% <math>\downarrow</math> in PC in BM aspirate and trephine biopsy, if performed, maintained <math>\geq 6</math> weeks</li> <li>● 25–49% <math>\downarrow</math> in size of soft tissue plasmacytomas (by radiology or clinical examination)</li> <li>● No <math>\uparrow</math> in size or number of lytic bone lesions (development of a compression fracture does not exclude MR)</li> </ul>
No change	Not meeting the criteria of either MR or progressive disease
Plateau	Stable values (within 925% value at the time response is assessed), maintained for $\geq 3$ months
Relapse from CR	<p>At least one of the following:</p> <ul style="list-style-type: none"> <li>● Reappearance of serum or urine paraprotein on immunofixation or electrophoresis, confirmed by at least 1 other study</li> <li>● <math>\geq 5\%</math> PC in BM aspirate or trephine biopsy</li> <li>● Development of new lytic bone lesions or soft tissue plasmacytomas or definite <math>\uparrow</math> in size of residual bone lesions</li> <li>● Development of hypercalcaemia not attributable to other cause</li> </ul>

*(continued)*

**Table 8.11** EBMT response criteria for MM<sup>15</sup> (continued)

Response category	Definition
Progressive disease	<p>1 or more of the following:</p> <ul style="list-style-type: none"> <li>● &gt;25% ↑ in serum paraprotein, which must also be an absolute ↑ of ≥5g/L, confirmed by ≥1 repeated study</li> <li>● &gt;25% ↑ in 24h LC excretion, which must also be an absolute ↑ of ≥200mg/24h, confirmed by ≥1 repeated study</li> <li>● &gt;25% ↑ in PC in BM aspirate or trephine biopsy, which must also be an absolute ↑ of at least 10%</li> <li>● Definite ↑ in size of existing bone lesions or soft tissue plasmacytomas</li> <li>● Development of new bone lesions or soft tissue plasmacytomas</li> <li>● Development of hypercalcaemia not attributable to other cause</li> </ul>

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**Table 8.12** International Myeloma Working Group uniform response criteria<sup>14</sup>

Response subcategory	Response criteria <sup>a</sup>
Stringent complete response (sCR)	<p>CR as defined below, plus:</p> <ul style="list-style-type: none"> <li>● Normal SFLC ratio</li> <li>● Absence of clonal cells in BM<sup>b</sup> by immunohistochemistry or immunofluorescence<sup>c</sup></li> </ul>
Complete response (CR)	<p>All of the following:</p> <ul style="list-style-type: none"> <li>● –ve immunofixation on the serum and urine</li> <li>● Disappearance of any soft tissue plasmacytomas</li> <li>● ≤5% PC in BM<sup>b</sup></li> </ul>
Very good partial response (VGPR)	<p>One of the following:</p> <ul style="list-style-type: none"> <li>● Serum and urine M-protein detectable by immunofixation but not on electrophoresis</li> <li>● ≥90% ↓ in serum M-protein plus urine M-protein level &lt;200mg/24h</li> </ul>
Partial response (PR)	<ul style="list-style-type: none"> <li>● ≥50% ↓ of serum M-protein and ↓ in urinary M-protein by ≥90% or to &lt;200mg/24h</li> <li>● If the serum and urine M-protein are unmeasurable, a ≥50% ↓ in the difference between involved and uninvolved SFLC levels is required in place of the M-protein criteria.</li> <li>● If serum and urine M-protein are unmeasurable, and SFLC assay is also unmeasurable, ≥50% ↓ in PC is required in place of M-protein, provided baseline BM PC percentage was ≥30%.</li> </ul> <p>In addition to the above listed criteria, if present at baseline, ≥50% ↓ in size of soft tissue plasmacytomas is also required.</p>

(continued)

**Table 8.12** International Myeloma Working Group uniform response criteria<sup>14</sup> (continued)

Response subcategory	Response criteria <sup>a</sup>
Stable disease (SD) <i>Not recommended for use as an indicator of response; stability of disease is best described by providing the time to progression estimates</i>	Not meeting criteria for CR, VGPR, PR, or progressive disease
Progressive disease <i>To be used for calculation of time to progression and progression-free survival end-points for all patients including those in CR (includes primary progressive disease and disease progression on or off therapy)</i>	Any one or more of the following: <ul style="list-style-type: none"> <li>● ↑ of ≥25% from baseline in: <ul style="list-style-type: none"> <li>• Serum M-component and/or (absolute ↑ must be ≥5g/L)<sup>d</sup></li> <li>• Urine M-component and/or (absolute ↑ must be ≥200mg/24h)</li> <li>• Only in patients without measurable serum and urine M-protein levels: the difference between involved and uninvolved SFLC levels (absolute ↑ must be &gt;100mg/L) <ul style="list-style-type: none"> <li>• BM PC percentage (absolute % must be ≥10%)<sup>e</sup></li> </ul> </li> </ul> </li> <li>● Definite development of new bone lesions or soft tissue plasmacytomas or definite ↑ in size of existing bone lesions or tissue plasmacytomas</li> <li>● Development of hypercalcaemia (corrected serum calcium &gt; 2.65mmol/L (&gt;11.5mg/dL)) solely attributable to the PC proliferative disorder</li> </ul>
Clinical relapse <i>Not used in calculation of time to progression or progression-free survival but is listed as something that can be reported optionally or used in clinical practice</i>	Any one or more of the following direct indicators of increasing disease and/or end organ dysfunction (CRAB features): <sup>d</sup> <ul style="list-style-type: none"> <li>● Development of new soft tissue plasmacytomas or bone lesions</li> <li>● Definite ↑ in size of existing plasmacytomas or bone lesions; definite ↑ is defined as a 50% (and ≥1cm) ↑ as measured serially by sum of the products of the cross-diameters of measured lesion</li> <li>● Hypercalcaemia (&gt;2.65mmol/L (≥11.5mg/dL))</li> <li>● Decrease in haemoglobin of ≥2g/dL</li> <li>● Rise in serum creatinine by ≥177 micromol/L (≥2mg/dL)</li> </ul>

(continued)

**Table 8.12** International Myeloma Working Group uniform response criteria<sup>14</sup> (continued)

Response subcategory	Response criteria <sup>a</sup>
Relapse from CR <i>To be used only if the end-point studied is DFS<sup>f</sup></i>	Any one or more of the following: <ul style="list-style-type: none"> <li>● Reappearance of serum or urine M-protein by immunofixation or electrophoresis</li> <li>● Development of <math>\geq 5\%</math> PC in the BM</li> <li>● Appearance of any other sign of progression (i.e. new plasmacytoma, lytic bone lesion, or hypercalcaemia)</li> </ul>

<sup>a</sup> All response categories require 2 consecutive assessments made at any time before the institution of any new therapy; all categories also require no new evidence of progressive or new bone lesions if radiographic studies were performed. Radiographic studies are not required to satisfy these response requirements.

<sup>b</sup> Confirmation with repeat BM not needed.

<sup>c</sup> Presence/absence of clonal cells is based upon the  $\kappa/\lambda$  ratio. An abnormal  $\kappa/\lambda$  ratio by immunohistochemistry and/or immunofluorescence requires a minimum of 100 plasma cells for analysis. An abnormal ratio reflecting the presence of an abnormal clone is  $\kappa/\lambda$  of  $>4:1$  or  $<1:2$ .

<sup>d</sup> For progressive disease, serum M-component increases of  $\geq 10\text{g/L}$  are sufficient to define relapse if starting M-component is  $\geq 50\text{g/L}$ .

<sup>e</sup> Relapse from CR has the 5% cut-off versus 10% for other categories of relapse.

<sup>f</sup> For purposes of calculating time to progression and progression-free survival, CR patients should also be evaluated using criteria listed above for progressive disease.

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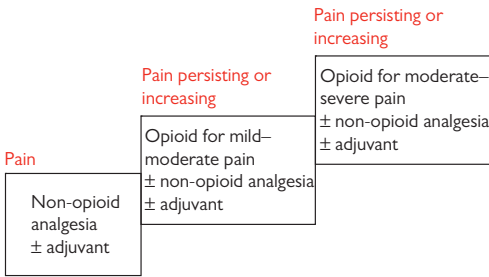
## Management

Partial and complete responses are achieved in a high proportion of patients with MM using a range of chemotherapeutic regimens but these responses are transient. Further responses are commonly achieved with the same or alternative regimens. MM is not curable with conventional regimens and only a small number of patients may have been cured by allogeneic SCT. The recent introduction of novel agents has changed the treatment options in MM and more than doubled OS in the last decade. Risk-adapted therapy is now feasible as new prognostic factors more clearly define risk groups.

### Initial management considerations and general aspects

#### Pain control

- Titrate simple analgesia (e.g. paracetamol 1g 4–6-hourly) for mild-to-moderate pain, weak opioids (co-codamol 2 tabs 6-hourly or dihydrocodeine 30–60mg up to 4-hourly) for moderate pain and strong opioids (morphine sulfate solution 5–10mg 4-hourly converting to slow release preparation, when daily requirement established with 5–10mg morphine sulfate solution prn for ‘breakthrough’ pain) for moderate-to-severe pain; commence simple laxative with opioids. (See Fig. 8.5.)
- Avoid NSAIDs (if essential, use caution and monitor renal function).
- Local radiotherapy (8–30Gy) often extremely effective.
- A spinal support corset often helpful for severe back pain.
- Neuropathic pain may be relieved by non-analgesic adjuvants, e.g. amitriptyline, carbamazepine, or gabapentin.



**Fig. 8.5** WHO pain treatment ladder. Reproduced with permission from the World Health Organization, *Palliative Care: Symptom management and end of life care*, p.12.

#### Renal impairment

- All patients should be instructed to maintain a high fluid intake ( $\geq 3\text{L}/\text{d}$ ); use caution with nephrotoxic drugs, including NSAIDs.
- Prompt intervention to correct early renal impairment may prevent long-term renal damage.
- Aim for urine output  $>3\text{L}/\text{d}$ , and rapid treatment of hypercalcaemia, infection and hyperuricaemia may improve renal function.
- Seek advice of nephrologist if renal failure does not improve within 48h; peritoneal or haemodialysis may be required ( $<5\%$ ).
- Renal impairment related to MM: bortezomib (and dexamethasone)-based regimen are used followed by PBSC harvest and high-dose melphalan (HDM) in renally reduced dose ( $140\text{mg}/\text{m}^2$ ) with autologous SCT in younger patients; after response EFS and OS same as other patients; allo-SCT not recommended for patients undergoing haemodialysis.

#### Hypercalcaemia

- Vigorous rehydration with IV saline ( $3\text{--}6\text{L}/\text{d}$  IV) with close monitoring of fluid balance and renal function.
- Loop diuretics (furosemide) increase  $\text{Ca}^{2+}$  excretion and maintain fluid balance.
- IV bisphosphonate (pamidronate disodium  $30\text{--}90\text{mg}$  IVI over 2h or zoledronic acid  $4\text{mg}$  IV over 5min; ↓ dose and infusion rate in renal impairment); zoledronic acid gives higher CR rate (50% by d4) and duration of effect; repeat if necessary.
- IV corticosteroids if refractory; chemotherapy to treat underlying cause (MM).

#### Bone disease

- Local radiotherapy for localized pain.
- Fixation of fractures/potential fractures followed by radiotherapy.
- Vertebroplasty or kyphoplasty may be considered in patients with persistent back pain and vertebral collapse.
- Long-term bisphosphonates: prevention of further damage.

#### Infection

- Prompt and vigorous broad-spectrum antibiotic therapy.

- Prophylactic antibiotics are currently not recommended.
- Annual influenza immunization and immunization against *Streptococcus pneumoniae* and *Haemophilus influenzae* is recommended.

#### Anaemia

- Blood transfusion for symptomatic anaemia; use caution if high paraprotein due to risk of hyperviscosity.
- EPO can be used if Hb persistently  $\leq 10\text{g/dL}$  (10,000IU tiw or 30,000IU once weekly;  $\sim 70\%$  response rate with  $\geq 2\text{g/dL}$  rise in Hb); not recommended until response to chemotherapy assessed then consider 6–8 week trial in patients with symptomatic anaemia on chemotherapy; discontinue if Hb has not risen by  $1\text{--}2\text{g/dL}$  after 6–8 weeks.

#### Hyperviscosity

- May develop in patients with high serum paraprotein levels, IgM  $>$  IgA  $>$  IgG causing cerebral, pulmonary, and renal manifestations and bleeding. Patients with levels of IgG  $> 60\text{g/L}$ , IgA  $> 40\text{g/L}$  and IgM  $> 30\text{g/L}$  are at particular risk.
- Symptomatic patients should be treated by plasmapheresis (3L plasma exchange) followed by prompt chemotherapy. Initial isovolaemic venesection with saline replacement can be used as a holding measure if plasmapheresis is not immediately or readily available.

#### Cord compression

- Medical emergency; requires immediate treatment
- Commonly presents with sensory loss, paraesthesiae, limb weakness, difficulty walking, and sphincter disturbance (cauda equina syndrome).
- Urgent MRI scan to define lesion (CT if MRI unavailable or contra-indicated).
- Commence oral dexamethasone stat. Dosing schedules vary. The 2013 BCSH guidelines recommend 40mg dexamethasone for 4d.
- Local radiotherapy should be commenced within 24h.
- Surgery is indicated if there is spinal instability.

#### Haemorrhage

- Uncommon; several possible mechanisms: direct inhibition of fibrin polymerization, heparin-like anticoagulants, acquired von Willebrand's syndrome and, in the presence of AL amyloid, factor X deficiency; treatment must be individualized.

### Specific treatment of MM

The aim of treatment is: (i) to control disease, (ii) to maximize quality of life, and (iii) to prolong survival. Chemotherapy and good supportive care are both essential to these aims. It is important that, wherever possible, patients are treated within clinical trials to determine the optimum combination and sequence of the effective new agents that have radically altered treatment options in recent years.

An early decision must be whether the patient is a candidate for high-dose therapy (HDT) and autologous SCT, based on age, co-morbidities, and risk-assessment. Advanced age and renal dysfunction are not absolute contraindications to SCT. An initial regimen that minimizes stem cell toxicity (non-alkylator regimen) must be selected for potential candidates for SCT.



Many agents and combinations are available and approved for use in the treatment of MM. Even a geographical difference for regimens used exists (different preferences in the USA and UK). Treatment must be tailored for specific patients and response followed closely.

#### *Treatment on clinical trial recommended*

##### *Initial therapy (treatments are not discussed in any particular order)*

No regimen is clearly superior. VAD, formerly widely used, has been superseded, and is not discussed. CTD is used in many UK centres as standard 1st-line treatment, whereas in the USA bortezomib regimens are mainly used in 1st line.

##### *Cyclophosphamide, thalidomide, and dexamethasone (CTD)*

- CTD (C 500mg PO/IV once weekly; T 50–200mg/d; D 40mg PO d1–4 and 15–18; 21d cycle) × 4–6 courses. Effective and well tolerated initial therapy pre-HDT. Has superseded previously used C-VAD. Prophylactic anticoagulation recommended in high risk patients; pregnancy prevention programme mandatory. 1st-choice treatment pre-SCT in many centres.

##### *Cyclophosphamide, thalidomide, and dexamethasone—attenuated (CTDa)*

- C 500mg PO once weekly; T 50–200mg/d; D 20mg PO d1–4 and d15–18; 28d cycle). Effective and well-tolerated initial therapy in elderly patients; Antithrombotic therapy recommended; pregnancy prevention programme mandatory.

##### *Cyclophosphamide<sup>16</sup>*

- ‘C-weekly’ (300–500mg PO once weekly) may be used as a single agent for patients intolerant of melphalan or other schedules due to persistent cytopenia and thalidomide; durable plateau phases achieved. Well tolerated.

##### *Melphalan and prednisolone (MP)<sup>17</sup>*

- Historically and until recently standard initial therapy for elderly patients with MM (since 1960); (M 6–9mg/m<sup>2</sup>/d PO; P 40–100mg/d PO for 4d every 4–6 weeks); achieves ≥50% reduction of paraprotein in 50–60% patients; response often slow; continue to maximum response (9–12 months); CR uncommon (<5%); well tolerated; side effects myelosuppression and steroid toxicity.
- Median duration 12–18 months.
- At progression after durable plateau patients may further respond to MP. Melphalan resistance ultimately develops in all patients.
- Median survival 36 months.
- Option for patients unable or unwilling to tolerate thalidomide.

##### *Melphalan, prednisolone, and thalidomide (MPT)<sup>18,19</sup>*

- Thalidomide is an immunomodulatory agent with multiple actions on MM-cells and the microenvironment: inhibition of angiogenesis, inhibition of growth and survival of MM cells, altered cytokine production, altered expression of adhesion molecules and T-lymphocyte stimulation;
- Side effects of thalidomide: constipation, sedation, rash, dizziness, and peripheral neuropathy; VTE is a risk when used in combination and patients at high risk of, or with a history of VTE should receive VTE prophylaxis; pregnancy prevention programme mandatory.

- MPT (MP + T 100–400mg/d) shows superior response (OR 76% vs 48%; CR+nCR 28% vs 7%) and progression-free survival (PFS: 54% vs 27% at 2 years) vs MP in 2 randomized trials; 1 also demonstrated superior median OS (52 months vs 33 months) and 3-year OS (48% vs 25%).
- MPT has higher toxicity than MP (48% vs 25%): thromboembolism 12% vs 2%; neuropathy 10% vs 1%; infections 10% vs 2%; GI events 6% vs 1%; thromboembolic events markedly reduced by introduction of LMWH prophylaxis.
- Doses and administration differ; optimum schedule unclear.
- Well tolerated in elderly patients.

#### *Bortezomib (Velcade) and dexamethasone (Vel/Dex) (± Cyclo)*

- Bortezomib is a 1st-in-class proteasome inhibitor; multiple effects on both MM-cells and the microenvironment; side effects are transient thrombocytopenia, peripheral neuropathy, neuropathic pain, constipation, nausea, vomiting, and neutropenic fever.
- Bortezomib (1.3mg/m<sup>2</sup> SC 2 × weekly (d1, 4, 8, 11) for 2 weeks in 3-week cycle × 4–8) approved as 2nd-line treatment in relapsed/refractory MM; ~40% OR in newly diagnosed patients.
- Cyclophosphamide can be added: improved RR.
- Vel/Dex: Dex 40mg/d usually added on day of and day after Vel.
- ORR 90%, 19% CR + nCR; rapid response 50% after cycle 2; 75% required Dex, improving response in 64%; manageable toxicities; SC harvest not compromised.<sup>20,21</sup>
- Mayo Clinic guidelines: bortezomib-containing regimen is considered in patients with high-risk features (t(4;14), t(14;16), or del(17p)).

#### *Bortezomib (Velcade), thalidomide, and dexamethasone (VTD)*

- VTD has been compared with Thal/Dex in a randomized trial in 187 patients giving a better CR + nCR rate (38% vs 7%) with similar toxicity apart from ↑ grade 3 skin rashes and was not adversely affected by poor-risk cytogenetic abnormalities; better CR + nCR rate persisted after SCT (57% vs 28%).<sup>22</sup>
- A rapid response rate was demonstrated for VTD in a study of 38 untreated patients with 16% CR and 87% ≥ PR after ≤3 cycles.<sup>23</sup>

#### *Bortezomib (Velcade), doxorubicin, and dexamethasone (PAD)*

- Initial therapy: Vel 1.3 (PAD1) or 1.0 (PAD2) mg/m<sup>2</sup> d1, 4, 8, 11, Dox 9mg/m<sup>2</sup> d1–4, Dex 40mg/d d1–44: CR + VGPR in 62% (PAD1) and 42% (PAD2), translating to 81% and 53% after HDM + SCT;
- Median responses of 29 and 24 months and 2-year OS of 95% and 73% respectively.<sup>24</sup>

#### *Bortezomib (Velcade), lenalidomide, and dexamethasone (VRD)*

- High response rates with 28% CR + nCR and 98%.<sup>25</sup>

#### *Bortezomib (Velcade), melphalan, and prednisolone (VMP)*

- Vel 1.3mg/m<sup>2</sup> SC 2 × weekly. Advantage for VMP over MP in RR (89% vs 42%), 16 months EFS (83% vs 51%), and 16 months OS (90% vs 62%); a phase 3 study in 680 patients was terminated early when VMP showed superiority over MP in all measures: CR 35% vs 5%; duration of CR 24 months vs 13 months, median time to progression 24 months vs 13 months; similar adverse effects.

*Melphalan, prednisolone, and Revlimid (lenalidomide) (MPR)*<sup>26</sup>

- M 0.18mg/kg/d and P 2mg/kg/d, R d1–4, 10mg/d, d1–21 every 28d for 9 cycles. 81% PR, 48% VGPR, 24% CR, and 1-year EFS and OS of 92% and 100%; grade 3+4 toxicity: 52% neutropenia, 24% thrombocytopenia; 5% VTE.

*High-dose dexamethasone (Dex)*<sup>27,28</sup>

- Single agent Dex (40mg/d PO on d1–4, 9–12, and d17–20; 28d cycle) Responses  $\geq$  PR achieved in 43–63%.

*Thalidomide and dexamethasone (ThalDex)*<sup>29,30</sup>

- Addition of thalidomide (200mg/d PO) to Dex improved RRs (63% vs 41%) but added toxicity (45% vs 21%), in particular DVT (17% vs 3%), also rash, bradycardia, and neuropathy; prophylactic anticoagulation recommended; pregnancy prevention programme mandatory.

*Lenalidomide (Revlimid) and dexamethasone (Rev/Dex)*

- Lenalidomide is a thalidomide analogue, immunomodulatory drug (IMiD); multiple effects on both MM-cells and the microenvironment and different side effect profile; main side effects—constipation, cytopenia, VTE, and possible birth defects.
- Rev/Dex (Len 25mg/d PO d1–21 and Dex 40mg/d PO d1–4, 9–12, and 17–20; 28-d cycle) is approved as therapy for relapsed/refractory MM.
- 91% RR with 6% CR and 32% VGPR; prophylactic anticoagulation recommended.<sup>31,32</sup>

*Carfilzomib*

- Next-generation proteasome inhibitor. FDA approved in 2nd relapse. ORR 225; Median duration of response 7–8 months. Combination studies pending

*Pomalidomide*

- Immune-modulatory drug. RR seen in patient refractory to bortezomib and lenalidomide. Further trial results awaited.

**Other therapy*****Bisphosphonates***<sup>33–36</sup>

- Inhibit OC activation; patients on long-term therapy experience less bone pain and fewer new bone lesions and fractures; evidence of improved QoL and possible prolonged survival.
- All patients with active MM should receive bisphosphonate therapy.
- No evidence for superiority of either monthly pamidronate disodium (90mg IV) or zoledronic acid (4mg IV).
- Zoledronic acid, check creatinine before each infusion, ensure hydration; not recommended if creatinine  $>265$ micromol/L.
- Monitor for osteonecrosis of jaw with zoledronate (risk factor poor dentition).
- Role on asymptomatic myeloma not clear and currently not recommended.

**Thromboprophylaxis<sup>37</sup>**

- Risk of VTE in malignancy ↑ (see Table 8.13).
- Thalidomide-containing regimens: associated with ↑ VTE incidence. Thromboprophylaxis indicated in high-risk patients and patients with a history of thrombosis or other risk factors.
- Lenalidomide monotherapy no ↑ risk and thromboprophylaxis not indicated; ↑ incidence with Rev/Dex (up to 23%) and Rev/cyclophosphamide (14%).
- *LMW heparin*: reduces VTE frequency in several Thal combinations; may be preferable due to shorter half-life and lower risk of haemorrhage but generally not recommended in renal failure.
- *Aspirin 75mg/d* reduces VTE risk with several Thal or Rev combinations, but not Thal + doxorubicin or multiagent chemotherapy; convenient and may be useful in lower-risk patients.
- *Risk factors*: initial therapy, high tumour load, hyperviscosity, high-dose Dex, doxorubicin, multiagent chemotherapy, concurrent EPO; age, obesity, history of VTE, diabetes, infection, renal disease, cardiac disease, surgery, immobility, HRT, thrombophilia.
- Unless contraindicated, prophylaxis for all patients receiving Thal or Rev in combination with other agents especially as initial therapy; continue for 4–6 months or duration of Thal or Rev therapy; consider longer if additional patient- or treatment-related factors.
- Select prophylaxis on individual patient basis, balancing risks of VTE and haemorrhage.
- Treat VTE according to standard protocol; Thal or Rev should be briefly discontinued and may be cautiously resumed when full anticoagulation established; ~10% risk of 2nd DVT; continue anticoagulation for duration of Thal or Rev therapy; discontinue 1 month after completion if no further DVT (minimum 3 months therapy).<sup>37</sup>

**Table 8.13** Proposed risk assessment model for VTE management in MM patients treated with thalidomide or lenalidomide<sup>37</sup>

Risk factors	Actions
<i>Individual</i> <ul style="list-style-type: none"> <li>● Obesity</li> <li>● Previous VTE</li> <li>● Central venous catheter or pacemaker</li> </ul>	If no risk factor or any one risk factor present: <ul style="list-style-type: none"> <li>● Aspirin 75–325mg od</li> </ul>
<i>Associated disease</i> <ul style="list-style-type: none"> <li>● Cardiac disease</li> <li>● Chronic renal disease</li> <li>● Diabetes</li> <li>● Acute infection</li> <li>● Immobilization</li> </ul>	If $\geq 2$ risk factors present: <ul style="list-style-type: none"> <li>● LMWH heparin (enoxaparin 40mg od or equivalent)</li> </ul> <b>(or)</b> <ul style="list-style-type: none"> <li>● Full dose warfarin (target INR 2–3))</li> </ul>
<i>Surgery</i> <ul style="list-style-type: none"> <li>● General surgery</li> <li>● Any anaesthesia</li> <li>● Trauma</li> </ul>	
<i>Medications</i> <ul style="list-style-type: none"> <li>● EPO</li> </ul>	
<i>Blood clotting disorders</i>	
<i>Myeloma-related factors</i> <ul style="list-style-type: none"> <li>● Diagnosis</li> <li>● Hyperviscosity</li> </ul>	
<i>Myeloma therapy</i> <ul style="list-style-type: none"> <li>● High-dose dexamethasone (&gt;480mg/month)</li> <li>● Doxorubicin</li> <li>● Immunomodulatory drug combinations</li> </ul>	If any of these therapies used: <ul style="list-style-type: none"> <li>● LMWH (enoxaparin 40mg od or equivalent)</li> </ul> <b>(or)</b> <ul style="list-style-type: none"> <li>● Full dose warfarin (target INR 2–3))</li> </ul>
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## Radiotherapy

- Important modality of treatment in myeloma at all stages of disease; local radiotherapy (8–30Gy) often a rapidly effective treatment for bone pain associated with pathological fracture or lytic lesions.
- Urgent radiotherapy is indicated for patients with spinal cord compression.

## Follow-up

- Monitor response to therapy with serial quantitation of serum paraprotein, SFLC, or BJP (+ FBC, renal biochemistry and serum  $\text{Ca}^{2+}$  group) at 4–6 weekly intervals to plateau (stable values  $\pm 25\%$  over 3 months) or SC mobilization.
- Patients in established plateau phase should be monitored for progression at 6–8 week intervals.

### Autologous stem cell transplantation (ASCT)<sup>38–42</sup>

- ASCT not curative but prolongs median OS by ~12 months; in the era of the new agents and targeted treatment its role might change but currently ASCT remains recommended to younger/fit patients in 1st response.
- High-dose melphalan (HDM; 200mg/m<sup>2</sup>) + ASCT achieves high CR rates (25–80%) after initial therapy. Median response duration 2–3 years; treatment of choice for patients <65 years; best responders have best survival, median >5 years; improved PFS (32 vs 20 months) and OS (54 vs 42 months) vs conventional therapy in randomized study.
- Side effects: myelosuppression, infection, delayed regeneration.
- No convincing benefit for 'double/tandem' ASCT but benefit for those converted from PR to CR after 2nd procedure.
- No benefit from SC purging procedures.
- Same OS whether ASCT early (after induction) or delayed (as salvage after relapse).
- 2nd autologous transplant has a role in patients who have achieved a durable response post 1st autograft and who have achieved a further response with 2nd-line treatment.

### Allogeneic stem cell transplantation<sup>43–45</sup>

- Allogeneic SCT should be considered for eligible patients with progressive disease after ASCT.
- Applicable to fit patients ≤50 years; transplant-related mortality with standard conditioning regimens is high (~33%) due to infection and GvHD; 35–45% long-term survival (>5 years); ~33% chance of durable remission and possible cure; ~33% chance of survival with recurrence.
- Reduced-intensity regimens have a lower toxicity, increase the age limit, but reduce the response rate.
- Reduced-intensity allogeneic SCT after ASCT has achieved 52–83% CR with 2–3 year, with an OS of 62–78% and PFS ~55% and may be a useful approach as either consolidation or salvage therapy; poor OS associated with chemoresistant MM, >1 prior SCT, and no chronic GvHD.
- Allogeneic SCT should be discussed with patients <50 years with a suitable sibling donor or young patients with a matched unrelated donor. Morbidity and mortality rates remain high.
- There is evidence of a graft-versus-myeloma effect; DLI can re-induce responses in patients with recurrence after allogeneic SCT.
- Preferentially in the context of a clinical trial.

### Maintenance therapy

- Maintenance chemotherapy does not prolong response or survival but adds toxicity (BM suppression); maintenance dexamethasone not convincingly beneficial.
- Thalidomide:
  - Trials of thalidomide maintenance post ASCT showed significantly benefit in 3-year EFS and 4-year OS; median duration 15 months; median dose 200mg; 39% discontinued due to toxicity; side effects neuropathy 68%, fatigue 34%; constipation 20%.<sup>46</sup>


- 50–100mg dose recommended; thromboprophylaxis not required unless individual risk factors are present.
- Guidelines<sup>12</sup> suggest maintenance therapy with thalidomide in high-risk patients who fail to achieve a CR post autograft.
- Lenalidomide or bortezomib maintenance: remains investigational.

### Treatment of primary refractory disease

- Patients who are refractory to their 1st-line treatment should be switched to an alternative regimen if appropriate. Many schedules are approved for 1st-line and 2nd-line use. Response criteria are listed in Tables 8.11 and 8.12.
- Poor prognosis.
- Consolidate with ASCT if possible after response achieved with 2nd-line treatment. Patients with 1<sup>o</sup> progressive myeloma may achieve good response to ASCT; 1-year PFS of 70% reported and compares to 83% in responders to initial therapy.

### Salvage therapy for disease progression

Treatment of disease progression before the development of novel therapies consisted of progressively shorter responses to available agents with the ultimate development of drug resistance and evolution of adverse features. The development of novel agents has significantly improved the outlook for patients with relapsed myeloma. Generally speaking patients will switch to a treatment regimen they did not receive before. (See Table 8.14.)

- Patients who achieve a durable response ( $\geq 18$  months) to initial therapy may respond to the same regimen.
- Patients who relapse early after initial therapy (<6 months) will require a different regimen to achieve a further response.
- Patients who relapse after prolonged response to HDM and ASCT (>18 months) with a PBSC harvest sufficient for 2 procedures or with a further successful harvest may benefit from a 2nd HDM after re-induction with an alternative regimen.
- Allogeneic SCT should be considered for eligible patients with progressive disease after ASCT.
- Alternative salvage regimens are generally given. The list of treatment schedules is the same as listed on  pp.351–353. Choice of regimen will be dependent on patient-specific factors as well as on disease characteristics.
- Cyclophosphamide (50–100mg/d PO) is a well-tolerated palliative therapy for patients with advanced refractory disease or cytopenia who are intolerant of thalidomide, dexamethasone or unable to tolerate other schedules such as Vel/Dex or Rev/Dex.

**Table 8.14** Salvage treatment options for relapsed MM outside a clinical trial

Relapse after initial treatment	Early <12 months	Consider alternative regimen
	Late ≥18 months	Consider same regimen or alternative regimen
Relapse after ASCT	Early <12 months	Consider alternative regimen and allo-SCT if appropriate/eligible.
	Late ≥18 months	Consider alternative regimen Consider allo-SCT or 2nd ASCT
Relapse after allo-SCT		Consider DLI

ASCT autologous stem cell transplantation; allo-SCT allogeneic stem cell transplantation; DLI donor lymphocyte infusion.



## Prognosis

Analysis of patient outcome showed improved median OS from relapse for patients relapsing after 2000 (24 months) compared to those relapsing before 2000 (12 months).

Patients diagnosed between 2000 and 2010 had a 50% improvement in overall survival (45 months vs 30 months). The improvements during that time are due predominantly to the introduction of novel agents and in part to improved supportive care.

With high-dose therapy followed by autologous stem cell transplantation, the median survival in 2003 was 4.5 years, compared to a median of approximately 3.5 years with 'standard' therapy. With the advent of new targeted treatments and regimens this number will have improved but mature data is pending.

The International Staging System helps predict survival (➔ p.344). Median survival is 62 months for stage 1 disease, 44 months for stage 2 disease, and 29 months for stage 3 disease, but it has to be kept in mind this data was analysed on patients treated before many new agents were widely available. It is likely prognosis will continue to improve. Younger patients have much longer survival rates.

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## Variant forms of myeloma

### Non-secretory myeloma

- 1% of MM cases.
- No detectable serum or urine paraprotein by immunofixation, but SFLC-ratio abnormal in 75%.
- Clonal plasma cells  $\geq 10\%$  in BM or plasmacytoma on biopsy; myeloma-related end-organ damage.
- Treatment as on  p.351; response rates comparable to secretory MM; SFLC assay provides a sensitive marker for monitoring response and identifying relapse in most patients; alternatives in others are surrogate markers ( $\beta_2$ -M, CRP) and repeated BM assessment.

### IgD myeloma

- ~1% of cases of MM; very small or no visible monoclonal spike on routine electrophoresis; younger mean age (50–60 years).
- High rate of Bence Jones proteinuria and associated higher frequency of acute and chronic renal failure; tendency to present with other poor prognostic features (high  $\beta_2$ -M; low Hb); extramedullary involvement and amyloidosis may be more frequent.
- Treat as for other myeloma.

### IgM myeloma

- Very rare; <0.5% of MM; 1% of all IgM gammopathies; plasma cell infiltrate in BM as opposed to lympho-plasmacytoid infiltrate characteristic of WM; aberrant PC phenotype; characterized by CD20 –ve, CD56 –ve, CD117 –ve phenotype and t(11;14).<sup>1</sup>
- Often associated with osteolytic lesions; no lymphadenopathy or splenomegaly.
- Overall outcome not established; may have inferior outcome to IgG or IgA MM.
- Differential diagnosis with WM essential.

### IgE myeloma

- Rarest form of MM; younger age; high incidence of plasma cell leukaemia; possible shorter survival.

### Plasma cell leukaemia

- Defined as PB plasma cells  $>2 \times 10^9/L$  or 20% of differential count; may occur *de novo* at presentation or in the terminal stages of otherwise typical MM.
- Aggressive disease associated with BM failure and organomegaly; poor response to conventional dose therapy; few survive >6 months; better responses to HDM.

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# Cryoglobulinaemia

## Classification and pathogenesis

- 3 types:
  - Type I (10–15%) single monoclonal Ig (usually IgM, less often IgG, occasionally LC-only). Associated with haematological disorders (plasma cell dyscrasia, NHL).
  - Type II (50–60%) polyclonal IgG plus monoclonal IgM with rheumatoid factor (RF) activity (i.e. anti-IgG).
  - Type III (25–30%) polyclonal IgG and IgM usually with small monoclonal component on high resolution assay.
- Types II and III referred to as mixed cryoglobulinaemia (MC):
  - ~75% associated with HCV infection.
  - Chronic stimulus may initiate multistep process of B-cell clonal expansion leading to LPDs.
  - Serology reflects progression from polyclonal to oligoclonal to monoclonal IgM-RF and clonal expansion.
- Associated with wide range of conditions:
  - Lymphoproliferative and haematological disorders.
  - Auto-immune diseases.
  - Infections (esp. HCV).
  - Renal and liver disorders.

## Clinical features and diagnosis

- Type I rarely associated with vasculitis but with signs of peripheral vessel obstruction and hyperviscosity: purpura, acrocyanosis, Raynaud's phenomenon, dystrophic manifestations, leg ulcers, gangrene; usually clinically indistinguishable from Waldenström macroglobulinaemia (WM), MM, or CLL; rare complication of paraprotein precipitation at low temperature.
- MC characterized by purpura, weakness, and arthralgias with multisystem involvement including chronic hepatitis, glomerulonephritis or peripheral neuropathy due to small and medium vessel vasculitis; biopsy of cutaneous lesions shows vasculitis.
- Diagnose by precipitation at 2–4°C in serum prepared from blood collected and allowed to clot at 37°C; immunofixation for assessment of clonality and typing; serum immunoglobulins, plasma viscosity, complement levels, and RF activity.
- Exclude underlying LPD in cases with monoclonal IgM with type I or II cryoglobulinaemia.

## Treatment

- Type I: treat underlying MM, WM or CLL and avoid the cold.
- HCV-related MC: interferon alfa ± ribavirin to eradicate HCV; may be followed by regression of established lymphoma.
- Non-HCV-related MC: mild–moderate symptoms, low dose prednisolone and analgesia; severe symptoms, Cyclophosphamide ± prednisolone ± plasmapheresis; rituximab may control B-cell clone and also improve renal function in HCV-related disease.

## Further reading

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## POEMS syndrome (osteosclerotic myeloma)

### Clinical features

- Synonym: Crow–Fukase syndrome.
- Clonal plasma-cell dyscrasia.
- Polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin changes: rare paraneoplastic syndrome 2° to a plasma cell neoplasm.
- Other features: papilloedema, extravascular fluid overload (oedema, pleural effusions, ascites), lymphadenopathy, diabetes mellitus, male gynaecomastia and impotence, female amenorrhoea, hypertrichosis and hyperpigmentation, sclerotic bone lesions, thrombocytosis, Castleman disease, polycythaemia, and clubbing.
- Association of osteosclerotic plasmacytoma with chronic inflammatory demyelinating progressive polyneuropathy causing predominantly motor disability.
- Endocrinopathy occurs in ~84% usually hypogonadism then thyroid abnormalities, diabetes and adrenal insufficiency; majority multiple.
- 11–30% have Castleman disease or Castleman-like disease with angiofollicular lymph node hyperplasia which may be unifocal or multifocal and associated with B-symptoms, auto-immune phenomena, more subtle sensory neuropathy, and polyclonal gammopathy.

### Diagnosis

- Confirm diagnosis by demonstrating monoclonal plasma cells in osteosclerotic plasmacytoma (>95% have monoclonal  $\lambda$  plasmacytoma or BM infiltration) and elevated plasma or serum levels of VEGF. (See Table 8.15.)
- BM usually <5% plasma cells (almost always monoclonal  $\lambda$ ); low-level paraprotein, usually IgG $\lambda$  or IgA $\lambda$  (median 11g/L, rarely >30g/L).
- Anaemia, hypercalcaemia, and renal impairment are rare.
- Differential diagnosis:
  - Chronic inflammatory demyelinating polyneuropathy (CIDP).
  - Guillain–Barré syndrome.
  - Monoclonal gammopathy-associated peripheral neuropathy.
  - AL amyloidosis.

### Treatment

- Treat solitary sclerotic bone lesion with aggressive radiotherapy (45Gy)  $\pm$  surgery ( $\geq$ 50% response); widespread lesions or diffuse BM involvement requires systemic therapy; treatments as for myeloma used.
- Autologous peripheral blood stem cell transplantation is the 1st-line treatment for younger patients with normal organ function. High response rate and durable remission. Transplantation-related morbidity and mortality significantly reduced over the past 5 years.
- Melphalan and dexamethasone: effective and well-tolerated treatment for older patients or those with organ dysfunction.

- Systemic symptoms and skin changes may respond in 1 month, neuropathy may begin to respond at 3–6 months with maximum benefit often taking 2–3 years.
- Prognosis is good with median survival 14.7 years; number of features does not affect survival; respiratory symptoms predict adverse outcome.

**Table 8.15** Criteria for the diagnosis of POEMS syndrome<sup>1</sup>

Major criteria	<ol style="list-style-type: none"> <li>1. Polyneuropathy<sup>a</sup></li> <li>2. Monoclonal plasma cell neoplasm (almost always <math>\lambda</math>)<sup>a</sup></li> <li>3. Sclerotic bone lesions</li> <li>4. Castleman disease</li> <li>5. VEGF elevation</li> </ol>
Minor criteria	<ol style="list-style-type: none"> <li>1. Organomegaly (splenomegaly, hepatomegaly, or lymphadenopathy)</li> <li>2. Extravascular volume overload (oedema, pleural effusion, or ascites)</li> <li>3. Endocrinopathy (adrenal, thyroid,<sup>b</sup> pituitary, gonadal, parathyroid, pancreatic<sup>b</sup>)</li> <li>4. Skin changes (hyperpigmentation, hypertrichosis, glomeruloid haemangiomas, plethora, acrocyanosis, flushing, white nails)</li> <li>5. Papilloedema</li> <li>6. Thrombocytosis/polycythaemia<sup>c</sup></li> </ol>
Other symptoms and signs	Clubbing, weight loss, hyperhidrosis, pulmonary hypertension/restrictive lung disease
Possible associations	Arthralgias, cardiomyopathy (systolic dysfunction) and fever

<sup>a</sup> Polyneuropathy and monoclonal plasma cell neoplasm present in all patients; diagnosis requires at least 1 other major criterion and 1 minor criterion.

<sup>b</sup> Because of high prevalence of diabetes mellitus and thyroid abnormalities, this diagnosis alone insufficient to meet this minor criterion.

<sup>c</sup> Anemia and/or thrombocytopenia are distinctly unusual in POEMS syndrome unless Castleman disease present.

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## Plasmacytoma

### Solitary plasmacytoma of bone (SPB)

#### *Clinical features and diagnosis*

- Solitary area of lytic bone destruction due to clonal plasma cells in an otherwise asymptomatic patient.
- Generally no serum/urine paraprotein though there may be small monoclonal band.
- BM from uninvolved site contains <5% plasma cells.
- Otherwise ↔ skeletal survey and MRI of spine and pelvis (exclusion of other lesions on MRI).
- No myeloma-related organ or tissue impairment (end-organ damage).
- ~5% of plasma cell neoplasms: ♂:♀ ratio 2:1; median age 55 years.
- Lesion usually in axial skeleton; 66% in spine.
- Generally presents with bony pain; may cause cord/root compression.
- Diagnosis requires biopsy or FNA.
- Differential diagnosis of MM and other malignancy.
- Serum/urine paraprotein detected in 24–72%; generally low level.
- Adverse prognostic factors for progression to MM include persistence of paraprotein >1 year after radiotherapy, immunoparesis and lesion >5cm.
- –ve MRI of spine is good prognostic feature.

#### *Treatment and prognosis*

- Treat with fractionated radical radiotherapy 40Gy (50Gy for lesions >5cm); local control 80–95%; curative in 50% if solitary lesion; DFS ~40% at 5 years.
- Treat non-responders and those with a rising paraprotein or other evidence of symptomatic MM with MM treatment/chemotherapy.
- Patients who meet diagnostic criteria for SPB but with evidence of clonal BM involvement should be treated with radiotherapy then monitored for disease progression as for MGUS (<10% BM PCs or SMM ≥10% BM PCs).
- Regular follow-up to monitor paraprotein; disappears in 25–50% (often slowly over several years).
- 75% progress to MM (~50% within 2 years of diagnosis); treat as *de novo* MM. High response rate; median survival 63 months.
- Some patients develop multiple solitary recurrences; treat each with local radiotherapy.
- Median survival >10 years; DFS 25–50% at 10 years.

### Solitary extraosseous plasmacytoma (SEP)

#### *Clinical features and diagnosis*

- Extramedullary tumour of clonal plasma cells.
- Rare; may occur anywhere but 90% in head and neck; most in upper airways: tonsils, nasopharynx, or paranasal sinuses.
- Generally no serum/urine paraprotein though may have small monoclonal band.
- BM from uninvolved site contains <5% plasma cells.
- ↔ skeletal survey and MRI of spine and pelvis normal.
- No myeloma-related organ or tissue impairment (end-organ damage).



- Diagnosis requires biopsy or FNA of the lesion.
- Differential diagnosis of MM and other malignancy.
- <25% have serum or urine paraprotein.

#### *Treatment and prognosis*

- Treat with radical radiotherapy (40Gy; 50Gy if lesion >5cm) including regional lymph nodes if possible (e.g. cervical).
- Radical surgery only for SEP outside head and neck, then usually followed with radiotherapy.
- Patients who meet diagnostic criteria for SEP but with evidence of clonal BM involvement should be treated with radiotherapy then monitored for disease progression as for MGUS (<10% BM PCs or SMM  $\geq$ 10% BM PCs).
- Most cured; <5% local recurrence; relapse <30%.
- Treat non-responders and those with a rising paraprotein or other evidence of symptomatic MM with MM treatment/chemotherapy.
- If monoclonal protein persists or reappears, patient may need further radiotherapy; if plasmacytoma shrinks but does not disappear and/or paraprotein persists, follow closely; persistent or rising paraprotein may indicate progression to MM and require treatment as such.
- >70% survival at 10–14 years; DFS 70–80% at 10 years.

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## Waldenström macroglobulinaemia

WM is an uncommon indolent chronic B-cell LPD characterized by BM infiltration by lymphoplasmacytic cells and an IgM paraproteinaemia. It is classified as lymphoplasmacytic lymphoma (LPC-L) in the WHO classifications, a B-cell neoplasm (➔ See also p.191.).

### Epidemiology

- Incidence 0.3 per 100,000 per annum; incidence higher in Caucasians; rare in black population.
- Median age 63–68; rare <40; ♂:♀ ~2:1.
- Cause unknown; no clear link to environmental exposures.
- Several familial clusters described; ~20% of patients have at least 1 1st-degree relative with B-cell disorder; tend to be younger, have higher % BM involvement and higher IgM level at diagnosis.
- Main risk factor for WM is pre-existing IgM MGUS (46 × relative risk).

### Pathophysiology

WM appears from a late stage of B-cell differentiation, possibly a memory B-cell, arrested after somatic hypermutation in the germinal centre and before terminal differentiation to a plasma cell.

Most cases have a normal karyotype; most frequent chromosomal abnormality del(6q21–23). Presence of del(6q) may be associated with more aggressive disease. Del(6q) has not been found in IgM MGUS and may be associated with disease progression; limited data at present.

IL-6 is upregulated in WM explaining the elevated serum C-reactive protein; high levels of IL-6 may contribute to anaemia.

Slowly progressive accumulation of clonal cells; symptoms may be due to infiltration of BM (BM failure), spleen (splenomegaly), or liver (hepatomegaly), or to hyperviscosity due to ↑ serum levels of monoclonal IgM.

### Clinical features and presentation

- Occasional diagnosis following routine ESR/SPEP/FBC/blood-film; otherwise usually insidious onset of weakness and fatigue.
- Patients often present with symptoms of anaemia, epistaxis, recurrent infection, dyspnoea, CCF, and weight loss.
- Usually no bone pain and no evidence of destructive bone disease.
- Symptoms of hyperviscosity (headache, dizziness, visual upset, bleeding, ataxia, CCF and somnolence, stupor, and coma) 15–20%.
- Peripheral neuropathy—usually sensory or sensorimotor (~20%); distal, symmetrical, slowly progressive, usually lower extremities.
- Hepatomegaly (~25%); splenomegaly and lymphadenopathy less frequent.
- Fundoscopy reveals distended sausage-shaped veins, retinal haemorrhage ± papilloedema.
- Cryoglobulinaemia (<5% present with symptoms though detectable in 20%) may cause Raynaud syndrome, arthralgia, purpura, and skin ulcers.
- Haemorrhagic symptoms (e.g. epistaxis or easy bruising) may develop as a result of abnormalities of platelet function or coagulation due to the paraprotein.

- Amyloidosis may occur (<5%) causing cardiac, renal, hepatic, or pulmonary dysfunction, or macroglossia.
- Autoimmune disorders may also develop due to the paraprotein: cold agglutinin disease, Schnitzler syndrome (IgM monoclonal gammopathy, urticaria, fever, and arthralgia), neuropathy due to antimyelin associated glycoprotein (MAG) activity, glomerulonephritis, angioedema, and acquired von Willebrand syndrome.

### Investigations and diagnosis

- No disease-defining morphological, immunophenotypic, or chromosomal abnormalities for WM (Table 8.16).
- **FBC and film:** normochromic normocytic anaemia 80% (often spuriously low due to ↑ plasma volume); rarely lymphocytosis or pancytopenia; blood film shows rouleaux or agglutination (cold agglutinins ~5%); may see lymphoplasmacytic cells.
- **ESR/plasma viscosity:** ↑ in almost all patients, often markedly (ESR commonly >100mm/h); risk of hyperviscosity symptoms when PV >4cP (5–10% at diagnosis); most have symptoms when PV >6cP; most with PV <4cP will not have symptoms of hyperviscosity; PV often correlates well for symptoms in an individual though not between patients.
- **Biochemistry:** renal impairment rare; LFTs may be abnormal in advanced disease or cryoglobulinaemia; urate and LDH may be ↑.
- **Serum  $\beta_2$ -microglobulin:** ↑ in 33%; prognostic factor.
- **C-reactive protein:** ↑ in ~66%; due to ↑ IL-6 production.
- **Hepatitis C serology:** association with WM.
- **Serum immunoglobulins:** ↑ IgM; may be mild immunoparesis of IgG (60%) and IgA (20%).
- **Serum protein electrophoresis, immunofixation and densitometry:** to characterize and quantify IgM paraprotein.
- **Urine electrophoresis:** scanty Bence Jones protein in ~50%.
- **BM aspirate:** often hypocellular; may show infiltration by lymphoplasmacytic cells with variable differentiation; mast cells may be ↑.
- **BM trephine biopsy:** essential; usually hypercellular; demonstrates intertrabecular infiltrate (diffuse, interstitial, or nodular) of lymphoplasmacytic cells (NB paratrabecular infiltrate suggests follicular NHL); immunochemistry demonstrates LC restriction.
- **BM immunophenotyping:** useful in differentiating WM from other B-cell disorders; characteristically pan B-cell marker (CD19, CD20, CD22, CD79) +ve (cf. myeloma plasma cells); LC restricted surface IgM; CD10 –ve (cf. FL), CD23 –ve (cf. CLL); 5–20% express CD5 (must differentiate from CLL and MCL); NB CD5+ does not rule out diagnosis of WM; CD103 and CD138 rarely +ve.
- **Cytogenetics:** optional; most have ↔ karyotype; no consistent defect; most commonly del(6q;21–22.1) by FISH in up to 55% patients with WM; +3, +5, and –8 also described; presence of IgH translocations (14q) suggests myeloma; may help rule out NHL.
- **CT neck chest abdomen and pelvis:** to detect organomegaly and lymphadenopathy.

- **Other potentially useful tests:** cryoglobulins, cold agglutinins, coagulation screen, Congo red stain for amyloid on BM or fat aspirates, haemolysis screen, ECG, EEG, peripheral blood flow cytometry, EMG, or lymph node/organ biopsy may be useful in some patients.
- WM may be divided by the presence or absence of symptoms attributable to either the IgM paraprotein (e.g. hyperviscosity or neuropathy) or tumour infiltration (BM failure or symptomatic organomegaly) into:
  - Symptomatic WM.
  - Asymptomatic WM (~25%).

**Table 8.16** Diagnostic criteria for WM<sup>1</sup>

- IgM monoclonal gammopathy of any concentration.
- BM infiltration by small lymphocytes, plasmacytoid cells, and plasma cells.
- Intertrabecular pattern of BM infiltration.
- Immunophenotype: monoclonal surface IgM+ (5:1 κ:λ ratio), CD19+, CD20+, CD5±, CD10-, CD19+, CD20+, CD22+, CD23-, CD25+, CD27+, FMC7+

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### Differential diagnosis

- IgM MGUS: IgM monoclonal protein <30g/L; Hb >12g/dL; no BM infiltrate (<10% clonal BM cells); no organomegaly or lymphadenopathy; no end-organ symptoms; 6q- is not seen in IgM MGUS; risk of progression 1.5% per year.
- IgM-related disorders: IgM monoclonal protein; no overt evidence of lymphoma; symptomatic cryoglobulinaemia, peripheral neuropathy, cold agglutinin disease, or amyloidosis; presence of constitutional or hyperviscosity symptoms, lymphadenopathy or organomegaly more suggestive of WM.
- Other B-cell LPDs: IgM monoclonal protein can be demonstrated in CLL/SLL and NHL (MCL, FL, MZL); generally very low levels; no lymphoplasmacytic BM infiltration; hyperviscosity rare; features of other LPD e.g. phenotype, e.g. splenic marginal zone lymphoma (SMZL) associated with splenomegaly, BM infiltration, and IgM paraprotein.
- IgM myeloma: very rare; BM contains plasma cells (cytoplasmic IgM+, CD20-, CD38+, CD138+) not lymphoplasmacytic cells; myeloma-associated cytogenetic abnormalities (esp. 14q translocations) and lytic bone lesions frequent.

### Prognostic factors

*IPSS is used. See Table 8.17.*

*Predictors of early progression in asymptomatic WM*

- Hb <11.5g/dL.
- β<sub>2</sub>-microglobulin ≥3mg/L.
- IgM >30g/L.

*Predictors of shorter survival in WM*

- Age ≥60 years.
- Hb <10g/dL.

- High  $\beta_2$ -microglobulin.
- Other less consistently identified factors—cytopenias: WBC  $<4.0 \times 10^9/L$ ; neutrophils  $<1.8 \times 10^9/L$ ; platelets  $<150 \times 10^9/L$ ;  $\downarrow$  serum albumin;  $\sigma$  sex; constitutional symptoms.

**Table 8.17** International Prognostic Scoring System (IPSS) for WM<sup>2</sup>

Adverse risk factors	<ul style="list-style-type: none"> <li>• Age <math>&gt;65</math> years</li> <li>• Serum <math>\beta_2</math>-microglobulin <math>&gt;3\text{mg/L}</math></li> <li>• Monoclonal protein <math>&gt;70\text{g/L}</math></li> <li>• Haemoglobin <math>\leq 11.5\text{g/dL}</math></li> <li>• Platelets <math>\leq 100 \times 10^9/L</math></li> </ul>
Low risk	$\leq 1$ adverse risk factors, except age
Median 5-year survival	87%
Intermediate risk	2 adverse risk factors or age $>65$ years
Median 5-year survival	68%
High risk	$>2$ adverse risk factors
Median 5-year survival	36%

## Management

The aims of treatment in WM are to relieve symptoms, reduce the risk of organ damage, and improve QoL and survival duration with minimal adverse effects. Treatment should be reserved for symptomatic patients and continued to maximal response. There is no indication for therapy in asymptomatic WM but regular review (3–6-monthly) of clinical and laboratory features is required. Consistently monitor paraprotein by densitometry (more reliable than IgM nephelometry).

### Indications for therapy

Initiation of therapy should not be based simply on IgM level alone as this does not correlate directly with clinical manifestations but for:

- Constitutional symptoms: recurrent fever, night sweats, fatigue due to anaemia, weight loss.
- Progressive symptomatic lymphadenopathy or splenomegaly.
- Haemoglobin  $\leq 10\text{g/dL}$  due to BM infiltration.
- Platelets  $<100 \times 10^9/L$  due to BM infiltration.
- Symptomatic hyperviscosity.
- Severe peripheral neuropathy.
- Systemic AL.
- Symptomatic cryoglobulinaemia.
- Cold agglutinin disease.

NB Avoid red cell transfusion simply to correct low Hb (plasma volume  $\uparrow$  causing spuriously low Hb; low Hb protects against clinical effects of hyperviscosity).

NB Although therapy should not be initiated on IgM level alone, serum monoclonal protein  $>50\text{g/L}$  carries a high risk of hyperviscosity; exclude early symptoms and signs of hyperviscosity by thorough history and examination including fundoscopy; do not postpone therapy.

At this time there are insufficient data to recommend one 1st-line therapy over another. Choice of initial therapy is based on patient age and co-morbidities, need for rapid disease control, cytopenias, and eligibility for SCT.

Treatment recommendations and response criteria follow the NCCN 2013 guidelines. (See Table 8.18.)

Although response rates may be higher with combination regimens these regimens may not be appropriate 1st-line therapies for patients with indolent disease and the long-term benefit has yet to be demonstrated by direct comparison with single agent therapy.

**Table 8.18** Updated response criteria from the 6th International Workshop on WM and NCCN guidelines 2013<sup>3</sup>

Response	Criteria
Complete response (CR)	IgM in normal range, and disappearance of monoclonal protein by immunofixation; no histological evidence of bone marrow involvement, and resolution of any adenopathy/organomegaly (if present at baseline), along with no signs or symptoms attributable to WM. Reconfirmation of the CR status is required by repeat immunofixation studies
Very good partial response (VGPR)	A 90% reduction of serum IgM and decrease in adenopathy/organomegaly (if present at baseline) on physical examination or on CT scan. No new symptoms or signs of active disease
Partial response (PR)	A 50% reduction of serum IgM and decrease in adenopathy/organomegaly (if present at baseline) on physical examination or on CT scan. No new symptoms or signs of active disease
Minor response (MR)	A 25% but < 50% reduction of serum IgM. No new symptoms or signs of active disease
Stable disease (SD)	A <25% reduction and <25% increase of serum IgM without progression of adenopathy/organomegaly, cytopenias, or clinically significant symptoms due to disease and/or signs of WM
Progressive disease (PD)	A 25% increase in serum IgM by protein electrophoresis confirmed by a 2nd measurement or progression of clinically significant findings due to disease (i.e. anaemia, thrombocytopenia, leukopenia, bulky adenopathy/organomegaly) or symptoms (unexplained recurrent fever 38.4°C, drenching night sweats, 10% body weight loss, or hyperviscosity, neuropathy, symptomatic cryoglobulinaemia or amyloidosis) attributable to WM

Many treatments now exist for WM/LPC-lymphoma. Guidelines list treatments in alphabetical order. No standard treatment exists. Treatment must be tailored to the patient. If adequate and durable responses are achieved patients could receive similar/same treatment at relapse (see Tables 8.19–8.21).

#### Plasmapheresis<sup>4</sup>

- Indicated in acute management of patients with symptoms of hyperviscosity; 1–1.5 volume exchange efficiently reduces plasma viscosity (PV) (80% of large IgM molecule is intravascular) with a 60–75% reduction in plasma IgM and 50% reduction in PV; 1–2

procedures will reduce PV to near-↔ and prevents return of paraprotein to pre-treatment levels for several weeks.

- Rarely required on a regular basis in treatment of neuropathic or chemo-intolerant patients.
- In an emergency, if plasmapheresis is unavailable, venesection and exchange transfusion will ↓ plasma viscosity.

#### Alkylating agents

- *Chlorambucil* previously frequently used alkylator; range of dosage schedules, from 0.1mg/kg/d continuously to 0.4mg/kg/d × 2d every 14d ± prednisolone 60mg/m<sup>2</sup>; 6–10mg/d (0.1mg/kg/d) for 7–14d every 28d. Responses usually slow; 31–75% achieve ≥50% reduction in paraprotein; CR uncommon.
- Addition of steroids does not affect response rate or survival; beneficial if associated autoimmune phenomena.
- Continue to maximum response; optimum duration of therapy not established; no evidence that maintenance improves survival.
- Duration of response 2–4 years; median survival ~5 years.
- Re institute therapy when paraprotein approaches previously symptomatic levels; often effective on several occasions; resistance ultimately develops; side effects; myelosuppression; AML.
- *Cyclophosphamide* ± prednisolone may achieve comparable results; no directly comparative data.
- Risk of myelodysplasia and stem cell toxicity make alkylating agents less attractive in younger patients; tolerability and ease of administration render them appropriate therapy for elderly patients.

#### Purine analogues<sup>5–7</sup>

- *Fludarabine* most widely used purine analogue (40mg/m<sup>2</sup> PO × 5d or 25mg/m<sup>2</sup> IV × 5d repeated monthly for 4–6 cycles); response rate 40–86% in previously untreated patients; response duration 40–50 months; patients who achieve response >1 year, may respond again at progression; as salvage treatment, 17–50% responses.
- *Cladribine* (0.1mg/kg continuous infusion × 7d) response rate 64–90% with generally 2–4 cycles in previously untreated patients; as salvage treatment, 38–63% responses; recommended limit of 2 cycles due to risk of myelosuppression; autoimmune complications occur as in CLL.
- These agents generally achieve more rapid responses in untreated WM than chlorambucil.
- Some studies found higher response rates in 1° refractory than refractory relapsed WM.
- Fludarabine and cladribine are cross-resistant.
- Side effects: myelosuppression (may be severe: >60% ≥ grade 3 neutropenia) and profound immunosuppression; need *Pneumocystis jirovecii* prophylaxis and irradiated blood products; consider antimicrobial and antiviral prophylaxis.
- Purine analogues are appropriate for initial and subsequent treatment of WM but should be avoided as initial therapy in patients eligible for SCT; no consensus on which agent is superior or optimum duration of therapy.

**Rituximab<sup>8,9</sup>**

- Monoclonal anti-CD20 antibody (375mg/m<sup>2</sup> IV × 4) achieves ~27% ≥MR in untreated and previously treated WM; improves haematocrit and platelet counts in >50%; useful in patients with marked cytopenia.
- Better responses if paraprotein <40g/L or IgM <60g/L.
- Responses occur at median ~3 months but best response may be delayed for several months.
- Response duration generally 9–16 months; administration of 8 doses over 4 weeks initially, repeated at 12 weeks, improves response rate (44–48%) and may extend duration.
- Abrupt elevation in serum paraprotein and PV may occur after rituximab therapy in up to 50% ('rituximab flare'); patients should be closely monitored and may require plasmapheresis to prevent hyperviscosity.
- May be beneficial for symptomatic neuropathy associated with anti-MAG antibodies.
- Use of rituximab monotherapy discouraged in patients with paraprotein >50g/dL due to risk of 'flare' effect.
- Role of maintenance Rituximab and should be considered.

**Combination regimens<sup>10–15</sup>**

- Higher response rates are achieved with combination regimens but ↑ toxicity.
- Fludarabine (30mg/m<sup>2</sup> IV d1–3) + cyclophosphamide (CTX; 300mg/m<sup>2</sup> IV d1–3) (FC) achieved 78% responses ≥MR with median duration >2 years; cladribine + CTX achieved 58% ≥MR but with significant myelosuppression.
- FC + rituximab (FCR) achieved responses ≥MR in 5/9; pentostatin (4mg/m<sup>2</sup> IV) + CTX (600mg/m<sup>2</sup>) ± rituximab (375mg/m<sup>2</sup>) every 21d in 14 patients achieved 65% responses ≥MR, including 12%CR; cladribine, CTX + rituximab achieved 94% ≥PR (18%CR) in 17 untreated patients with no relapse after median follow-up of 21 months. *Standard 1st-line treatment in many centres.*
- R-CVP: *standard in many centres.*
- R-CHOP × 6 with rituximab maintenance achieved ≥MR in 11/13 and ≥PR in 10/13.
- Rituximab, dexamethasone, and CTX active (78%≥MR) and well tolerated; response in most patients without myelosuppression or immunosuppression.
- Bortezomib, rituximab, and dexamethasone highly active (100% OR after median 4 cycles) and well tolerated. Active in relapsed/ refractory WM with 85% achieving ≥MR; responses prompt (median 1.4 months) with ~8 months median TTP; discordance between BM and serum IgM responses was observed in some patients. Becoming more popular.

**Newer agents<sup>16,17</sup>**

- *Thalidomide* has shown activity in WM, with ↑ RR with added rituximab. Active with 5/20 achieving ≥MR with <3 months TTP; may have role ± steroids in 1st-line failures with pancytopenia.
- *Alemtuzumab* achieves responses but with significant toxicity and is rapidly going out of fashion.
- *Ofatumumab*: has a role in patients intolerant of rituximab.



- *Everolimus*: mTOR inhibitor with promising results in WM.
- *Bendamustine*: an old drug coming back into fashion. Adequate response rated seen; added benefit with rituximab.

#### *High dose therapy and autologous or allogeneic SCT<sup>6,18</sup>*

- Limited data due to age of most patients with WM; high response rates (generally PR) achieved after autograft but relapse rate high.
- HDT and ASCT have a role in 1<sup>o</sup> refractory or relapsed disease in eligible patients; patients in whom SCT is planned should have limited prior exposure to alkylator and purine analogue therapy.
- The role of allografts in WM remains uncertain but due to the reduced TRM with RIC allogeneic transplant, they should be considered in younger fit patients.

#### *Treatment of relapse*

- If response of >1 year achieved most patients will respond to same therapy.

**Table 8.19** Options for 1st-line and salvage therapy: updated from the NCCN guidelines 2013

1st-line therapy	Salvage therapy
<i>Non-stem cell toxic</i>	<i>Non-stem cell toxic</i>
Bortezomib ± rituximab	Alemtuzumab
Bortezomib/dexamethasone	Bortezomib ± rituximab
Bortezomib/dexamethasone/ rituximab	Bortezomib/dexamethasone
Cyclophosphamide/doxorubicin/ vincristine/prednisone/rituximab	Bortezomib/dexamethasone/ rituximab
Rituximab	Cyclophosphamide/doxorubicin/ vincristine/prednisone/rituximab
Rituximab/cyclophosphamide/ prednisone	Everolimus
Rituximab/cyclophosphamide/ dexamethasone	Ofatumumab
Thalidomide ± rituximab	Rituximab
	Rituximab/cyclophosphamide/ prednisone
	Rituximab/cyclophosphamide/ dexamethasone
	Thalidomide ± rituximab
<i>Possible stem cell toxicity and/or risk of transformation (or unknown)</i>	<i>Possible stem cell toxicity and/or risk of transformation (or unknown)</i>
Bendamustine ± rituximab	Bendamustine ± rituximab
Cladribine ± rituximab	Cladribine ± rituximab
Chlorambucil	Chlorambucil
Fludarabine ± rituximab	Fludarabine ± rituximab
Fludarabine/cyclophosphamide/ rituximab	Fludarabine/cyclophosphamide/ rituximab
	<i>Stem cell transplant (in selected cases)</i>
	HDT and auto-SCT
	Allo-SCT

Order of schedules alphabetical and does not indicate preference.

**Table 8.20** NCCN practice guidelines for WM (2013)

Initial treatment <sup>a</sup>	Response	Advise
Combination chemo or mono-R <sup>a</sup>	CR	Observe Consider maint-R <sup>b</sup>
Combination chemo or mono-R	PR & asymptomatic	Observe Consider maint-R
Combination chemo or mono-R	PR and symptoms	Relapse strategy
Combination chemo or mono-R	SD/PD	Relapse strategy

<sup>a</sup> Monotherapy rituximab. <sup>b</sup> Maintenance rituximab

**Table 8.21** NCCN practice guidelines for WM for relapse strategy (2013)

Time since response	Advise
Relapse <12 months	Choose alternative strategy
Relapse ≥12 months	Choose previous treatment or consider alternative therapy
No response	Choose alternative therapy

### Follow-up<sup>19</sup>

- Review FBC, quantitative immunoglobulins, and paraprotein quantitation after every 2 treatment cycles with PV in symptomatic patients; repeat CT at 3–6-month intervals if abnormal at diagnosis.
- After response to therapy, patients should be monitored every 2–3 months without maintenance therapy.

### Prognosis<sup>5–7,20</sup>

- Median time to progression of asymptomatic WM ~7 years.
- Median survival of patients with WM ~5 years with 10% alive at 15 years.
- Patients who achieve CR with chlorambucil have median survival of ~11 years.
- Up to 20% die of unrelated causes and 33% from infection; others from disease progression, transformation and bleeding.
- Indolent course may be interrupted by transformation to diffuse large B-cell NHL; worsening constitutional symptoms, profound cytopenias, ↑ serum LDH, lymphadenopathy, extramedullary disease, and organomegaly; often poorly responsive to treatment; poor tolerance of aggressive treatment due to poor marrow reserve.

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## Heavy chain disease

Uncommon lymphoplasmacytic cell neoplasms characterized by production of incomplete Igs comprising HCs without LCs.

### $\alpha$ -HCD (= IPSID)

- IPSID: immunoproliferative small intestinal disease (WHO 1978).
- Variant of extranodal marginal zone lymphoma of MALT type.
- Most frequent; more frequent in the Mediterranean and Middle East; generally 10–30 years; associated with low socioeconomic group, poor hygiene, recurrent infectious diarrhoea, and chronic parasitic infection.
- Commonly presents with diarrhoea, steatorrhoea, weight loss, abdominal pain and vomiting; mild-to-moderate anaemia; low serum albumin; hypokalaemia, and hypocalcaemia (tetany).
- $\alpha$ -HC protein detectable in serum of most patients; SPEP normal in 50%; monoclonal  $\alpha$ -chain on immunofixation of serum or in concentrated urine, otherwise diagnose by biopsy.
- Villous atrophy and infiltrative lesions in duodenum and jejunum in most patients; histology ranges from lymphoplasmacytic infiltration of mucosa (stage A) to immunoblastic lymphoma invading entire intestinal wall.
- Mesenteric lymph nodes sometimes involved but peripheral lymph nodes, liver, spleen and BM rarely involved.
- Progressive course without treatment; early stage (A) treated with oral metronidazole and tetracycline for 6 months; treat non-responders and stage B or C with RCHOP-type regimen.
- Variable clinical course; some die within 1–2 years, others have long remissions lasting many years.

### $\gamma$ -HCD (Franklin disease)

- Usually elderly ♂, associated with RA, Sjögren's, SLE, hypereosinophilic syndrome and autoimmune haemolytic anaemia.
- Present with palatal oedema, lymphadenopathy, hepatosplenomegaly, fever and recurrent infection; pancytopenia; eosinophilia and atypical lymphocytes/plasma cells in blood  $\pm$  BM; monoclonal  $\gamma$  chain in serum and/or urine on immunofixation confirms diagnosis.
- Usually behaves as aggressive lymphoma; survival  $\sim$ 1 year.

### $\mu$ -HCD

- Rare LPD, resembles CLL; presents with weight loss, fever, anaemia, and recurrent infection.
- Hepatosplenomegaly; abdominal nodes generally involved; osteolytic lesions and pathological fractures in 40%.
- Vacuolated BM plasma cells in 66%; serum protein electrophoresis generally  $\leftrightarrow$  or shows hypogammaglobulinaemia; BJP 10–15%.
- Variable clinical course; some develop AL; no specific treatment: observation or alkylating agents according to course.



## AL (1° systemic) amyloidosis

AL (1° systemic) amyloidosis is a clonal plasma cell disorder in which systemic disease results from organ dysfunction due to extracellular deposition of a fibrillar protein. It can be associated with and complicate most clonal B-cell lymphoplasmacytic disorders, notably myeloma, WM, MGUS, and lymphoma.

### Incidence

Estimated incidence 0.5–1 per 100,000 per annum; ♂:♀ ratio 2:1; most cases aged 50–70 years; <10% <50 years; ~1% <40 years; 15% of patients with MM develop amyloid (lower % for MGUS and WM).

### Pathophysiology

In AL amyloidosis the fibrillar deposits are composed of the variable regions of immunoglobulin light-chains (VL) in association with glycosaminoglycans and amyloid P component derived from the ↔ plasma protein serum amyloid P (SAP) component. More commonly derived from λ LCs. Unique amino acid insertions may render the proteins amyloidogenic and lead to deposits. The underlying clonal neoplasm (producing the Ig/LC) is usually small and <20% of patients meet diagnostic criteria for MM at diagnosis. Subsequent progression to MM is rare but may reflect short survival. The t(11;14) IgH translocation occurs more frequently in AL amyloidosis than in MM or MGUS.

Without treatment, amyloid deposition progressively accumulates in viscera, notably kidneys, heart, liver, and peripheral nervous system causing increasingly severe dysfunction. Under favourable circumstances, further amyloid deposition can be prevented, deposits possibly regress, and improvement in organ dysfunction can occur.

### Clinical features and presentation

- Renal involvement is the predominant feature in 33% with nephrotic syndrome (oedema, fatigue, and lethargy) ± renal impairment (usually mild).
- Cardiac symptoms predominate in 20–30%: CCF due to restrictive cardiomyopathy notably with right-sided features (↑ JVP, peripheral oedema, and hepatomegaly).
- Peripheral neuropathy occurs in 20%; 10–15% present with isolated neuropathic symptoms; typically painful sensory polyneuropathy; autonomic neuropathy may cause postural hypotension, impotence and disturbed GI motility in ~10%.
- Carpal tunnel syndrome in ~20%.
- GI involvement may be focal or diffuse: malabsorption, perforation, haemorrhage, and obstruction may occur; hepatomegaly 25%.
- Haemorrhage occurs at some time in up to 33% of patients; usually non-thrombocytopenic purpura, often periorbital causing characteristic 'raccoon eyes' appearance.
- Vocal cord infiltration may cause dysphonia; large joint arthropathy; adrenal and thyroid infiltration may cause endocrine dysfunction; cutaneous plaques and nodules usually on face or upper trunk; pulmonary infiltration rarely symptomatic.
- Macroglossia 10%.

## Investigations and diagnosis

- High index of suspicion required; consider in patient with nephrotic syndrome, cardiomyopathy, peripheral neuropathy, hepatomegaly, autonomic neuropathy, or macroglossia.
- Confirm diagnosis by histological examination of biopsy of affected organ (Table 8.22) or subcutaneous fat aspirate, rectal biopsy, or labial salivary gland biopsy stained with Congo red for red–green birefringence under polarized light; confirm AL amyloidosis by immunochemistry for  $\kappa$  or  $\lambda$  LCs (50% are –ve).
- Assess severity of organ involvement:
  - **FBC:**  $\downarrow$  Hb suggests probable myeloma.
  - **Serum chemistry:** to evaluate renal and hepatic function.
  - **$\beta_2$ -microglobulin:** prognostic indicator in MM.
  - **Coagulation screen:** may be coagulopathy due to absorption of factor X and sometimes FIX by the amyloid-fibrils.
  - **Serum protein electrophoresis, immunofixation and densitometry:** to detect, type, and quantitate any paraprotein (~70%; usually only modest quantity).
  - **Serum immunoglobulins:** immunoparesis suggests MM.
  - **SFLC assay:** identifies excess of  $\kappa$  or  $\lambda$  LC in >95%; provides best assessment of response to therapy in most patients.
  - **Creatinine clearance and 24h quantitative proteinuria:** to assess renal dysfunction and severity of albuminuria.
  - **Urine electrophoresis:** to detect, type and quantify paraprotein (85%; 90% have albuminuria).
  - **BM aspirate and trephine biopsy:** usually only mild  $\uparrow$  in % plasma cells; overt MM in <20%; immunohistochemistry for  $\kappa$  and  $\lambda$  LCs; Congo red for amyloid.
  - **CXR, ECG, and echocardiography:** low-voltage ECG; echo shows concentrically thickened ventricles, normal-to-small cavities, and a normal or mild reduction in ejection fraction.
  - **SAP (serum amyloid protein) scan:** radiolabelled serum amyloid P component allows detection and quantification of amyloid deposition and assessment of extent of organ involvement by scintigraphy (done at amyloidosis diagnostic and treatment centres).
  - **Skeletal survey:** only if MM suspected.

## Differential diagnosis

Exclude plasma cell dyscrasia (MM, WM), reactive (AA) amyloidosis (history of chronic inflammatory disorder), and familial amyloidosis (family history).

## Prognostic factors

### Poor prognostic features

- CCF.
- Multisystem involvement.
- Renal failure.
- Jaundice.
- High total body amyloid load on SAP scan.

**Table 8.22** Organ involvement: biopsy of affected organ or an alternate site<sup>a</sup>

Kidney	<ul style="list-style-type: none"> <li>• 24h urine protein &gt;0.5g/d, predominantly albumin</li> </ul>
Heart	<ul style="list-style-type: none"> <li>• Echo: mean wall thickness &gt;12mm, no other cardiac cause</li> <li>• NT-proBNP &gt; 332 ng/L in absence of RF or atrial fibrillation</li> </ul>
Liver	<ul style="list-style-type: none"> <li>• Total liver span &gt;15cm in the absence of heart failure or ALP &gt;1.5 × ULN</li> </ul>
Nerve	<ul style="list-style-type: none"> <li>• Peripheral: clinical; symmetrical lower extremity sensorimotor peripheral neuropathy</li> <li>• Autonomic: gastric emptying disorder, pseudo-obstruction, voiding dysfunction not related to direct organ infiltration</li> </ul>
GI tract	<ul style="list-style-type: none"> <li>• Direct biopsy verification with symptoms</li> </ul>
Lung	<ul style="list-style-type: none"> <li>• Direct biopsy verification with symptoms</li> <li>• Interstitial radiographic pattern</li> </ul>
Soft tissue	<ul style="list-style-type: none"> <li>• Tongue enlargement</li> <li>• Clinical arthropathy</li> <li>• Claudication, presumed vascular amyloid</li> <li>• Skin</li> <li>• Myopathy by biopsy or pseudohypertrophy</li> <li>• Lymph node (may be localized)</li> <li>• Carpal tunnel syndrome</li> </ul>

<sup>a</sup> Alternate sites available to confirm histological diagnosis of amyloidosis: fine needle abdominal fat aspirate and/or biopsy of the minor salivary glands, rectum or gingiva.

Revised consensus criteria for amyloidosis involvement from the XII International Symposium on Amyloidosis.

## Management

The current aim of treatment is to suppress underlying plasma cell neoplasia and paraprotein production to reduce further deposition of amyloid and permit regression resulting in improvement in organ dysfunction.

Most treatment regimens have been adapted from those used in MM but patients with AL amyloidosis often experience greater toxicity. In contrast to MM where CR is the goal, a partial clonal response may halt amyloid deposition and even lead to its regression. Thus treatments with a significant risk of severe toxicity or death may be inappropriate. Many patients with AL amyloidosis may not require prolonged treatment schedules and a risk-adapted approach should be adopted. Good supportive care is essential.

*Where possible, patients should be treated within a clinical trial.*

## Supportive care

- Nephrotic syndrome: loop diuretic + salt ± fluid restriction.
- Renal failure: peritoneal or haemodialysis if required; rigorous control of hypertension.
- CCF: diuretic + ACE inhibitors if tolerated; digoxin hypersensitivity common; Ca<sup>2+</sup> channel blockers and β-blockers contraindicated; cardiac transplantation should be considered in appropriate patients.



## Chemotherapy

There is insufficient data to indicate the optimal treatment for amyloidosis. Treatment will depend on patient-specific factors, underlying disease and disease localization. Table 8.23 lists the therapeutic options.

**Table 8.23** Therapeutic options in AL amyloidosis

Oral melphalan + dexamethasone (M-Dex)
Cyclophosphamide, thalidomide and dexamethasone (CTD)
Bortezomib ± dexamethasone
Bortezomib, melphalan, and dexamethasone
Cyclophosphamide, bortezomib, and dexamethasone (CVD)
Thalidomide + dexamethasone
Lenalidomide + dexamethasone
Interferon alfa + dexamethasone
Modified-dose or high-dose melphalan + ASCT
Best supportive care

**Intermediate dose:** 1st line treatment with combination treatment for most patients. Commonly used drug combinations include CTD (cyclophosphamide, thalidomide, and dexamethasone) and CVD (cyclophosphamide, bortezomib (Velcade) and dexamethasone), or melphalan, bortezomib (Velcade) and dexamethasone.

**Low dose:** melphalan/dexamethasone; thalidomide or lenalidomide with dexamethasone; rarely IFN.

**High dose:** high-dose melphalan + auto-SCT. Rarely recommended as 1st line treatment. Sometimes only treatment required, but can be preceded by intermediate dose treatment.

The most used schedules are discussed here.

- **Melphalan/Dex:** melphalan + high-dose dexamethasone (M 0.22mg/kg/d and Dex 40mg/d d1–4, every 28d); 67% haematological responses (33% CR) in median 4.5 months; improved organ function in 48% responsive patients; well tolerated; long-term follow-up reported 4.9 year median clonal remission in 9/15 CRs.
- **CTD:** thalidomide is poorly tolerated in doses used in MM; combination with cyclophosphamide and dexamethasone in risk-adapted CTD achieved 74% haematological responses (21% CR) and median OS 41 months; organ responses 31%; 3-year estimated OS 100% in CRs and 82% in PRs; TRM 4%. Thalidomide dose not to exceed 50 mg ON.
- **CVD:** bortezomib active in AL amyloid with 77% responses ≥PR. High discontinuation for toxicity; lower doses may be more tolerable.
- **Lenalidomide/Dex:** 67% ≥ haematological PR; myelosuppression occurred in 35% and VTE in 9%; lower doses required.
- **High-dose melphalan and autologous SCT:** limited availability as <20% are genuinely low risk for treatment-related mortality (TRM); 41% achieve haematological CR (superior to 20% achieved by best intermediate dose regimen); procedure-related mortality 10–12%; improves organ

function in up to 60%; long-term follow-up in 80 patients reported median OS 57 months; median OS exceeds 10 years if in CR after HDT. Not clearly superior to intermediate regimens such as M-Dex or CTD; HDT better tolerated if  $\leq 2$  organ systems involved; younger patients with good performance status and good renal and cardiac function do best; cardiac involvement or elevated creatinine are adverse prognostic indicators;  $\downarrow$  melphalan dose to 100–140mg/m<sup>2</sup> in high-risk patients; GI haemorrhage a frequent complication. HDT should ideally be undertaken in context of a clinical trial.

- Note: stem cell mobilization associated with mortality and morbidity due to cardiac complications (avoid cyclophosphamide), oedema and splenic rupture (low doses of G-CSF recommended).
- Careful selection criteria recommended: see Tables 8.24 and 8.25.
- **Allogeneic SCT:** isolated reports of small numbers of patients treated with allografts with high TRM (40%); reduced-intensity conditioning reduces this figure; should be restricted to clinical trials in highly selected patients in experienced centres.

**Table 8.24** Mayo Clinic risk stratification model to predict progression of primary amyloidosis<sup>1</sup>

Risk group	Median survival of patients undergoing SCT (months)	Median survival of patients not undergoing SCT (months)
<i>Low risk</i> (cardiac troponin <0.035mcg/L and NT-proBNP <332ng/L)	Not reached at 40 months	26.4
<i>Intermediate risk</i> (any 1 factor abnormal)	Not reached at 40 months	10.5
<i>High risk</i> (cardiac troponin $\geq 0.035$ mcg/L and NT-proBNP $\geq 332$ ng/L)	8.4	3.5

Adapted from Rajkumar, S.V. et al. (2006) Monoclonal gammopathy of undetermined significance, Walden-ström macroglobulinemia, AL amyloidosis and related plasma cell disorders: diagnosis and treatment. *Mayo Clin Proc*, **81**, 693–703., with permission of Elsevier.

**Table 8.25** Criteria for eligibility for ASCT in UK amyloidosis treatment trial<sup>2</sup>

ECOG performance status 0 or 1  
 No greater than NYHA class I or II heart failure  
 No more than 2 organs involved by amyloid by consensus guidelines  
 Age  $\leq 65$  years  
 Creatinine clearance  $\geq 0.8$ mL/sec (50mL/min)  
 Bilirubin  $\leq 1.5 \times$  and alkaline phosphatase  $\leq 2 \times$  ULN  
 Inter-ventricular and left ventricular posterior wall thicknesses  $\leq 15$ mm by echocardiography  
 Absence of clinically important amyloid related autonomic neuropathy  
 Absence of clinically important amyloid related gastrointestinal haemorrhage

## Response assessment

Consensus response criteria have been published.<sup>3,4</sup> Separate haematological and organ responses have been defined. The latter occur slowly and are generally dependent on the former which is a strong predictor of survival. (See Tables 8.26 and 8.27.)

**Table 8.26** Hematological response criteria<sup>3,4</sup>

Complete response (CR)	<ul style="list-style-type: none"> <li>• Serum and urine –ve for paraprotein by immunofixation</li> <li>• Free LC ratio ↔</li> <li>• BM plasma cells &lt;5%</li> </ul>
Very good partial response (VGPR)	<ul style="list-style-type: none"> <li>• Free LC &lt; 40mg/L</li> </ul>
Partial response (PR)	<ul style="list-style-type: none"> <li>• LC decrease ≥50%</li> </ul>
Progression	<ul style="list-style-type: none"> <li>• From CR, any detectable paraprotein or abnormal free LC ratio (LC must double)</li> <li>• From PR or stable response, 50% ↑ in serum paraprotein to &gt;5g/L or 50% ↑ in urine paraprotein to &gt;200mg/d; a visible peak must be present</li> <li>• Free LC ↑ of 50% to &gt;100mg/L</li> </ul>
Stable	<ul style="list-style-type: none"> <li>• No CR, no PR, no progression</li> </ul>

**Table 8.27** Organ response criteria<sup>3,4</sup>

	Response	Progression
Heart	Mean interventricular septal thickness ↓ by 2mm, 20% ↑ in ejection fraction, improvement by 2 NYHA classes without ↑ diuretic use and no ↑ in wall thickness, decrease of NT-proBNP of ≥30%	Interventricular septal thickness ↑ by 2mm compared with baseline, ↑ in NYHA class by 1 grade with a ↓ in ejection fraction ≥10%
Kidney	50% ↓ (≥0.5g/d) of 24h urine protein (must be >0.5g/d pretreatment)	50% ↑ (≥1g/d) of 24h urine protein to >1g/d or 25% worsening of serum creatinine or creatinine clearance
Liver	50% ↓ in abnormal alk phos; ≥2cm ↓ in liver size radiographically	50% ↑ of alk phos above the lowest value
Nerve	Improvement in EMG nerve conduction (rare)	Progressive neuropathy by EMG or nerve conduction velocity

## Follow-up

Response to therapy should be monitored by quantitation of the SFLCs or in the minority with measurable levels, the serum paraprotein; SAP scintigraphy; ECG, echocardiography, and assessment of other organ dysfunction should be reviewed every 6 months.

### Prognosis

- Median survival 1–2 years; 4–6 months if CCF at diagnosis.
- Most common cause of death cardiac, progressive congestive cardiomyopathy, or sudden death due to VF or asystole.
- Others succumb to uraemia or other complications.

### Other causes of amyloid<sup>5</sup>

#### Acquired

- AA amyloidosis: reactive systemic amyloidosis associated with chronic inflammatory diseases, e.g. rheumatoid arthritis, TB; due to AA fibrils derived from serum amyloid A protein (SAA); treat underlying condition.
- Senile systemic amyloidosis due to transthyretin deposition; no therapy.
- Endocrine amyloidosis, associated with APUD-omas.
- Haemodialysis associated amyloidosis, localized to osteoarticular tissues or systemic due to  $\beta_2$ -microglobulin deposition.
- Non-familial Alzheimer disease, Down syndrome due to  $\beta$ -protein.
- Sporadic Creutzfeldt–Jakob disease, due to prion protein deposition.
- Type 2 diabetes mellitus due to islet amyloid polypeptide.


#### Hereditary

- Numerous syndromes with characteristic patterns of peripheral or cranial neurological involvement or visceral or cardiac involvement due to a variety of proteins.
- Familial mutant ATTR due to mutant transthyretin.
- Familial Alzheimer disease due to a  $\beta$ -protein.
- Familial Mediterranean fever due to AA derived from SAA; treat with colchicine.

### References

1. Rajkumar, S.V. et al. (2006) Monoclonal gammopathy of undetermined significance, Waldenström macroglobulinemia, AL amyloidosis and related plasma cell disorders: diagnosis and treatment. *Mayo Clin Proc*, **81**, 693–703.
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# Haematopoietic stem cell transplantation

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## Haematopoietic stem cell transplantation (SCT)

Haematopoietic SCT achieves reconstitution of haematopoiesis by the transfer of pluripotent haematopoietic stem cells. In allogeneic SCT stem cells are obtained from a donor, e.g. a matched sibling or normal matched volunteer unrelated donor (VUD); in syngeneic SCT the donor is a monozygotic (identical) twin. For autologous SCT the patient acts as his/her own source of stem cells. Umbilical cord stem cells have become a useful source of stem cells for some patients where no HLA-identical donor is found/available.

Note that stem cell transplants/bone marrow transplants should only be performed in specialized and accredited centres under supervision by transplant physicians. Centres in Europe are accredited by JACIE, the Joint Accreditation Committee of the ISCT (International Society for Cellular therapy) and the EBMT (European Society for Blood and Marrow Transplantation). This regulatory body mandates standards and inspection programmes to assure appropriate facilities, staff, experience, and protocols are in place. Outcome measures such as d100 outcome/mortality, 1-year survival, and relapse rates are also monitored.

The decision for referral for a patient to a transplant centre is based on indications (discussed on pp.392–396), patient-specific features, and multidisciplinary team discussions.

### The aim of SCT is:

- To permit haematopoietic reconstitution after potentially curative but myeloablative doses of chemotherapy or chemoradiotherapy (high-dose therapy (HDT)) in the treatment of malignant disease;

or

- To replace congenital or acquired life-threatening abnormal BM or immune function with a normal haematopoietic and immune system.

The therapeutic effect of allogeneic SCT is not restricted to the conditioning as there can be an additional graft-versus-disease effect (e.g. graft-versus-leukaemia (GvL)), mediated by the incoming donor immune system that contributes significantly to the curative potential of the procedure, though this is linked to its major toxicity, graft-versus-host disease (GvHD). No GvL effect occurs in syngeneic or autologous SCT and in allogeneic SCT it can be abrogated by intensive efforts to prevent GvHD (see Tables 9.1 and 9.2).

Stem cells may be obtained from BM (bone marrow transplant (BMT)) by multiple aspirations under general anaesthesia (BM harvest), or obtained from peripheral blood after 'mobilization' and collection by apheresis (peripheral blood stem cell harvest and transplant (PBSCH and PBSCT)). Whether used fresh from donor harvest or thawed after cryopreservation, stem cells are re-infused IV. PBSCT carries the advantages of avoiding general anaesthesia for the donor and more rapid engraftment (~7d) but may be associated with a higher incidence of chronic GvHD (cGvHD). In the autologous setting not all previously treated patients will mobilize adequate numbers of stem cells.

Stem cell collection for autologous SCT with curative intent in diseases involving the BM should be undertaken after a complete response has been achieved by initial therapy. In some settings, e.g. myeloma where HDT is being used with the aim of disease control rather than with curative intent, low-level BM involvement is often accepted.

The patient receives 'conditioning' comprising of high-dose chemotherapy or chemo-radiotherapy  $\pm$  antibodies (e.g. alemtuzumab) which ablates the recipient's BM and immune system. Reduced intensity conditioning (RIC) is now more often used, relying more heavily on the graft-versus-disease effect. After conditioning is completed, BM or PBSC are infused IV. After a period of profound myelosuppression (7–25d), engraftment occurs with production of WBCs, platelets, and RBCs. Immunosuppression is required after allogeneic transplantation to prevent graft failure, GvHD, and graft rejection.

**Table 9.1** Comparison of autologous and allogeneic SCT

Autologous	Allogeneic
Wide age range, generally $\leq 70$ years	Age range generally $\leq 60$ years, though higher age limit now that RIC is used
No need for donor search if BM clear	Sibs have ~1 in 4 chance of match
Not feasible if BM involved	May be used in patients with BM disease
Risk of tumour cell re-infusion	No tumour contamination of graft
Not all patients can be mobilized	Donor search may impose delay
No GvHD	GvHD mortality and morbidity
No immunosuppression	Graft-versus-disease effect (e.g. GvL)
Low early treatment related mortality (2–5%)	Higher early treatment-related mortality from GvHD and infection (10–20%)
Risk of long-term MDS from BM injury	Lower risk of late MDS

**Table 9.2** Factors influencing outcome of haemopoietic SCT

Time	Patient	Donor
<i>Pre-transplant</i>		
Disease	Diagnosis, subtype, stage	
Individual	Age, sex, ethnicity, organ status, viral status, Performance status	Age, sex, sex-mismatch, ethnicity, viral status, histocompatibility Major histocompatibility antigens Minor histocompatibility antigens ABO Cytokine polymorphisms
Environment	Geography Economic status	
<i>Peri-transplant</i>		
Conditioning	Intensity: RIC?	
GvHD prevention	Prevention method	
Graft product	Stem cell source, graft composition	
<i>Post-transplant</i>		
Disease	Relapse	
Individual	Immune reconstitution	Availability for DLI or retransplant
Environment	Retreatment possibilities	

Reproduced from Gratwohl, A. (2007). Risk assessment in haemopoietic stem cell transplantation. *Best Pract Res Clin Haematol*, **20**, 119–24, with permission from Elsevier © 2007.

## Early complications of the transplant procedure

### Chemo-radiotherapy

- Nausea/vomiting.
- Reversible alopecia.
- Fatigue.
- Dry skin.
- Mucositis.
- Organ failure.
- VOD (veno-occlusive disease).

### Infection

- Bacterial (Gram –ve and +ve).
- Viral—HZV.
- CMV (particularly pneumonitis)\*.
- Fungal—*Candida*, *Aspergillus*\*, *Mucor*\*.
- Atypical organisms—*Pneumocystis* (PCP)\*, *Toxoplasma*\*, *Mycoplasma*\*, *Legionella*\*.

(\*Low risk in autologous SCT; significant to high risk in allogeneic SCT.)




## GvHD

May occur in recipients of allogeneic SCT due to tissue incompatibility between donor and recipient undetected by standard tissue-typing tests. Acute and chronic forms. Higher incidence of severe GvHD following VUD SCT, and mismatched or haploidentical grafts. Acute or chronic.

## Late complications of transplantation

- Complications of chronic GvHD.
- Infertility (both sexes).
- Hypothyroidism.
- 2<sup>o</sup> malignancy.
- Late sepsis due to hyposplenism (in myeloablative regimens).
- Cataracts (where TBI used).
- Psychological disturbance.

## Follow up and post-transplant surveillance

Lifelong follow-up is required. The particular risks and monitoring required depend on the type of graft and whether TBI was used. Suitable conditioning regimens are outlined in  Stem cell transplant conditioning regimens, p.414.

## Further reading

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## Indications for haemopoietic SCT

The Accreditation Subcommittee of the European Group for Blood & Marrow Transplantation (EBMT) reports current practice for HSCT in Europe (Tables 9.3 and 9.4).

**Table 9.3** EBMT indications for HSCT for adults (recommendation from 2010, adopted and unchanged 2012)<sup>1</sup>

Disease	Disease status	Allo			Auto
		Sibling donor	Matched unrelated /1 antigen mismatched related	Mismatch unrelated/ >1 antigen mismatched related	
AML	CR1 (low risk)	CO	D	GNR	CO
	CR1 (int risk)	S	CO	D	S
	CR 1 (high risk)	S	S	CO	CO
	CR2	S	S	CO	CO
	CR3, incipient relapse	S	CO	D	GNR
	APML molecular persistence	S	CO	GNR	GNR
	APML molecular CR2	S	CO	GNR	S
	Relapse/refractory	CO	D	D	GNR
ALL	CR1 (standard/int risk)	D	GNR	GNR	D
	CR1 (high risk)	S	S	CO	D
	CR2, incipient relapse	S	S	CO	GNR
	Relapse/refractory	CO	D	D	GNR
CML	1st CP, failed imatinib	S	S	CO	D
	AP, >1st CP	S	S	CO	D
	Blast crisis	CO	CO	CO	GNR
Myelofibrosis	1°, 2° with high/int Lille score	S	S	D	GNR
Myelodysplastic syndrome	RA, RAEB	S	S	CO	GNR
	RAEBt, sAML	S	S	CO	CO
	CR1, CR2 More advanced stages	S	CO	D	GNR
CLL	High-risk disease	S	S	D	CO
Diffuse large B-cell NHL	CR1 (int/high-risk at Dx)	GNR	GNR	GNR	CO
	Chemosensitive relapse; ≥CR2	CO	CO	GNR	S
	Refractory	D	D	GNR	GNR

(continued)

**Table 9.3** (continued) EBMT indications for HSCT for adults (recommendation from 2010, adopted and unchanged 2012)<sup>1</sup>

Disease	Disease status	Allo			Auto
		Sib	Matched	Mismatched	
Mantle cell lymphoma	CR1	CO	D	GNR	S
	Chemosensitive relapse; $\geq$ CR2	CO	D	GNR	S
	Refractory	D	D	GNR	GNR
Lymphoblastic and Burkitt lymphoma	CR1	CO	CO	GNR	CO
	Chemosensitive relapse; $\geq$ CR2	CO	CO	GNR	CO
	Refractory	D	D	GNR	GNR
Follicular NHL	CR1 (int/high risk at dx)	GNR	GNR	GNR	CO
	Chemosensitive relapse; $\geq$ CR2	CO	CO	D	S
	Refractory	CO	CO	D	GNR
T-NHL	CR1	CO	D	GNR	CO
	Chemosensitive relapse; $\geq$ CR2	CO	CO	GNR	D
	Refractory	D	D	GNR	GNR
HL—classical	CR1	GNR	GNR	GNR	GNR
	Chemosensitive relapse; $\geq$ CR2	CO	CO	CO	S
	Refractory	D	D	GNR	CO
HL—NLPHL	CR1	GNR	GNR	GNR	GNR
	Chemosensitive relapse; $\geq$ CR2	GNR	GNR	GNR	CO
	Refractory	GNR	GNR	GNR	CO
Myeloma		CO	CO	GNR	S
Amyloidosis (primary; AL)		CO	CO	GNR	CO
Severe aplastic anaemia	Newly diagnosed	S	CO	GNR	GNR
	Relapsed, refractory	S	S	CO	GNR
PNH		S	CO	CO	GNR
<b>Solid tumours</b>					
Germ-cell tumours	Sensitive relapses	GNR	GNR	GNR	CO
	3rd line refractory	GNR	GNR	GNR	S
Breast ca	Adjuvant high risk	GNR	GNR	GNR	CO
	Metastatic respond	D	D	GNR	D/CO
Ovarian ca	CR/PR	GNR	GNR	GNR	D
	Platinum sensitive relapse	D	GNR	GNR	CO
Medulloblastoma	Post-surgery	GNR	GNR	GNR	D
Small cell lung	Limited stage	GNR	GNR	GNR	D
Renal cell ca	Metastatic, cytokine refr	CO	CO	GNR	CO
Soft cell sarcoma	Metastatic responsive	D	GNR	GNR	D

(continued)

**Table 9.3** (continued) EBMT indications for HSCT for adults (recommendation from 2010, adopted and unchanged 2012)<sup>1</sup>

Disease	Disease status	Allo			Auto
		Sib	Matched	Mismatched	
<b>Auto immune diseases</b>					
Immune cytopenias		CO	D	D	CO
Systemic sclerosis		D	GNR	GNR	CO
R.A.		CO	GNR	GNR	CO
Multiple sclerosis		D	GNR	GNR	CO
Lupus erythematosis		D	GNR	GNR	CO
Crohn's disease		CO	GNR	GNR	CO
CIDP		CO	GNR	GNR	D

S = standard of care, generally indicated in suitable patients; CO = clinical option, can be carried out after careful assessment of risks and benefits; D = developmental, further trials are needed; GNR = generally not recommended.

*NB* This classification does not cover patients for whom a syngeneic donor is available. A syngeneic twin donor is generally considered S for all indications except, by definition, congenital disorders.

Donor type is divided into:

- HLA identical sibling donor.
- Matched unrelated: there is no uniformly accepted definition. A  $\geq 10/10$  antigen matched donor is unequivocally considered as matched.
- Mismatched donor: there is no uniformly accepted definition. A  $< 8/8$  antigen matched unrelated and a  $> 1$  antigen mismatched family donor is unequivocally considered mismatched.

**Table 9.4** EBMT indications for HSCT for children (recommendation from 2010, adopted and unchanged 2012)<sup>1</sup>

Disease	Disease status	Allo			Auto
		Sibling donor	Matched unrelated /1 antigen mismatched related	Mismatch unrelated/ >1 antigen mismatched related	
AML	CR1 (low risk)	GNR	GNR	GNR	GNR
	CR1 (high risk)	S	CO	GNR	S
	CR1 (very high risk)	S	S	CO	CO
	CR2	S	S	S	S
	> CR2	CO	D	D	GNR
ALL	CR1 (low risk)	GNR	GNR	GNR	GNR
	CR1 (high risk)	S	S	CO	GNR
	CR2	S	S	CO	CO
	> CR2	S	S	CO	CO
CML	CP	S	S	D	GNR
	AP	S	S	CO	GNR
NHL	CR1 (low risk)	GNR	GNR	GNR	GNR
	CR1 (high risk)	CO	CO	GNR	CO
	CR2	S	S	CO	CO
HL	CR1	GNR	GNR	GNR	GNR
	1st relapse, CR2	CO	D	GNR	S
MDS		S	S	D	GNR
Primary ID		S	S	S	N/A
Thalassaemia		S	CO	GNR	N/A
Sickle cell disease	High risk	S	CO	GNR	N/A
Aplastic anaemia		S	S	CO	N/A
Fanconi anaemia		S	S	CO	N/A
Diamond–Blackfan anaemia		S	CO	GNR	N/A
CGD		S	S	CO	N/A
Kostman's disease		S	S	GNR	N/A
MPS-1H Hurler		S	S	CO	N/A
MPS-1H Hurler-Scheie	Severe	GNR	GNR	GNR	N/A
MPS-VI Maroteaux–Lamy		CO	CO	CO	N/A
Osteopetrosis		S	S	S	N/A
Other storage diseases		GNR	GNR	GNR	N/A

(continued)

**Table 9.4** (continued) EBMT indications for HSCT for children (recommendation from 2010, adopted and unchanged 2012)<sup>1</sup>

Disease	Disease status	Allo			Auto
Autoimmune disorders		GNR	GNR	GNR	CO
Germ cell tumour		GNR	GNR	GNR	CO
Ewing sarcoma	High risk or >CR1	D	GNR	GNR	S
Soft tissue sarcoma	High risk or >CR1	D	D	GNR	CO
Neuroblastoma	High risk	CO	GNR	GNR	S
Neuroblastoma	>CR1	CO	D	D	S
Wilms tumour	> CR1	GNR	GNR	GNR	CO
Osteogenic sarcoma		GNR	GNR	GNR	D
Brain tumours		GNR	GNR	GNR	CO

S = standard of care, generally indicated in suitable patients; CO = clinical option, can be carried out after careful assessment of risks and benefits; D = developmental, further trials are needed; GNR = generally not recommended.

NB This classification does not cover patients for whom a syngeneic donor is available. A syngeneic twin donor is generally considered S for all indications except, by definition, congenital disorders.

Donor type is divided into:

- HLA identical sibling donor.
- Matched unrelated: there is no uniformly accepted definition. A  $\geq 10/10$  antigen matched donor is unequivocally considered as matched.
- Mismatched donor: there is no uniformly accepted definition. A  $< 8/8$  antigen matched unrelated and a  $>1$  antigen mismatched family donor is unequivocally considered mismatched.

## Reference

1. Ljungman, P. *et al.* (2010). Allogeneic and autologous transplantation for hematological diseases, solid tumours and immune disorders: current practice in Europe 2009. *Bone Marrow Transplant*, **45**, 219–34.



## Allogeneic SCT

### Patient and donor selection

- Recipients should be in good physical condition. Due to the advent of RIC, transplants are being done more and more in older patients with acceptable toxicity, treatment-related mortality (TRM), and morbidity. Generally  $\leq 70$  years old; 'biological' age is more important than chronological age; the development of reduced-intensity conditioning regimens (RIC) associated with less procedure-related toxicity, permits an increase in the age-limit for SCT (see Table 9.5).
- Donor and recipient should be fully or closely HLA-matched to reduce the risk of GvHD or graft rejection.
- Risk of graft failure and GvHD related to degree of donor–recipient HLA-mismatching; the least mismatched donor should be selected.
- Greatest chance of full HLA match is a sibling transplant; each sibling has ~1:4 chance of full HLA-match.
- Matched VUDs may be sought from donor registries (e.g. the Anthony Nolan trust).
- Haplo-identical sibling or mismatched UD may be considered as a donor for patients in whom no matched sibling or volunteer donor is available and where the  $\uparrow$  risks of the procedure are acceptable (e.g. poor risk adult AML, Ph-positive adult ALL).
- Patient and donor CMV status should be considered: with +ve donors preferred for +ve patients and -ve donors preferred for -ve patients.
- Male donors are preferred for all recipients; VUDs aged  $\leq 40$  years preferred to older VUDs; ABO-matched donors preferred for heavily pre-transfused patients.

**Table 9.5** HLA hierarchy for best choice of donor

1. HLA-identical sibling = phenotypically HLA-matched related donor<sup>a</sup> = HLA-identical related donor umbilical cord blood<sup>b</sup>
2. 10/10 molecularly HLA-matched VUD = 6/6 HLA-matched umbilical cord blood<sup>b</sup>
3. 9/10 HLA-matched VUD = 5/6 HLA-matched related donor = 4/6 VUD umbilical cord blood<sup>b</sup>
4. 8/10 matched VUD = haploidentical related donor<sup>c</sup>

Options 3 and 4 should only be considered in poor risk patients for whom alternative treatment is unavailable. Option 4 usually requires in vitro T-cell depletion. Mismatched related donors may be preferred over UDs in certain disorders.

<sup>a</sup> HLA-matched donor where one haplotype is genetically identical but the other is not.

<sup>b</sup> With nucleated cell dose  $> 3 \times 10^7$ /kg recipient weight.

<sup>c</sup> Most haploidentical donors for children are parents.

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## Indications for allogeneic SCT

See Tables 9.3 and 9.4.

### Source of stem cells

- Donors should be allowed to choose between donating BM or PBSCs after non-directive counselling.
- BM preferred to PB in SCT for non-malignant conditions.

### Outline of allogeneic SCT procedure

- Patient receives conditioning therapy with high-dose chemoradiotherapy (traditionally in myeloablative regimens cyclophosphamide (CTX) 120mg/kg + 14Gy fractionated total body irradiation (TBI)) or chemotherapy (e.g. cyclophosphamide 120mg/kg + busulfan 16mg/kg).
- RIC regimens are increasingly used; moderate doses of chemotherapy and immunosuppression used to achieve engraftment with lower transplant-related mortality and morbidity; no TBI used; shifts balance between risks of transplant-related mortality (TRM) and relapse; lower toxicity allows ↑ age range for allogeneic SCT; also allows SCT in some patients with comorbidities; permits use of adoptive immunotherapy with donor lymphocyte infusion (DLI) for residual disease post-transplant; good results but full-intensity conditioning remains the treatment of choice in younger patient without co-morbidities; RIC discouraged in patients with progressive or refractory disease.
- In patients receiving UD or haploidentical SCT, alemtuzumab (humanized anti-CD52 monoclonal antibody) is often administered prior to conditioning for T-cell depletion as an immunosuppressant to ↓ the risk of graft rejection.
- For patients with aplastic anaemia less intensive conditioning is used (CTX combined with antithymocyte globulin).
- Because of sensitivity to alkylating agents in Fanconi anaemia, less intensive conditioning is used.
- In severe combined immunodeficiency (SCID), it is possible to engraft selected cell lineages without conditioning therapy.
- 1d after completing conditioning treatment, BM or PBSC are harvested from donor and infused into the patient IV through a central line.
- After 7–21d of severe myelosuppression, haematopoietic engraftment occurs.
- Reverse barrier nursing in a filtered air environment, prophylactic anti-infectives (antifungal and aciclovir prophylaxis; consider antibiotic prophylaxis.) reduce the risk of infective complications.
- Immunosuppression is required to prevent GvHD and graft rejection; generally MTX (in the early engraftment phase) + ciclosporin (for 3–6 months).

### Mechanism of cure: evidence for GvL effect

- ↓ risk of relapse in patients with acute and chronic GvHD.
- ↑ risk of relapse after syngeneic SCT (no GvHD).
- ↑ risk of relapse after T-lymphocyte-depleted SCT.
- Delayed clearance of minimal residual disease detected post SCT.
- Induction of remission by DLI after relapse post-SCT.

### Early complications of allogeneic SCT

- Overall transplant related mortality for matched sibling allografts is ~5% for VUDs may reach 5-20%.
- Infection: (severe) myelosuppression together with immune dysfunction from delayed reconstitution or GvHD predisposes to a wide variety of potentially fatal infections with bacterial, viral, fungal, and atypical organisms. Both HSV and HZV infections are common—may present with fulminant extensive lesions. Main causes of infective death post-transplant are: CMV pneumonitis and invasive fungal infections with moulds, e.g. *Aspergillus*.
- GvHD: acute GvHD occurs ≤100d of transplant and chronic >100d.
- Other complications:
  - Endocrine: infertility (both sexes), early menopause, and occasionally hypothyroidism.
  - Cataract (TBI induced) >12 months post-transplant.
  - 2° malignancies (esp. skin).
  - EBV-associated lymphoma/PTLD.
  - Psychological disturbances common (serious psychoses rare).

### Follow-up treatment and post-transplant surveillance

Immunosuppression requires careful monitoring to avoid toxicity. Unlike solid organ transplant recipients, lifelong immunosuppression is not required and weaning of ciclosporin is usually started at 3 months post-transplant with the aim to discontinue at 6 months. Prophylaxis against pneumococcal sepsis 2° to hyposplenism, CMV reactivation and *P. jirovecii* infections required. Most patients return to an active, working life without the need for continuing medication.

### Umbilical cord transplants

- Umbilical cord blood donation post-delivery is shown to be safe for mother and child.
- Cord blood stem cells are immunologically immature and may be more permissive of HLA donor/recipient mismatches.
- Less risk of GvHD.
- Successful grafts in children.
- Due to cell dose required single cord donation often insufficient for adult grafts, hence dual cord transplant required with matching between both cords and recipient.
- Typed 6/6 (due to immunological immaturity). Matching 4/6 between recipient and cords, as well as between 2 cord donations required.
- Longer time to engraftment, hence ↑ risk of infective complications.
- Ultimately 1 cord persists/'survives'.
- Due to source of cells no possibility for DLI.



## Autologous SCT

### Patient selection

Patients should be in good physical condition; age range for some procedures can be extended up to ~70 years. BM should be uninvolved or in CR at the time of harvest/mobilization unless disease control rather than cure is the primary intent such as in multiple myeloma.

### Indications

➔ See Tables 9.3 and 9.4, pp.392–396.

### Outline of autologous SCT procedure

- Haematopoietic stem cells harvested are processed, frozen, and stored in liquid N<sub>2</sub>. Stem cells are harvested from the PB.
- SCT may take place within days of harvest or several years later after treatment for recurrent disease. In some conditions enough stem cells for 2 autografts are collected for possible relapse planning.
- Different conditioning chosen for underlying indication, e.g. high-dose melphalan for MM or BEAM for NHL or HL.
- After completion of conditioning, the stem cell product is thawed rapidly and infused IV (>24h after last chemotherapeutic agent). Bags are thawed by transfer directly from liquid N<sub>2</sub> into a water-bath at 37–43°C. Product is infused IV rapidly through indwelling central line.
- There is a period of myelosuppression (7–25d) followed by WBC, platelet and RBC engraftment.

### Early complications of the transplant procedure

- Overall transplant related mortality is 3–5%.
- Morbidity from conditioning regimens: nausea from chemotherapy, mucositis, oral ulceration, buccal desquamation, oesophagitis, gastritis, abdominal pain, and diarrhoea may all be features.
- Spectrum of infective organisms seen is similar to allografts but severity and mortality are ↓.

### Late complications of autologous SCT

- Single commonest long-term complication is relapse of underlying disease.
- Other late complications similar to allografts, but less frequent and less severe.
- Higher increase of MDS compared to allograft.

### Follow-up treatment and post-transplant surveillance

- Regular haematological follow-up is mandatory and psychological support from the transplant team, family, and friends are important for readjustment to normal life.
- Prophylaxis against specific infections required including *Pneumococcus*, CMV and *P. jirovecii*. Most patients return to an active, working life without continuing medication.



## Investigations for BMT/PBSCT

### Hematology

- FBC, blood film, reticulocytes, ESR.
- Serum B<sub>12</sub> and red cell folate, ferritin.
- Blood group, antibody screen, and DAT.
- Coagulation screen, PT, APTT, fibrinogen.
- BM aspirate for morphology (cytogenetics if relevant).
- BM trephine biopsy (not always indicated).

### Biochemistry

- U&Es, LFTs.
- Ca<sup>2+</sup>, phosphate, random glucose.
- LDH.
- Thyroid function tests.
- Serum and urine Igs.
- EDTA clearance if renal impairment.

### Virology

- Hepatitis and HIV serology.
- CMV IgG, and IgM.
- EBV, HSV, and VZV IgG.

### Immunology

- Autoantibody screen.
- HLA type, (if not known) in case HLA matched platelets are subsequently required.
- HLA and platelet antibody screen (if previously poor increments to platelet transfusions).
- CRP.

### Bacteriology

- Baseline blood cultures (peripheral blood and Hickman line).
- Routine admission swabs: nose, throat, perineum, central line site.
- MSU, stool cultures if diarrhoea.
- Syphilis serology.
- Toxoplasma serology.

### Cardiology

- ECG.
- Echocardiogram, to include measurement of systolic ejection fraction.

### Respiratory

- Lung function tests.

### Radiology

- CXR.
- Other imaging as indicated

### Cytogenetics

- Blood for donor/recipient polymorphisms (allografts only).

**Other**

- Consider semen storage/egg harvesting.
- Dental review.
- Psychiatric opinion if previous history of psychological problems.

## Pretransplant investigation of donors

### Hematology

- FBC, reticulocytes, ESR.
- Blood group, antibody screen, and DAT.
- Coagulation screen, PT, APTT, fibrinogen.

### Biochemistry

- U&Es.
- LFTs.
- Thyroid function tests.
- Urinalysis: dipstick.

### Virology

- Hepatitis and HIV serology.
- CMV IgG. and IgM.
- EBV, HSV. and VZV IgG.
- HTLV-I serology.

### Immunology

- Autoantibody screen.
- CRP.

### Microbiology

- Syphilis serology.
- Toxoplasma serology.

### Cardiology

- BP.
- ECG if indicated.

### Molecular biology

- DNA fingerprinting for chimerism studies.





## Bone marrow harvesting

### Preoperative preparations

Elective procedure done under general anaesthetic; requires operator, anaesthetist/anaesthesiologist, and available theatre.

*Within 30d before the harvest procedure, arrange the following virological investigations:*

- Hepatitis and HIV serology.
- HTLV-I serology.
- Syphilis serology.
- Spare serum stored.

### Harvest procedure

- Check donor FBC and U&E.
- Cross-match 2–3U blood (CMV –ve) for donor. If harvest is on healthy donor, offer autologous blood collection to donor.
- Check consent is in place.
- Prepare harvest bag adding ACD (acid citrate dextran) with a dilution factor of 1:7–10 for the prospective marrow volume; Ensure the ratio of marrow to ACD is maintained at 7–10:1.
- Attach one 500-micron filter, followed by the 200-micron filter to the collection bag.
- Mix 50mL sodium chloride 0.9% with 50,000 units of heparin in a receiver and flush several 20mL syringes and the harvest needles with the heparin solution.
- Donor in prone position.
- Begin with posterior superior iliac crests, limiting the number of skin entry points, the aspirate needle should be manoeuvred to collect as much marrow as possible with 5–10mL maximum from each penetration of the bone. Each aspirate is deposited in the sterile harvest bag and syringe rinsed in the heparinized saline prior to re-use. Gently agitate bag at intervals.
- Midway through harvest (or 500mL) a sample should be sent for FBC to determine the adequacy of the harvest.
- The aim is to collect between  $2.0$  and  $4.0 \times 10^8$  nucleated cells/kg weight of the recipient. The marrow volume necessary to achieve this is usually between 500–800mL. The marrow collection should not exceed 1000mL collection (see Box 9.1).

**Box 9.1 The volume of marrow required may be calculated as follows:**

Total volume required for autograft

$$= 2.0 \times \text{recipient weight (kg)} / (\text{collector bag WBC} \times 10)$$

e.g. recip. 100kg and the collector bag contains WBC  $20 \times 10^9$ /L then volume required:

$$= (2.0 \times 100) / (20 \times 10) = 1.0\text{L}$$

Volume still needed to be harvested = total volume – volume already taken at time of count + ~10%

*Notes*

1. The extra 10% compensates for reduced harvesting efficiency and the ACD.
2. The formula works at whatever volume you choose to do the first WBC but is a more accurate prediction at >500mL.
3. If need to harvest >1L, remember to add additional ACD in the same 1 in 7 ratio.
4. For allograft calculations, substitute 3.0 for 2.0 in the formula.

If the yield is not adequate from the posterior iliac crests, other sites may be considered (e.g. anterior superior iliac crests and sternum). Review puncture sites the following morning for signs of local infection or continuing bleeding. For healthy normal donors, offer out-patient follow-up appointment as additional safeguard and provide access to counselling services.

## Peripheral blood stem cell mobilization and harvesting

### Properties of stem cells

- Stem cells are defined as the most primitive haemopoietic precursor cell.
- Unique property is capability of both infinite self-renewal and differentiation to form all mature cells of the haemopoietic and immune systems.
- In the resting state almost all stem cells reside in the BM although a minority circulate in the peripheral blood.
- Stem cells in marrow can migrate into the blood after treatment with chemotherapy and/or haemopoietic growth factors.
- Once circulating, they can easily be harvested using a cell separator machine.
- Stem cell levels in peripheral blood can be assessed by CD34 immunophenotype analysis.
- >1d of apheresis may be necessary to achieve required yield.
- The yield can be assessed for engraftment potential.

### Protocols

Mobilization and harvesting protocols differ between diseases. The following illustrate the principal types of schedule:

#### *Mobilization after standard chemotherapy*

- G-CSF given at standard time post chemotherapy.
- Harvest times determined by WBC and platelet recovery, and CD34 count.
- Yields variable. If insufficient harvest, further harvest with chemotherapy/growth factor can be done.

*Suitable for:*

- NHL, e.g. post RCHOP, R-ICE, R-DHAP, Nordic chemotherapy.

#### *Mobilization with chemotherapy and haemopoietic growth factors*

The most common schedule and the best evaluated. Harvest timing and yields more predictable.

#### *Typical protocol for NHL*

- Day 0 cyclophosphamide 1.5g/m<sup>2</sup> IVI with Mesna.
- Day +4 to day +10 G-CSF 5mcg/kg/d SC continued until last day of harvesting.
- Harvest ~day +10 when CD34 >10 × 10<sup>6</sup>/L and/or WBC >10 × 10<sup>9</sup>/L.

#### *Typical protocol for myeloma*

- Day 0 cyclophosphamide 4g/m<sup>2</sup> IVI with Mesna.
- Day +1 to day +10–14, G-CSF 5–10mcg/kg/d SC.
- Harvest day +10 to 14 when CD34 > 10 × 10<sup>6</sup>/L and/or WBC >10 × 10<sup>9</sup>/L.

### *Mobilization with haemopoietic growth factor alone*

- Suitable for normal volunteers (allograft donors).
- G-CSF 5–10mcg/kg/d SC for 4–5d.
- Harvest d4–5 (CD34 >10 × 10<sup>6</sup>/L and/or WBC >10 × 10<sup>9</sup>/L ideally).
- Multiple harvest days might be required.

### *Mobilization with plerixafor<sup>1,2</sup>*

- Salvage method.
- Immunostimulant used to mobilize haemopoietic stem cells in patients who have not mobilized sufficient stem cells with standard methods (15–20% patients).
- Suitable for normal volunteers (allograft donors).
- Approved for use in lymphoma and myeloma patients.
- Orphan drug status in USA and Europe for the mobilization of haematopoietic stem cells.
- Plerixafor should be administered approximately 11h prior to initiation of apheresis for up to 4 consecutive days. It is given with preceding G-CSF injections for 4d.
- Dose: Plerixafor 0.24mg/kg bodyweight SC. The patient's actual body weight can be used. The dose should not exceed 40mg/d.

### **Yield evaluation and target**

The yield is calculated by the number of CD34+ cells. It is a quantitative assessment of engraftment potential expressed per kg of recipient weight. The daily apheresis and G-CSF should continue until the collection reaches 5–10 × 10<sup>6</sup>/kg CD34+ cells.

### **References**

1. DiPersio, J. *et al.* (2009). Plerixafor and G-CSF versus placebo and G-CSF to mobilize hematopoietic stem cells for autologous stem cell transplantation in patients with multiple myeloma *Blood*, **113**, 5720–6.
2. Tanhehco, Y.C. *et al.* (2013). The evolving role of plerixafor in hematopoietic progenitor cell mobilization. *Transfusion*, **53**(10), 2314–26.

## Microbiological screening for stem cell cryopreservation

Infective agents, particularly viruses, can be transmitted through stem cell preparations as through blood products and may cause significant morbidity and mortality in the recipient. It has been demonstrated that transmission of hepatitis B virus has occurred following common storage in a liquid nitrogen tank that contained 1 patient's hepatitis B surface antigen +ve BM.

### The following tests should be performed on all patients in whom it is planned to cryopreserve stem cells

- Hepatitis B and C serology.
- HIV Ag and AB testing.
- HTLV-1 serology.
- VDRL = syphilis serology.
- Additional serum for storage for retrospective analysis.

These results must be available to transplant laboratories before cryopreservation. Since many of these patients will be receiving blood products as part of their ongoing treatment, they must be performed within 30d of cryopreservation to prevent false -ve antibody tests due to the interval between exposure and seroconversion. In practice these constraints dictate that samples should be taken between 7 and 30d prior to cryopreservation.

Patient samples shown to be -ve for all the above infectious agents should have stem cells stored in a dedicated liquid nitrogen freezer conventionally in the liquid phase.

Patient samples shown to be +ve for any of the above agents should be double bagged and stored in a separate liquid nitrogen freezer in the vapour phase (to reduce transmissibility). Data on all stem cell product samples must be registered in a secure environment on a computerized database with a logical inventory and retrieval system. No material should be imported to the freezers unless a complete negative virological audit storage trail can be demonstrated.



## Stem cell transplant conditioning regimens

Conditioning is the treatment the patient undergoes immediately prior to a SCT. The purpose is to reduce the burden of residual disease; in allogeneic transplant recipients, it also acts as an immunosuppressant to prevent rejection of the graft. Many different protocols using chemotherapy alone or in combination with TBI have been developed. None has proven to be superior. Fractionation of radiation results in less toxicity but the total dose has to be higher to achieve similar immunosuppression. Unrelated transplants might require immunosuppression with anti-T-cell monoclonal antibodies or ATG. Reduced intensity regimens can reduce transplant-related mortality and offers otherwise ineligible patients a potentially curative treatment.

### Examples

See Tables 9.6 and 9.7.

**Table 9.6** Commonly used transplant conditioning regimens by diseases<sup>1,2</sup>

Disease	Regimen	Total dose	Days
AML and CML	Cy/TBI		
	Cyclophosphamide	120mg/kg	-6, -5
	Total body irradiation	12-14Gy	-3 to -1
	Bu/Cy		
	Busulfan	16mg/kg	-7 to -4
	Cyclophosphamide	120mg/kg	-3, -2
ALL	Cy/TBI		
	Cyclophosphamide	120mg/kg	-6, -5
	Total body irradiation	12-14Gy	-3 to -1
	TBI/VP		
	Total body irradiation	12-13.2Gy	-7 to -4
	Etoposide	60mg/kg	-3
	Cy/VP-16/TBI		
	Cyclophosphamide	120mg/kg	-6, -5
	Etoposide	30-60 mg/kg	-4
Total body irradiation	12-13.2Gy	-3 to -1	
Severe aplastic anaemia	Cy/ATG		
	Cyclophosphamide	200mg/kg	-5 to -2
	ATG	90mg/kg	-5 to -3
Thalassaemia	Bu/Cy		
	Busulfan	14-16 mg/kg	-9 to -6
	Cyclophosphamide	200mg/kg	-5 to -2

(continued)



**Table 9.6 (continued)** Commonly used transplant conditioning regimens by diseases<sup>1,2</sup>

Disease	Regimen	Total dose	Days
Fanconi anaemia	Flu/Bu/Cy		
	Busulfan	6mg/kg	-9 to -6
	Fludarabine	100mg/m <sup>2</sup>	-5 to -2
	Cyclophosphamide	40mg/kg	-5 to -2
	ATG <sup>a</sup>		-4 to -1
Myeloma	High dose melphalan	140–200mg/m <sup>2</sup>	-3, -2
Lymphoma	BEAM (LEAM if Lomustin used)		
	Carmustine (BCNU)	300mg/m <sup>2</sup>	-6
	Etoposide	800mg/m <sup>2</sup>	-5 to -2
	Cytarabine	800–1600mg/m <sup>2</sup>	-5 to -2
	Melphalan	140mg/m <sup>2</sup>	-1

<sup>a</sup> ATG dose depends on specific brand of ATG.

**Table 9.7** Examples of reduced intensity conditioning regimens<sup>1,2</sup>

Regimen	Treatment	Total dose
Flu/TBI	Fludarabine	90mg/m <sup>2</sup>
	Total body irradiation	2Gy
Flu/Bu ± ATG	Fludarabine	150mg/m <sup>2</sup>
Flu/Bu ± thiotepa	Busulfan	8–10mg/kg
	ATG <sup>a</sup>	
	Thiotepa	5mg/m <sup>2</sup>
Flu/Mel ± alemtuzumab	Fludarabine	150mg/m <sup>2</sup>
	Melphalan	140mg/m <sup>2</sup>
	Alemtuzumab	140–180mg/m <sup>2</sup>
Flu/Cy	Fludarabine	150mg/m <sup>2</sup>
	Cyclophosphamide	140g/m <sup>2</sup>

<sup>a</sup> ATG dose depends on specific brand of ATG.

## References

- Aschan, J. (2007). Risk assessment in haematopoietic stem cell transplantation: conditioning. *Best Practice Res Clin Haematol*, **20**, 295–310.
- Apperley, J. et al. (2012). *The EBMT-ESH Handbook on Haemopoietic Stem Cell Transplantation*, revised ed. Paris: EBMT.

## Infusion of cryopreserved stem cells

### Equipment

- Dedicated liquid nitrogen freezer containing stem cells in liquid N<sub>2</sub>.
- Water bath heated to 37–40°C.
- Tongs.
- Protective gloves.
- Patient's notes.
- Trolley with: syringes, needles, ampoules of 0.9% saline, blood giving sets, sterile dressing towels and gloves, chlorhexidine spray, bags of 500mL N/saline.

### Ensure the patient is consented and confirm identification

#### Method

1. Write up the stem cell infusion on the blood product infusion chart.
2. 30min before re-infusion, ensure water bath is filled and heated to 37–40°C and give (chlorphenamine) 10mg IV and paracetamol 1g PO.
3. When ready to return the stem cells take the equipment trolley to the patient's bedside.
4. Check the patient's vital signs.
5. Set up a standard blood giving set with micro-aggregate filter. Never use additional filters. Prime with 500mL 0.9% saline, connect to the patient and ensure good flow before starting to thaw any cells.
6. Check the water bath is 37–40°C and using the protective gloves and large tongs remove a bag of cells from liquid nitrogen container. Carefully remove from the outer sleeve and place in water bath and allow 1min. DMSO (dimethyl sulfoxide) cryopreservative is very toxic to cells once thawed so it is important to go straight from rapid thaw to infusion.
7. Remove bag of cells from water bath using the tongs, spray with chlorhexidine and allow it to dry. Check patient identification number and date of birth with the patient and connect to the giving set.
8. Cells should be returned as quickly as possible. Each bag contains approximately 100–150mL. Providing the flow is good, start thawing the next bag. Only thaw the next bag if you are able to finish the previous bag within the next minute. Check the patient's details on every bag.
9. Check the patient's observations at 15min intervals.
10. If the patient complains of abdominal pain, nausea, or feeling faint, slow down the IVI for a short time. If symptoms persist or patient develops chest tightness or wheezing—stop the infusion. O<sub>2</sub> ± nebulized salbutamol may be required. Anaphylaxis rare. Reinfusion must continue.

*Special considerations*

- ▶▶ If the bag splits/leaks do not re-infuse—contents will not be sterile. Very rarely, a bag could start to expand rapidly upon thawing if all the air is not removed from the bag before freezing. A sterile needle may be used to pierce the bag if release of pressure appears essential.
- ▶▶ Acute anaphylaxis is very rare but epinephrine (adrenaline) (1mL of 1:1000) should be available in the patient's room for SC or IM administration.
- ▶▶ Central access required for reinfusion.

## Infusion of fresh non-cryopreserved stem cells

Ensure patient is consented and understands procedure. In general, BM will be in a larger volume than an apheresis product.

### Procedure

1. A medical staff member must be available to start the infusion and stay with the patient for the first 30min.
2. Give paracetamol 1g PO and chlorphenamine 10mg IV 30min prior to start of infusion.
3. Prime blood giving set without an in-line filter with 500mL 0.9% saline and connect to the patient—check there is a good flow.
4. Check details of patient and donation.
5. Check BP, pulse, and chest auscultation before the infusion. Check observations regularly (every 15–30min). Patient on saturation monitor.
6. Cells are given fresh (not frozen). Give stem cells as slowly as possible for the first 15min, then increase the rate to 100mL in 60min. If after 2h the patient is tolerating infusion without problems, increase to 200mL/h until completion.
7. Watch for fluid overload—give diuretic if necessary.

### Complications of stem cell infusion

- Microemboli occasionally cause dyspnoea and cyanosis. O<sub>2</sub> should be available. Slow down or stop the stem cell infusion if dyspnoea.
  - Pyrexia, rash, and rigors can occur—treat with further hydrocortisone 100mg IV and chlorphenamine 10mg IV. More frequently seen in blood group mismatched transplant.
  - More severe reactions can be seen in blood group mismatched transplants due to the red cells present in the donation.
  - Hypertension may occur (especially if patient fluid overloaded). Usually responds to diuretic.
- ▶▶ Acute anaphylaxis is very rare but adrenaline (1mL of 1:1000) should be available in the patient's room for SC or IM administration.
- ▶▶ Central access required for reinfusion.



## Blood product support for SCT

### Irradiation

- All cellular blood products given to allogeneic and autologous SCT patients must be irradiated to prevent transfusion-associated GvHD due to transfused T lymphocytes.
- Transfusion-associated GvHD is often fatal particularly in allografts.
- Irradiation protocol is standard 2500cGy.
- Commence blood product irradiation 2 weeks prior to allogeneic SCT until 1 year post-SCT or off all immunosuppression, whichever is later.
- Commence blood product irradiation 2 weeks prior to autologous SCT until 6 months post-SCT.
- Cell-free blood products, e.g. FFP, cryoprecipitate, or albumin do not need to be irradiated.
- Marrow or blood stem cell transplant itself is never irradiated.

### CMV status of blood products

- CMV is not destroyed by irradiation.
- *Due to leucodepletion of all donations some countries have now switched to no longer requiring CMV -ve products and hence use CMV unscreened products. Further recommendations on CMV are therefore partly historical but remain widely used. These recommendations should therefore be read in conjunction with local guidelines/protocols.*
- Transplant recipients should ideally receive CMV -ve red cells and platelet transfusions regardless of their own CMV status if sufficient CMV -ve blood products available. Transfused CMV carried in donor white cells may cause disease post-transplant regardless of the CMV status of the patient. CMV -ve recipients must always have -ve products.
- Should CMV -ve platelets not be available at any time, it is acceptable to use unscreened leucodepleted red cells or platelets (all donations are now leucodepleted as a standard). This is because CMV is carried predominantly in leucocytes.
- For allograft recipients, additional preventive measures are taken against CMV reactivation (➔ see CMV prophylaxis and treatment, p.446).

### Indications for RBC and platelet transfusions

Identical to those for patients undergoing intensive chemotherapy.

### Management of ABO incompatibility

ABO incompatibility between donor and recipient does not affect the long-term success of the transplant or the incidence of graft failure or GvHD. However, major ABO incompatibility transfusion reactions will occur unless specific steps are taken to manipulate the graft where donor and recipient are ABO mismatched. Furthermore, additional care must be given post-transplant in providing appropriate ABO matched products.

### ABO mismatched definitions

- *Major* ABO mismatch. This is where the recipient has anti-A or anti-B antibody to donor ABO antigens, e.g. group O recipients with group A donor.
- *Minor* ABO mismatch. This is where the donor has antibodies to recipient ABO antigens, e.g. group A recipient with group O donor.
- *Bidirectional* ABO mismatch. Presence of reactive allo-agglutinins in both recipient and donor plasma, e.g. recipient group B, donor group A.

### Management of major ABO mismatch

*Manipulation of donor marrow/stem cells:* red cells are removed in the transplant laboratory by starch sedimentation or Ficoll centrifugation, prior to infusion of the graft.

#### *Choice of red cell and platelet transfusions after HSCT*

- Red cells of group O or recipient's own ABO group should be given. Switched to donor ABO group once ABO antibodies to donor RBC no longer detected and DCT/DAT negative.
- Plasma and platelets should be of donor-type blood group.
- The choice of platelet group is less critical and may be affected by availability. 1st, 2nd, and 3rd choice groups for platelet transfusions are shown in Table 9.8.

### Management of minor ABO mismatch

*Manipulation of donor stem cells:* prior to infusion, the graft will have been plasma reduced in the transplant laboratory by centrifugation to remove antibody that could be passively transferred. Delayed immune haemolysis, which may be severe and intravascular, can occur after minor ABO mismatch due to active production of antibody by engrafting donor lymphocytes. Maximum haemolysis occurs 9–16d post-transplant.

#### *Choice of red cell and platelet transfusions after HSCT*

- Red cells of donor ABO group should be given.
- Plasma and platelets should be of recipient-type blood group. Switched to donor group once ABO antibodies to donor RBC no longer detected and DCT/DAT negative.
- Platelet transfusions 1st, 2nd, and 3rd choice group is shown in Table 9.8.

### Management of bidirectional ABO incompatibility

#### *Choice of red cell and platelet transfusions after HSCT*

- Give group O red cells. Switch to donor ABO group once ABO antibodies to donor RBC no longer detected and DCT/DAT –ve.
- Plasma and platelets group AB until RBC of recipient group no longer detected, then switch to donor group.

**Table 9.8** Initial choice of ABO group of blood/platelets in ABO mismatch BMT (see text for products given once ABO antibodies to donor RBC no longer detected and DCT/DAT negative)

Donor	Recipient	Red cells	Platelets		
			1st choice	2nd choice	3rd choice
<i>Major ABO mismatch</i>					
A	O	O	A	B	O
B	O	O	B	A	O
AB	O	O	A	B	O
A	B	B/O	A	B <sup>a</sup>	O <sup>a</sup>
B	A	A/O	B	A <sup>a</sup>	O <sup>a</sup>
AB	A	A/O	A	B <sup>a</sup>	O <sup>a</sup>
AB	B	B/O	B	A <sup>a</sup>	O <sup>a</sup>
<i>Minor ABO mismatch</i>					
O	A	O	A	B <sup>a</sup>	O <sup>a</sup>
O	B	O	B	A <sup>a</sup>	O <sup>a</sup>
O	AB	O	A <sup>a</sup>	B <sup>a</sup>	O <sup>a</sup>
A	AB	A	A <sup>a</sup>	B <sup>a</sup>	O <sup>a</sup>
B	AB	B	B <sup>a</sup>	A <sup>a</sup>	O <sup>a</sup>

<sup>a</sup> Risk of haemolysis but do not withhold.

### RhD mismatch

Anti-D is not a naturally occurring antibody but may be induced by sensitization with RhD +ve cells through pregnancy or previous incompatible transfusion. It is essential both recipient and donor serum are screened for the presence of anti-D.

- When either donor or recipient serum contains anti-D, RhD –ve blood products should always be given post-transplant.
- *Note:* in the situation where an RhD +ve recipient receives a graft from a donor whose serum contains anti-D, immune haemolysis may occur despite plasma reduction of the donor marrow due to active production of donor lymphocyte derived anti-D. This cannot be prevented but is rarely severe.
- Provided neither donor nor recipient have anti-D in the serum, specific pre-transplant manipulation of the product is only required in the situation of RhD +ve donor going into RhD –ve recipient where red cell depletion is required pre-transplant.
- It will occasionally be necessary to give RhD +ve platelet support when RhD –ve is preferable simply due to lack of availability of RhD –ve platelet products.
- If RhD +ve platelets have to be given, anti-D 250IU SC can be given post-transfusion.





## Graft-versus-host disease (GvHD) prophylaxis

*In vitro* T-cell depletion of graft or *in vivo* T-cell depletion with alemtuzumab or antithymocyte globulin (ATG) are successful in reducing both the incidence and severity of GvHD in graft recipients. However there is an association with an ↑ risk of relapse. Both are used in VUD and haplo-identical grafts where the risk of severe GvHD is ↑. The 'Seattle protocol' is most commonly used post graft infusion and consists of a combination of ciclosporin and stat pulses of IV MTX. Ciclosporin is initially given IV (od) from before stem cell infusion and later switched to oral dosing (bd). Some RIC regimens use oral ciclosporin from the outset.

### MTX dosage

- MTX: IV bolus 15mg/m<sup>2</sup> on day +1, then 10mg/m<sup>2</sup> days +3, +6 and +11.
- Folinic acid rescue 15mg/m<sup>2</sup> IV tds may be given 24h after each MTX injection for 24h (rescue protocol designed to reduce mucositis), but not always needed.
- MTX dose reduced depending on renal/hepatic function.
- See Table 9.9.

**Table 9.9** Methotrexate dosage reductions

Creatinine (micromol/L)	MTX dose (%)
<145	100
146–165	50
166–180	25
>180	omit dose
Bilirubin (micromol/L)	MTX dose (%)
<35	100
36–50	50
51–85	25
>85	Omit dose

### Side effects

Although reduced by folinic acid rescue, mucositis may remain severe and require IV diamorphine and occasionally omission of dose.

### Ciclosporin administration

Powerful immunosuppressant with profound effects on T-cell suppressor function. Available for IV and oral use.

### IV regimen

Commence on day -1. Dosing schedules vary. Range from 5mg/kg OD IV on day -1 followed by 3mg/kg from day 0, to 1.5 mg/kg IV BD from day -1. Given in 100mL 0.9% saline/2h. If flushing, nausea, or pronounced tremor, slow infusion rate 4–6h/dose. Following loading, adjust ciclosporin dosage based on plasma ciclosporin level together with renal and hepatic function.

### Oral regimen

Switch IV → oral when patient can tolerate oral medication and is eating and when there are no clinical concerns regarding absorption. Dosage conversion is 2 × the IV dose. If od IV schedule is used, this is changed to bd dosing once oral. Some RIC conditioning regimens use oral ciclosporin from the start, commencing on day -2.

### Monitoring ciclosporin levels

- Ciclosporin is toxic and renal impairment is the most frequent dose-limiting toxicity.
- Ciclosporin levels should be monitored at least twice weekly. Levels taken just before the next dose is due (trough level).
- Never take blood for ciclosporin levels from the central catheter through which ciclosporin has been given as ciclosporin adheres to plastic and falsely high levels will be obtained. One lumen should be marked for ciclosporin administration and another lumen marked for blood levels testing.

Instruct patient to delay the morning dose of ciclosporin until after the blood level has been taken. The optimum blood ciclosporin level is not known and varies between regimens and centres. Target range generally 100–300ng/mL. Aim towards the top of the therapeutic range in the early post-transplant period and lower part of the range at later times. In practice, the dose is often limited by a rise in serum creatinine. If serum creatinine >130micromol/L—adjust dose.

### Dosage adjustment

Ciclosporin has a very long  $t_{1/2}$ .

- To ↓ ciclosporin level omit 1–2 doses and make a 25–50% reduction in ongoing maintenance dose, recheck levels at 48h.
- To ↑ levels, give 1 additional dose, increase maintenance dose by 25–50%, recheck level in 48h.
- Monitor renal function and LFTs daily. Check serum calcium and magnesium twice weekly.

### Ciclosporin toxicity

- Nephrotoxicity. Worse with concurrent use of nephrotoxic drugs (e.g. aminoglycosides, vancomycin, and amphotericin).
- Hypertension—often associated with fluid retention and potentiated by steroids. Treat initially with diuretic to baseline weight.
- Neurological syndromes, esp. grand mal seizures (usually if untreated hypertension/fluid retention).
- Anorexia.
- Nausea and vomiting.
- Tremor (almost always occurs—if severe suggests overdosage).
- Hirsutism and gum hypertrophy with prolonged usage.
- Hepatotoxicity—less common than nephrotoxicity. Usually intrahepatic cholestatic picture on LFTs. Potentiated by concurrent drug administration, e.g. macrolide antibiotics, norethisterone, and the azole antifungals.
- Hypomagnesaemia commonly occurs. Potentiated by combination with amphotericin. Give 20mmol IVI if levels <0.5micromol/L or if symptoms develop. Oral supplements often needed and given throughout.

- If concerns regarding malabsorption (patient with diarrhoea), consider switching back to IV ciclosporin temporarily to maintain adequate therapeutic levels.
- The same concern arises in patient with severe mucositis who are unable to take their ciclosporin orally.
- Post-transplant TTP 2° to ciclosporin. Emergency and potentially life-threatening. Requires instant intervention. Neurological symptoms, renal impairment, and raised LDH raise the suspicion; MAHA on blood film confirms the diagnosis. Ciclosporin should be stopped immediately and an alternative agent started.

### Ciclosporin drug interactions

See Table 9.10. *Levels might need to be checked more frequently.*

**Table 9.10** Ciclosporin drug interactions

Drugs that ↑ ciclosporin levels	Drugs that ↓ ciclosporin levels
Azole antifungals	Rifampicin → major effect
Digoxin	Phenytoin → major effect
Macrolide antibiotics, especially erythromycin	Sulfonamides
	Carbamazepine
Imipenem/meropenem	
Ca <sup>2+</sup> channel blockers	
Oral contraceptives	

### Drugs worsening ciclosporin nephrotoxicity

- Aminoglycosides.
- Amphotericin.
- Ciprofloxacin.
- Co-trimoxazole.
- ACE inhibitors.
- Other nephrotoxic drugs.

### Mycophenolate mofetil (MMF)

- Immunosuppressant.
- Reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH) in purine (guanine) biosynthesis which is necessary for the growth of T cells and B cells.
- Guideline starting dose 1g bd IV. Comes in oral preparation as well.
- MMF/CsA appears to have equivalent activity to CsA/MTX in control of acute GvHD with reduced mucositis and duration of neutropenia but a possible ↑ risk of viral infection, myelosuppression, and gut ulceration.
- 2nd choice of agent if ciclosporin has to be discontinued.

**Tacrolimus**

- Calcineurin inhibitor that prevents early T-cell activation; mechanism of action, pharmacology, drug interactions, and toxicity similar to ciclosporin.
- Renal impairment, neurotoxicity, and cardiomyopathy.
- 0.03mg/kg/d by continuous IV infusion from day -2; once weaning starts taper 20% every 2 weeks. Monitor blood level and toxicities and modify dose accordingly.

*Methylprednisolone, sirolimus, alemtuzumab, cyclophosphamide occasionally used.*

## Acute GvHD

The major cause of early transplant-related mortality. Median incidence of clinically significant (grade II–IV) acute GvHD is ~40%. Risk factors for acute GvHD include: older recipients, older donors, ♂ recipient of ♀ SCT (↑ risk with previous donor pregnancies), matched unrelated donors, haploidentical sibling donor, PBSC graft (> BM > cord blood).

*Defined as GvHD occurring within 1st 100d post-transplant (most frequently between d7 and d28 post-transplant). Ranges from mild self-limiting condition to extensive disease and may be fatal. Characterized by fever, rash, abnormal LFTs, diarrhoea, suppression of engraftment and/or viral reactivation, particularly CMV.*

Classified according to the Seattle system by a *staging* for each organ involved (skin, liver, gut) and overall clinical *grading* based on the organ staging (Table 9.11).

*Skin* involved in >90% cases. May be mild and unremarkable maculopapular rash (esp. palms of hands and soles of feet, but can affect any part of the body). May be pruritic and/or painful. In more severe cases, erythroderma and extensive desquamation and exfoliation can occur.

*Liver* typical pattern of LFT abnormalities is intrahepatic cholestasis with ↑ bilirubin and alkaline phosphatase (relative sparing of transaminases). *Note:* this picture often does not discriminate between other causes of post-transplant liver dysfunction (e.g. drugs, infection—particularly CMV and fungal).

*Gut* may occasionally be only organ involved, with anorexia, nausea and vomiting. Diarrhoea, typically green and watery. In severe cases can contain fresh blood and mucosa and is accompanied by abdominal pain and on occasion paralytic ileus.

### Diagnosis

- Skin biopsy. Do not delay treatment if strong clinical suspicion.
- Sigmoid/rectal biopsy; helpful to distinguish infective from pseudo-membranous colitis but beware risk of bleeding and bacteraemia—perform only if it will alter management.
- Where GI symptoms are predominantly upper GI, gastroscopy with oesophageal, gastric, and duodenal biopsies may be helpful (e.g. to distinguish between CMV and fungal oesophagitis and gastritis).
- Liver biopsy is hazardous and should only be performed where other convincing diagnostic guides are not available. It should be performed only by the transjugular route by an experienced operator and covered appropriately with blood products.

**Table 9.11** Consensus criteria for grading acute GvHD

Stage	Skin	Liver	Gut
1	Rash <25% body surface	Bilirubin 34–50micromol/L	Diarrhoea >0.5L/day
2	Rash 25–50% body surface	Bilirubin 51–102micromol/L	Diarrhoea 1–1.5L/day
3	Generalized erythroderma (>50%)	Bilirubin 103–255micromol/L	Diarrhoea >1.5L/day
4	Generalized erythroderma with bullae and desquamation	Bilirubin >255micromol/L	Severe abdominal pain ± ileus
<b>Overall clinical grading for the patient</b>			
<i>Grade</i>			
I	Stage 1–2	None	None
II <sup>a</sup>	Stage 1–3	Stage 1	and/or Stage 1
III <sup>b</sup>	Stage 2–3	Stage 2–3	and/or Stage 2–3
IV <sup>c</sup>	Stage 2–4	Stage 2–4	and/or Stage 2–4

<sup>a</sup> Mild decrease in clinical performance.

<sup>b</sup> Marked decrease in clinical performance.

<sup>c</sup> Extreme decrease in clinical performance.

## Treatment

### General measures

Good nutrition and weight maintenance important. TPN may be necessary. IV antibiotics and antifungals often necessary in the absence of neutropenia and signs of infection may be masked by steroids. Continue ciclosporin during acute GvHD. CMV-PCR to exclude reactivation.

### Specific treatment

Should always be discussed with an experienced transplant haematologist. Mild GvHD confers a GvL effect; hence in mild forms GvHD may require no treatment. Patient will require very close monitoring and must be instructed to contact the transplant centre instantly if symptoms worsen or new symptoms arise.

### 1st-line treatment

Steroid treatment is the mainstay of acute GvHD management. If there is gut GvHD or diarrhoea switch ongoing ciclosporin IV.

- Patients with grade IIa GVHD (not progressing rapidly): methylprednisolone (MP) 1 mg/kg ± beclometasone, 1–2mg 4 times a day, ± budesonide 3mg twice a day.
- Grades IIb to IV GVHD: 2mg/kg (± beclometasone and budesonide).
- Continue 7–14 days then tapered slowly.
- If no response consider adding other agent (no evidence for higher doses of steroids).

### *Side effects of steroid treatment*

- Gastric/peptic ulceration; proton pump inhibitor preferred over H<sub>2</sub> blocker.
- Hyperglycaemia, particularly when TPN in use. Monitor blood sugars closely.
- Hypertension may be potentiated by ciclosporin and by fluid retention; treat with diuretics.
- Insomnia and psychosis.

### **Supportive care**

- Very important; can include GI rest, parenteral nutrition, replacement of enteral fluid loss, pain control and infection prophylaxis.
- Very close monitoring.
- Requires antifungal prophylaxis.

### **Failure of response to high-dose methylprednisolone**

Defined by:

- Progression after 3d.
- No change after 7d.
- Incomplete response after 14d.

The prognosis of steroid refractory GvHD is very poor. Response to 2nd-line therapy ranges from 35–70% but 6–12-month survival is poor because of infectious complications and/or recurrence of GvHD. Long-term survival 25–35%. There are no systematic randomized studies; various alternative/2nd-line treatments have been and are used. There is no consensus on standard 2nd-line treatment. Options for treatment of steroid refractory GvHD are listed in Table 9.12

### **Extracorporeal photopheresis (ECP)**

Photodynamic treatment: blood is initially treated with 8-methoxypsoralen (a photoactivable agent) which binds to DNA of nucleated cells following photoactivation. Via an apheresis technique the blood leaves the patient and is exposed to UV light (the photoactivation) before returning back into the patient; this photoactivation leads to photochemically damaged T cells, hence treating GvHD. Used in treatment of skin and liver GvHD.

### **Prognosis**

Skin involvement is more likely to respond than liver or gut acute GvHD. Viral, bacterial, and fungal infections are the most common cause of death in patients with severe acute GvHD. The outcome depends on:

- Overall grade of acute GvHD.
- Time of onset.
- Response to treatment.



**Table 9.12** Treatments used in steroid refractory acute GvHD<sup>1</sup>

Organ with predominant GVHD manifestation	2° therapy
Skin	ATG Denileukin diftitox Monoclonal antibodies (anti-CD25, anti-CD3, anti-CD52) Phototherapy (PUVA, ECP) Chemotherapy (MMF, calcineurin inhibitors, pentostatin, sirolimus)
Liver	ATG Denileukin diftitox Monoclonal antibodies Chemotherapy (sirolimus, pentostatin, calcineurin inhibitors) Phototherapy (ECP)
GI tract	Non-absorbable steroids (beclomethasone, budesonide) ATG TNF $\alpha$ blockade (infliximab, etanercept) Chemotherapy (sirolimus, pentostatin, calcineurin inhibitors) Octreotide

## Reference

1. Deeg J. (2007). How I treat GVHD. *Blood*, **109**, 4119–26.

## Chronic GvHD

The 1° cause of late morbidity and mortality after an allograft. By definition occurs >100d post-allogeneic transplant (most frequently d100–300). Median incidence 30–45%. The major risk factor is previous acute GvHD. Other risk factors are increasing recipient age (children less frequently affected), prior acute GvHD, CMV seropositivity of recipient and/or donor, higher degree of mismatch, donor age, ♀ donor to ♂ recipient, inadequate prophylaxis, busulfan conditioning, graft T-cell dose, and donor lymphocyte infusion (DLI). Preceding acute GvHD not always seen; acute GvHD may have resolved prior to onset of chronic GvHD.

### Diagnosis

- Diagnosis of chronic GvHD requires at least 1 diagnostic manifestation or at least 1 distinctive manifestation confirmed by the pertinent biopsy or test. See Table 9.13.
- Conventionally subdivided into limited or extensive chronic GvHD. Major clinical features are debility, weight loss with malabsorption, sclerodermatous reaction due to excessive collagen deposition, severe immunosuppression, and features of autoimmune disease. See Table 9.14.

### Limited chronic GvHD—clinical features

- Localized skin involvement <50% total surface.
- Hepatic dysfunction—portal lesions but lacking necrosis, aggressive hepatitis, or cirrhosis.
- Other localized involvement of eyes, salivary glands, and mouth.

### Extensive chronic GvHD—clinical features

- Generalized skin involvement >50% of surface—may include sclerodermatous changes and ulceration.
- Abnormal liver function—histology shows centro-lobular changes, chronic aggressive hepatitis, bridging necrosis, or cirrhosis.
- Liver dysfunction ± localized skin GvHD with involvement of eyes, salivary glands, or oral mucosa on labial biopsy.
- Involvement of any other major organ system.

**Table 9.13** Signs and symptoms of chronic GvHD<sup>1</sup>

Organ/site	'Diagnostic'	'Distinctive'	Other	Common (both acute and chronic)
Skin	Poikiloderma, lichen planus-like features, sclerotic features	Depigmentation	Sweat impairment, hypopigmentation, hyperpigmentation	Erythema, maculopopular rash, pruritis
Nails		Dystrophy, Brittle features		
Scalp		Alopecia	Premature grey hair	
Mouth	Lichen planus	Xerostomia		Mucositis
Eyes		Dry eyes, keratoconjunctivitis, sicca	Photophobia, blepharitis	
Genitalia	Lichen planus	Erosions, fissures, ulcers		
GI tract	Oesophageal web, strictures of oesophagus		Exocrine pancreatic insufficiency	Anorexia, weight loss, nausea, vomiting
Liver				Bilirubin or ALP/ALT >2 × ULN
Lung	Bronchiolitis obliterans			BOOP: bronchiolitis obliterans organizing pneumonia
Muscles, fascia, joints	Fasciitis, joint stiffness secondary to sclerosis	Myositis, polymyositis	Oedema, muscle cramps, arthralgia, arthritis	
Haemopoietic and immune			Thrombocytopenia, eosinophilia, lymphopenia, hypo- or hypergamma-globulinaemia	
Other			Peripheral neuropathy, myasthenia gravis, ascites, pericardial or pleural effusion	

**Table 9.14** Criteria for classification of chronic GvHD

Classification	Criteria
Subclinical	Histological evidence on screening biopsies without clinical signs or symptoms
Limited	Localized skin or single organ involvement not requiring systemic therapy
Extensive low risk	Platelet count $>100 \times 10^9/L$ and extensive skin disease or other organ involvement requiring systemic therapy
Extensive high risk	Platelet count $<100 \times 10^9/L$ and extensive skin disease or other organ involvement requiring systemic therapy

### Grading of chronic GvHD

The NIH Working Group<sup>2</sup> consensus for global grading of chronic GvHD encompasses both the number of organs/sites involved and the severity of chronic GvHD within each organ. See Table 9.15.

*Mild cGvHD* = only 1 or 2 sites involved (except lung) with a maximum severity score of 1.

*Moderate cGvHD* = 1 or 2 sites involved with score 2, or  $\geq 3$  organs with score 1, or lung involvement with score 1.

*Severe cGvHD* = score 3 in any number of sites or score 3 in lung.

**Table 9.15** NIH Consensus for global grading of cGvHD

Number of organs/sites	Mild	Moderate	Severe
1 site	Score 1	Score 2	Score 3
2 sites	Score 1	Score 2	Score 3
$\geq 3$ sites		Score 1	Score 3
Lung involvement		Score 1	Score 2

Score 0 = no symptoms.

Score 1 = mild symptoms.

Score 2 = moderate symptoms.

Score 3 = severe symptoms.

### Treatment

Always discuss with a senior member of transplant team. Limited chronic GvHD is much easier to treat than extensive chronic GvHD. Localized mucosal or skin involvement often responds to topical steroids.

### General measures

- Adequate nutrition, vitamin/calorie supplements may be required and severe cases may require TPN.
- Pneumococcal prophylaxis must be continued lifelong (infection with *S. pneumoniae* and *H. influenzae* common).
- Restart conventional prophylaxis with antiviral (aciclovir) and antibacterial agents.
- Antifungal prophylaxis essential in patients treated with prolonged courses of immunosuppression. Posaconazole superior to other agents.
- CMV surveillance is critical as reactivation is more common.
- *P. jirovecii* prophylaxis must be commenced with co-trimoxazole or nebulized pentamidine and continued for 6 months after immunosuppressive therapy is discontinued.
- Ursodeoxycholic acid may be given for cholestasis in patients with hepatic chronic GvHD.
- Artificial tears for patients with ocular involvement.
- Skin hydration/emollients to lubricate skin; avoid perfume and sun-exposure.
- Regular dental care for patients with oropharyngeal involvement as caries ↑.
- Bisphosphonates for patients on long-term steroids to prevent osteoporosis.
- Psychological support may be required to adjust to chronic disability.

### Specific treatment

- Commonest protocol used is the Seattle regimen using prednisolone and ciclosporin. If disease stable or improved after 2 weeks taper prednisolone by 25% per week to target dose of 1mg/kg every other day to minimize side effects.
- After successful completion of steroid taper, reduce ciclosporin slowly.
- If resolved completely at 9 months, slowly wean patient from both medications.
- Incomplete responses should be re-evaluated after 3 months more therapy; if fail to respond or progress then salvage therapy required.
- 2nd-line therapy includes ECP, azathioprine, thalidomide, tacrolimus, sirolimus inhibitors, mycophenolate, etanercept, imatinib and TLI. Efficacy remains limited and clinical trials remain urgently needed.

### Prognostic factors

Thrombocytopenia is an adverse prognostic factor for survival from diagnosis of chronic GvHD. Other factors associated with poor outcome are progressive onset, lichenoid skin rash, elevated bilirubin, poor performance status, and sex mismatched donor. Different prognostic models for chronic GvHD survival exist.

### Prognosis

In a retrospective series only 10–30% of patients with extensive chronic GvHD became long-term survivors. Prompt introduction of effective immunosuppressive therapy has improved the outcome for these patients.

## References

1. Apperley, J. et al. (2012). *The EBMT-ESH Handbook on Haematopoietic Stem Cell Transplantation*, revised ed. Paris: EBMT.
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## Veno-occlusive disease (*syn. sinusoidal obstruction syndrome*)

Presents clinically early post-transplant (usually within the first 14d). Pathophysiology poorly understood, probably due to damage to hepatic sinusoidal endothelial cells leading to venous occlusion and liver failure. A procoagulant state develops with low antithrombin and protein C levels, consumption of FVII, and ↑ levels of plasminogen activator inhibitor-1 (PAI-1). Multiorgan failure and death may follow. Mortality is very high.

Risk factors for severe VOD include: intensive/myeloablative conditioning regimens, pre-transplant hepatitis, and 2nd/subsequent transplants. VOD is characterized by a triad of hepatomegaly, jaundice and ascites. In addition there is fluid retention and weight gain as a result of this. It is much more commonly seen in allografts compared to autografts.

Diagnosis is largely and initially clinical, but may be supported by typical findings on Doppler ultrasound study of hepatic arterial and venous flows, or by elevated PAI-1 levels. The only definitive diagnostic investigation is transjugular liver biopsy, the risks of which must be weighed against the importance of the information obtained. Diagnostic criteria vary and are listed in Table 9.16.

### Differential diagnosis

- Acute GvHD.
- Sepsis with hepatic failure.
- Cholestasis due to medication or TPN.
- Haemolysis.
- CCF.

**Table 9.16** Criteria for diagnosis of VOD (SOS)

	McDonald (Seattle) 2 out of 3	Jones (Baltimore) hyperbilirubinaemia plus 2 out of 3
Hepatomegaly or right upper quadrant pain	+	+
Weight gain (>2% from pre-transplant baseline)	+	+
Hyperbilirubinaemia (>2mg/dL or 34 micromol/L)	+	+
Ascites	–	+

Adapted from Abinun, M. and Cavet, J. (2007). Gastrointestinal, respiratory and renal/urogenital complications of HSCT. In Cant, A.J. et al. (eds.) *Practical Haematopoietic Stem Cell Transplantation*. Blackwell Publishing, Oxford, pp.126–32, with permission.



## Risk assessment

See Table 9.17.

**Table 9.17** Scoring system for VOD

Clinical feature	Score
Progressive and persisting raise in bilirubin:	
● Serum bilirubin $\geq 34$ but $< 75$ micromol/L or	1
● Serum bilirubin $\geq 75$ micromol/L	2
Persisting hepatomegaly ( $> 2$ cm from baseline)	1
Ascites	1
Persisting weight gain (from baseline)	
● $\geq 5\%$ but $< 15\%$ or	1
● $\geq 15\%$	2
Raised PT or need to transfuse platelets regularly	1
Risk factors (1 or more present):	1
● Age $< 6$ months or	
● Raised ALT pre-SCT	
Associated organ system failure <sup>a</sup> (excluding liver failure):	
● Each system failure	1
● Maximum	2
Maximum total score	10
Calculate total score on daily basis:	
● Score of 4 suggests early or impending VOD	
● Score of 5–7 suggests moderate VOD and specific treatment is recommended	
● Score of 8–10 indicates severe VOD and a poor prognosis	

<sup>a</sup> Respiratory failure needing ventilation, circulatory failure needing inotropes, renal failure needing dialysis or need for abdominal paracentesis.

Adapted from Bajwa, R.P.S. et al. (2003). Recombinant tissue plasminogen activator for treatment of hepatic veno-occlusive disease following bone marrow transplantation in children: effectiveness of a scoring system for initiating treatment. *Bone Marrow Transplant*, **31**, 591–7, with permission, copyright 2003.

## Prophylaxis<sup>1</sup>

- Avoid hepatotoxic drugs.
- Ursodeoxycholic acid 600–900mg/d oral. Reduction in VOD and TRM.
- LMWH prophylaxis. May have some effect. Requires adequate platelet count (as per local protocol).
- Defibrotide. Can be used. Not routinely used in most centres.

### Treatment<sup>2</sup>

- Restriction of salt and water—strict fluid balance chart  $\pm$  diuretics.
- Maintain intravascular volume and renal perfusion.
- Careful and strict electrolyte balance management.
- Analgesics, platelet transfusion, and correction of deranged coagulation.
- Defibrotide 6.25mg/kg IV in 2h infusion 6-hourly for 14d. 50–55% CR in severe VOD with MOF and 47–60% survival at day +100 with no 2° effects. Defibrotide binds to vascular endothelium and stimulates fibrinolysis by increasing endogenous tPA function and decreasing PAI-1 activity; no significant toxicity.
- Antithrombotic and thrombolytic agents: alteplase (recombinant tissue plasminogen activator, r-tPA)  $\pm$  heparin has been used but high risk of fatal haemorrhage.
- Antithrombin III concentrate: occasionally used.
- Analgesia.
- Paracentesis/thoracocentesis.
- Haemodialysis/filtration if required.
- Mechanical ventilation if required.
- TIPS: transvenous intrahepatic portosystemic shunt.
- Surgical shunt.
- Liver transplantation.

### Prognosis

Patients with mild VOD have no apparent adverse effects and generally respond to treatment with analgesics and diuretics and have a good prognosis. Patients with moderate VOD require specific treatment for symptomatic hepatic failure. Severe VOD is associated with severe effects from hepatocellular failure and patients require specific treatment but may fail to respond. See options listed above. Can progress to multi-organ failure, require ITU support and have a mortality >90%.

### References

1. Corbaciogly, S. *et al.* (2010). Defibrotide prevents hepatic VOD and reduces significantly VOD-associated complications in children at high risk: final results of a prospective phase II/III multicenter study. *Bone Marrow Transplant*, **45** (Suppl 2), S1.
2. Ho, V.T. *et al.* (2008). Hepatic VOD after HSCT: update on defibrotide and other current investigational therapies. *Bone Marrow Transplant*, **41**, 229–37.



## Invasive fungal infections and antifungal therapy

Invasive fungal infections are an important cause of morbidity and mortality after allogeneic SCT with a frequency of 10–25% and mortality of >70%.

### Pathogenesis

- Majority of infections are due to *Candida* spp. and *Aspergillus* spp. though infections due to other opportunistic fungi are increasing (*Trichosporon* spp., *Fusarium* spp., *Bipolaris* spp., and *Zygomycetes*, amongst others).
- Invasive *Candida* infections classified as candidaemia or acute disseminated candidiasis, and arise from invasion of bloodstream from infected mucosal surfaces or via central venous catheters; ↓ incidence due to introduction of antifungal prophylaxis though ↑ non-albicans spp. (*glabrata* and *krusei*).
- Invasive *Aspergillus* infections can affect paranasal sinuses and lungs and arise from airborne exposure; ↑ incidence, particularly late after transplantation, as well as in patients with GvHD due to prolonged immunosuppression.
- Risk factors: prolonged and profound neutropenia; use of immunosuppressants, particularly corticosteroids.

### Prophylaxis

- Prophylaxis: high efficiency (>90%) particulate air (HEPA) filtration or positive pressure ventilation.
- Prophylaxis with antifungal agents. Fluconazole 400mg/day (does not cover *Aspergillus* spp., *C. glabrata*, or *C. krusei*), itraconazole 200–400mg/day (poor absorption from capsules; extremely unpalatable liquid preparation) and posaconazole 200mg tds used. Posaconazole has superior cover and efficacy.

### Management

- If a febrile neutropenic transplant patient is unresponsive to 2nd-line antibiotics after 48–72h and/or there is a suspicion of possible fungal infection (unwell; chest symptoms; peripheral nodules, halo sign or cavitation on CT chest, evidence of candidaemia), then antifungal therapy should be started.
- Empirical therapy should be initiated with an agent active against *Aspergillus* spp. as this is the major risk in adult patients with neutropenic sepsis.

### Treatment of invasive aspergillosis

#### *Liposomal amphotericin*

- Follow local guidelines.
- AmBisome achieves responses in ~50% of patients with proven/probable invasive aspergillosis at a dose of 3mg/kg/d with 12-week survival of 72% in a large randomized trial. It is better tolerated than amphotericin B lipid complex (Abelcet) and more effective and better tolerated than amphotericin B colloidal dispersion though no trials have undertaken direct comparisons.

- Commence liposomal amphotericin (AmBisome) at 3mg/kg/d with initial 1mg test-dose. In practice round up or down to standard vial size to avoid wastage and minimize cost.
- The dosage can be ↑ to a maximum of 5mg/kg AmBisome in patients who have either a confirmed mycological diagnosis or a fever that does not respond within 72h on the lower dose.
- Serum Mg<sup>2+</sup> and LFTs must be monitored.

#### Voriconazole

- A 2nd-generation triazole. 48% response rate. Shown to be superior to standard amphotericin in antifungal efficacy, safety, and survival in a randomized trial and widespread treatment of choice for invasive aspergillosis due to more favourable toxicity profile.
- Dose 6mg/kg IV bd d1 then 4mg/kg IV bd maintenance converting to oral 200mg bd (may commence orally with 400mg bd loading dose on d1).
- IV voriconazole is contraindicated in renal insufficiency.
- Side effects: visual disturbances, rash, elevated LFTs and with IV administration, flushing, fever, tachycardia, and dyspnoea.

#### Caspofungin

- An echinocandin which targets the fungal cell wall and is active against *Candida* and *Aspergillus* spp. Higher response rate demonstrated in comparison to amphotericin in treatment of invasive candidiasis.
- Loading dose of slow IV infusion of 70mg on d1 followed by maintenance dose of 50mg/d (lower maintenance in moderate liver insufficiency).
- It may also be used for treatment of invasive aspergillosis refractory to amphotericin preparations. Caution with concomitant ciclosporin: monitor LFTs.
- Side effects: phlebitis, fever, headache, rash, abdominal pain, nausea, diarrhoea.

#### Combination therapy

- A combination of voriconazole and caspofungin as salvage therapy after failure of amphotericin B provided a substantially improved 3-month survival in allogeneic SCT recipients compared with a historical control group receiving voriconazole monotherapy.

*Total duration of treatment is difficult to determine. General principles are that therapy should continue for at least 2 weeks, until neutrophil recovery and until there are no signs of progression radiologically.*

## Recommendations of ECIL Working Group<sup>1</sup>

### Treatment of invasive aspergillosis

#### 1st-line therapy

Voriconazole was strongly recommended as 1st-line therapy for pulmonary aspergillosis in patients with acute leukaemia or after SCT by a Working Group at the European Conference on Infections in Leukaemia (ECIL). Liposomal amphotericin and amphotericin lipid complex were graded as alternatives when voriconazole is contraindicated or unavailable.

Amphotericin colloidal dispersion and standard amphotericin B deoxycholate were not recommended. There were insufficient data in this setting to grade caspofungin, itraconazole and posaconazole.

#### Salvage therapy

No data are available for any agent in the context of voriconazole failure. Caspofungin and posaconazole are options. Voriconazole is an option for patients who did not receive it as 1st-line therapy. Combinations of caspofungin and voriconazole or caspofungin and liposomal amphotericin were scored as options.

#### Optimal duration of therapy

Therapy must be long enough to achieve complete response and to allow recovery from immunosuppression. No fixed duration can be proposed.

Note: as with all protocols check local policies since these may differ from those outlined in this handbook.

### Treatment of invasive candidiasis

- Treatment is initiated on detection of *Candida* spp. on blood culture before species and susceptibility are identified.
- Liposomal amphotericin, caspofungin, or voriconazole generally used as the patient has generally been on fluconazole, itraconazole, or posaconazole prophylaxis.
- Caspofungin is the drug of choice for the treatment of confirmed *C. glabrata* or *C. krusei* infections. Voriconazole is an alternative and a switch to oral voriconazole can be considered when a patient is stable and able to take oral medication.
- Removal of indwelling IV catheters is recommended in patients with candidaemia.
- Neutropenic patients should receive antifungal therapy for 14d after the last +ve blood culture and resolution of signs and symptoms.

#### Recommendations of BCSH guidelines for invasive fungal infection<sup>2</sup>

- Empirical therapy for antibiotic resistant febrile neutropenia discouraged.
- Antifungal therapy for possible invasive fungal infection should be justified through CT scans and mycological tests.
- Choice of empirical therapy should be between liposomal amphotericin (not in escalated doses) and caspofungin to minimize toxicity.
- Proven intestinal fungal infection may be treated with liposomal amphotericin, invasive or voriconazole; voriconazole is recommended in intracerebral aspergillosis.

### References

1. Herbrecht, R. et al. (2007). Treatment of invasive candida and invasive aspergillus infections in adult hematological patients. *Eur J Cancer*, **5**(Suppl), 49–59.
2. Prentice, A.G. et al. (2008). *BCSH Guidelines on the Management of Invasive Fungal Infections during Therapy for Hematological Malignancy*. [http://www.bcsghguidelines.com/documents/fungal\\_infection\\_bcsgh\\_2008.pdf](http://www.bcsghguidelines.com/documents/fungal_infection_bcsgh_2008.pdf)

### Further reading

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## CMV prophylaxis and treatment

Most transplant recipients who are CMV seronegative continue to receive CMV -ve blood products, although since leucodepletion at source is now standard this is likely no longer needed. Reactivation of the patient's own latent virus is now the main source of CMV disease in recipients of allogeneic SCT (80% of previously seropositive patients reactivate). CMV status in the donor is an important consideration in donor selection. CMV disease has been a major cause of death in allogeneic SCT recipients except where both donor and recipient are seronegative.

### CMV surveillance

- All allograft patients and CMV sero-positive autograft recipients should receive CMV surveillance as active CMV infection may remain asymptomatic and does not always progress to disease.
- Progression to CMV disease is predicted by detection of CMV viraemia with high viral loads by quantitative PCR in PB (EDTA sample).
- 5mL EDTA blood should be sent weekly on the above cohort of transplant patients from admission until d100. Screening of allograft recipients should continue until 1 year post-transplant although the frequency of testing may be reduced in the absence of appropriate symptoms. Urine and throat washings are not sent routinely for CMV detection.

### CMV prophylaxis

Indicated in all allograft patients. Not recommended for autograft recipients.

#### *Suggested protocol*

- Aciclovir 400mg bd or 200mg qds.
- Some centres continue to use higher doses: 800mg tds IV from day -5 to discharge, then 800mg tds PO for 3 months although graft suppression of this dose of aciclovir may be dose limiting.

### Treatment of CMV infection

A +ve CMV identification in PB by surveillance should be treated even if the patient is asymptomatic:

- Ganciclovir: 5mg/kg IV bd for 14–21d then continue maintenance dose 5mg/kg/d IV daily or 6mg/kg/d 5d a week as outpatient.
- Valganciclovir 900mg bd for 21 days oral then 900mg od oral valganciclovir 900mg bd is equivalent to IV ganciclovir 5mg/kg bd.
- Stop aciclovir when ganciclovir or valganciclovir is commenced.
- Side effects:
  - Myelosuppressive, may be abrogated by G-CSF.
  - Nephrotoxic; renal function must be monitored and dose reductions implemented accordingly.
  - Abnormal LFTs may occur.
  - Fever, rashes, and headaches.



- Alternative: foscarnet 90mg/kg IV bd for 14–21d minimum. Administer through a central line as IVI over 2h; may be given as a peripheral IVI but should be given concurrently with a fast running 1L 0.9% saline.
- Side effects: nephrotoxic and hepatotoxic.

### Treatment plan

On a 1st episode of CMV antigenaemia, start with valganciclovir or ganciclovir. Failure to become CMV antigen –ve by the end of the 2-week course would lead to immediate progression to foscarnet. If ganciclovir is used this can be switched to oral valganciclovir once response is seen to aid discharge.

### CMV-related disease

May cause PUO, pneumonitis, oesophagitis, gastritis, enteritis (diarrhoea), hepatitis, retinitis, delayed engraftment, and myelosuppression. Where CMV antigenaemia is accompanied by symptoms or signs of CMV disease high titre anti-CMV Immunoglobulins 200mg/kg IV can be used and administered on d1, 3, 5, and 7 of antiviral therapy, together with ganciclovir or foscarnet. Bronchoalveolar lavage (BAL) should be performed to establish the presence of CMV locally in the lung. If diarrhoea is a presenting symptom, flexible sigmoidoscopy is needed to obtain biopsy to establish a diagnosis (differential diagnosis with GvHD).

## Post-transplant vaccination programme and foreign travel

### General

The subject of revaccination post-transplant remains a contentious topic. The general principles are that live vaccination is contra-indicated, most likely for the lifetime of the patient. Secondly, antibody and T-cell responses to vaccination in the 1st year following transplantation are suboptimal. In allogeneic SCT recipients, immune reconstitution continues beyond 1 and up to 2 years post-transplant. These general considerations have been used to suggest the policy listed below.

### Allogeneic transplants

No immunizations should be given in the presence of acute or chronic GvHD. In the absence of these conditions, proceed as follows:

#### *At 12 months post-matched sibling donor SCT (18 months after others)*

- Diphtheria tetanus toxoid and acellular pertussis course (3 doses at monthly intervals).
- 1st course of inactivated polio vaccine (3 doses at monthly intervals).
- Conjugated 7-valent pneumococcal vaccine (2 doses at 8-week interval).
- Conjugated *Haemophilus influenzae* B (3 doses at monthly intervals).
- Conjugated meningococcal C (2 doses at monthly intervals).
- Influenza vaccine (and annually thereafter).

The vaccinations should be staggered with only diphtheria and tetanus being administered concurrently. It is reasonable to leave a gap of several days between each vaccination. Not only may this enhance antibody responses but it will easily identify the cause if there are any reactions.

#### *At 18 months post-matched sibling donor SCT (24 months after others)*

- MMR.

#### *At 2 years post-matched sibling donor SCT (30 months after others)*

- HiB and conjugated meningococcal C vaccine.

#### *At 25 months post-matched sibling donor SCT (31 months after others)*

- MMR and conjugated 7-valent pneumococcal vaccine.

#### *At 30 months post-matched sibling donor SCT (3 years after others)*

- Polysaccharide pneumococcal vaccine.

### **Autologous SCT for lymphoma or myeloma—1 year post-transplant**

Consider:

- Tetanus booster.
- Inactivated polio vaccine booster.
- Pneumovax II (repeated every 6 years).
- *Haemophilus influenzae* B.
- Meningococcal C.
- Influenza vaccine (repeated annually).

### **Foreign travel**


All transplant recipients should take medical advice from their transplant team before travelling abroad:


- Typhoid, cholera, hepatitis A/B, and meningococcal vaccines are safe.
- Yellow fever and Japanese B encephalitis are not safe.
- Remember malaria prophylaxis.

*Avoid live vaccines. e.g.:*

- Yellow fever.
- BCG.
- Oral polio.
- Oral typhoid.

## Longer term effect post-transplant

Patients must be monitored indefinitely. GvHD has been discussed on  pp.428–437. Long-term effects/side effects are common and must be part of counselling pre-transplant and must be monitored for post-transplant.

- Endocrine: hypothyroidism may occur post-transplant. Check TFTs at 3-monthly intervals initially and yearly thereafter.
- Respiratory: check lung function tests at 6 months and 1 year if TBI has been given.
- Skin: advise about sun protection (following TBI avoid the sun). If exposure is unavoidable, total sun block factor 15 or higher is essential for at least 1 year.
- Fertility: most patients will be infertile after transplant (almost invariably if TBI given). Since this cannot be absolutely guaranteed, contraceptive precautions should be taken until the confirmatory tests have been performed.
  - ♂: check sperm counts at 3 and 6 months post-transplant. Zero motile sperm on both samples confirms infertility.
  - ♀: check FSH, LH, and oestradiol at 3 months. FSH and LH levels should be high and oestradiol levels low if no ovulation is occurring.
- Menopause: women may have an early menopause due to the treatment and may experience symptoms such as hot flushes, dry skin, dryness of the vagina, and loss of libido. Women can have hormone replacement therapy and must be counselled about HRT problems.
- Cataracts: patients who have had TBI are at risk of developing cataracts. Refer for ophthalmological assessment at 1 year post BMT.
- Immunizations at 12–30 months post-transplant ( see Post-transplant vaccination programme, p.448).
- Late cardiac complications.
- Late vascular complications.
- Late metabolic complications: HT, hyperlipidaemia, diabetes.
- Chronic kidney disease.
- Late liver complications and iron overload.
- Osteoporosis.
- 2° haematological malignancies.
- 2° solid tumours.

## Treatment of relapse post-allogeneic SCT

Recurrence of leukaemia, myeloma, or lymphoma after an allogeneic SCT may be treated by DLI, a 2nd transplant (in those patients with a durable 1st response who are fit enough to withstand a 2nd allograft; ideally from a different donor), or conventional dose or palliative treatment.

### Donor lymphocyte infusion

- May be used in CML, AML, ALL, NHL, HL, and myeloma.
- DLI can promote full donor chimerism in patients with mixed chimerism or residual tumour after reduced intensity non-myeloablative conditioning.
- Patient should discontinue ciclosporin and steroid therapy at least 2 weeks before DLI and chemotherapy at least 24h before DLI.
- Donor lymphocytes are collected by leucapheresis; a typical collection of 150mL contains  $\sim 50 \times 10^8$  T lymphocytes.
- Escalating doses are generally used to limit GvHD:
  - 1st dose  $10^7$  donor lymphocytes followed by
  - 2nd dose (12 weeks later if no response):  $5 \times 10^7$  cells
  - 3rd dose (12 weeks later if no response):  $10^8$  cells
  - Subsequent doses  $>10^8$  cells.
  - Lower initial doses and increments are utilized in VUD SCT.
- Where possible, e.g. CML or AML, molecular monitoring may be undertaken and DLI may be utilized for molecular relapse with molecular monitoring of response.
- The main adverse effect of DLI is acute or chronic GvHD especially if administered early after SCT. The incidence of these complications has been  $\downarrow$  by the adoption of an escalating dose regimen but is  $\uparrow$  with VUD SCT.

## Discharge and follow-up

### Criteria for discharge

Blood counts should ideally be: Hb  $>10.0\text{g/dL}$  (but may require transfusions), neutrophils  $>1.0 \times 10^9/\text{L}$ , platelets  $>25 \times 10^9/\text{L}$ , and patients should be able to maintain a fluid intake of 2–3L/d, tolerating diet and oral medications particularly in patients on ciclosporin.

### Counsel patients

- Possible need for blood/platelet transfusions.
- Check temperature and report immediately if febrile.
- Fatigue post-transplant in irradiated patients due to the late TBI effect usually 6–10 weeks post-transplant. Post-TBI somnolence.
- Risk of HZV (explain the early symptoms).
- To continue with mouth care.
- To report any new symptoms.

### Blood tests—initially twice weekly

- FBC, reticulocytes, and blood film (to exclude MAHA).
- Biochemistry including LFTs.
- Ciclosporin levels pre-dose (EDTA sample)—allografts only.

### Initially once a week

- Magnesium
- CMV-PCR (EDTA) weekly
- Stool culture: allografts if relevant symptoms.

### Drugs

- Ciclosporin capsules: allografts only.
- Aciclovir prophylaxis 6 months.
- Phenoxymethylpenicillin 250mg bd PO should be given to all patients post ablative/TBI regimens. Erythromycin 250mg od PO if penicillin allergic.
- Consider ciprofloxacin 250mg bd PO if neutrophils  $<1.0 \times 10^9/\text{L}$ .
- Co-trimoxazole 480mg bd PO Monday, Wednesday, Friday for 1 year minimum and until CD4 count  $>500$ . Co-trimoxazole should be started when neutrophils  $>1.5 \times 10^9/\text{L}$  and platelets  $>50 \times 10^9/\text{L}$ . Until then, use nebulized pentamidine 300mg every 3 weeks.
- Ongoing mouthwashes and good oral hygiene.
- Antiemetics PRN.

### Follow-up

- Monitor closely for first 100d (twice-weekly blood tests and weekly clinical review) for engraftment, transfusion requirements, GvHD, CMV, opportunistic infection, side effects of therapy, chimerism, psychosocial needs and relapse.
- Review 4–8 weekly for 1st year after 100d if uncomplicated.
- Review intervals may be extended after 1st year if uncomplicated.

# Haemostasis and thrombosis

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## Assessing haemostasis

### Haemostatic capacity is assessed by:

- History.
- Examination.
- Laboratory investigations.

### Haemophilia

Describes a tendency to bleeding and may be heritable (often congenital) or acquired. Acquired haemophilia due to anticoagulant drugs is very common in the UK as 1 in 100 of the population is receiving oral anticoagulant therapy with warfarin.

### Thrombophilia

Describes a tendency to thrombosis and may be heritable (but usually late onset in adult) or acquired. Acquired thrombophilia is common in hospitalized patients by virtue of immobility, surgery, and multiple medical illness (VTE including DVT and PE).

The haemostatic system maintains the integrity of the vasculature through a complex network of cellular, ligand-receptor, and enzymatic interactions. It is essential that blood remains fluid within the circulation but clots at sites of vascular injury. This is achieved by the equilibrium between:

- Procoagulant and anticoagulant systems.
- Fibrinolytic and antifibrinolytic systems.

In response to endothelial damage there is rapid thrombin generation and antifibrinolysis at the site of injury. At adjacent areas of healthy intact endothelium there is enhanced natural anticoagulant activity and fibrinolytic activity to limit the clot to the site of injury and thus prevent thrombosis (a blood clot within the lumen of a blood vessel). Regulation of the haemostatic network in such a way results in localized clot formation with minimal loss of vascular patency. Pathological disruption of the network results in thrombosis or bleeding, or both; the most extreme example of haemostatic pathology is a complete breakdown as occurs in disseminated intravascular coagulation (DIC).





## The coagulation system

The initiation of coagulation is triggered by tissue factor, a cell membrane protein which binds activated factor VII (factor VIIa). Although there is a small fraction of circulating factor VII in the activated state it has little or no enzymatic activity until it is bound to tissue factor.

Once bound to tissue factor, factor VIIa activates factor IX and factor X (to IXa and Xa respectively) leading to thrombin generation and clot formation (Fig. 10.1).

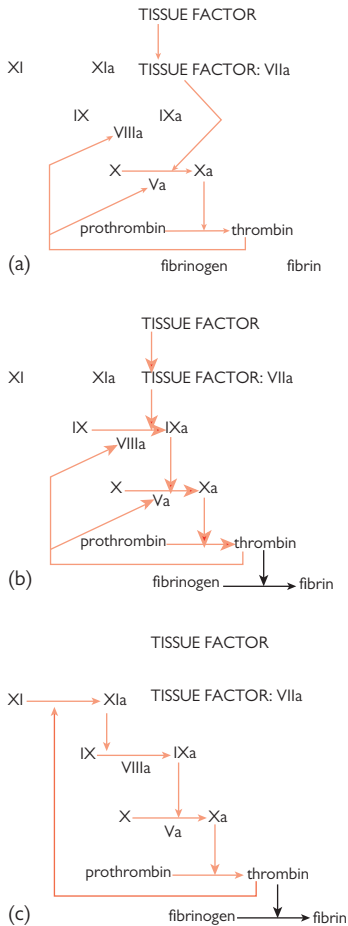
Thrombin converts soluble fibrinogen into insoluble fibrin monomers which spontaneously polymerize to form the fibrin mesh that is then stabilized and crosslinked by activated factor XIII (factor XIIIa).

### Further reading

Baglin, T. (2005). The measurement and application of thrombin generation. *Brit J Haem*, **130**, 653–61.

Hoffman, M. and Monroe, D.M. (2001). A cell-based model of hemostasis. *Thromb Haemostasis*, **85**, 958–65.

Monroe, D. M. and M. Hoffman (2006). What does it take to make the perfect clot? *Arteriosclerosis Thrombosis and Vascular Biology*, **26**, 41–8.



**Fig. 10.1** Thrombin generating mechanism.

The thrombin explosion occurs in 3 phases, the first (Fig. 10.1a: initiation) in which TF:VIIa generates small amounts of thrombin which enhances platelet activation (in conjunction with exposed subendothelial collagen at the site of vascular injury) and V and VIII to their active forms. The activation of Va and VIIIa opens the factor IX-dependent pathway to thrombin generation (Fig. 10.1b: amplification) and increasing concentrations of thrombin then lead to back activation of factor XI (Fig. 10.1c: propagation). The activated forms of factors V and VIII are non-enzymatic cofactors which assemble and orientate the enzymes (IXa and Xa) on the negatively charged surface of activated platelets.

## Laboratory tests

- Prothrombin time (PT).
- Activated partial thromboplastin time (APTT).
- Fibrinogen (Fgn) level.
- Thrombin time (TT).
- Reptilase time (RT).
- Factor assays.
- Mixing studies.
- Thromboelastography (TEG).
- Tests of fibrinolysis.

Coagulation tests are typically performed on plasma that has been separated from a blood sample by centrifugation. Thrombin generation takes place on phospholipid surfaces (provided by platelets normally) and so an artificial lipid preparation is added as the platelets are removed by the centrifugation. Most routine clotting tests use the time taken for a clot to appear as the endpoint of the assay. Blood is usually taken into tubes containing citrate, which chelates  $\text{Ca}^{2+}$  and thereby prevents clotting. Blood is centrifuged and the plasma is removed and recalcified during the clotting assay.

### PT

The PT is the time in seconds for a blood sample to clot after recalcification and addition of thromboplastin (a preparation of tissue factor which is the protein that binds to factor VIIa and initiates thrombin generation). The normal PT is about 11–14sec, depending on the type of thromboplastin used.

*The PT is prolonged by:*

- Oral anticoagulant therapy with a VKA (Vitamin K antagonist such as warfarin).
- Vitamin K deficiency.
- Liver disease.
- DIC.
- Factor VII deficiency (congenital isolated VII deficiency is rare).

### APTT

The APTT is the time taken in seconds for a citrated blood sample to clot after recalcification and addition of a contact factor activator, such as kaolin.

The normal APTT is about 32–38sec, depending on the type of contact factor activator used.

*The APTT is prolonged by:*

- Unfractionated heparin (LMWH has minimal effect at therapeutic levels).
- Oral anticoagulant therapy with a VKA (mildly, the PT is much more sensitive).
- Vitamin K deficiency (mildly, the PT is much more sensitive).
- Liver disease (mildly, the PT is much more sensitive).
- DIC.
- Dilutional coagulopathy (massive blood transfusion).
- Severe and moderate deficiencies of factors VIII, IX, or XI.
- Antiphospholipid antibodies (known as lupus anticoagulant activity).
- Contact factor deficiency (including factor XII and prekallikrein).

## Fgn level

*Fgn levels are low in:*

- DIC.
- Dilutional coagulopathy (massive blood transfusion).
- Advanced liver disease.
- Following thrombolytic therapy.
- Congenital hypofibrinogenaemia (very rare).
- Acquired dysfibrinogenaemias.

## TT

The TT (thrombin time) is the time in seconds for a citrated blood sample to clot after addition of thrombin.

*The TT is prolonged by:*

- Unfractionated heparin.
- Hypofibrinogenaemia (➔ see Rare congenital coagulation disorders, p.482 for low Fgn levels)
- Fibrin degradation products (high levels may occur in DIC and after thrombolysis).

## RT

The RT (reptilase time) is a snake venom-based test. It is prolonged by low Fgn levels but not by heparin and so comparison of the TT and RT is useful for determining if a prolonged APTT is due to heparin (long TT with ↔ RT = heparin).

## Factor assays

Individual factor assays are useful in patients with a bleeding history and are guided by PT and APTT results. The cascade model of coagulation (Fig. 10.2) is no longer considered to represent the physiological process involved in coagulation. The cascade model was derived from observation of results using the PT and APTT assays which are not 'physiological' tests. Therefore, whilst the cascade model may not be 'physiologically true' it is still a useful framework for interpreting PT and APTT results. For example, in a patient with a bleeding history with a ↔ PT and a long APTT (not due to heparin or a lupus anticoagulant) there may be deficiency of factor VIII, IX, or XI.

## Mixing studies

If the PT or APTT are prolonged then mixing with ↔ plasma and repeating the test will indicate if the prolongation is likely due to a factor deficiency (the mix corrects the abnormality) or an inhibitor such as heparin or a specific factor inhibitor (the mix does not correct the abnormality).

## Thromboelastography

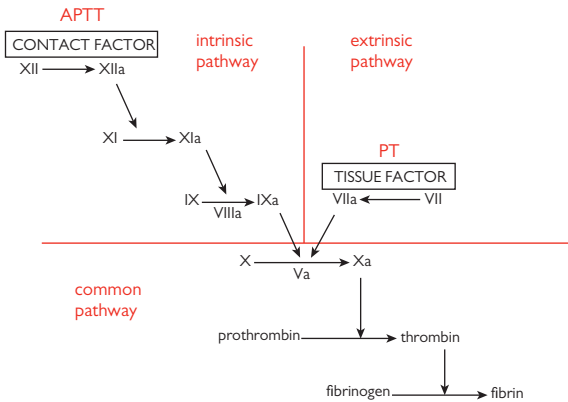
This test measures the physical strength of a clot as it forms. Its role in clinical practice is limited. It is often used as a near-patient testing device during surgical procedures with potentially massive blood loss (such as liver transplantation) when it is used to guide transfusion therapy. It is useful for identifying severe hyperfibrinolysis, as occurs during liver transplantation—an indication for using an antiplasmin drug such as tranexamic acid.

### Tests of fibrinolysis

These tests are not performed routinely. Defects of fibrinolysis have not been linked with a predisposition to thrombosis and bleeding disorders due to defective regulation of fibrinolysis being exceptionally rare. The fibrinolytic system may have a 1<sup>o</sup> role in clearing clot as part of wound healing rather than in the immediate haemostatic response to injury. Nevertheless pharmacological doses of fibrinolytic activators (e.g. recombinant tissue factor activator) have a very useful clinical role in rapidly lysing a newly formed clot—‘clot-busting drugs’.

### Practical application of coagulation tests

Clotting tests are indicated in patients with a personal or family history of bleeding. They are not generally indicated as routine preoperative screening tests as they have very low sensitivity and specificity for surgical bleeding in unselected patients. Preoperative assessment of bleeding risk is better determined by identification of a personal or family bleeding history, which should then be investigated accordingly.



**Fig. 10.2** Cascade model of coagulation.

The concept of the cascade model involves an extrinsic pathway (triggered by tissue factor and measured by the PT) and an intrinsic pathway (triggered by contact factor activation and measured by the APTT) joining in a common pathway (measured by both PT and APTT). The utility of this concept involves comparing the results of the PT and APTT to determine where there is a deficiency in the cascade, e.g. a  $\leftrightarrow$  PT with a prolonged APTT indicates a defect in the intrinsic pathway before it joins the common pathway, e.g. a deficiency of factor VIII.

## Platelets

↔ platelet survival 7–10d. Platelets are activated by multiple agonists through numerous intracellular 2nd-messenger pathways. The activation pathways converge on activation of the fibrinogen receptor, glycoprotein IIb-IIIa (integrin  $\alpha_{IIb}\beta_3$ ), such that an induced conformational change results in fibrinogen/fibrin binding (see Fig. 10.3).

As a result of occupation of the Fgn receptor (GPIIb-IIIa) 'outside-in signalling' consolidates the platelet activation process by upregulating 2nd-messenger pathways and hence providing a +ve feedback loop. In addition to adhesion and aggregation at sites of vascular injury a major role of platelets in coagulation is the provision of an anionic phospholipid surface required for assembly of the coagulation factor enzymes and cofactors required for thrombin generation.

Following vascular injury platelets adhere to subendothelial collagen via the von Willebrand factor (vWF) ligand. Platelets then aggregate to form a platelet mass at the site of injury.

### Laboratory tests

Blood collection needs to be with a non-traumatic venepuncture, rapid transport to the lab with storage at room temperature, and testing within a limited time.

- Platelet count and platelet size.
- Blood film for platelet morphology.
- Platelet function at high shear rate (PFA-100).
- Platelet function at low shear rate (platelet aggregation).
- Platelet granules and nucleotide quantification.
- Bleeding time is no longer recommended as a routine test.<sup>1</sup>

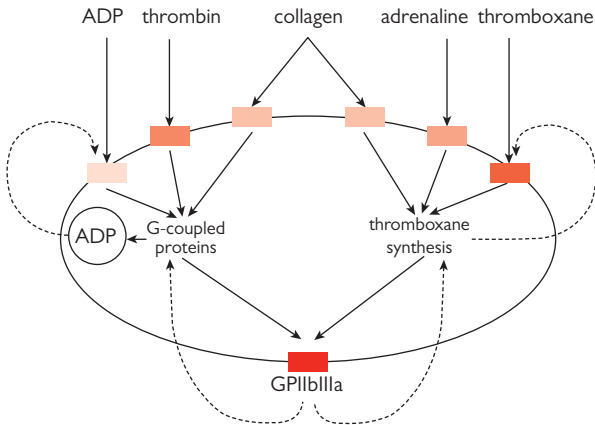
### Platelet count

- Normal range  $150\text{--}450 \times 10^9/\text{L}$ .
- Beware pseud thrombocytopenia—due to platelet clumping typically in EDTA (1% of blood counts)—check film for clumps and if suspicious repeat count on citrated or heparinized sample.
- Adequate function is maintained until platelet count  $<80 \times 10^9/\text{L}$ .
- With platelet count  $<20 \times 10^9/\text{L}$  there is often easy bruising and petechial haemorrhages and more serious bleeding can occur.
- The degree of bleeding is influenced by the cause of the thrombocytopenia—more bleeding with marrow failure syndromes (aplastic anaemia and post-chemotherapy)—less bleeding with peripheral platelet destruction, as in immune thrombocytopenic purpura (ITP).

### Reference

1. Harrison, P. (2005). Platelet function analysis. *Blood Rev*, **19**, 111–23.





**Fig. 10.3** Mechanisms of platelet activation.

Platelets are activated by multiple agonists through numerous intracellular 2nd-messenger pathways. The activation pathways converge on activation of the fibrinogen receptor, glycoprotein IIb-IIIa, such that an induced conformational change results in fibrinogen/fibrin binding. Platelet activation is completed by occupation of the fibrinogen receptor resulting in 'outside-in signalling' and upregulation of 2nd-messenger pathways.

### Platelet size

Large platelets are often biochemically more active; high mean platelet volume (MPV >10fL) is associated with less bleeding in patients with severe thrombocytopenia, acquired BM disorders are a less likely cause of thrombocytopenia the higher the MPV (especially MPV >10fL).

Reticulated platelets can be counted by some new blood count analysers and may prove to be useful in assessing thrombocytopenia (often reported as 'immature platelet fraction' (IPF)).

### Altered platelet size is seen in inherited platelet disorders

- High MPV (>10fL) in May–Hegglin anomaly, grey platelet syndrome.
- Low MPV (<7fL) in Wiskott–Aldrich syndrome (WAS), X-linked thrombocytopenia.

### Morphology

Pseudothrombocytopenia may be due to *in vitro* platelet clumping and clumps may be visible in blood film. Platelet morphology may be abnormal and white cells may contain inclusions in some inherited thrombocytopenias, e.g. May–Hegglin anomaly.

### Platelet function at high shear rate (PFA-100)

Automated technique that measures the ability of platelets to occlude an aperture at high shear rate. Minimal sample manipulation. Test performed on citrated blood sample within 4h of sample collection. Abnormal in VWD and platelet function defects.

### Platelet function at low shear rate (platelet aggregation)

Performed on platelet-rich plasma prepared by slow centrifugation of citrated blood sample. Performed within 4h of sample collection. Poor correlation with bleeding tendency except in specific congenital disorders with severe platelet function abnormality, e.g. Glanzmann thrombasthenia (GT), Bernard–Soulier syndrome (BSS).

- Agonists used for aggregation studies include ADP, collagen, arachidonic acid, adrenaline (epinephrine).
- Response to ristocetin is an agglutination response dependent on induced conformational change of platelet membrane proteins, e.g. glycoprotein Ib-IX-V, promoting interaction with vWF.
- Ristocetin-induced platelet agglutination (RIPA) is carried out at high (1.2mg/mL) and low ristocetin concentrations (0.5mg/dL). +ve RIPA at 0.5mg/dL is an abnormal result and is observed in type 2B VWD and with very high vWF levels, e.g. pregnancy.

### Platelet granules and nucleotides

Platelet storage pool disorders (SPD) are characterized by absent platelet granules.  $\alpha$  granule proteins include  $\beta$ -thromboglobulin ( $\beta$ -TG) and platelet factor 4 (PF4) which can be measured by ELISA—deficient in  $\alpha$ -granule storage pool defects. Nucleotides (ADP/ATP) can be measured by a variety of techniques including HPLC and are deficient in dense granule  $\delta$ -storage pool disorders. These are beyond the scope of the routine laboratory.

### **Clinical utility of platelet function tests**

Main role is in diagnosis of inherited platelet functional defects. In acquired platelet dysfunction 2° to drugs (aspirin, clopidogrel), and disorders such as renal and hepatic disease, DIC, platelet function is rarely tested as the clinical utility is not established in this setting, i.e. no established correlation between test results and bleeding tendency. Therefore, platelet function testing is generally restricted for the investigation of possible congenital bleeding disorders.

### **Drugs impair platelet function**

Many drugs impair platelet function, e.g. aspirin, clopidogrel, dipyridamole, glycoprotein IIb/IIIa inhibitors such as abciximab, and NSAIDs. Many other drugs have subtle effects on platelet function that are rarely of clinical importance. Drug-induced thrombocytopenia is a more common and clinically important drug effect.

## Bleeding

### Major issues to be determined

- Is haemostatic capacity reduced or is there a non-haematological cause for bleeding?
- If haemostatic capacity is reduced is it due to a heritable defect with late clinical onset or is it the result of a newly acquired defect?
- If newly acquired is it due to an anticoagulant drug?
- If not due to reduced haemostatic capacity then what are the likely circumstances that resulted in abnormal bleeding?

### Causes of bleeding

Surgery, trauma, non-accidental injury, coagulation disorders (including anticoagulant drugs), platelet dysfunction (including drug induced, e.g. aspirin), vascular disorders.

### Clinical features

Is there a lifelong bleeding history, has the patient been previously challenged by surgery or dental extraction, is this an isolated symptom? Type of bleeding problem that led to presentation, e.g. mucocutaneous, easy bruising, spontaneous, post-traumatic. Duration and time of onset. Menstrual history is important—menorrhagia has a low +ve predictive value for a bleeding disorder but absence of menorrhagia has a high -ve predictive value. Absence of obstetric bleeding does not exclude a bleeding disorder as haemostatic capacity increases significantly in pregnancy.

- **Systemic enquiry:** do symptoms suggest a systemic disorder, BM failure, infection, liver disease, renal disease?
- **Past medical history:** previous episode, previous known disorder, e.g. ITP, exposure to trauma, surgery, dental extraction, or pregnancies?
- **Family history:** similar bleeding tendency in other family members? Pattern of inheritance—autosomal dominant, sex-linked?
- **Drugs:** thrombocytopenia (➡ Thrombocytopenia (acquired), p.490), platelet dysfunction (➡ Acquired disorders of platelet function, p.502), cause not always obvious—aspirin, warfarin? Drug reaction— allergic purpura.
- **Physical examination:** signs of systemic disease—anaemia, lymphadenopathy ± hepato-splenomegaly? Assess bleeding site—check palate and fundi. Check size, e.g. petechiae (pinhead); purpura (larger = 1cm); bruises (ecchymoses) >1cm— measure them. Joints—swelling or other signs of chronic arthritis, joint destruction, or muscle contractures from previous bleeds?
- **Vascular lesions:** purpura, e.g. allergic purpura, Henoch–Schönlein purpura (➡ Henoch–Schönlein purpura, p.504), senile purpura, steroid-related, hypergammaglobulinaemic purpura, hereditary haemorrhagic telangiectasia (HHT)—capillary dilatations which blanch on pressure, vasculitic lesions, autoimmune disorders, hypersensitivity reactions.
- **Preoperative history:** is an important aspect of identifying clinically significant bleeding risk. If abnormal bleeding does occur during operation then exclude surgical bleeding and take blood for testing. Results may not be reliable in immediate postoperative period due to

effect of blood products and stress response of surgery (high vWF, factor VIIIc and Fgn). Full investigation several weeks after surgery is recommended.

- *The most common cause* of an acquired bleeding disorder is anticoagulant therapy (➔ see Anticoagulant drug therapy, p.514).

### Antiplatelet drugs

Individual response to antiplatelet therapy is extremely variable and even aspirin or clopidogrel will produce a significant bleeding tendency in some patients.

*Treatment:* stop antiplatelet therapy. Platelet transfusion for acute severe bleeding.

### Anticoagulants

Approximately 1 in 100 of the population of some countries are now receiving long-term oral anticoagulant therapy with a VKA and overanticoagulation—often due to intercurrent illness and antibiotic use—is probably responsible for the majority of life-threatening bleeds due to antithrombotic therapy with VKA. New oral anticoagulant drugs (NOACs) which are oral direct inhibitors of thrombin and factor Xa are now approved as anticoagulant drugs. The first 3 drugs to complete phase III clinical trials are dabigatran etexilate, rivaroxaban, and apixaban which are given at fixed dose and do not require monitoring. Increasingly these drugs will be taken by patients and may contribute to or be the cause of bleeding.

### Surgical bleeding

Essential to examine the drug and infusion charts and check that the dose of any drug that may affect haemostasis is correct. Determine if the site of surgery is the only site of bleeding. If this is the case, e.g. there is no bleeding from venepuncture sites or an endotracheal tube and there is no history of previous abnormal bleeding, then depending on the results of coagulation tests it is important to keep the possibility of anatomical surgical bleeding as a likely possibility. In some cases of severe bleeding the patient may have to return to theatre to look for a bleeding point.

### Critically ill patients

Many potential acquired disorders of haemostasis in critically ill patients. A coagulopathy due to vitamin K deficiency occurs within a few days in critically ill patients with no oral intake.

*Treatment:* IV vitamin K should be used routinely to prevent bleeding. Many critically ill patients develop disseminated intravascular coagulation (DIC).

### Disseminated intravascular coagulation

(➔ see Disseminated intravascular coagulation, p.494.) Major manifestations of DIC are end-organ damage due to microvascular thrombosis but the most readily apparent clinical manifestation is often bleeding due to the consumptive coagulopathy. DIC is a clinical diagnosis supported by the results of laboratory investigations.

**Treatment:** most important aspect of treatment is treating the underlying cause, e.g. sepsis or obstetric emergency such as placental abruption. FFP and platelet concentrates are used to treat bleeding or prevent haemorrhage associated with planned invasive procedures.

### Massive transfusion

Dilutional coagulopathy resulting in deficiency of clotting factors and platelets will cause abnormal bleeding in patients receiving large amounts of plasma expanders and RBCs even in the absence of DIC.

**Treatment:** give replacement therapy with FFP and platelet concentrates guided by repeated measurement of the PT, APTT, and platelet count.

### Thrombocytopenia

Many drugs result in a reversible idiosyncratic thrombocytopenia. In most cases drug-induced thrombocytopenia is mild and does not cause bleeding. Notable exceptions are quinine and gold-induced thrombocytopenia which are severe. Evaluation of drug history and cessation of possibly implicated drugs is essential in patients with acquired bleeding who are found to be thrombocytopenic. Cytotoxic drugs produce a dose-dependent suppression of BM platelet production and thrombocytopenic bleeding is common in oncology practice. BM suppression and BM failure syndromes, such as aplastic anaemia and myelodysplasia, often result in production of dysfunctional platelets and the bleeding tendency is significantly greater than in patients with thrombocytopenia and an uncompromised marrow, such as occurs in ITP.

### Acquired inhibitors

Rare and most often autoantibodies. Platelet autoantibodies result in shortened platelet survival and thrombocytopenia (immune thrombocytopenic purpura (ITP)). The bleeding manifestations of ITP are variable and often mild, as compared with thrombocytopenia associated with inadequate platelet production (➔ see Thrombocytopenia (acquired), p.490). Treatment-immunosuppression. Rarely, an autoantibody to a specific clotting factor, such as factor VIII, produces a severe acquired bleeding disorder.

**Treatment:** consult haematologist.

## Bleeding: laboratory investigations

- FBC (platelet count).
- Blood film examination.
- PT, APTT.
- ESR.
- Biochemistry—creatinine, LFTs, and immunoglobulins.
- Factor assays and platelet function tests depending on type of bleeding.

When PT or APTT abnormal investigate accordingly (➔ see Laboratory tests, p.458). Testing strategy depends on history as ↔ PT and APTT do not exclude moderate factor deficiency sufficient to cause abnormal bleeding tendency. LMWH, VWD, platelet abnormality, or very rare factor deficiency such as factor XIII. VWD often missed because PT and APTT and platelet count are ↔. If history suggestive of VWD then plasma level of vWF must be measured. The bleeding time is not a reliable test and is rarely performed. Fill blood sample tube to the mark to ensure correct anticoagulant concentration. Repeat test if result abnormal. Check patient not on anticoagulants. Family studies should be considered dependent on history.

### Further investigations

- Check blood film for red cell fragments, platelet morphology, TT and RT (if abnormal APTT checking for heparin effect), Fgn, fibrin degradation products—XDPs/D-dimer.
- Vitamin K deficiency: give vitamin K and repeat 24h later; consider checking II, VII, IX, or X level (vitamin K-dependent) and comparing with factor XI or V (not vitamin K-dependent).
- Liver disease: check LFTs; will not correct to ↔ with vitamin K but may improve due to associated vitamin K deficiency.
- Isolated factor deficiency: assay as indicated by PT/APTT results.
- Inhibitor: lupus anticoagulant tests, check anticardiolipin antibodies, other factor-specific assays.
- Heparin: abnormal APTT (↑ APTT ratio), PT ↔ if heparin with APTT ratio <2.5. Prolonged TT with ↔ RT indicates heparin effect.
- Warfarin: PT prolongation > than APTT, low vitamin K-dependent factors.
- VWD: diagnosis of VWD requires measurement of vWF level and function.

## Bleeding: therapeutic products

### Fresh frozen plasma (FFP)

Dose is 10–20mL/kg. Solvent-detergent virally-inactivated FFP is now produced and is used for rare bleeding disorders and patients with TTP who will require repeated exposure to FFP. Methylene blue-treated single donor unit FFP is also available as a virally-inactivated product.

### Platelet transfusion

Typically used for thrombocytopenic bleeding, rarely required for abnormality of platelet function.

### Desmopressin

A vasopressin analogue that increases the plasma levels of FVIII and vWF (mainly by release from stores) and directly activates platelets. Desmopressin is usually given subcutaneously or intravenously but the SC route reduces the severity of side effects of headache, flushing, and tachycardia. Tachyphylaxis (a progressively diminished response) can occur. Water retention and hyponatraemia may complicate therapy and for this reason it should not be used in very young children. Fluid intake should not exceed 1L in the 24h following treatment and with repeated doses the serum sodium should be monitored. Desmopressin will also shorten the bleeding time in patients with renal or liver failure.

### Tranexamic acid

Inhibits the binding of plasminogen and t-PA to fibrin and effectively blocks conversion of plasminogen to plasmin—fibrinolysis is thus retarded. Used for menorrhagia and after prostatic surgery. Tranexamic acid may also reduce bleeding in VWD and FVIII deficiency after dental extraction where it is normally used in combination with desmopressin or clotting factor concentrate. May be of value in thrombocytopenia (idiopathic or following cytotoxic chemotherapy) reducing requirement for platelet transfusion. Contraindicated in patients with haematuria as it will prevent clot lysis in the urinary tract and can result in clot colic.

In the CRASH-2 study treatment with tranexamic acid 1g 8-hourly within 3h of trauma reduced death due to bleeding.<sup>1</sup>

### Aprotinin

Naturally-occurring inhibitor of plasmin and other proteolytic enzymes which has been used to limit bleeding following open heart surgery with extracorporeal circulation or liver transplantation. Currently unlicensed for this indication.

### Clotting factor concentrates

Bleeding due to deficiency of specific coagulation factors is treated by either elevating the deficient factor—e.g. treatment of mild FVIII deficiency with desmopressin—or replacement of the missing factor. Recombinant factor VIII and IX are now available in some countries for routine use in patients with congenital deficiencies. For patients with rare coagulation factor deficiencies or multiple acquired deficiencies, as occurs with liver disease, massive blood loss with dilutional coagulopathy or DIC, replacement therapy still requires donor-derived FFP.



### Recombinant VIIa and FEIBA

Products used to treat patients with inhibitory antibodies to factor VIII or IX. Recombinant factor VIIa also used for factor VII deficiency and GT. rVIIa is not recommended for treatment of severe bleeding due to other causes.

### Reference

1. CRASH-2 (2011). The importance of early treatment with tranexamic acid in bleeding trauma patients: an exploratory analysis of the CRASH-2 randomised controlled trial. *Lancet*, **377**, 1096–101.

## von Willebrand disease

Commonest heritable bleeding disorder. Autosomal inheritance. Low vWF level or reduced vWF function, affects both sexes but presents more frequently in ♀ because of menorrhagia. Incidence of 1 per few hundred.

### Pathophysiology

vWF, produced in endothelial cells and megakaryocytes, is a protein of 250kDa molecular weight. Initial dimerization and subsequent removal of propeptide allows polymerization and secretion of large multimers. The higher molecular weight (HMW) multimers, up to  $20 \times 10^6$ Da, are particularly haemostatically active. vWF has 2 main functions:

- Its principal haemostatic function is to act as a ligand for platelet adhesion and when this activity is reduced bleeding tendency increases.
- It has a 2<sup>o</sup> function as a carrier protein for factor VIII protecting it from proteolysis. In most patients with vWD the associated mild reduction in factor VIII level is not the cause of the haemostatic defect.

Many cases of heritable/congenital vWD are currently thought to be caused by genetic mutations at the vWF locus but some may be due to defects in other genes which affect vWF levels (epigenetic factors such as ABO blood group). Increasingly, vWF is considered a continuous variable with low levels associated with an ↑ bleeding tendency.

### VWD is classified into 3 main types

(See Table 10.1.)

- Type 1—quantitative deficiency of vWF (75% of cases).
- Type 2—qualitative deficiency of vWF (20% of cases).
- Type 3—complete deficiency of vWF (rare).

### Clinical features

Presentation varies markedly but typically a mild-to-moderate bleeding tendency. Symptoms may be intermittent possibly related to fluctuating vWF levels. It may be necessary to test on repeated occasions to establish that vWF levels may be abnormally low on occasion.

- Bleeding type—mucocutaneous, easy bruising, nose bleeds, prolonged bleeding from cuts, dental extractions, trauma, surgery, and menorrhagia. Haemarthroses do not typically occur (except in type 3).
- Patients often present in 2nd or 3rd decade after prolonged bleeding after dental extraction or surgery.
- Type 2B causes thrombocytopenia.

### Laboratory diagnosis

vWF is an acute phase protein—increasing with stress, oestrogens, pregnancy, malignancy, thyrotoxicosis. vWF levels are dependent on ABO blood group being lower in group O than non-O.

- Because vWF is the carrier protein for FVIII protecting it from proteolysis there is a 2<sup>o</sup> deficiency of FVIII associated with vWF deficiency, but it is a low vWF level that is primarily responsible for bleeding in most patients.
- vWF level and function typically mildly or moderately reduced.

**Table 10.1** Classification of vWD

Type	VIII	vWF Ag	vWF activity	RIPA low dose	HMW multimer
1	↔/↓	↓	↓	↓/↔	↔
2A	↔/↓	↔/↓	↓	↓/↔	↓
2M	↔/↓	↔/↓	↓	↓/↔	↔
2B	↔/↓	↔/↓	↓	↑	↓
2N	↓	↔	↔	↔	↔
3	↓↓	↓↓	↓↓	↓↓	Usually undetectable

- FVIII may be ↔, or low in some patients. Factor VIII is seldom low enough to cause the joint bleeds seen in haemophilia except in type 3 which is a severe bleeding disorder.
- In type 1 vWD the PT, APTT, and platelet count are often ↔ (a 50% reduction in vWF is sufficient to reduce platelet adhesion and cause bleeding but the associated 50% reduction in FVIII is not sufficient to prolong the APTT).
- Bleeding time is often ↔ and is no longer used as a routine test. It has been largely replaced by automated *in vitro* platelet function analysis.
- When mild, the condition may be difficult to diagnose as many of the tests are ↔, including the VIII and variably the vWF level. Repeat testing is necessary. Family testing is useful.
- vWF activity measured as ristocetin cofactor activity (vWF:RCo) or collagen binding activity (vWF:CB), which is measured in plasma and is not the same as RIPA (↻ see p.464) which is the ability of ristocetin to agglutinate platelet-rich plasma.
- Type 2 often diagnosed when vWF level is low and vWF:RCo/vWF:Ag ratio <0.7.
- The main subtypes of type 2 are 2A and 2M—in 2A there is a qualitative defect with absent HMW multimers and in 2M there is a qualitative defect but with HMW multimers present.
- RIPA is ristocetin-induced platelet agglutination performed on a patient's platelet rich plasma. Only use of RIPA test is for detection of type 2B when RIPA is low due to high affinity variant vWF which produces thrombocytopenia and reduced circulating level of vWF.
- Type 2N is rare autosomal recessive variant in which the FVIII:C carrier function of vWF is reduced. May be misdiagnosed as haemophilia A but the clue is that ♀ are affected as well as ♂ and autosomal recessive inheritance.
- Majority of patients will have type 1 disease which rarely causes life-threatening bleeds; may have little impact on quality of life/life expectancy.
- Management with vWF rich VWF/FVIII concentrates as for severe haemophilia should enable patients with severe vWD to have good QoL.

### Treatment

- Mainstay of treatment is desmopressin. Avoid aspirin and NSAIDs. Mild bleeding symptoms—easy bruising, bleeding from cuts may settle with local pressure. Tranexamic acid is a useful antifibrinolytic drug (15mg/kg oral tds), e.g. for dental extraction in conjunction with desmopressin.
- Vaccination against HBV and HAV recommended for all patients.
- Inhibitors infrequent—usually type 3 disease.

### Moderate disease and minor surgery

- Desmopressin (0.3mcg/kg SC or by slow IV injection/infusion). Fewer side effects with SC route.
- Most responders have type 1 VWD but may work in some type 2 patients. *Avoid in type 2B* (may reduce platelets).

### Major surgery, bleeding symptoms, or severe disease

- If desmopressin insufficient use vWF (Wilfactin) or vWF/VIII concentrate (intermediate purity VIII concentrate), e.g. Alphanate, BPL 8Y, Haemate P.
- A high purity vWF concentrate (Wilfactin) avoids extremely high FVIII levels when repeated dosing is required, e.g. surgery in type 3 patient.
- Monitor treatment with vWF:RCo or vWF:Ag assay.
- Bleeding time or PFA-100 (↻ see p.464) may not correct despite good clinical response. Treat post-op for 7–10d.

### Pregnancy

FVIII and vWF ↑ in pregnancy so rarely presents a problem for type 1. Post-partum vWF declines quickly so watch out for PPH in moderate/severely affected women. Give desmopressin or vWF concentrate to maintain levels >0.30U/mL if clinical problem. In type 2B abnormal HMW multimers can cause platelet aggregation and thrombocytopenia in pregnancy. *Avoid tranexamic acid in pregnancy/type 2B as there may be risk of thrombosis.*

### Menorrhagia

May be major problem. Tranexamic acid for first 3d of the menstrual period can reduce blood loss by 50%. Combined oral contraceptive pill is useful. Levonorgestrel (hormone impregnated) coil very effective in some patients.

### Blood group O-associated low vWF levels

Major modifier of plasma vWF concentration is ABO blood group with vWF levels ranging from 0.35 to 1.50U/mL in group O and 0.50–>2.00U/mL in non-O. Many people with vWF levels of 0.35–0.50U/mL do not bleed but minor bleeding in the general population is common which may be due to lowish vWF levels in some people.

Distinguishing vWD from low- $\leftrightarrow$  vWF levels can be difficult. Some clinicians restrict diagnosis of vWD to patients with levels  $<0.30\text{U/mL}$  or a known mutation in the vWF gene and label other patients with bleeding history and vWF level between  $0.30\text{--}0.50\text{U/mL}$  as 'low-vWF-related bleeding tendency'.

### Further reading

Laffan, M. *et al.* (2004). The diagnosis of von Willebrand disease: a guideline from the UK Haemophilia Centre Doctors' Organization. *Haemophilia*, **10**(3), 199–217.

Pasi, K.J. *et al.* (2004). Management of von Willebrand disease: a guideline from the UK Haemophilia Centre Doctors' Organization. *Haemophilia*, **10**(3), 218–31.

Sadler, J.E. (2003). Von Willebrand disease type 1: a diagnosis in search of a disease. *Blood*, **101**(6), 2089–93.

## Haemophilia A and B

Congenital bleeding disorders with low levels of factor VIII (haemophilia A, classical haemophilia) or IX (haemophilia B, Christmas disease). Sex-linked inheritance. Males typically affected, female carriers are rarely symptomatic (see Table 10.2).

### Clinical presentation

Haemophilia A and B are clinically indistinguishable. Symptoms depend on the factor level. Bleeds into joints particularly ankles, knees, and elbows and also muscles, spontaneous bleeding into arms, legs, iliopsoas, or any site—may lead to nerve compression, compartment syndrome, muscle contractures—look for these. Haematuria is common (1 or 2 episodes per patient per decade), retroperitoneal and CNS bleeds are life-threatening.

### Chronic arthropathy

Repeated joint bleeds preventable but older patients often have arthropathy. Blood is highly irritant to synovium and causes synovial hypertrophy with hyperaemia and a tendency to rebleed. Rapid degenerative arthritis with features both of OA (mechanical pain—intermittent, worse on movement) and RA (inflammatory pain—constant but variable, morning stiffness, worse after rest) affecting predominantly ankles, knees, and elbows.

### Pseudotumours

Progressive cystic enlargement of an encapsulated haematoma. Due to recurrent subperiosteal bleeding and reactive new bone formation leading to destruction of bone, usually long bones in adults. Muscles also affected—iliopsoas.

### Pathophysiology

Factor VIII activated by thrombin, and factor IX activated by the TF/factor VIIa complex, together activate factor X, leading to thrombin generation and conversion of soluble Fgn to insoluble fibrin. Haemophilia A and B are disorders characterized by inability to generate cell surface-associated factor Xa.  $\frac{1}{3}$  of haemophilia B patients have dysfunctional molecule (type II defect).

### Genetic abnormalities

Include inversions within intron 22 of factor VIII gene in 50%, point mutations, and deletions. Gross gene alterations common in haemophilia A but infrequent in haemophilia B. This may account for low frequency of inhibitors in haemophilia B.

Carrier detection and antenatal diagnosis now possible in many cases by direct gene mutation detection. Affected family member useful for identifying gene mutation. Linkage analysis no longer recommended as 1st-line method.

### Epidemiology

- Haemophilia A occurs in 1:10,000, in  $\frac{1}{3}$  cases no family history as mutation is new. May present in neonatal period in previously unaffected family with prolonged bleeding from the cord or cephalhaematoma. May be erroneously diagnosed as 'non-accidental injury'.
- Haemophilia B occurs in 1:50,000 (i.e.  $5 \times$  less frequent than haemophilia A); no striking racial distribution.

**Table 10.2** Classification of severity of haemophilia

Severe disease (plasma level <0.01U/mL)	Usually presents in the 1st years of life with easy bruising and bleeding out of proportion to injury
Moderate disease (0.01–0.05U/mL factor level)	Intermediate and variable severity
Mild disease (>0.05U/mL)	May only present after trauma/surgery in later life
General features	Haemarthrosis; spontaneous bleeding into joints (knees>elbows>ankles>hips>wrists) produce local tingling, pain; later—swelling, limitation of movement, warmth, redness, severe pain

### Diagnosis

Assess duration, type of bleeding, exposure to previous trauma/surgery and family history. Look for bruising, petechial haemorrhages, early signs of joint damage. Exclude acquired bleeding disorders.

### Laboratory tests

PT ↔, APTT prolonged depending on degree of deficiency (*note*: a normal APTT does not exclude mild disease). Assay FVIII first, then FIX. Exclude VWD.

### Radiology

Acute bleed—US or CT scan if in doubt. In established disease—chronic synovitis, arthropathy, and other pathological changes seen.

### Complications

#### Factor VIII inhibitors

- Suggested by ↑ frequency of bleeding and/or reduced response to concentrates; occurs in 15–25% haemophilia A patients following treatment, usually after 5–20 treatment episodes (IX inhibitors are uncommon; <2%). Familial tendency, inhibitors occurring more often in patients with deletions or mutations within factor VIII gene. The antibody acts against part of the amino-terminal component of the A2 domain or the carboxyterminal part of the C2 domain of the VIII molecule. Inhibitor is detected by inhibitor screen and quantified by Bethesda assay.
- In 50% of patients inhibitors are transient and low titre (<5BU/mL) being noted incidentally on review.
- In 50%, however, a permanent high-titre inhibitor develops (>5BU/mL) and is a major complication.

### Diagnosis

- Prolonged APTT with failure to correct with ↔ plasma.
- Inhibitor screen—APTT of mix of patient plasma and ↔ plasma compared immediately after mixing and after 2h incubation at 37°C.

- Inhibitor assay—factor assays performed on serial dilutions of patient's plasma in ↔ plasma after 2h incubation (Bethesda assay)—inhibitor titre (BU/mL) calculated from dilution that produces 50% reduction of ↔ factor VIII level.

### Treatment

Requires a 'FVIII bypassing agent' for treatment of acute bleeds (rVIIa or FEIBA) and desensitization by immuntolerance with FVIII for high titre inhibitors. Various immune tolerance regimens have been tried—successful in up to 80% of patients but may take up to 2 years to eliminate inhibitor. Treatment programme requires supervision in comprehensive care centre or similar expert centre with regular pharmacokinetic studies.

*Example of immuntolerance programme:*

- *Phase 1:* wait until FVIII titre <10BU/mL treating acute bleeds with bypassing agent.
- *Phase 2:* commence 100U/kg FVIII od or bd until inhibitor undetectable in Bethesda assay.
- *Phase 3:* perform half-life ( $t_{1/2}$ ) study 4–6-weekly after 3d wash out when inhibitor undetectable. When  $t_{1/2}$  >6h switch to 50U/kg 3 × weekly and continue to monitor  $t_{1/2}$ .
- *Phase 4:* When  $t_{1/2}$  >6h for 3 months reduce to standard prophylaxis 25U/kg 3 × weekly.

Asymptomatic patients with low titre inhibitor—observation may be all that is necessary as the inhibitor may disappear.

### Transmission of HBV, HCV, and HIV

Not a risk with recombinant products. Viral inactivation procedures for plasma-derived products in mid 1980s limited transmission.

### Treatment

General regular medical and haemophilia review and lifelong support are essential. At presentation establish:

- Factor level.
- Inhibitor screen.
- Mutation (useful for subsequent carrier detection and/or antenatal diagnosis and some predictive value for risk of inhibitor development).
- FBC—exclude anaemia due to bleeds including iron deficiency.
- Blood group.
- Liver function.
- Baseline viral status (HIV, HCV including genotype and HCV RNA level, HBV, HAV).
- Early treatment of bleeding episodes is essential. Prophylaxis is preferable to demand treatment for many patients with severe haemophilia. Prophylaxis started in 1st or 2nd year of life can prevent most if not all joint damage and almost eliminate significant bleeding. Port-A-Cath may be required to deliver prophylaxis. Factor concentrate needs to be administered every 2 or 3d. If not on prophylaxis, home demand treatment is preferable to hospital demand treatment.
- Regularly check inhibitor status, LFTs, FBC.
- Avoid aspirin, antiplatelet drugs, and IM injections.
- Vaccinate against HBV and HAV if not immune.



### HIV management

Retroviral therapy requires HIV specialist involvement.

### HCV management

Requires hepatologist involvement. HCV infected most patients with haemophilia treated with pooled human factor concentrates before 1985. ~20% have chronic liver disease. HCV genotyping and RNA detection by PCR is used to identify patients at higher risk of progressive liver disease. Liver biopsy if required is not contraindicated as factor levels are readily normalized with replacement therapy—need for biopsy has diminished as genotyping and RNA detection has become more informative. Combined antiviral therapy superior to IFN alone.

### Variant CJD

No evidence as yet of transmission of vCJD by pooled human blood products.

### Haemophilia A-specific treatment

- Minor bleeds may stop without factor concentrate therapy.
- Desmopressin for minor surgery and bleeds that fail to settle (0.3mcg/kg SC or slow IVI/20min)—may also be given by nasal spray. 30min later take blood sample to check response (if required)—plasma level  $\uparrow$  3–4-fold. Reduced response with repeated exposure sometimes observed.
- Tranexamic acid (15mg/kg tds oral)—useful for cuts or dental extraction. *Do not use when haematuria.*
- Cryoprecipitate no longer recommended.
- Severe disease—factor VIII concentrate therapy necessary.
- Products—recombinant products are treatment of choice. 2nd generation recombinants do not contain any human material in product. Human donor-derived products are now subjected to multiple viral inactivation steps lyophilization (dry heat), pasteurization (wet-heat), solvent-detergent, nanofiltration)—good record of viral safety. High and intermediate purity human donor-derived products available for patients not receiving recombinant therapy. No particular advantage for high purity over intermediate except possibly useful in patients with allergic reactions to intermediate purity. Factor concentrate not available for majority of patients worldwide.
- Principle of treatment—raise factor VIII to haemostatic level (0.15–0.25U/mL for minor bleeds, 0.25–0.50U/mL for moderate bleeds, >0.50U/mL for severe bleeds, >0.40U/mL minor ops, >0.50U/mL for major surgery with achievement of 1.00U/mL for major surgery and life-threatening bleeds). *Formula:* 1U/kg body weight raises plasma concentration by about 0.02U/mL (2U/dL).  $t_{1/2}$  6–12h. Spontaneous bleeds usually settle with single treatment if treated early. In major surgery replacement therapy required for up to 10d.
- Do not give IM injections when factor is low.

### Haemophilia B-specific treatment

- General approach as for haemophilia A. Desmopressin typically of no value.
- Products—recombinant factor IX treatment of choice. If not recombinant then high purity factor IX preferable to intermediate (intermediate known as prothrombin complex concentrate) as high risk of thrombosis with intermediate purity product. *Formula:* 1U/kg body wt raises plasma concentration by 0.01U/mL (1U/dL).  $t_{1/2}$  12–24h.

### Special considerations

#### *Antenatal diagnosis*

Carrier detection ideally by genetic mutation analysis. Phenotypic detection by FVIII:vWF ratios is unreliable. Antenatal diagnosis in carriers ideally performed by chorionic villus sampling (CVS) with mutation analysis at 10–12 weeks' gestation if termination preferred—rarer nowadays because of improved treatment and prognosis. Issue is complex and counselling/testing should be at comprehensive care centre or similar expert centre.

#### *Home treatment*

Home treatment has transformed QoL of patients. Parents, the local GP, the boy himself from age 6–7 years onwards can be trained to give IV factor concentrates at home. Treatment usually starts in 1st or 2nd year of life and Port-A-Cath may be needed until age 4 years or more.

#### *Prophylaxis*

E.g. 3 × weekly injections of concentrate (average dose 15–25U/kg) given at home.

#### *Multidisciplinary support required*

Physiotherapy plays key role in preservation of muscle and joint function in patients with haemarthroses. Combined clinics with orthopaedic surgeons, dental surgeons, hepatologists, paediatricians, HIV physicians, and geneticists are required to give comprehensive care.

*Clinical trials* of gene therapy and long-acting factor concentrates (Fc-fusion and PEGylated proteins) are under way.

### Further reading

Key, N.S. and Negrier C. (2007). Coagulation factor concentrates: past, present, and future. *Lancet*, **370**, 439–48.



## Rare congenital coagulation disorders

### Clotting factor deficiencies

Deficiency of coagulation factors other than vWF, FVIII, and FIX is rare with a prevalence of 1–2 per million. Autosomal recessive inheritance, the deficiency either due to reduced level (type 1) or production of a variant protein (type 2).  $t\frac{1}{2}$  of factors vary and will determine the frequency and ease of treatment.

Rare factor deficiencies rarely produce haemarthrosis, except factor XIII deficiency, and may only present at time of surgery. Many patients with inherited coagulation deficiencies will not bleed unless exposed to surgery or trauma, and may seldom require treatment. When bleeding arises or cover for surgery is needed, the aim is to achieve a plasma factor concentration at least as high as the minimal haemostatic value and make sure it does not drop below this until haemostasis is secure.

Factor concentrates should be considered when available, e.g. factor XI, factor XIII, recombinant VIIa for FVII deficiency. FFP can be used, e.g. when appropriate concentrate not available, and virally inactivated plasma should be used when available. FFP is a source of all coagulation factors but large volumes may be required and even with viral inactivation there is risk of disease transmission.

### Specific conditions

#### Factor XI deficiency

Deficiency more common in certain ethnic groups such as Ashkenazi Jews. Clinically of variable severity, often mild—even low factor levels may not produce symptoms whilst significant bleeding can occur with mild deficiency.

- **Diagnosis:**  $\leftrightarrow$  PT,  $\leftrightarrow$  APTT unless factor XI  $<0.40\text{U/mL}$ . Therefore necessary to measure factor XI level to make diagnosis in many cases.
- **Treatment:** tranexamic acid and desmopressin for oral and dental surgery. Factor XI concentrate sometimes available. Otherwise, use virally-inactivated FFP.

#### Hypo/afibrinogenaemia

$\leftrightarrow$  range 2.0–4.0g/L. Produced by liver; acute phase protein, raised in inflammatory reactions, pregnancy, stress, etc. Converted to fibrin by the action of thrombin and is a key component of a clot. Abnormalities of Fgn are more often acquired than inherited. Inherited defects are usually quantitative and include heterozygous hypofibrinogenaemia or homozygous afibrinogenaemia.

Bleeding usually only in afibrinogenaemic patients, hypofibrinogenaemia usually asymptomatic. Bruising, bleeding usually after trauma or operations will depend on the concentration and are more severe when  $<0.5\text{g/L}$ . Afibrinogenaemia (Fgn  $<0.2\text{g/L}$ ) is a moderate-to-severe disorder with spontaneous bleeding; umbilical stump bleeding common, cerebral and GI haemorrhage and haemarthrosis less common. Epistaxis, menorrhagia and postpartum bleeding common.

- *Diagnosis:* prolonged (unclottable in afibrinogenemia) PT, APTT, and TT. Fgn level measured by Clauss assay. Acquired hypofibrinogenemia needs to be excluded (DIC, liver disease) and family studies are necessary.
- *Treatment:* Fgn has a long  $t_{1/2}$  (3–5d), severe deficiency managed by repeated (twice weekly) prophylactic injections with Fgn concentrates, FFP or cryoprecipitate. Fgn levels should be raised to 0.5–1.0g/L to achieve haemostasis.

### *Dysfibrinogenemia*

Qualitative defects of Fgn—the dysfibrinogenemias—are inherited as incomplete autosomal dominant traits with >200 reported Fgn variants. Defective fibrin polymerization or fibrinopeptide release may occur. Most patients are heterozygous and asymptomatic, bleeding symptoms are not typical and usual clinical manifestation if any is arterial and/or venous thrombosis with some variants (although very rare at ~1 per million of population, <1% of patients with venous thrombosis). More likely to present with pregnancy-associated thrombosis.

- *Diagnosis:* possible prolongation of PT and APTT with variable abnormalities of TT and RT and Fgn-dependent platelet function may be defective. Diagnosis usually by demonstrating normal immunological Fgn concentrations with reduced functional activity in patient with a prolonged TT.

### *Factor VII deficiency*

Vitamin K-dependent factor playing a pivotal role in initiating coagulation but low level required. The  $t_{1/2}$  is short (4–6h). In severe deficiency (<0.05U/mL), bleeding symptoms (similar to haemophilia) are not common but spontaneous intracerebral haemorrhage at a young age has been reported.

- *Diagnosis:* prolonged PT with ↔ APTT. Assay factor VII to assess severity.
- *Treatment:* recombinant VIIa (20mcg/kg) if available. Repeated doses required. Very short  $t_{1/2}$  makes management difficult, requiring repeated doses. If using FFP, give initial IV injection (10–15mL/kg) and check response with subsequent monitoring and repeat infusion.

### *Factor XIII (fibrin stabilizing factor) deficiency*

Characteristically produces delayed postoperative bleeding (6–24h later). Neonatal umbilical stump bleeding more common than with other deficiencies. High risk of cerebral haemorrhage.

- *Diagnosis:* PT and APTT both normal so diagnosis will be missed unless specifically looked for. Screening tests are insensitive (clot solubility test with thrombin and acetic acid recommended if performed). Factor XIII antigen ( $\alpha$ -subunit) level can be measured by ELISA.
- *Treatment:* very low levels required for haemostasis;  $t_{1/2}$  is very long. Severe deficiency should be treated with once-monthly prophylactic replacement with factor XIII concentrate.

**Other deficiencies**

Factor II, V, X deficiencies very rare. Bleeding less severe with factor V deficiency than with factor X or prothrombin deficiency. Consider consanguinity with rare autosomal recessive bleeding disorders.

**Multiple defects**

Rare familial coagulation factor deficiencies described; often involves factor VIII and another factor, e.g. combined FV and FVIII deficiency. Other combinations seen.

**Further reading**

Bolton-Maggs, P.H. *et al.* (2004). The rare coagulation disorders—review with guidelines for management from the United Kingdom Haemophilia Centre Doctors' Organisation. *Haemophilia*, **10**, 593–628.

## Congenital thrombocytopenias

Family history useful but –ve family history does not exclude inherited abnormality, usually autosomal recessive.

### Non-inherited neonatal thrombocytopenia

*Infection in the neonate*: relatively common cause of neonatal thrombocytopenia. Also as a result of maternal toxoplasma, CMV, rubella when neonatal thrombocytopenia resolves over several months.

*Maternal ITP*: autoantibody crosses placenta and destroys fetal platelets, however severe thrombocytopenia rare and bleeding exceptional (unlike in NAIT see below). Recovery within 1–3 weeks.

*Drugs*: drugs taken by mother can cause thrombocytopenia, neonatal thrombocytopenia usually resolves rapidly without any haemorrhage.

*Neonatal alloimmune thrombocytopenia (NAIT)*: similar mechanism to rhesus haemolytic disease of the new born (HDN) but IgG antibody is to platelet antigen, usually in HPA-1 +ve fetus of HPA-1 –ve mother. Associated with severe bleeding including intracranial haemorrhage which may occur before delivery. Requires urgent diagnosis and transfusion of selected antigen –ve platelets. Recovery may take several weeks.

*Marrow infiltration*: congenital leukaemia very rare.

### Inherited neonatal thrombocytopenia

Very rare. Platelet size and presence or absence of other clinical features help to classify.

↓ *platelet size*: WAS, X-linked, eczema, susceptibility to infection, death at early age from bleeding, infection or lymphoma.

↔ *platelet size*: amegakaryocytic thrombocytopenia including TAR syndrome (thrombocytopenia with absent radius). Schulman–Upshaw syndrome—neonatal TTP.

↑ *platelet size*: commonest inherited thrombocytopenias, Mediterranean thrombocytopenias (asymptomatic), May–Hegglin anomaly (Dohle bodies in granulocytes, mild neutropenia, major haemorrhage rare, presence of other clinical features such as deafness, cataracts, nephritis identifies variants, all due to mutations of *MYH9* gene) BSS (associated with functional defect, ↻ see Congenital platelet function deficits, p.486), grey platelet syndrome (reduced  $\alpha$ -granules), type 2B VWD (↻ see von Willebrand disease, p.472) and pseudo-VWD (platelet type VWD due to mutation in platelet membrane GPIb-IX).

## Congenital platelet function defects

Rare. Acquired platelet dysfunction is much more likely to be a cause of bleeding or easy bruising.

### 2 main hereditary qualitative defects are found:

- Defective platelet membrane glycoproteins (GPs)—GT, abnormal GPIIb/IIIa in which there is severe bleeding, and absent aggregation response to all agonists except  $\leftrightarrow$  agglutination with ristocetin, BSS, abnormal GPIb-IX-V complex, receptor for vWF, defective adhesion, variable bleeding can be severe, absent response to ristocetin.
- Abnormalities of platelet granules—storage pool disease (SPD)— $\alpha$ -granules (grey platelet syndrome),  $\delta$ -granules (May–Hegglin anomaly, Hermansky–Pudlak syndrome, Chediak–Higashi syndrome, TAR syndrome).

### Laboratory

- Platelet count.
- Platelet size.
- Blood film (examine granulocytes as well as platelets).
- PFA-100.
- Platelet aggregation.
- Flow cytometry for detection of platelet membrane glycoproteins.

### Treatment

- Avoid antiplatelet drugs.
- Use pressure to control bleeding from minor cuts.
- Desmopressin.
- Tranexamic acid (15–25mg/kg body weight) 8-hourly for 7–10d for minor surgery and dental work.
- Platelet transfusions are effective in major surgery and severe bleeding but alloimmunization may occur causing platelet refractoriness.
- Recombinant VIIa considered for severe defects, e.g. GT.
- Splenectomy has been used in WAS.
- Allogeneic BMT has been performed (GT and WAS).



## Congenital vascular disorders

### **Hereditary haemorrhagic telangiectasia (Osler-Weber-Rendu (OWR) syndrome)**

Autosomal dominant, multiple mucocutaneous and skin telangiectasia which bleed easily. Recurrent epistaxis, menorrhagia, and GI bleeds causing Fe deficiency. Presentation may not be until later life. Development of pulmonary and cerebral AV malformations. Pulmonary AVMs may enlarge in pregnancy. Diagnosis by recognition of typical telangiectases and family history. Beware, another cause of bleeding may coexist in a OWR patient.

#### *Laboratory*

- FBC and film may show Fe-deficient picture, i.e. microcytic, hypochromic anaemia, ↓ MCV, raised platelets, ↓ serum ferritin.
- Angiography of mesenteric circulation when recurrent bleeding.
- ENT examination.
- CT scan to identify pulmonary AV malformations or desaturation on exercise.

#### *Treatment*

- Fe supplementation if Fe deficient.
- Tranexamic acid if menorrhagia.
- Antibiotics for surgical/dental procedures as risk of cerebral abscess due to bacteraemia and shunting in lungs.
- Consider interventional procedure, e.g. embolization (if angiography +ve).
- Oestrogen may reduce frequency of bleeding episodes.

### **Giant cavernous haemangioma**

Venous malformation, may cause DIC-like features due to activation of coagulation system within haemangioma, spontaneous regression may occur.

## Haemorrhagic disease of the newborn

- Caused by deficiency of the vitamin K-dependent factors.
- Poor vitamin K transfer across placenta.
- Significant cause of bleeding in the neonatal period unless prevented by vitamin K.
- Three forms described—early, classic, and late.

### Early

Due to maternal ingestion of drugs that interfere with vitamin K metabolism—warfarin, phenytoin, barbiturates, rifampicin. Presents in 1st day of life. Umbilical cord and skin bleeding, post circumcision bleeding, ICH is rare. Prevented by maternal administration of vitamin K.

### Classic

Presents at 2–5d of birth, almost exclusively a disease of breast-fed babies, often full term and healthy, incidence 0.5/1000 deliveries. Human milk has less vitamin K than cows' and formula milk.

- Bruising.
- Bleeding from venepuncture sites.
- GI bleeding.
- ICH.

### Late

Presents at 2–12 weeks but can occur up to 6 months of age. Can present in breast-fed infants who did not receive vitamin K, may occur with chronic diarrhoea but cholestatic liver disease is often present—biliary atresia, cystic fibrosis,  $\alpha$ -1 antitrypsin deficiency. ICH common.

#### Diagnosis—laboratory findings

- $\uparrow$  PT and APTT—may be markedly  $\uparrow\uparrow$ .
- $\leftrightarrow$  TT, Fgn, D-dimer/FDPs (cf. DIC).
- Factor assay (II, VII, IX, X) if in doubt.
- Correction of coagulation abnormality in 72h with parenteral vitamin K confirms diagnosis.

#### Differential diagnosis

Exclude other causes of bleeding in the neonatal period, e.g. DIC, thrombocytopenia (platelets are  $\leftrightarrow$  in haemorrhagic disease of the newborn), haemophilia.

#### Radiology

Scan for ICH/internal bleeding as required.

#### Treatment

Prevention is indicated, e.g. 1mg IM vitamin K<sub>1</sub> at birth (or multiple oral doses but not as effective as IM).

- Treatment should not be delayed when diagnosis suspected—1mg SC or IV vitamin K<sub>1</sub> (will correct PT and APTT to  $\leftrightarrow$  for age within 72h).
- If significant bleeding give PCC (factor concentrate of II, VII, IX, X) or FFP 15mL/kg.
- Previous concerns of a causal link between parenteral vitamin K administration to the neonate and childhood cancer have been shown to be unfounded.



## Thrombocytopenia (acquired)

➔ See also Congenital thrombocytopenias, p.486 and beware pseudo-thrombocytopenia (➔ Platelets, p.462). Thrombocytopenia defined as platelet count  $<150 \times 10^9/L$ .

### May be due to:

- ↓ bone marrow production.
- Short platelet survival—↑ consumption, destruction, or sequestration (or combination).
- Dilution.

Platelet counts  $>100 \times 10^9/L$  are not usually associated with bleeding. Purpura, easy bruising, and prolonged post-traumatic bleeding are increasingly common as the platelet count falls  $<50 \times 10^9/L$ . Although there is no platelet count at which a patient definitely will or will not experience spontaneous bleeding, the risk is greater in patients with a platelet count  $<20 \times 10^9/L$  and increases further in those with a count  $<10 \times 10^9/L$ .

### ↓ bone marrow production of platelets

- *Marrow failure*: aplastic anaemia (➔ see Aplastic anaemia, p.102).
- *Marrow infiltration*: leukaemias, myelodysplasia, myeloma, myelofibrosis, lymphoma, metastatic carcinoma.
- *Marrow suppression*: predictable with cytotoxic drugs, radiotherapy. Idiosyncratic response—e.g. chloramphenicol.
- *Megaloblastic anaemia* (➔ see Vitamin B<sub>12</sub> deficiency, p.46).
- *Amegakaryocytic*: ethanol, drugs (phenylbutazone, co-trimoxazole; penicillamine), chemicals, viral infection (e.g. HIV, parvovirus).
- *Hereditary non-congenital*: Fanconi syndrome (➔ see Fanconi anaemia, p.594).

### Short platelet survival

#### Consumption

- DIC (➔ see Disseminated intravascular dissemination, p.494).
- TTP/HUS (➔ see Thrombotic thrombocytopenic purpura, p.538).
- HIT (➔ see Heparin-induced thrombocytopenia, p.542).
- Haemangioma (Kasabach–Merritt syndrome).
- Congenital/acquired heart disease.
- Cardiopulmonary bypass.

#### Destruction

- Immune:
  - 1°: immune thrombocytopenia.
  - 2° ITP: other autoimmune states (SLE, CLL, lymphoma)—drug-induced (heparin—also HIT (➔ see Heparin-induced thrombocytopenia, p.542), gold, quinidine, quinine, penicillins, cimetidine, digoxin, vancomycin).
  - Infection (HIV, other viruses, malaria).
  - PTP (➔ see Specific thrombocytopenic syndromes, p.492).

#### Sequestration

- Hypersplenism (➔ see Specific thrombocytopenic syndromes, p.492).

### Dilution

- Gestational thrombocytopenia.
- Massive transfusion (↻ see Massive blood loss, p.506).
- Exchange transfusion.

### Clinical assessment

- *History*: drugs, symptoms of viral illness.
- *Examination*: signs of infection, lymphadenopathy, hepatosplenomegaly.
- *Investigations*: FBC, is thrombocytopenia isolated or not? MPV—acquired BM disorders are a less likely cause of thrombocytopenia the higher the MPV (especially MPV >10fL).
- *Blood film*: platelet clumps (pseudothrombocytopenia), RBC
- *fragmentation* (DIC, TTP, HUS), white cells (atypical lymphocytes/blasts).
- *Others*: ANA, dsDNA, monospot, HIV serology, creatinine, LFTs.
- *BM examination*: if clearly not due to short platelet survival.

### Further reading

Provan, D. *et al.* (2010). International consensus report on the investigation and management of primary immune thrombocytopenia. *Blood*, **115**, 168–86.

## Specific thrombocytopenic syndromes

### ITP

2° thrombocytopenia (SLE and LPDs esp. low-grade NHL and CLL) may present with isolated thrombocytopenia and underlying disorder may only be discovered on further investigation. Often refractory to therapy. Those with LPDs will require treatment of underlying condition.

### ITP in pregnancy

Fetal thrombocytopenia may occur due to placental transfer of IgG anti-platelet antibodies in a pregnant woman with ITP but rare. Risk of ICH in fetus during delivery is very low. No good predictor for fetal thrombocytopenia. Treatment with prednisolone, or IVIg should be administered to the mother with thrombocytopenia severe enough to constitute a haemorrhagic risk to her. Avoid splenectomy—high rate of fetal loss. Severe maternal haemorrhage at delivery is rare but may require platelet transfusion, IVIg and possibly splenectomy. Special antenatal treatment of the fetus is unnecessary but avoid prolonged and complicated labour. Ensure paediatric support at delivery and check neonatal platelet count—monitor for several days (delayed thrombocytopenia). IVIg, prednisolone or exchange transfusion may be required.

*Differential diagnosis*—gestational thrombocytopenia (common), pre-eclampsia, all other causes of thrombocytopenia.

### Gestational thrombocytopenia

Benign thrombocytopenia (platelets  $>80 \times 10^9/L$ ) occurs in 5% of pregnancies. No treatment is indicated. Neonatal count  $\leftrightarrow$ .

### Neonatal alloimmune thrombocytopenia

➡ See Neonatal alloimmune thrombocytopenia, p.586.

### Post-transfusion purpura

Rare but life-threatening. Causes severe haemorrhage due to thrombocytopenia 1 week after transfusion of blood or blood products. Thrombocytopenia may persist for several days. Occurs most commonly in platelet antigen HPA-1a -ve recipient (2% of population) of red cell or platelet transfusion from HPA-1a +ve donor. Patient usually previously sensitized by pregnancy.

### Hypersplenism

Thrombocytopenia primarily due to platelet pooling in enlarged spleen. If haemorrhagic complications, consider splenectomy if the underlying cause is unknown or if treatment of underlying disorder has been ineffective.

### Drug-induced thrombocytopenia

Many drugs cause idiosyncratic thrombocytopenia, largely through  $\uparrow$  destruction—usually immune mechanism—patient may have been using the drug for several weeks/months, thrombocytopenia may be severe ( $<20 \times 10^9/L$ ), e.g. with gold or quinine. Drugs—heparin, quinine, quinidine, gold, sulfonamides, trimethoprim, penicillins, cephalosporins, cimetidine, ranitidine, diazepam, sodium valproate, phen-

acetin, rifampicin, thiazides, (furosemide), chlorpropamide, tolbutamide, digoxin, methyldopa, vancomycin, and teicoplanin. If drug-induced thrombocytopenia suspected, discontinue the suspected drug. If the patient is bleeding platelet transfusion should be administered. IVIg may be helpful. Thrombocytopenia usually resolves quickly but may persist for a prolonged period notably that due to gold which may be permanent. Implicated drugs should be avoided by that patient in future.

## Disseminated intravascular coagulation

A syndrome which complicates a range of illnesses. Characterized by systemic activation of coagulation resulting in the generation of fibrin clots that cause organ failure and consumption of platelets and coagulation factors resulting in bleeding.

### Conditions associated with DIC

- Sepsis and severe infection.
- Trauma.
- Organ necrosis, e.g. pancreatitis.
- Malignancy—solid tumours and leukaemia.
- Obstetric—amniotic fluid embolism, placental abruption, pre-eclampsia.
- Vascular abnormalities—large haemangiomas, aortic aneurysm.
- Severe liver failure.
- Toxic—snake bites, recreational drugs including ‘Ecstasy’.
- Immunological challenges—ABO transfusion incompatibility, transplant rejection.

These conditions induce systemic activation of coagulation either by activating cytokines as part of a systemic inflammatory response or by causing exposure to procoagulant components, ↑ tissue factor expression, suboptimal function of natural anticoagulant systems, dysregulation of fibrinolysis and ↑ anionic phospholipid availability.

### Diagnosis

No single laboratory test that can establish or rule out the diagnosis of DIC—therefore assess the whole clinical picture, taking into account the clinical condition of the patient, the diagnosis, and all available laboratory results.

Laboratory tests need to both keep pace with the rapidly changing condition of the patient.

- Thrombocytopenia.
- ↑ fibrin degradation products (inc. D-dimer).
- ↑ PT.
- ↑ aPTT.
- ↓ Fgn.

Platelet count is a sensitive (*but not specific*) marker of DIC and thrombocytopenia is a feature in up to 98% cases, with platelet count  $<50 \times 10^9/L$  in ~50% patients.

### A scoring system aids diagnosis of overt DIC

Risk assessment—does the patient have an underlying disorder known to be associated with overt DIC?

*If yes:* proceed.

*If no:* do not use scoring algorithm.

- Platelet count ( $>100 = 0$ ;  $<100 = 1$ ;  $<50 = 2$ ).
- Elevated FDPs, e.g. D-dimer (no increase = 0; moderate increase = 2; strong increase = 3).



- Prolonged PT (<3sec = 0; >3 but <6sec = 1; >6sec = 2).
- Fgn level (>1g/L = 0; <1g/L = 1).

**Calculate score:**

- $\geq 5$  compatible with overt DIC (repeat score daily).
- $< 5$  suggestive for non-overt DIC (repeat next 1–2d).

**Treatment**

- Rapid treatment of the underlying disorder. In many cases the DIC will spontaneously resolve when the underlying disorder is treated, e.g. antibiotics and/or surgical drainage in patients with DIC due to severe infection and sepsis.
- Supportive treatment to minimize or correct coagulation abnormalities may be required.
- Transfusion of platelets or plasma (components) should not primarily be based on laboratory results but should in general be reserved for patients with bleeding.
- In patients with bleeding or at high risk of bleeding (e.g. postoperative patients or patients due to undergo an invasive procedure) and a platelet count of  $< 50 \times 10^9/L$  transfusion of platelets should be considered.
- In non-bleeding patients a platelet threshold for transfusion is  $10\text{--}20 \times 10^9/L$ . In patients at high risk of bleeding platelets may be transfused at higher levels, e.g.  $50 \times 10^9/L$ .
- In bleeding patients with a prolonged PT and APTT administration of FFP should be considered.
- If transfusion of FFP is not possible in patients with bleeding who are fluid overloaded, consider using factor concentrates such as PCC (prothrombin complex concentrate).

Anticoagulant therapy not routinely indicated. Consider using heparin in patients with thrombotic complications, e.g. large vessel arterial or venous thromboembolism, severe purpura fulminans associated with skin ischemia or skin infarction.

In critically ill, non-bleeding patients with DIC, prophylaxis for venous thromboembolism with prophylactic dose LMWH is recommended.

Antifibrinolytic agents not routinely indicated. If 1<sup>o</sup> hyperfibrinolytic state (e.g. acute promyelocytic leukaemia) and bleeding then tranexamic acid can be considered.

**Further reading**

Levi, M. (2004). Current understanding of disseminated intravascular coagulation. *Brit J Haematol* **124**, 567–76.

## Liver disease

Most coagulation factors, including the vitamin K-dependent factors, are made exclusively in the liver. Any damage to the liver may cause rapid reduction of levels and resultant coagulopathy due to their short  $t_{1/2}$ . Associated thrombocytopenia is common in established liver disease (30% of patients have count  $70\text{--}100 \times 10^9/\text{L}$ ) and platelet function is reduced, increasing the risk of bleeding.

### Pathophysiology

Haemostasis is a balance between procoagulant and anticoagulant mechanisms. Because of its central role in the production of these factors, haemostasis is often disturbed in liver disease. Clotting tests become abnormal and are useful monitors of liver function, especially PT. Low grade DIC may be present. Fibrinolysis may be  $\uparrow$  in chronic liver disease (cf. during liver transplantation when it is  $\uparrow$  causing bleeding). Dysfibrinogenaemia due to  $\uparrow$  sialic acid content of the Fgn molecule is described. In obstructive jaundice, impaired bile flow leads to malabsorption of vitamin K, a fat soluble vitamin. A degree of intrahepatic obstruction  $2^\circ$  to hepatocyte swelling and fibrosis may also have this effect. Administration of IV vitamin K may partially correct clotting abnormalities. Thrombocytopenia may be due to portal hypertension, splenic pooling, alcohol, viral infection, drugs, or DIC. Altered platelet function with a prolonged bleeding time may occur.

### Clinical features

Many patients with established hepatic dysfunction will have abnormal PT and thrombocytopenia but may be asymptomatic. Bleeding becomes a problem when other complications arise such as oesophageal varices, thrombocytopenia, surgery, liver biopsy, and infection.

### Types of bleeding

- Ecchymoses.
- GI bleeding.
- Varicella bleeding.
- Epistaxis.
- Oozing from venepuncture sites and operative sites.

### Laboratory

- Vitamin K deficiency— $\uparrow$  PT  $>$  APTT.
- $\downarrow$  Fgn with advanced liver disease.
- $\uparrow$  TT due to  $\downarrow$  Fgn, DIC with  $\uparrow$  FDPs, dysfibrinogenaemia.
- Thrombocytopenia.

### Treatment

- IV or SC vitamin K, 10mg daily  $\times$  3d.
- FFP (correction of clotting factor deficiency with partial correction of PT and APTT).
- Cryoprecipitate (for hypofibrinogenaemia).
- PCC (prothrombin complex concentrate).
- Platelets (if bleeding or high risk and very low count, e.g.  $<10 \times 10^9/\text{L}$ ).

- Desmopressin (improves platelet function).
- Red cell transfusion for anaemia.
- Stop NSAIDs, heparin flushes, and other drugs that may interfere with haemostasis.

Asymptomatic patients do not require treatment other than that directed at the underlying condition. Give vitamin K 10mg IV for 3d to exclude and correct vitamin K deficiency. Complete correction of the PT confirms this diagnosis—partial correction indicates combined hepatocellular dysfunction and vitamin K deficiency.

#### *Liver biopsy*

Aim for INR <1.4 and platelet count  $>50 \times 10^9/L$ . Check on day of biopsy. Give FFP 10–15mL/kg. Check INR and repeat FFP dose until PT is satisfactory—not always achieved. Consider PCC (no longer contraindicated, previous concerns of DIC and/or thrombosis but low clinical risk). Platelet transfusion  $>50 \times 10^9/L$ .

#### *Active bleeding*

Blood transfusion, vitamin K, FFP, platelets as for liver biopsy with monitoring and further therapy as required, may need repeating 6–12-hourly, surgical intervention for oesophageal bleeding (Sengstaken tube, sclerotherapy, desmopressin, TIPS). DIC is a feature of fulminant liver failure and after liver surgery and transplantation. Control underlying condition, support with platelet/FFP as required. Consider aprotinin or rVIIa for uncontrollable bleeding.

## Renal disease

### Uraemic bleeding

Bleeding common due to platelet dysfunction in patients with uraemia due to chronic renal failure correlating with the severity of the uraemia—exacerbated by anaemia. vWF levels  $\leftrightarrow$  but vWF function may be impaired. Abnormal bleeding time and abnormal platelet aggregation previously documented in studies but of little or no value in routine practice in individual patients. Bleeding time does not predict risk of haemorrhage and is not indicated. PFA-100 requires evaluation as a predictor of risk of bleeding in this situation.

### Treatment

- Intensive dialysis.
- Maintain haemoglobin  $>10\text{g/dL}$  with transfusion/EPO.
- Desmopressin ( $0.3\text{mcg/kg}$  SC or slow IV injection, beware hyponatraemia).
- Cryoprecipitate (mechanism unknown, may be affected due to HMW vWF content, effect may last up to 36h, not always clinically effective).
- Conjugated oestrogens (oral Premarin  $10\text{--}50\text{mg/d}$  or daily IV Emopremarin  $0.6\text{mg/kg}$ ).

Often mucocutaneous bleeding but can be ICH and pericardial haemorrhage.

## Acquired anticoagulants and inhibitors

The development of inhibitors against coagulation factors is uncommon other than antiphospholipid antibodies (➔ see Antiphospholipid syndrome p.534). Factor VIII antibodies, either spontaneous or in treated patients with haemophilias, are a major clinical problem. Acquired VWS may be due to antibodies (seen particularly in paraproteinaemias) or consumption when high platelet turnover (MPDs particularly essential thrombocythaemia). Inhibitors to other clotting factors and heparin-like inhibitors are very rare.

### Factor VIII inhibitors

#### *Alloantibodies*

Occur in 15–25% haemophilia A patients following treatment, usually after 5–20 treatment episodes in severely affected patients (FVIII<0.01U/mL) (➔ see Haemophilia A complications, p.477). FIX inhibitors in haemophilia B patients are rare.

#### *Autoantibodies*

Spontaneous development of FVIII inhibitors in non-haemophilia patients is reported in 1 per million population. Antibody is usually IgG, occasionally IgM or IgA and will neutralize the functional VIII protein or result in ↑ clearance or both. Acquired FVIII inhibitors develop in the elderly, during pregnancy, in association with autoimmune and malignant disease, various skin disorders (psoriasis, pemphigus, erythema multiforme) infections, and drug therapy (penicillin, aminoglycosides, phenothiazines). Symptoms include bleeding (postoperatively this can be major), easy bruising. Haemarthrosis is unusual. Mortality is significant, as many as 25% patients with persisting VIII inhibitors will die from bleeding.

### Diagnosis

- Prolonged APTT with failure to correct with ↔ plasma.
- Low plasma FVIII level.
- Inhibitor screen and inhibitor assay as for detection of FVIII inhibitor in patient with congenital haemophilia A (➔ see p.476), however assay is not quantitative in patients with acquired FVIII inhibitors and poor correlation between titre and bleeding.
- Differential diagnosis of spontaneous inhibitors—need to exclude non-specific inhibitors, e.g. myeloma paraproteins which bind non-specifically to coagulation plasma proteins. Ensure sample not contaminated with heparin.

### Further reading

Collins, P. W. (2007). Treatment of acquired hemophilia A. *J Thromb Haemostasis*, **5**, 893–900.

## Treatment of spontaneous FVIII inhibitor

- Prednisolone (1mg/kg/d)—may take weeks to work.
- Cyclophosphamide can be used or added to prednisolone initially or if no response after 6 weeks.
- Increasingly rituximab (375mg/m<sup>2</sup> weekly × 4 weeks) used with good response rate. Treat active bleeding with 'FVIII bypassing agent' (rVIIa or FEIBA).
- In past porcine FVIII was available and if no cross-reactivity porcine VIII treatment was often effective. A recombinant product is under development.
- Mild bleeding may respond to local pressure, tranexamic acid, or desmopressin.

### Acquired VWS

Rare disorder presenting in later life, has a variable bleeding pattern similar to the inherited condition. An associated monoclonal gammopathy/LPD is common but the condition may be autoimmune or idiopathic. Bleeding symptoms vary from mild to major, e.g. massive GI haemorrhage requiring frequent blood transfusion.

### Diagnosis

- ↔ PT.
- ↑ APTT.
- ↓ VIII:C.
- ↓ vWF:Ag and vWF:RCo, *in vitro* evidence of the vWF inhibitor not always demonstrable.
- Paraprotein, often IgG.

### Treatment

- High-dose immunoglobulin (1g/kg/d × 2d) is often effective when IgG paraprotein, not effective when IgM. Regular treatment may be required, e.g. monthly.
- Bleeding treated with desmopressin, high purity vWF concentrate (Wilfactin), vWF containing factor VIII concentrate (e.g. Alphanate).
- Platelet transfusions may help if refractory major bleeding.

### Other coagulation inhibitors

Inhibitors, spontaneous or post-treatment, are reported against most other coagulation factors (V, IX, and prothrombin, XI, VII, and X)—all very rare. Factor V antibodies may arise in congenitally deficient patients following treatment or spontaneously following antibiotics, infection, blood transfusion. Postoperative autoantibodies may develop as a result of exposure to haemostatic agents contaminated with bovine factor V, e.g. fibrin glue. Most are low titre and transient. Treat with FFP and platelets (a source of factor V).

Heparin-like inhibitors are reported in patients with malignant disease, following chemotherapy and may cause bleeding. Protamine sulfate neutralization *in vitro* and *in vivo* is a feature of this inhibitor.

### Diagnosis

- Screening tests (PT, APTT, TT) will give abnormal results depending on the factor involved, with failure to correct with ↔ plasma.
- Defining the specific factor requires factor assays.
- Exclude acquired deficiencies, e.g. factor X deficiency in amyloidosis.
- Treatment for actively bleeding patients since acquired inhibitors may not give rise to symptoms.
- 1st-line treatment is often FFP but large volumes may be required and efficacy may be limited.
- Some specific concentrates are available.
- Recombinant VIIa can be considered in many cases.
- Treatment of the underlying condition may cause the inhibitor to disappear.

## Acquired disorders of platelet function

### Drugs associated with platelet dysfunction

- Aspirin, clopidogrel, dipyridamole, epoprostenol, IIb/IIIa antagonists.
- NSAIDs.
- $\beta$ -lactam antibiotics: penicillins and cephalosporins.
- 'Antiplatelet agents': epoprostenol, dipyridamole.
- Heparin.
- Plasma expanders: dextran, hydroxyethyl starch.
- Other drugs: antihistamines, local anaesthetics,  $\beta$ -blockers.
- Food additives: fish oil.

Easy bruising, epistaxis, haematomas, haemorrhage after surgery especially in patients with a pre-existing bleeding tendency. NSAIDs cause reversible inhibition of cyclooxygenase. Effect on bleeding and platelet aggregation is brief (only as long as circulating drug present—up to 24h after ingestion) and less likely to cause clinical bleeding in patients without a prior bleeding disorder.  $\beta$ -lactam antibiotics affect platelet function by lipophilic attachment to cell membrane in dose-dependent manner—only after sustained high dosage though effect may last 7–10d. A diet rich in fish oils (omega-3 fatty acids) can cause mild prolongation of bleeding time but rarely bleeding. Ethanol has direct inhibitory effect on platelet function.

Aspirin should be avoided in patients with bleeding tendency. A patient on aspirin should discontinue the drug at least a week prior to a surgical procedure. Desmopressin or platelet transfusion may be used when bleeding due to aspirin-induced platelet function defect. In most cases discontinuation of drug is sufficient.

### Systemic conditions which affect platelet function

- Renal failure.
- Liver failure.
- Glycogen storage disorders types Ia and Ib.
- Cardiopulmonary bypass surgery—abnormal platelet function and thrombocytopenia are frequently seen in patients subjected to cardiopulmonary bypass surgery. Impaired aggregation studies *in vitro* occur in proportion to duration of the bypass procedure. Possibly due to platelet activation and fragmentation in the extracorporeal loop. Desmopressin and platelet transfusion used in patients with bleeding.
- DIC.
- Valvular heart disease, renal allograft rejection, cavernous haemangioma.

### Paraproteinaemias and antiplatelet antibodies

- Myeloma and Waldenström macroglobulinaemia—haemorrhage is more commonly due to thrombocytopenia or hyperviscosity. Plasmapheresis to remove circulating protein may be tried.
- Autoimmune disorders (but thrombocytopenia usually more important).



**Haematological conditions with production of abnormal platelets**

- Chronic MPDs—especially essential thrombocythaemia—acquired VWS may also be contributory due to depletion of HMW vWF.  
↑ whole blood viscosity in patients with polycythaemia causes bleeding.
- Myelodysplasia—abnormal morphology with ↓ granules, acquired storage pool defects.
- Leukaemia.

## Henoch–Schönlein purpura

Immune complex disease characterized by a leucocytoclastic vasculitis. Purpura is not due to thrombocytopenia or impaired platelet function. Predominantly affects children aged 2–8 years. Clear preponderance in the winter. Commonly presents 1–3 weeks after upper respiratory tract illness. Various infections, toxins, physical trauma, possibly insect bites, and allergies have all been postulated as triggers of the disease but no clear causation established. May also occur with malignancy.

### Clinical features

- Rapid onset usual.
- Classically a palpable purpuric rash over buttocks/legs (extensor surfaces).
- Urticarial plaques and haemorrhagic bullae seen, often symmetrical.
- Abdominal pain due to mesenteric vasculitis.
- Arthritis, particularly knees and ankles.
- Renal involvement—haematuria  $\pm$  proteinuria, may lead to either acute or chronic renal failure.

### Diagnosis

- Presence of typical findings above and exclusion of other causes.
- FBC and film  $\leftrightarrow$ .
- Platelet numbers and function are  $\leftrightarrow$ . ESR raised.
- Other markers of autoimmune disorders may be present.

### Treatment and prognosis

- Spontaneous resolution within a month is commonest outcome in children.
- Long-term sequelae more common in adults, e.g. chronic renal failure.
- Steroids may be of benefit particularly if joint pains are troublesome.



## Perioperative bleeding and massive blood loss

70–90% of abnormal intraoperative or postoperative bleeding is ‘surgical’ (e.g. bleeding vessel due to failed ligature cautery) rather than haemostatic failure.

### Distinguishing surgical bleeding and haemostatic failure

#### Features suggesting surgical bleeding

- Bleeding from a single site with other potential sites not bleeding.
- Sudden onset of massive and/or rapid bleeding.
- Bright red or pulsatile bleeding from an identifiable source.

#### Features suggesting haemostatic failure

- Multiple simultaneous bleeding sites—surgical incisions, vascular access sites, mucosal membranes, skin, haematuria.
- Slow persistent ooze of blood from a non-identifiable source.
- Delayed haemorrhage following initial haemostasis.

### Investigation

- Review drug chart—anticoagulants, antiplatelet drugs, NSAIDs.
- Were preoperative tests ↔ (PT, APTT, platelets if done, LFTs U&Es)?
- Take fresh samples for:
  - Platelet count.
  - PT and APTT (Fgn if either abnormal).
  - D-dimer if clinical suspicion of DIC and PT, APTT, and platelet count consistent with diagnosis (➔ see Disseminated intravascular coagulation, p.494).
- If liver transplantation consider hyperfibrinolysis—thromboelastography useful.
- Obtain history again from patient or relative regarding personal or family history of bleeding.
- Is patient septicæmic—DIC?
- Does patient have multiorgan failure—DIC?
- Was surgery or anaesthesia prolonged—acidosis, hypothermia?
- Was patient transfused—dilutional coagulopathy?
- Estimate bleeding rate for later comparison.

### Treatment

- Maintain tissue perfusion and oxygenation—fluid volume replacement and red cell transfusion if anaemic—aim to maintain Hb >8g/dL.
- Correct acidosis and hypothermia by restoring perfusion—use blood warmer if necessary for blood products.
- Explore surgical site when possible and when surgical bleeding suggested by clinical and laboratory findings.
- Correction of coagulopathy (PT and APTT ratios <1.5 × ↔) with FFP, platelet transfusion, and cryoprecipitate when necessary.
- Empirical administration of desmopressin.
- Empirical administration of antifibrinolytic drugs—tranexamic acid, aprotinin.

- In life-threatening cases not responding to treatment consider rVIIa use after consultation with haematologist. Ensure Fgn  $>1\text{g/dL}$ , platelets  $>50 \times 10^9/\text{L}$ , PT and APTT corrected as far as possible with FFP.

### Further reading

Stainsby, D. et al. (2006). Guidelines on the management of massive blood loss. *Brit J Haematol*, **135**, 634–41.

## Massive blood loss

- Total blood volume replaced within 24h, or
- 50% blood loss within 3h, or
- Blood loss at a rate of 150mL/min.

### Treatment

- Maintain tissue perfusion and oxygenation.
- Correct surgical bleeding.
- Blood component therapy as for surgical bleeding—maintain Hb >8g/dL, platelets  $>75 \times 10^9/L$ , PT and APTT ratios  $<1.5 \times$   $\leftrightarrow$ , Fgn  $>1g/L$ .
- Contact key personnel—e.g. anaesthetist, blood bank.
- Consider use of VIIa.



## Heparin

Heparin remains the most widely used parenteral antithrombotic.

- Naturally occurring glycosaminoglycan. Pharmaceutical preparation is extracted from porcine (usually) or bovine mucosa.
- Heterogeneous mixture of straight chain anionic mucopolysaccharides spanning 20–100 monosaccharide units, alternating chains of uronic acid and glucosamine, sulphated to varying degrees.
- LMWH manufactured from unfractionated heparin (UFH) by chemical or enzymatic methods—no evidence that these differences in chemical structure affect biological function.
- The anticoagulant action of heparin is due to a combination of indirect antithrombin and anti-Xa activity.
- Elimination of heparin from the plasma appears to involve a combination of zero-order and 1st-order processes, the effect of which is that the plasma  $t_{1/2}$  alters disproportionately with dose, being 60min after 75U/kg and 150min after 400U/kg. LMWHs are less protein bound and have a predictable dose response profile. They also have a longer  $t_{1/2}$  than standard heparin preparations.
- Heparin is used when it is necessary to achieve immediate anticoagulation.
- Therapeutic dose UFH given either as a continuous IVI or by injection under the skin. Treatment is monitored by the APTT ratio, aiming to prolong the APTT to  $2.0 \times$  ↔ (range 1.5–2.5). ↔ starting dose is 15–20U/kg/h. The APTT should be measured within 6–12h of starting treatment and the dose of heparin adjusted accordingly. Thereafter, the APTT should be measured (preferably on a daily basis) and the dose of heparin adjusted each day. UFH is still used in some circumstances as the short duration of action can be advantageous when it is necessary to vary the intensity of anticoagulation over a short time period, e.g. in a high thrombosis risk patient undergoing surgery.
- Nowadays the heparin of choice for prevention and treatment of DVT and PE and unstable coronary disease is LMWH, given once daily SC injection without the need for monitoring or dose adjustment. Lower risk of HIT. Patients with acute VTE can be treated as outpatients.
- Osteoporosis may complicate long-term use. It is dose-related and is most frequently observed with use in pregnancy. The relative risk with LMWH is not yet established but appears less.
- Hypersensitivity reactions and skin necrosis (similar to that seen with warfarin) occur but are rare. Transient alopecia has been reported.

### Bleeding due to heparin

- More common with impaired hepatic or renal function, with carcinoma, and in patients >60 years of age.
- An APPT ratio >3.0 is associated with an ↑ risk of bleeding.



### Treatment

- Effect of UFH is short and so reversal with protamine sulfate is seldom required, except after extracorporeal perfusion for heart surgery.
- Protamine sulfate, 1mg by slow IV neutralizes 80–100U of UFH.
- The dose given is usually estimated from the dose of heparin given and its expected  $t_{1/2}$ .
- Protamine itself has some anticoagulant effect and overdosage must be avoided.
- The maximum dose must not exceed 50mg.
- Its clinical effectiveness in patients treated with LMWH is unknown.

### Further reading

Baglin, T. *et al.* (2006). Guidelines on the use and monitoring of heparin. *Brit J Haematol*, **133**, 19–34.

## Heparin induced thrombocytopenia/with thrombosis (HIT/T)

HIT (↻ see Heparin-induced thrombocytopenia (HIT), p.542) with or without thrombosis (HIT/T) is due to an autoantibody against heparin complexed with PF4, causing platelet activation. It occurs most commonly with heparin derived from bovine lung. Thrombosis occurs in <1% of patients treated with LMWH but is associated with a mortality and limb amputation rate in excess of 30%.

### Diagnosis

- Suspected in any patient in whom the platelet count falls by 50% or more after starting heparin.
- Usually occurs after ≥5d of heparin exposure (or sooner if the patient has previously been exposed to heparin).

### Treatment

- When suspected all heparin (UFH and LMWH) stopped and an alternative thrombin inhibitor, such as argatroban should be given.
- In patients with adequate renal function fondaparinux is often used as there is a very low risk of antibody cross-reactivity to this synthetic pentasaccharide.
- Warfarin should not be started until adequate anticoagulation has been achieved with one of these agents and the platelet count has returned to ↔.



## Oral anticoagulant therapy (warfarin, VKAs)

Warfarin is a coumarin derivative that inhibits vitamin K epoxide reductase. Leads to intrahepatic depletion of the reduced form of vitamin K which is a necessary cofactor for the post-translational modification of vitamin K-dependent proteins. Warfarin is the most widely used oral vitamin K antagonist. Rapidly accumulates in the liver with  $t_{1/2} > 40\text{h}$  with a prolonged dose-dependent terminal phase of elimination with detectable warfarin levels 120h after a single dose. Average dose required to achieve an INR of 2.5 is between 3–5mg, although some patients require as little as 1mg and some as much as 30mg, or more. Warfarin is particularly effective for the prevention and treatment of venous thromboembolism and prevention of embolization in association with atrial fibrillation or prosthetic heart valves.

- Oral vitamin K antagonists (VKAs) take several days to produce an anticoagulant effect, hence the need for an immediate acting anticoagulant, such as a heparin, in the first few days of therapy if rapid anticoagulation is required.
- Response to warfarin, and other coumarins, varies within and between individuals and therefore the dose must be monitored regularly.
- Pharmacokinetics (absorption and metabolism) and pharmacodynamics (haemostatic effect) are influenced by vitamin K intake and absorption, by heritable functional polymorphisms affecting metabolism, by rates of synthesis, and clearance of coagulation proteins and by drugs.
- Anticoagulant therapy with oral VKAs is monitored by the international normalized ratio (INR). The INR is derived from the PT ratio and is a standardized method of reporting which permits comparability between laboratories. The ratio of the patient's PT is divided by the geometric mean  $\leftrightarrow$  PT (GMNPT) and the ratio is then raised to the power of the International Sensitivity Index (ISI) of the thromboplastin used to measure the PTs. Thus:

$$\text{INR} = \left( \frac{\text{PT (patient)}}{\text{GMNPT}} \right)^{\text{ISI}}$$

For example using a thromboplastin with an ISI of 1.2 and a GMNPT of 13.5sec a patient taking a daily maintenance dose of warfarin producing a PT of 27sec would have an INR of  $(27 \div 13.5)^{1.2} = 2.3$ .

For *rapid induction of anticoagulation* (e.g. treatment of VTE) commence loading dose (e.g. 5 or 10mg with daily or alternate day INR measurement and appropriate dose adjustment) whilst treating with immediate acting anticoagulant, e.g. heparin. Daily maintenance dose is predicted by the INR on d4.

For *slow induction of anticoagulation* (e.g. atrial fibrillation) a low dose can be started, e.g. 2mg of warfarin daily for 1 week and INR then measured—additional immediate acting anticoagulant not required. Daily dose of warfarin  $\uparrow$  by 1mg each week, with weekly monitoring of the INR, and when the INR is  $> 2.0$  maintenance dose has been reached.

- Maintain as stable a level of anticoagulation as possible.
- Adopt the lowest effective target INR.
- Educate patients regarding risk, particularly that associated with additional drug use.

## Bleeding due to oral VKA (warfarin)

Risk of bleeding greatest with:

- Age >65 years.
- A history of stroke.
- History of GI bleeding.
- Other medical illness, including renal failure and anaemia.

Warfarin embryopathy, central nervous system bleeding and fetal bleeding complicate administration of warfarin and other oral VKAs to pregnant women. Therefore:

- Warn women of childbearing age.
- Indicate need for early pregnancy test (within 6 weeks) and cessation of therapy with switch to heparin if +ve.

Acenocoumarol has a shorter  $t_{1/2}$  than warfarin. Indications, contraindications, and side effects are similar to warfarin. The dose required to produce the same intensity of anticoagulation as warfarin is about 50% of the warfarin dose.

Phenindione is an oral VKA that is not a coumarin derivative. It is an indanedione. It is more toxic than the coumarin derivatives and is associated with an ↑ risk of hypersensitivity reactions, including exfoliative dermatitis, renal and liver damage, and agranulocytosis. For this reason it is rarely used. It also produces taste disturbance and pink/orange urine. It is also teratogenic.

## Management of overanticoagulation and/or bleeding due to oral VKA (warfarin)

- *No bleeding, INR <8.0:* ↓ warfarin dose or stop warfarin and restart appropriate new dose when INR <5.0 (response to warfarin dose change is delayed so INR will continue to fall after restarting).
- *No bleeding or minor bleeding, INR > 8.0:* stop warfarin (restart when INR <5.0), consider oral vitamin K (0.5–2.5mg) especially if other risk factors for bleeding.
- *Major bleeding:* stop warfarin, give factor concentrate (II, VII, IX, and X) or FFP, give 5–10mg IV vitamin K.

## Perioperative management of oral anticoagulation

- Unless high risk of thromboembolism stop warfarin. Complete reversal of anticoagulation will require stopping 5d before surgery, for partial reversal 3d.
- Anticoagulation does not need to be stopped before dental surgery, including extraction. Safe to proceed as long as INR <4.0.
- Check INR preoperatively.
- Short term risk of thromboembolism low in patients with mechanical prosthetic valves, mitral > aortic risk. When necessary to continue anticoagulation, e.g. metal mitral valve, bridge with heparin, e.g. therapeutic dose LMWH starting 3d after stopping warfarin, omitting in 24h before surgery, continuing postoperatively until INR >2.0 in response to reintroduction of warfarin.

## Further reading

Keeling, D. et al. (2011). Guidelines on oral anticoagulation—fourth edition. *Br J Haematol*, **154**, 311–24.

## New oral anticoagulant drugs

Oral direct inhibitors of thrombin and factor Xa are now approved as anticoagulant drugs. Inhibitors of thrombin and factor Xa have now been shown to be selective, orally active, safe, and at least as effective as low-dose LMWH for thromboprophylaxis and as effective as, if not superior to, warfarin for long-term prevention of thromboembolism. Dabigatran, rivaroxaban and apixaban are the first thrombin and X-a inhibitors to be licensed for prevention of thromboembolism in patients with atrial fibrillation. For some patients these drugs offer substantial benefits over oral VKAs. For the majority of patients these drugs are prescribed at fixed dose without the need for monitoring or dose adjustment. There are no food interactions and very limited drug interactions. The rapid onset of anticoagulation and short  $t_{1/2}$  make initiation and interruption of anticoagulant therapy considerably easier than with VKAs. As with all anticoagulants produced so far there is a correlation between intensity of anticoagulation and bleeding. Consequently, the need to consider the balance of benefit and risk in each individual patient is no less important than with VKA therapy.

### Dabigatran

- Dabigatran is an oral anticoagulant with a short  $t_{1/2}$ .
- The risk of intracranial haemorrhage is significantly less than with warfarin.
- Licence includes prevention of CVA and systemic embolism in patients with non-valvular atrial fibrillation.
- Given at a fixed dose without monitoring and without dose adjustment.
- INR not used.
- Dose in patients with AF 150mg bd under 75 years (in patients  $\geq 75$  years a dose of 110mg bd should be considered in the presence of risk factors that increase the risk of bleeding, in patients  $\geq 80$  years a dose of 110mg bd is recommended regardless).
- Food has no significant effect on dabigatran.
- Use of dabigatran and the following drugs is contraindicated: ciclosporin, itraconazole, ketoconazole, tacrolimus.
- No dose adjustment is needed with digoxin.
- No interaction with commonly used antibiotics or statins.
- Caution required with amiodarone, verapamil, quinidine (e.g. reduce dose and take advice).
- Avoid aspirin and NSAIDs as with warfarin.
- Dabigatran does not affect the action of proton pump inhibitors. (PPI). When using antacids in combination with dabigatran, it is advised to take dabigatran 2h before the antacid.
- 10% of patients suffer indigestion; PPI can be given.
- At  $eGFR < 30$  mL/min do not prescribe.
- At  $eGFR 30-50$  mL/min suggest take advice.
- Suggest check  $eGFR$  once yearly (twice yearly if  $eGFR < 60$ ).
- Contraindicated if liver disease with coagulopathy, i.e. baseline PT  $> 13$  sec.

- For surgical, endoscopy and dental procedures including extraction omit doses of dabigatran on day of procedure (i.e. last dose 24h beforehand).

### Rivaroxaban

- Rivaroxaban is an oral anticoagulant with a short  $t_{1/2}$ .
- The risk of intracranial haemorrhage is significantly less than with warfarin.
- Licence includes: (i) prevention of CVA and systemic embolism in patients with non-valvular atrial fibrillation and (ii) treatment and prevention of DVT and PE.
- Given at a fixed dose without monitoring and without dose adjustment.
- INR not used.
- Dose 20mg od all ages for treatment of DVT and prevention of stroke and embolism in AF.
- Food has no significant effect on rivaroxaban.
- Use of rivaroxaban and the following drugs is contraindicated: ketoconazole and other azole-antimycotics, HIV protease inhibitors.
- No dose adjustment is needed with digoxin.
- No interaction with antibiotics or statins.
- At eGFR <15mL/min do not prescribe.
- At eGFR 15 to 30 mL/min dose should be 15mg od.
- Suggest check eGFR once yearly (twice yearly if eGFR <60).
- Contraindicated if liver disease with coagulopathy, i.e. baseline PT >13sec.
- For surgical, endoscopy and dental procedures including extraction omit dose of rivaroxaban on day of procedure (i.e. last dose 24h beforehand).

### Further reading

Baglin, T. (2013). Clinical use of new oral anticoagulant drugs: dabigatran and rivaroxaban. *Br J Haematol*, **163**, 160–7.

## Thrombosis

Thrombosis is defined as coagulation within the circulation. Thrombosis may occur in any part of the circulation including the heart, arteries, veins, and the microcirculation. The results of thrombosis are:

- Local obstruction of the circulation.
- Embolization of clot.
- Consumption of haemostatic factors (if thrombosis is extensive).

### Venous thrombosis

DVT and PE are different clinical manifestations of a spectrum of disease termed collectively venous thromboembolism (VTE). Most patients presenting with PE have asymptomatic DVT and most patients presenting with symptoms of DVT alone have asymptomatic PE.

- Annual incidence of 1/1000 increasing with age.
- Case fatality rate range 1–5%.
- Post-thrombotic syndrome—characterized by chronic pain, swelling and sometimes ulceration of the skin of the leg occurs in up to 1/3 of patients. Can occur early or up to 10 years. Cumulative frequency 30% at 5 years.
- Chronic thromboembolic pulmonary hypertension develops in 2% of patients presenting with symptomatic PE. May require pulmonary artery endarterectomy.
- DVT usually starts in the calf but by the time symptoms develop 80% of patients have proximal DVT (popliteal vein and above).

### Risk factors for VTE

➔ See VTE risk assessment and thromboprophylaxis, p.524.

### DVT

#### Diagnosis

Investigation of suspected DVT is now frequently on combination of clinical assessment (pretest probability (PTP) score, Table 10.3) and result of D-dimer measurement (see Box 10.1). D-dimer is a fibrin degradation product. Test can be performed on plasma. Thrombosis results in high D-dimer (+ve result) *but not specific*, i.e. most patients with +ve D-dimer do not have thrombosis (infection, inflammation, malignancy, tissue trauma, pregnancy). Therefore, D-dimer cannot be used to diagnose DVT it can only be used to exclude it (depending on PPT probability score).



**Table 10.3** Clinical probability (PTP) assessment (Well's score) score

Active cancer (treatment ongoing or within previous 6 months or palliative)	1
Paralysis, plaster cast	1
Bed >3d or surgery within 4 weeks	1
Tenderness along veins	1
Entire leg swollen	1
Calf swollen >3 cm	1
Pitting oedema	1
Collateral veins	1
Alternative diagnosis likely	-2

Low: 0 or less; moderate: 1 or 2; high: 3 or more. 5%, 20% and 75% of patients with low, moderate or high pretest probability, respectively, have DVT.

#### *D-dimer assay*

Measurement of D-dimer can then be used to determine which patients require imaging (now usually compression ultrasound or CT angiography) and which patients do not.

- D-dimer should not be measured until after clinical score (pretest probability score).
- -ve D-dimer has a role in excluding VTE.
- D-dimer affected by heparin administration—potentially giving false -ve result.
- D-dimer may be false -ve if the patient has had symptoms for >2 weeks.
- D-dimer assay with appropriately high sensitivity must be used.<sup>1</sup>

#### **Box 10.1**

Low PTP + -ve D-dimer = DVT *excluded*.

Moderate PTP + -ve D-dimer = US—if -ve DVT *excluded*.

Moderate PTP + +ve D-dimer = US—if -ve repeat US in 1 week.

High PTP = US.

### Radiology

Compression US is now preferred imaging technique. Inability to compress the vein lumen is the principal diagnostic criterion and other findings do not increase sensitivity. Sensitivity for proximal DVT of 97%. Advantage over conventional venography is the ability of US to identify alternative diagnoses of leg pain and swelling. The 1st test will detect any proximal thrombosis, a calf vein thrombus may remain undetected but a repeat scan 1 week later will pick up clinically important calf DVT that has extended. It is safe to withhold anticoagulant treatment from patients with clinically suspected DVT who have -ve US and -ve D-dimer or -ve US and +ve D-dimer and -ve repeat US 1 week later. If D-dimer not used it is safe to dispense with the follow-up US with a -ve 1st US in patient with a low PTP.

### Treatment

- Therapeutic dose LMWH for 3–5d (as outpatient for majority of patients but ⚡ see Contraindications to outpatient therapy, p.520).
- Loading dose of oral VKA when diagnosis confirmed, using loading nomogram with prediction of daily maintenance dose for target INR 2.5. Particular risk of overanticoagulation when elderly (>70 years), liver disease, alcohol abuse, body weight <50 kg, congestive heart failure.
- Oral anticoagulation for 3–6 months for first episode of VTE, longer if persistent risk factors (evidence of heritable thrombophilia not usually an indication of itself for prolonged anticoagulation).
- Treatment with rivaroxaban (15mg bd for 3 weeks then 20mg daily) is alternative to treatment with LMWH + VKA. Dabigatran and apixaban likely to be licensed for treatment of VTE in future.
- Compression stocking exerting pressure of at least 30mmHg at ankle (European Class 2, British Class 3 stocking). 50% reduction in post-thrombotic syndrome (PTS).
- Education of patient regarding risk avoidance and need for thromboprophylaxis at times of high risk.
- Testing for acquired thrombophilia as indicated. Antiphospholipid syndrome (APS, ⚡ see Acquired thrombophilia, p.534), MPD including PNH.
- Review of family history with view to consideration of testing for heritable thrombophilia (⚡ see Heritable thrombophilia, p.530) (may require completion of anticoagulant therapy for full assessment).

*Contraindications to early/immediate outpatient treatment:* coexistent serious medical pathology, severe acute venous obstruction (phlegmasia cerulea dolens), severe pain, active bleeding, or significant risk of bleeding, renal impairment (creatinine in excess of 200micromol/L), liver disease (PT >2sec beyond normal range), uncontrolled hypertension (diastolic >110mmHg, systolic >200mmHg), recent eye or CNS surgery (within 1 month); recent haemorrhagic stroke (within 1 month), known heparin allergy or previous HIT/T, active peptic ulceration, thrombocytopenia (<100 × 10<sup>9</sup>/L), suspected problems compliance.

Advantages of LMWH over UFH: once daily SC dose calculated from patient weight, no monitoring of APTT required (platelet count at beginning and on d3 or 4), low risk of HIT/T (⚡ see Heparin-induced thrombocytopenia, p.542), interpatient and inpatient variability in

UFH dosage requirement results in the APTT being in the therapeutic range in only 50% of time.

## PE

- PE carries a higher mortality than DVT. Whilst many patients will require hospital admission there is a subgroup of patients who present with peripheral PE (pleuritic chest pain and/or haemoptysis) who can safely be treated on an outpatient basis.
- Right heart strain and right ventricular muscle ischaemia causes release of troponin—sometimes used as prognostic indicator.

## Diagnosis

All patients with possible PE should have clinical probability assessed.

### Clinical probability (PPT)

Some assessment systems try and define PE as likely or unlikely but require complex scoring systems (see Box 10.2). A simple pragmatic approach is:

- Patient has clinical features compatible with PE—dyspnoea and/or tachypnoea with or without chest pain and/or haemoptysis (a).
- Absence of another diagnosis (b).
- Presence of a major risk factor (c).

### Box 10.2 Probability score for PE

- *High probability* = (a) + (b) + (c) = CTPA.
- *Moderate probability* = (a) + (b) or (c) = CTPA or high sensitivity D-dimer, and CTPA only if D-dimer +ve.
- *Low probability* = (a) = D-dimer and CTPA if +ve.

## D-dimer

Same considerations of assay as for DVT.

## Radiology

- CT pulmonary angiography is now recommended imaging method for non-massive PE. V/Q scan if CTPA not available and CXR is ↔ and no significant pre-existing cardiopulmonary disease and non-diagnostic result is followed by further imaging.
- CTPA or echocardiography will reliably diagnose massive PE.
- Good quality normal CTPA excludes PE.

## Treatment

- Thrombolysis is 1st-line treatment for massive PE—e.g. 10mg tPA over 2min followed by 90mg over 2h. May be started on clinical grounds if cardiac arrest imminent, e.g. 50mg tPA IV bolus.
- Heparin should be started before imaging if moderate or high clinical probability.
- IV bolus of UFH recommended after thrombolysis for massive PE followed by LMWH.
- LMWH alone for non-massive PE.

- Oral VKA as for DVT when diagnosis confirmed—loading nomogram, target INR 2.5, 3–6 months' duration for 1st episode of VTE.
- Treatment with rivaroxaban (15mg bd for 3 weeks then 20mg daily) is alternative to treatment with LMWH + VKA. Dabigatran and apixaban likely to be licensed for treatment of VTE in future.

#### Vena cava (VC) filters<sup>2</sup>

- VC filters are indicated to prevent PE in patients with VTE who have a contraindication to anticoagulation.
- Anticoagulation should be considered in patients with a VC filter when a temporary contraindication to anticoagulant therapy is no longer present.
- VC filters are not indicated in unselected patients with VTE who will receive conventional anticoagulant therapy.
- VC filter insertion may be considered in selected patients with recurrent PE despite therapeutic anticoagulation. Alternative treatment options such as long-term high-intensity oral anticoagulant therapy (INR target 3.5) or LMWH should be considered prior to VC filter placement, particularly in patients with thrombophilic disorders (e.g. antiphospholipid syndrome) or cancer.
- VC filter insertion may be considered in pregnant patients who have contraindications to anticoagulation or develop extensive VTE shortly before delivery (within 2 weeks). Retrievable filters should be considered.
- Free floating thrombus is not an indication for insertion of a VC filter.
- Thrombolysis is not an indication for filter insertion.
- VC filters should be considered in any preoperative patient with recent VTE (within 1 month) in whom anticoagulation must be interrupted. Retrievable VC filters should be considered in this situation where a temporary contraindication to anticoagulation exists.

**IV drug users**—management of iliofemoral venous thrombosis in injecting drug users is problematic because of poor venous access, non-compliance with prescribed treatment, ongoing IV drug use, and co-existent sepsis. Treatment with a LMWH results in a satisfactory clinical outcome. Use of a NOAC would likely achieve similar results.

**Cancer**—oral VKA therapy is inferior to therapeutic dose LMWH for treatment of VTE in patients with cancer. Therapeutic dose LMWH should be given for 4 weeks after which a 25% dose reduction can be made.

#### References

1. Keeling D. et al. (2006). on behalf of the Haemostasis and Thrombosis Task Force of the British Committee for Standards in Haematology. The management of heparin-induced thrombocytopenia. *Brit J Haematol*, **133**, 259–69.
2. Baglin, T.P. et al. (2006). Guidelines on use of vena cava filters. *Br J Haematol*, **134**, 590–5.

#### Further reading

Keeling, D.M. et al. (2004). The diagnosis of deep vein thrombosis in symptomatic outpatients and the potential for clinical assessment and D-dimer assays to reduce the need for diagnostic imaging. *Brit J Haematol*, **124**, 15–25.



## Risk assessment and thromboprophylaxis

Many studies have been performed to determine the incidence of DVT and PE in various groups of patients and to identify risk factors. The risk of VTE in a hospitalized patient depends not only on the reason for admission (procedural risk) but also on pre-existing patient-related factors (patient risk). Decision as to whether patient requires thromboprophylaxis depends on procedural and patient risks, and choice of prophylactic intervention depends also on procedural and patient bleeding risk.

### Thrombosis risk factors

#### *Procedural risk*

- Major orthopaedic surgery to lower limb, e.g. hip or knee replacement.
- Abdominal or pelvic surgery lasting >30min under general anaesthetic.
- Major trauma is also a risk factor with hip fracture being associated with a very high risk of DVT.

#### *Patient risk*

- Age >40 years and particularly >60 years.
- Obesity—BMI >30kg/m<sup>2</sup> and particularly >35kg/m<sup>2</sup>.
- Previous VTE.
- Known thrombophilia (a predisposing state which may be heritable).
- Malignancy.
- Heart failure.
- Respiratory disease.
- Severe infection.
- Oestrogen therapy and high-dose progestogens.
- Pregnancy and the postpartum.
- Immobility.

### Bleeding risk factors

#### *Procedural risk*

- Neurosurgery.
- Eye surgery.
- Other procedures with a high bleeding risk.

#### *Patient risk*

- Haemophilia and other bleeding disorders.
- Thrombocytopenia (platelets <100 × 10<sup>9</sup>/L).
- Recent cerebral haemorrhage (in previous month).
- Severe hypertension.
- Severe liver disease (↑ PT or oesophageal varices).
- Peptic ulcer.
- Endocarditis.

For each patient an assessment of risk should be undertaken on admission and reviewed periodically during hospitalization; depending on the degree of risk patients should receive advice and treatment to reduce risk. Patients should be mobilized early and low-dose heparin (LMWH or UFH) and graded pressure stockings, or alternative mechanical devices, should be considered for patients at moderate-to-high risk.

## Methods for prevention of VTE

Can be divided into mechanical and pharmacological methods:

*Mechanical methods include:*

- GCS.
- IPC.

The advantage of mechanical methods is the absence of an effect on coagulation so that there is no ↑ risk of bleeding. Disadvantages include cost (IPC), compliance (IPC and GCS), exacerbation of arterial insufficiency in patients with peripheral arterial disease, and possibly lower efficacy (GCS) than pharmacological methods.

*Pharmacological methods include:*

- LMWH.
- NOACs
- UFH.

The advantage of pharmacological methods is established efficacy and compliance, i.e. it is known when a dose is given. The disadvantage is bleeding due to an effect on coagulation and idiosyncratic drug reactions including heparin allergy, skin necrosis, and HITT.

UFH at a dose of 5000U, 2 or 3 × daily by SC injection reduces the risk of DVT and PE by >50%. The risk of non-fatal haemorrhage is more than doubled but the absolute excess is small as most patients do not suffer any appreciable bleeding and there is no increase in life-threatening haemorrhage. The use of LMWH has the advantage of once daily injection, and a lower risk of bleeding relative to efficacy. It is at least as effective as standard heparin and probably more effective. The combination of graded pressure stocking plus heparin appears to be more effective than either alone. LMWH reduces the risk of DVT and PE in high-risk medical patients by >50% when given at a high-risk patient dose, e.g. (enoxaparin 40mg, dalteparin 5000 units).

## Suggested options for risk assessment and prophylaxis

### *Thromboprophylaxis in high-risk medical patients*

*Risk assessment can be difficult and risk assessment methods are not fully validated.*

- Age >60 years and likely hospitalization with immobility for >3d.
- Or age >60 years and a diagnosis of heart failure, respiratory disease, sepsis, or inflammatory disease.
- Or hospitalization with condition known to be associated with high risk of thrombosis, e.g. SLE with a lupus anticoagulant.
- Prophylaxis—if low bleeding risk LMWH at high-risk dose (e.g. enoxaparin 40mg, dalteparin 5000 units), if high bleeding risk graded pressure stockings.

### *Thromboprophylaxis in high-risk non-orthopedic surgery*

*Risk assessment is easier.*

- Abdominal or pelvic surgery lasting >30min under general anaesthetic.
- Or low risk procedure but 1 or more patient-related risk factors.

- Prophylaxis—if low bleeding risk LMWH at standard risk dose (e.g. enoxaparin 20mg, dalteparin 2500 units), if high bleeding risk appropriate mechanical intervention.

*Thromboprophylaxis in high-risk orthopaedic surgery*

- Major orthopaedic surgery to lower limb, e.g. hip or knee replacement.
- Or hip fracture or major trauma.
- Prophylaxis—if low bleeding risk LMWH at high risk dose (e.g. enoxaparin 40mg, dalteparin 5000 units), or a NOAC if high bleeding risk appropriate mechanical intervention.
- Or low risk procedure but 1 or more patient-related risk factors.
- Prophylaxis—if low bleeding risk LMWH at standard or high-risk dose depending on perceived risks of thrombosis and bleeding.





## Example of VTE risk assessment

- Risk assessment is mandatory for all patients on admission to hospital. This example of a risk assessment model is not for use in pregnant women or children less than 18 years old.
- All patients should be assessed on admission and periodically during inpatient stay as risk may change. Reassessment after 48–72h is recommended and outcome and appropriate action must be recorded in the medical record.

### Step one

- Review the patient-related factors shown on the assessment sheet (see Table 10.4) against thrombosis risk, ticking each box that applies (more than 1 box can be ticked). Use the highest category of risk if more than 1 box is ticked (e.g. if both moderate and high risk are ticked, use guidance for high-risk patients).
- Any tick for thrombosis risk should prompt thromboprophylaxis. The choice of thromboprophylaxis will be determined by local policy but will likely utilize a combination of mechanical and pharmacological methods (➔ see Suggested options for risk assessment and prophylaxis, p.524).
- The risk factors identified are not exhaustive. Clinicians may consider additional risks in individual patients and offer thromboprophylaxis as appropriate.
- If the nurse or doctor completing this assessment is uncertain of risks, e.g. duration of surgery, procedural bleeding risk, then medical staff should be consulted.

### Step two

- Review the patient-related factors shown against bleeding risk and tick each box that applies (more than 1 box can be ticked).
- Any tick for bleeding risk should prompt medical staff review and to consider if bleeding risk is sufficient to preclude pharmacological intervention.

### Step three

- If no boxes are ticked, then the patient is at low risk of VTE and no intervention is indicated.

### Further reading

This topic draws on the VTE risk assessment guidance from the Department of Health, *Venous thromboembolism (VTE) risk assessment*, September, 2008.

**Table 10.4** VTE risk assessment

Mobility—all patients (tick one box)	Tick	Tick	Tick
Surgical patient	Medical patient expected to have ongoing reduced mobility relative to normal state	Medical patient NOT expected to have significantly reduced mobility relative to normal state	

**Assess for thrombosis and bleeding risk below Risk assessment now complete**

#### Thrombosis risk

Patient-related	Tick	Admission-related	Tick
Active cancer or cancer treatment		Significantly reduced mobility for 3 days or more	
Age >60		Hip or knee replacement	
Dehydration		Hip fracture	
Known thrombophilias		Total anaesthetic + surgical time >90min	
Obesity (BMI >30kg/m <sup>2</sup> )		Surgery involving pelvis or lower limb with a total anaesthetic + surgical time >60min	
One or more significant medical comorbidities (e.g. heart disease; metabolic, endocrine or respiratory pathologies; acute infectious diseases; inflammatory conditions)		Acute surgical admission with inflammatory or intra-abdominal condition	
Personal history or first-degree relative with a history of VTE		Critical care admission	
Use of hormone replacement therapy		Surgery with significant reduction in mobility	
Use of oestrogen-containing contraceptive therapy			
Varicose veins with phlebitis			
Pregnancy or <6 weeks post partum (see NICE guidance for specific risk factors)			

#### Bleeding risk

Patient-related	Tick	Admission-related	Tick
Active bleeding		Neurosurgery, spinal surgery or eye surgery	
Acquired bleeding disorders (such as acute liver failure)		Other procedure with high bleeding risk	
Concurrent use of anticoagulants known to increase the risk of bleeding (such as warfarin with INR >2)		Lumbar puncture/epidural/spinal anaesthesia expected within the next 12h	
Acute stroke		Lumbar puncture/epidural/spinal anaesthesia within the previous 4h	
Thrombocytopenia (platelets < 75 × 10 <sup>9</sup> /L)			
Uncontrolled systolic hypertension (230/120mmHg or higher)			
Untreated inherited bleeding disorders (such as haemophilia and von Willebrand's disease)			

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## Heritable thrombophilia

Blood tests can be done after an episode of DVT or a PE to test for evidence of a genetic predisposition for heritable thrombophilia.

- Thrombophilia explains why some individuals suffer a blood clot.
- The risk of a recurrent blood clot is not significantly greater in most individuals with thrombophilia than it is in individuals without thrombophilia. Therefore, duration of treatment is not generally different in patients with and without laboratory evidence of thrombophilia.
- Treatment decisions are based on the circumstances that led to the thrombosis, e.g. whether or not the clot followed an operation and if there is a strong family history of DVT or PE.

The most common type of heritable predisposition to DVT and PE is a change in the genetic code known as a mutation. Some mutations are present in about 1 in 15 people. 2 common mutations, each present in 3.5% of the Caucasian UK population are:

- Factor V Leiden (FVL).
- F2G20210A, also known as the prothrombin gene mutation.

Less common mutations affect natural anticoagulants including antithrombin, protein C (PC), and protein S (PS) giving rise to low levels (type 1 defect) or impaired function (type 2 defects).

25% of unselected patients presenting with a 1st episode of VTE will have laboratory evidence of a heritable thrombophilic defect; 25–50% of patients with thrombosis and a +ve family history. The frequency of the commonly identified heritable major factors is shown in Table 10.5.

### PC and PS deficiency

Many patients are asymptomatic and will never have a VTE. Clinically PC and PS deficiency are similar in terms of risk of thrombophlebitis and VTE. In neonates with severe PC deficiency (homozygous) purpura fulminans is life threatening. This is due to microvascular thrombosis (DIC). Skin necrosis may complicate warfarin therapy in patients with PC or S deficiency—less likely if slow loading regimen or when heparin used in combination with rapid loading.

### Antithrombin (AT) deficiency

This was the first major familial defect described (1965). VTE thrombosis risk appears greater than PC/S deficiency particularly during pregnancy. Homozygous severe AT deficiency is probably incompatible with life.

**Table 10.5** Heritable thrombophilic defects

Defect	Relative risk of VTE	Patients with 1st VTE	VTE with family history
FVL	2–4	15–20%	15–25%
F2G20210A	2–4	5%	5–10%
AT deficiency	10–20	1–2%	2–5%
PC deficiency	10	1–2%	2–5%
PS deficiency	5–10	3–6%	6–8%

### FVL mutation/polymorphism

Coagulation system involves a complex network of interactions between clotting factors—the result of this interaction of clotting factors is the generation of enzymes (XIa, IXa, Xa, and thrombin—thrombin converts soluble Fgn → insoluble fibrin). These interactions take place on the surface of cells, e.g. on the surface of platelets, and require activated non-enzymatic cofactors—factors Va and VIIIa. Generation of thrombin is regulated by proteolysis of FVa by the activated form of PC (APC). APC inactivates membrane bound factor Va through proteolytic cleavage at 3 specific sites in the HC. >95% cases APC resistance due to factor V Leiden (mutation causes amino acid substitution glutamine to arginine at position 506). This reduces proteolysis promoting sustained thrombin generation.

- FVL is associated with an ↑ risk of a 1st episode of DVT.
- Risk of PE is not ↑ as much as DVT, if at all.
- Risk of recurrent VTE is no greater in carriers of FVL than in other patients with a history of VTE who are not carriers of FVL.
- Relative risk of DVT associated with COC is ↑ in carriers of FVL but this translates into an absolute risk of 15/10,000/year.
- Relative risk of DVT associated with HRT is ↑ in carriers of FVL but this translates into an absolute risk of 30/10,000/year.

### Pregnancy

Risk of VTE estimated from personal and family history. Determines whether antenatal or postnatal prophylaxis is required.

### Contraceptive pills

OCP-users have 4-fold VTE risk compared to non-users generally. The FVL mutation increase the risk a further 8-fold—the 30–35-fold ↑ risk in FVL +ve COC users compared to FVL –ve non-COC users translates into a low absolute risk, see Table 10.6.

These are overall risk estimates and some women with a strong family history of VTE may be at higher risk. Because thrombophilia is a polygenic disorder with significant environment–gene interaction a –ve thrombophilia result may give false reassurance of low risk.

**Table 10.6** Annual VTE risk for FVL mutation and COC users

	Annual VTE risk per 10,000 women-years
FVL – COC –	0.5
FVL – COC +	1–2
FVL + COC –	2–4
FVL + COC +	15–20

### Treatment

- Treatment as for any symptomatic VTE—heparin, warfarin thromboprophylaxis for high risk periods, e.g. surgery, pregnancy.
- AT deficiency—do not usually require higher heparin doses.
- Duration of anticoagulation following a 1st event will depend on the severity of the VTE and clinical risk factors for recurrence. Each patient needs to be individually assessed. Heritable thrombophilia is not an indication of itself for prolonged anticoagulation.
- Factor concentrates—AT and PC concentrates have been used in patients with heritable deficiency during surgical and pregnancy high risk periods but are not used routinely.
- PC concentrate should be used in fulminant neonatal thrombosis, including purpura fulminans, in severe homozygous deficiency.
- Thromboprophylaxis in pregnancy depends on family history and nature of thrombophilic defect if tested.

### Case-finding and counselling

Identifying asymptomatic individuals by testing relatives of symptomatic patients (case-finding) is not routinely recommended for FVL and F2G20210A as the annual risk of VTE is marginally elevated above the general population. The risk is greater in asymptomatic relatives of affected patients with AT, PC, or PS deficiency but not high enough (<2% per year) to justify lifelong anticoagulant treatment. Therefore, high risk periods should be covered with thromboprophylaxis. It may be more sensible to offer thromboprophylaxis to all potentially affected relatives rather than to only those found to have a deficiency. VTE risk has been shown to be higher even in non-carriers of families illustrating the polygenic basis of VTE and the potential false reassurance of a –ve test result. Before embarking on a search for heritable thrombophilia it is essential that careful thought be given to any possible value for the patient and family. As a general rule young children should not be tested.

### Further reading

Baglin T. (2010). Unravelling the thrombophilia paradox: from hypercoagulability to the prothrombotic state. *Journal of Thrombosis and Haemostasis*, **8**, 228–33.



## Acquired thrombophilia

### Antiphospholipid syndrome (APS)

Defined as the association between the persistent presence of circulating antiphospholipid antibodies, and a history of thrombosis  $\pm$  pregnancy morbidity including fetal loss. Antiphospholipid antibodies are a group of antibodies that react with proteins associated with -vely charged phospholipids, e.g.  $\beta_2$ -glycoprotein 1 ( $\beta_2$ -GPI). Some patients have LA, some have ACL, and some have both. Strongest correlation with recurrent thrombosis is with +ve LA result. Diagnosis requires 1 or more clinical criteria and a +ve antiphospholipid antibody assay result.

### Diagnostic criteria have been strictly defined:

*Clinical, either:*

- Thrombosis—1 or more clinical episode of arterial, venous, or small vessel thrombosis, *or*
- Pregnancy morbidity—either (i) unexplained fetal death after 10 weeks with normal fetal morphology, or (ii) 1 or more premature births before 34th week of gestation because of eclampsia or severe pre-eclampsia or placental insufficiency, or (iii) 3 or more unexplained consecutive spontaneous abortions before 10th week of gestation.

*Laboratory, either:*

- Lupus anticoagulant—on 2 or more occasions at least 12 weeks apart, *or*
- Anticardiolipin antibody—on 2 or more occasions at least 12 weeks apart at moderate or high concentration.
- APTT may be prolonged and does not correct with  $\leftrightarrow$  plasma.  $\leftrightarrow$  result does not rule out the condition as different reagents have different sensitivity. PT usually  $\leftrightarrow$  unless hypoprothrombinaemia is present.
- LA tests include dilute Russell's viper venom time (DRVVT), Kaolin clotting time (KCT also known as Exner test), silica clot time (SCT). Principle of these tests is that prolonged clotting time is not observed when an excess of phospholipid is added (addition neutralizes the antibodies).
- Other features may be present which are not diagnostic criteria:
  - Thrombocytopenia, livedo reticularis, heart valve disease (usually without haemodynamic effects), myelopathy, migraine, visual disturbances.
  - Transient antiphospholipid antibodies are found after infection and may persist with hepatitis C or syphilis infection. Infection-related antibodies are not associated with thrombosis or obstetric problems.
  - Referred to as 2° APS when present in association with other autoimmune disorders, such as SLE, and 1° when there is no apparent underlying condition.

### Lupus anticoagulant (LA)

The paradoxically named LA is arguably the most common clotting abnormality predisposing to thrombosis. It is an *in vitro* phenomenon and the term is a misnomer as it increases the risk of thrombosis not bleeding.



It is an IgG/IgM autoantibody which prolongs phospholipid dependent coagulation tests (hence the use of the term anticoagulant); bleeding is very rare despite the prolonged APTT. LA was first described in patients with SLE—hence the name. Other underlying disorders include the LPDs, HIV, other autoimmune disorders, and drugs (e.g. phenothiazines).

Thrombosis, the major defining feature, may be arterial (stroke, ocular occlusions, MI, limb thrombosis) or venous (DVT, PE, renal, hepatic, and portal veins). Fetal loss may be as high as 80% in women with APS. Catastrophic widespread intravascular thrombosis is reported (catastrophic APS).

### *Pathogenesis*

The antibody specificity is to  $\beta_2$ -GPI, a phospholipid membrane-associated protein. Antibodies to prothrombin may also be present but rarely cause hypoprothrombinaemia and bleeding. Mechanism of thrombosis is not clear but may involve dimerization of  $\beta_2$ -GPI by antibodies on cell surfaces leading to cellular activation, e.g. platelet activation and tissue factor expression by monocytes and endothelial cells.

## **Treatment**

### *Asymptomatic*

Patients with +ve antibody assays without clinical manifestations require no specific action; the risk of thrombosis is estimated at <1% per patient-year.

### *Thromboembolic prophylaxis*

Consider perioperative thromboprophylaxis.

### *APS*

Acute thrombotic events should be treated as appropriate with heparin/warfarin. Long-term anticoagulation is considered by many clinicians as recurrence risk may be high. Target INR usually 2.5 e.g. PE but may be higher if history of arterial or cerebral small vessel thrombosis. Target INR 3.0 or 3.5.

### *Recurrent abortion*

Subsequent pregnancies have been successfully achieved in women with APS with combined aspirin and heparin (UFH or LMWH) begun as soon as pregnancy is confirmed. Steroids are not indicated. Pregnancy in a woman with APS and a past history of thrombosis will require prophylactic anticoagulation with heparin (↻ see p.536).

## **Thrombophilia in pregnancy**

Pregnancy is a hypercoagulable state with an ↑ risk of thrombosis throughout and up to 6 weeks post-partum. In addition to ↑ venous stasis <sup>2°</sup> to abdominal pressure and reduced mobility, physiological prothrombotic changes—↑ Fgn, FVIII, vWF.

The risk of venous thromboembolic events (VTE) ↑ 10-fold in ↔ pregnancy—1/1000 deliveries, fatal PE 10/year in UK is the major cause of maternal death in pregnancy and the postpartum. Risk higher when the pregnancy is complicated (sepsis, prolonged bed rest, advanced maternal age, delivery by LSCS), previous VTE particularly in pregnancy, inherited/acquired thrombophilia.

*Indications for anticoagulation in pregnancy*

- Acute VTE presenting in pregnancy.
- Long-term anticoagulation for prosthetic heart valves/recurrent VTE.
- Previous VTE, particularly in pregnancy/post-partum.
- APS.
- Inherited thrombophilia  $\pm$  a history of VTE.

There are no universally accepted protocols for the management of anticoagulation in pregnancy. There are few controlled studies and much of the information relates to non-pregnant subjects. Both oral anticoagulants and heparin have advantages and disadvantages in pregnancy. LMWH is a significant advance in management. Warfarin crosses the placenta and is teratogenic in the 1st trimester. Exposure during weeks 6–12 can cause warfarin embryopathy with nasal hypoplasia, stippled epiphyses and other manifestations. Incidence ranges from <5% to 65% in reported series. Warfarin at any stage of pregnancy is associated with CNS abnormalities and  $\uparrow$  risk of fetal haemorrhage *in utero* and at delivery.

Heparin (UFH and LMWH) does not cross the placenta and poses no teratogenic or haemorrhagic threat to the fetus. Maternal complications include haemorrhage (severe in <2%), thrombocytopenia (severe in <1%) and osteoporosis, usually asymptomatic and reversible but rare cause of vertebral fractures. LMWH may have fewer complications than UFH.

*Treatment of VTE presenting in pregnancy*

- Heparin 5–7d, either monitored IV UFH; aim for APTT ratio 1.5–2.5 or therapeutic SC LMWH based on body weight then monitored therapeutic dose SC LMWH, od or bd.
- Continue heparin until delivery; omit heparin during labour.
- Recommence heparin after delivery and start warfarin if desired.
- Continue treatment for at least 6 weeks post-partum, stop heparin once INR in therapeutic range.

*Prophylaxis of thromboembolism in pregnancy*

National guidelines should be consulted and patients treated in centres with special expertise.

**Cardioversion**

A target INR of 2.5 is recommended for 3 weeks before and 4 weeks after cardioversion to prevent thromboembolism. To minimize cardioversion cancellations due to low INRs on the day of the procedure a higher target INR, e.g. 3.0, can be used prior to the procedure.

**Metal heart valve prostheses**

The frequency of thromboembolism is lower with modern valves than 1st generation valves but oral anticoagulation is still required. For patients in whom valve type and location are known specific target INRs are recommended—otherwise a target INR of 3.0 is recommended for valves in the aortic position and 3.5 in the mitral position (Table 10.7). NOACs should not be used to prevent thromboembolism in patients with mechanical heart valves.

**Table 10.7** Target INRs for cardiac valves

Valve	Target INR
Bileaflet aortic	2.5
Tilting disk aortic	3.0
Bileaflet mitral	3.0
Tilting disk mitral	3.0
Caged ball or caged disk aortic or mitral	3.5

### Peripheral arterial thrombosis and grafts

Antiplatelet drugs remain 1st-line intervention for 2<sup>o</sup> antithrombotic prophylaxis. If long-term anticoagulation is given to patients at high risk of femoral vein graft failure a target INR of 2.5 is recommended.

### Paroxysmal nocturnal haemoglobinuria

Long-term anticoagulation with a target INR of 2.5 is recommended if large PNH clones (PNH granulocytes >50%) and a platelet count  $>100 \times 10^9/L$ . Anticoagulation can also be considered for patients with smaller clones and platelet counts  $<100 \times 10^9/L$  dependent on additional risk factors for thrombosis and bleeding.

### Further reading

Greaves, M. et al. (2000). Guidelines on the investigation and management of the antiphospholipid syndrome. *Brit J Haematol*, **109**, 704–15.

Robertson, B. and Greaves M. (2006). Antiphospholipid syndrome: an evolving story. *Blood Rev*, **20**, 201–12.

## Thrombotic thrombocytopenic purpura (TTP)

TTP is rare (4 per million). Early diagnosis and treatment is essential. Most patients have ADAMTS13 activity less than 5% of normal. 80% of cases due to acquired antibody. Congenital TTP due to mutations in ADAMTS13 gene much rarer- but may present in adulthood. ADAMTS13 required for cleavage of ultra large VWF multimers. Failure of cleavage results in VWF-rich microthrombi in capillaries and arterioles of heart, kidney and brain.

### Clinical diagnosis:

- Thrombocytopenia and microangiopathic haemolytic anaemia not explained by another cause of thrombotic microangiopathy.
- Fluctuating neurological signs.
- Renal impairment.
- Fever.

Often insidious onset. Neurological impairment has multiple manifestations including headache, bizarre behaviour, transient sensorimotor deficits (TIAs), seizure, and coma. Presence of coma at presentation is a poor prognostic indicator. Additional features may be present e.g. gastrointestinal ischaemia causing abdominal pain, myocardial ischaemia (see Table 10.8).

### Laboratory investigations

FBC and blood film, reticulocyte count, PT, APTT, Fgn and D-dimer, U&E, LFTs, lactate dehydrogenase, urinalysis, direct antiglobulin test.

Measurements of ADAMTS13 activity (<5% indicates TTP likely to respond to plasma exchange therapy) and detection of inhibitor (antibody).

### Treatment

- Single-volume daily plasma exchange should be started within 24h of presentation.
- Plasma exchange using cryosupernatant may be more efficacious than FFP.
- Daily plasma exchange should continue for a minimum of 2d after complete remission.
- Steroid therapy should be given at a dose of at least 1mg/kg prednisolone; pulsed methylprednisolone 1g IV for 3d is often used.
- Low-dose aspirin 75mg daily should be commenced on platelet recovery (platelet count  $>50 \times 10^9/L$ ).
- For refractory disease, an alternative plasma product lacking HMW vWF multimeric forms can be considered—cryosupernatant or SD plasma.
- Vincristine can be added—1mg every 3–4d.
- Malignancy and BMT-associated TTP are often refractory to plasma exchange and are characterised by normal or low ADAMTS13 but not less than 5%.

**Table 10.8** Clinical subtypes of TTP

Congenital		
Acquired	Acute idiopathic	No identifiable precipitant, low relapse rate
	Secondary	Drugs (oral contraceptive pill, ticlopidine, ciclosporin)
		Bone marrow transplantation
		SLE
		Malignancy
		Pregnancy
Infection—HIV		
Intermittent	Recurrent episodes	

**Further reading**

Allford, S. L., *et al.* (2003). Guidelines on the diagnosis and management of the thrombotic microangiopathic haemolytic anaemias. *Brit J Haem*, **120**, 556–73.

## Haemolytic uraemic syndrome (HUS)

HUS is characterized by microangiopathic haemolytic anaemia, thrombocytopenia, and renal failure. There may be more extensive multi-organ disease (which may make distinction with TTP difficult). Epidemic form (D+) is associated with a prodromal illness often with bloody diarrhoea in contrast to the rare sporadic or atypical cases (D-) HUS. D+ seen increasingly following outbreaks of infection with verotoxin (VT)-producing organisms e.g. *E. coli* 0157:H7. After ingestion of contaminated food or water, the organisms bind to gut wall receptors and remain within the gut lumen. Toxin targets organs with specific globotriosyl ceramide (Gb) receptors, in particular glomerular microvascular endothelial cells.

### Poor prognostic features

- High neutrophil count.
- Severe thrombocytopenia uncommon.

Indicators of long-term renal disease are thrombocytopenia for >10d and proteinuria after 1 year.

### Treatment

- D+ HUS: fluid and electrolyte balance and BP control.
- Dialysis.
- Anti-motility drugs and antibiotics not indicated.
- FFP and therapeutic plasma exchange not effective.



## Heparin-induced thrombocytopenia (HIT)

HIT is caused by IgG antibody that recognizes multimolecular complexes of PF4 and heparin. PF4 is a 70-amino acid protein that self-associates to form tetramers of approximately 30kDa. HIT antibodies recognize a heparin-induced conformational change in the PF4 tetramer. The ability to induce the conformational change depends on the chain length and degree of sulphation of the glycosaminoglycan, which explains the differences in incidence of HIT observed with different heparins. PF4/heparin complexes bind to the platelet surface by binding to membrane Fc-receptors—this causes platelet activation.

- Incidence of HIT greater with bovine than with porcine heparin.
- Greater with UFH than with LMWH.
- All heparins used in the UK are of porcine origin.
- Frequency of HIT is greater in surgical than in medical patients.
- In orthopaedic patients given SC prophylactic heparin, the incidence is approximately 5% with UFH and 0.5% with LMWH.
- Risk is very low in obstetric patients given LMWH.
- Skin lesions occur at the site(s) of SC injection—range in appearance from indurated erythematous nodules or plaques to frank skin necrosis.
- If HIT develops the platelet count typically begins to fall 5–10d after starting heparin although in patients who have received heparin in the previous 3 months it can have a rapid onset because of pre-existing antibodies.
- Occasionally, the onset can occur after >10d of heparin exposure but it is rare after 15d.
- The platelet count normally falls by >50% and has a median nadir of  $50 \times 10^9/L$  but severe thrombocytopenia ( $<15 \times 10^9/L$ ) is unusual.
- Half of the patients who develop HIT will have associated thrombosis.
- In patients without thrombosis (isolated HIT) there is a high risk of thrombosis if heparin is not stopped and an alternative anticoagulant (direct-thrombin inhibitor or danaparoid) given in therapeutic dose.

### Diagnosis

Use pretest probability score to calculate likelihood of disease if HIT suspected (Table 10.9).

### Treatment

High score:

- Heparin stopped and full-dose anticoagulation with a direct thrombin inhibitor, such as argatroban commenced. Therapeutic dose fondaparinux can be used in patients without severe renal impairment.
- Warfarin should not be used until the platelet count has recovered—the direct thrombin inhibitor must be continued until the INR is therapeutic (INR >2.0) for 2 consecutive days.
- Platelets should not be given for prevention of bleeding due to thrombocytopenia.

Intermediate score:

- Heparin stopped and prophylactic-dose anticoagulation with a non heparin parented anticoagulant.



Low score:

- Monitor platelet count.
- Consider stopping heparin and substituting prophylactic-dose anticoagulation with a direct thrombin inhibitor.

**Table 10.9** Pretest probability score to calculate likelihood of disease if HIT suspected

Thrombocytopenia	0	Fall <30% or platelet count $<10 \times 10^9/L$
	1	30–50% fall or platelet count $10\text{--}19 \times 10^9/L$
	2	>50% fall or platelet count $20\text{--}100 \times 10^9/L$
Timing of thrombocytopenia	0	<5d with no previous heparin exposure
	1	Possibly d5–10 but unclear, e.g. missing platelet counts
	2	D5–10 or less if previous heparin exposure
Thrombosis	0	None
	1	Progressive or recurrent thrombosis or skin lesions or suspected but unproven new thrombosis
	2	New thrombosis, skin necrosis, acute systemic reaction following heparin injection
Other cause of thrombocytopenia not evident	0	Definite other cause present
	1	Possible other cause evident
	2	No other cause evident

Score 0, 1, or 2 for each of 4 categories, maximum possible score = 8

### Further reading

Keeling, D. *et al.* (2006). The management of heparin-induced thrombocytopenia. *Brit J Haematol*, **133**, 259–69.

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# Immunodeficiency

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## Congenital immunodeficiency syndromes

Incidence: rare, though as knowledge increases there is recognition of an increasing number of inherited defects in the complex human host defence system. The classical life-threatening disorders of specific immunity with major dysfunction or absence of T cells and/or B cells are all diseases that present in childhood, but milder variants may not be recognized until later life. 'Immunodeficiency' is a vague term that is generally taken to also encompass defects in opsonization and phagocytosis, so can be taken to include neutrophil and macrophage disorders of number, function, or both.

### Classification of inherited immune deficiency syndromes

- Affecting T cells, B cells, and neutrophils.
- Affecting B and T cells.
- Affecting T cells.
- Affecting B cells.
- Affecting neutrophils.

### Affecting T cells, B cells, and neutrophils

#### *Reticular dysgenesis*

A rare autosomal recessive or sometimes X-linked disorder where T cells, B cells, and granulocytes are absent. Such children present with serious infection at birth or shortly afterwards. They have no lymph nodes or tonsils, and the usual thymic shadow is absent. BM is hypoplastic, and there may also be thrombocytopenia and anaemia. It appears to be a pluripotential stem cell failure and carries a dire prognosis. The only curative therapy is BMT.

### Affecting T cells and B cells (combined immunodeficiency disorders)

#### *Severe combined immunodeficiency—SCID*

A mixed group of disorders that all have grossly impaired T- and B-cell function leading to death normally within the first years of life. They can be broadly classified into 5 groups depending on their clinical and pathological characteristics. Reticular dysgenesis is generally considered to be a SCID variant, accounting for 3% of the total. Other types are:

- Adenosine deaminase deficiency (16%).
- T- B- SCID (27%).
- T- B+ SCID (44%).
- T+ B+ SCID (9%).

#### *Adenosine deaminase deficiency*

A recessively inherited enzyme deficiency. ADA is rate limiting in purine salvage metabolism and is essential for the synthesis of nucleotides in cells incapable of *de novo* purine synthesis—including lymphocytes. The gene for ADA is on chromosome 20q12–q13.11, and many mutations have been defined. Gene deletion leads to very low ADA activity and a profound T and B lymphopenia with early onset of clinical symptoms. Other tissues are involved, and there may be bony defects and neurological disturbances. A similar rare syndrome is seen with deficiency of the enzyme purine nucleoside phosphorylase. It is less severe and presents later.

### *Other forms of SCID*

SCID with both T-cell and B-cell lymphopenia is a recessive disorder that also occurs without the enzyme deficiencies described above, but the commonest form of the disease is X-linked and shows a lack only of T cells. It appears to be due to a defect in the gene coding for the  $\gamma$  chain of the interleukin (IL)-2 receptor.

There are other rare SCID variants where T cells are present but dysfunctional, including MHC class II deficiency, where lymphocytes fail to express MHC class II molecules; and Omenn syndrome which presents in early infancy with the clinical features of acute widespread GvHD (skin rash, hepatosplenomegaly, diarrhoea, failure to thrive) coupled with persistent infections. Omenn syndrome is due to mutations in recombination activating genes 1 or 2 (*RAG1/2*) and is associated with absent B cells. In contrast to the more typical T-B-cell SCID defects, patients with Omenn syndrome have normal/elevated numbers of T and B cells. However, these cells lack the normal diverse T-cell repertoire, being oligoclonal in nature and having poor/absent proliferative capacity *in vitro* with no/low antibody responses to immunizations. The findings of activated T cells with  $\uparrow$  production of IL-4, IL-5, and IL-10 at least in part may explain some of the clinical and laboratory manifestations of this syndrome. Recently a subtype of X-linked dyskeratosis congenita has also been recognized to present with SCID (T+ B- NK- phenotype).

### *Treatment of SCID*

- Matched BMT is the treatment of choice for all varieties; a good outcome can be expected in >90%.
- No preconditioning needed for matched donors.
- Mismatched BMT results improving but donor marrow needs careful mature T-cell depletion and patients may need conditioning.
- ADA deficiency can be treated with regular enzyme replacement using a polyethylene glycol-linked ADA preparation.
- ADA deficiency has also been treated with gene replacement therapy, with so far only a transient effect, but the technique shows promise.
- X-linked SCID due to mutations in common  $\gamma$ -chain has been treated with gene replacement therapy in 19 patients with long-term efficacy.

### *Wiskott–Aldrich syndrome*

An X-linked congenital disorder with a triad of (i) eczema, (ii) thrombocytopenia with characteristically small platelets, and (iii) T- and B-cell dysfunction with susceptibility to infections, particularly otitis media and pneumonia. Due to a mutation in the gene encoding the Wiskott–Aldrich syndrome protein (WASP), important *inter alia* in regulating the cytoskeleton of haemopoietic cells.

- Presents in childhood.
- Tendency to immune cytopenias—compounding pre-existing thrombocytopenia and causing haemolytic anaemia.
- Herpes simplex, EBV, varicella, and CMV may be severe and life-threatening.
- Greatly  $\uparrow$  risk of lymphoid malignancy in adulthood for survivors.
- Splenectomy greatly  $\uparrow$  risk of fatal infection.
- Need prophylactic antibiotics and immunoglobulin replacement therapy.
- BMT now treatment of choice; early in childhood if possible.

### *Ataxia telangiectasia*

A recessive disorder with ↑ chromosome fragility and a single gene (*ATM*) defect on chromosome 11q22.3. This affects several systems. The first is neuromotor development with cerebellar ataxia appearing around 18 months of age and progressing to include dysarthria associated with degeneration of the Purkinje cells. Telangiectases appear between 2–8 years of age affecting the eyes, face, and ears. An immune deficiency is evident affecting both humoral and cellular immunity, though less severe than SCID. Affected children get:

- Sinopulmonary infections.
- Progressive failure of antibody production.
- Hypogammaglobulinaemia.
- CD4+ lymphopenia.
- Small thymus.
- ↑ incidence of lymphoid malignancies.

### **Affecting T cells**

#### *DiGeorge syndrome*

Absence or hypoplasia of the thymus and parathyroid glands with aortic arch anomalies, contralateral cardiac abnormalities or other congenital heart defects. This congenital anomaly of the 3rd and 4th branchial arches usually presents with hypocalcaemic fits or problems with a heart defect. Total thymic aplasia occurs only in a minority, with severe immunodeficiency and a high risk of transfusion-transmitted GvHD. Most have some T-cell function, and relatively minor problems with impaired immunity for which treatment is supportive.

### **Affecting B cells**

#### *X-linked agammaglobulinaemia (Bruton tyrosine kinase deficiency)*

Boys with this condition have mutations in the gene for Bruton tyrosine kinase (locus Xq22), resulting in a failure of B-cell development and lack of antibody production. Early infancy is not a problem because of maternally transmitted IgG, but by 2 years of age serious infections become apparent. These include bacterial invasion of the respiratory system, the GIT, meninges, joints, and skin. Viruses, particularly coxsackie and echo viruses, are also a major threat.

- Absent or very low numbers of B cells.
- Absent or low levels of all immunoglobulins.
- Treatment is by regular antibody replacement with polyvalent IVIg.

#### *Hyper IgM syndrome*

An X-linked disorder with B-cell dysfunction due to defective T-cell CD40 ligand production and thus lack of signalling to B-cell CD40 receptors. B cells are normal, but receive no instructions to generate isotypes of Ig other than IgM. Low levels of IgG, IgA, and IgE result. There is also deficient function of some tissue macrophages and a tendency to develop *Pneumocystis jirovecii* (previously known as *Pneumocystis carinii*) pneumonia. Treatment is with IVIg replacement therapy and cotrimoxazole prophylaxis.

### *IgA deficiency*

A relatively benign and common disorder affecting 1:500 individuals. They may develop anti-IgA antibodies in serum which can cause urticarial and anaphylactic reactions to blood product infusions. No replacement therapy is needed.

### **Affecting neutrophils, monocytes, and macrophages**

Inherited disorders of neutrophil function mostly present in childhood and are described in the paediatric section on congenital neutropenia (➔ p.600). 1° functional disorders of monocytes and macrophages are also described in the paediatric section under histiocytic syndromes (➔ p.628).

### **Poorly characterized 1° immune deficiency syndromes**

There are a number of syndromes where susceptibility to certain types of infection is not associated with a clear pattern of inheritance and where the clinical picture is variable. Few are as severe as the specific syndromes referred to earlier in this section. They include *chronic mucocutaneous candidiasis*, where there is persistent superficial skin and mucous membrane fungal infection, and where there may be defective T-cell regulation or dendritic cell function. CMC is also associated with a wide variety of autoimmune phenomena, particularly thyroid and adrenal disease, and different patterns of inheritance are seen in different kindreds.

There is also a heterogeneous group of disorders collectively referred to as *common variable immunodeficiency*. Defined by the clinical susceptibility to infection and in the absence of any other apparent cause, this collectively named syndrome is usually a diagnosis of exclusion and presents in adult life. Low rather than absent levels of several isotypes of Ig are usual, and the condition is rarely life-threatening.

## Acquired immune deficiencies

Clinically important defects in lymphocyte numbers and/or function can be seen as a complication of a variety of acquired diseases. They can also be due to drugs, both those given deliberately to suppress an auto-immune process and those given primarily for other reasons. Similarly neutrophils can be reduced by a large number of acquired disorders and a long list of drugs and toxins. An acquired susceptibility to infection also arises in patients with absent or poorly functioning spleens.

### Acquired hypogammaglobulinaemia

#### Causes

- Malignant LPDs including CLL and myeloma.
- Immunosuppressive therapy with, e.g. azathioprine.
- Maintenance therapy for ALL.
- Nephrotic syndrome.

#### Clinical features

Bacterial infections—recurrent chest infections (may lead to bronchiectasis), sinus, skin, and urinary tract infections common. Fulminant viral infections, especially measles, varicella.

#### Treatment

- May improve with treatment of the underlying disease.
- IVIg should not be used routinely as prophylaxis.
- High titre specific antibody can be given for serious zoster/varicella infections if available; polyvalent for measles.
- Patients with severe hypogammaglobulinaemia and recurrent infections may be considered for IVIg replacement therapy every 4 weeks (400–600mg/kg).

### Acquired T-lymphocyte abnormalities

#### Reduced numbers

- HIV infection (➡ see HIV infection and AIDS, p.552).
- High-dose steroids.
- ATG.
- Purine analogues especially fludarabine and cladribine.
- Pentostatin (deoxycoformycin) (adenosine deaminase inhibitor).
- After allogeneic SCT.

#### Reduced function

LPDs, HL, immunosuppressive agents, e.g. ciclosporin and steroids, burns, uraemia.

#### Clinical features

↑ risk of viral, fungal, and atypical infections including HSV, HZV, CMV, EBV, *Candida*, *Aspergillus*, *Mycoplasma*, PCP, toxoplasmosis, TB, and atypical mycobacteria.

#### Treatment

Treat specific infection where possible. Consider prophylaxis against HZV, CMV, PCP, and *Candida* in high-risk groups, e.g. post-allogeneic stem cell transplant.



## Combined B- and T-lymphocyte abnormalities

### Causes

- Chronic lymphocytic leukaemia.
- Intensive chemotherapy.
- Extensive radiotherapy.
- Severe malnutrition.

### Clinical features and treatment

➔ See Acquired T-lymphocyte abnormalities, p.550.

## Neutrophil/macrophage abnormalities

### Reduced numbers

➔ See Neutropenia, p.600.

### Abnormal function

➔ See Myelodysplasia, p.624, Myeloproliferative disorders, p.624, Histiocytic syndromes, p.628.

### Clinical features

Bacterial and fungal sepsis.

### Treatment

Treat specific infections and consider prophylaxis.

## Hyposplenism

Hyposplenism is an acquired immunodeficiency without lymphocyte or neutrophil abnormalities. It arises either following splenectomy, or due to functional deficiency as part of another disorder, especially sickle cell disease, inflammatory bowel disease, and following BMT. It gives rise to susceptibility to overwhelming infection with certain organisms due to lack of the spleen's function as a filter. These include:

- *Streptococcus pneumoniae*.
- *Neisseria meningitidis*.
- *Haemophilus influenzae* type B.
- Falciparum malaria.

The risk of hyposplenic infection is greatest in children in the first 6 years of life. It dwindles thereafter but the risk continues into adult life. All facing splenectomy should be vaccinated against HIB, pneumococcus, and meningococcus type B and all splenectomized children and young adults (and those with sickle cell disease) should take prophylactic phenoxymethylpenicillin 250mg bd (if allergic to penicillin use erythromycin).

## HIV infection and AIDS

Infection with HIV-1 or HIV-2 produces a large number of haematological effects and can simulate a number of haematological conditions during both the latent pre-clinical phase and once clinical syndrome of AIDS has developed. HIV infection divided into 4 stages.

### Stage 1: 1° infection

Entry of HIV-1 or HIV-2 through a mucosal surface after sexual contact, direct inoculation into the bloodstream by contaminated blood products, or IV drug abuse can be followed by a transient febrile illness up to 6 weeks later associated with oral ulceration, pharyngitis, and lymphadenopathy. Photophobia, meningism, myalgia, prostration, encephalopathy, and meningitis may also occur. FBC may show lymphopenia or lymphocytosis often with atypical lymphocytes, neutropenia, thrombocytopenia, or pancytopenia. Major differential diagnoses are acute viral meningitis and infectious mononucleosis. False +ve IM serology may occur. Specific IgM then IgG antibody to HIV appears 4–12 weeks after infection and routine tests for HIV may be –ve for up to 3 months. However, the virus is detectable in plasma and CSF from infected individuals during this period and the patient is highly infectious.

### Stage 2: pre-clinical HIV infection

Although viral titres fall in the circulation at this time there is significant and persistent virus replication within lymph nodes and spleen. The clinically latent period may last 8–10 years and circulating CD4 T-cell count remains normal for most of this period. However, there is a delayed, gradual, but progressive fall in CD4 T lymphocytes in most patients, who may remain asymptomatic for a prolonged period despite modest lymphopenia. A number of minor skin problems such as seborrhoeic dermatitis are characteristic of the end of the latent phase.

A patient with latent HIV infection may have isolated thrombocytopenia on routine blood testing. This is due to an immune mechanism and may be confused with ITP as there is frequently  $\uparrow$  platelet associated immunoglobulin.

### Stage 3: clinical symptoms

Marked by onset of symptoms, rising titre of circulating virus, and decline in circulating CD4 T-cell count to  $<0.5 \times 10^9/L$ . Wide variation in individual patient's rate of progression at this stage. A number of minor opportunistic infections are common: oral/genital candida, herpes zoster, oral leucoplakia. Lethargy, PUO, and weight loss occur frequently. Deepening lymphopenia ( $CD4 <0.2 \times 10^9/L$ ) invariably present when opportunistic infection occurs. Persistent generalized lymphadenopathy is a condition where lymphadenopathy  $>1cm$  at 2 or more extra-inguinal sites persists for  $>3$  months. It is a prodrome to severe immunodeficiency, opportunistic infection, and neoplasia.

### Stage 4: AIDS

AIDS is now defined as the presence of a +ve HIV antibody test associated with a CD4 lymphocyte count  $<0.2 \times 10^9/L$  rather than by the

development of a specific opportunistic infection or neoplastic complication. This final stage of HIV infection is associated with a marked reduction in CD4 T cells, severe life-threatening opportunistic infection, neoplasia, and neurological degeneration. Severity of these complications usually reflects the degree of immunodeficiency as measured by the CD4 T-cell count. However, there is evidence that prophylactic therapy reduces the incidence of complications and newer antiviral therapies slow the progression of this stage.

### Haematological features of HIV infection

- Lymphopenia—CD4 lymphopenia may be masked by CD8 lymphocytosis in stage 2; improved by antiviral therapy.
- Neutropenia—marrow suppression by virus or therapy; splenic sequestration.
- Normochromic/normocytic anaemia due to suppression of marrow by virus or therapy. Microangiopathic haemolysis associated with TTP.
- Thrombocytopenia—suppression of marrow by virus or therapy or shortened survival due to immune destruction (may respond to antiviral therapy), infection, TTP, or splenic sequestration.
- BM suppression—direct HIV effect or complication of antiretroviral therapy, ganciclovir, trimethoprim, or amphotericin therapy.
- BM infiltration—by NHL, HL, granulomas due to *M. tuberculosis*, and atypical mycobacteria or disseminated fungal disease.

### Complications of HIV infection

*Opportunistic infections* (see Table 11.1)

**Table 11.1** Complications of HIV infection

<b>Fungal</b>	<i>Pneumocystis jirovecii</i>	Pneumonia
	<i>Candida albicans</i>	Oro-oesophageal
	<i>Cryptococcus neoformans</i>	Meningitis
	<i>Histoplasma capsulatum</i>	Meningo-encephalitis, pneumonia
<b>Mycobacterial</b>	<i>M. avium intracellulare</i>	Disseminated, intestinal
	<i>M. tuberculosis</i>	Pulmonary, intestinal
<b>Parasitic</b>	<i>Cryptosporidium</i>	Hepatobiliary, intestinal
	<i>Isospora</i>	Colon, hepatobiliary
	<i>Toxoplasma gondii</i>	Multiple abscesses: CNS ocular, lymphatic
<b>Viral</b>	Cytomegalovirus	Retinal, hepatic, intestinal, CNS
	Herpes zoster	Mucocutaneous
	Herpes simplex	Mucocutaneous
	JC virus	CNS
<b>Bacterial</b>	<i>Haemophilus influenzae</i>	Meningitis
	<i>Streptococcus pneumoniae</i>	Pneumonia, meningitis, septicaemia

**Neoplasia**

- AIDS-related Kaposi sarcoma 20–30% of patients; multiple skin lesions; later lymph nodes, mucous membranes, and visceral organs. ?role of HHV8 (>95% +ve).
- NHL up to 10%; 65% diffuse large B-cell, 30% Burkitt-like; extranodal esp. small bowel and CNS; 1° effusion lymphomas; aggressive. ?role of EBV (100% +ve in 1° CNS NHL).
- Cervical carcinoma.
- Anal carcinoma.
- HL; advanced stage, extranodal sites.

**Direct effects of HIV infection**

- BM suppression—dysplastic appearance; pancytopenia.
- Small bowel enteropathy—malabsorption syndrome.
- CNS—dementia, myelopathy, neuropathy.



## Therapy of HIV infection

See Tables 11.2 and 11.3.

**Table 11.2** Infection prophylaxis, if severely immunocompromised

Drugs	Activity against
Fluconazole/itraconazole	Oro-oesophageal candidiasis ± cryptococcal meningitis
Co-trimoxazole	<i>Pneumocystis jirovecii</i> ± ocular/CNS toxoplasmosis
Dapsone/nebulized pentamidine <sup>a</sup>	<i>Pneumocystis jirovecii</i>
Rifabutin/azithromycin/clarithromycin	<i>M. avium-intracellulare</i>
Aciclovir	HSV and HZV

<sup>a</sup>If cannot take cotrimoxazole

### Antiviral therapy

- Combination antiretroviral therapy (also called highly active antiretroviral therapy, HAART) is standard of care. Initiation of HAART based predominantly on clinical staging (with stage 3 or 4) and CD4 count. ↑ trend to start HAART earlier (e.g. CD4 count ~ 350–500).
- Generally two drugs from nucleoside class of viral reverse transcriptase (RT) inhibitors: zidovudine (AZT), didanosine (ddl), lamivudine (3TC), abacavir, tenofovir (TDF). Used in combination with a non-nucleoside reverse transcriptase inhibitor (e.g. nevirapine (NVP) or efavirenz (EFV) or a protease inhibitor (e.g. lopinavir, ritonavir, darunavir, atazanavir).
- Specific therapy is followed within hours by rapid clearance of virions from the circulation and subsequently by reappearance of circulating T cells and a rising count over several weeks. Viral resistance develops with time on combination therapy if adherence is suboptimal.

**Table 11.3** Treatment of complications

<b>Oro-oesophageal candidiasis</b>	Systemic fluconazole or amphotericin then lifelong prophylaxis
<b><i>Pneumocystis pneumonia</i></b>	High-dose co-trimoxazole or pentamidine then lifelong prophylaxis; pyrimethamine, dapsone, and leucovorin weekly
<b>Tuberculosis</b>	Multiagent therapy (drug resistance common) $\pm$ lifelong isoniazid prophylaxis
<b>Fungal pneumonia</b>	Amphotericin then lifelong prophylaxis
<b>CMV pneumonitis/retinitis</b>	Ganciclovir/foscarnet then lifelong prophylaxis
<b>CNS toxoplasmosis</b>	Pyrimethamine then lifelong prophylaxis; atovaquone (has <i>in vitro</i> activity against cysts of <i>Toxoplasma gondii</i> and has been used as salvage therapy for cerebral toxoplasmosis.
<b>Cryptococcal meningitis</b>	Amphotericin/fluconazole
<b>AIDS-related Kaposi's sarcoma</b>	Limited disease: local DXT, cryotherapy, intralesional vincristine, interferon alfa; advanced disease: combination chemotherapy such as doxorubicin (adriamycin), bleomycin, and vincristine (ABV), liposomal daunorubicin, paclitaxel
<b>Non-Hodgkin lymphoma</b>	Poor prognosis; combination chemotherapy (often standard regimens at reduced dosage due to toxicity) 50% response, median survival <9 months; CNS lymphoma particularly poor prognosis; palliative dexamethasone DXT
<b>2° prophylaxis</b>	Sulfadiazine (2–4g daily in 4 divided doses) + pyrimethamine (25–50mg/d) is 1st choice for 2° prophylaxis. Folic acid (10–25mg/d) is given concurrently

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## Paediatric haematology

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## Blood counts in children

Blood counts in children are often different from adults, to varying degrees at different ages. The differences are greatest during the neonatal period.

### Red cells

The relatively hypoxic intrauterine environment means that the newborn is polycythaemic by adult standards, a phenomenon that self-corrects during the first 3 months of life by which time the normal infant is anaemic relative to adults. Neonatal red cells are also macrocytic by adult standards, a feature that also disappears during the first 6 months as HbA replaces HbF.

- Neonatal red cells show much greater variation in shape than those from adults, particularly in premature babies—alarming microscopists more used to adult blood films.
- Occasional nucleated red cells are normal in the first 24–48h of life.
- Fe lack is common around 12 months of age due to ↑ demand from ↑ red cell mass and (often) poor oral intake—cows' milk has virtually no Fe content. The MCV falls to what would be abnormally low levels for adults as a reflection of this.
- In healthy premature neonates all these red cell differences may be exaggerated, with a nadir Hb at 2–3 months of 8–9g/dL in those with birth weight 1–1.5kg.
- Children have slightly lower Hb than adults until puberty.

### White cells

The most striking difference between children and adults is the high lymphocyte count in infants and young children. This means that the normal differential WBC in those <4 years shows more lymphocytes than neutrophils. Otherwise most of the changes in WCC seen in children are similar to those seen in adults and due to the same causes, with a few exceptions:

- Healthy term babies show a transiently raised neutrophil count in the first 24h after birth ( $7\text{--}14 \times 10^9/\text{L}$ ) which returns to the normal (adult) range by 48h.
- Immature neutrophils (band cells and myelocytes) may comprise 5–10% of the total WBC in healthy neonates.
- Sick neonates with bacterial infections commonly show a paradoxical neutropenia, with or without an ↑ band cell count.
- Black children have lower neutrophil counts than other ethnic groups.
- Lymphocytoses with very high counts occur in children with specific infections—notably pertussis.

### Platelets

Platelet counts in children are essentially the same as adults as far as the lower limit is concerned, but there is greater volatility at the upper end and infants tend to produce high counts ( $>500 \times 10^9/\text{L}$ ) as part of an acute phase reaction more frequently. There is a statistically significant fall in the upper limit (95th centile) from 4 years onwards from around 500 to reach 350–400 by the end of childhood.

Cord blood platelets are less reactive to aggregating agents *in vitro* and have other features of hypofunction compared with mature platelets.

### Normal blood count values from birth to adulthood

See Table 12.1.

**Table 12.1** Normal blood count values from birth to adulthood<sup>1</sup>

Age	Hb (g/dL)	MCV (fL)	Neuts	Lymph	Platelets
Birth	14.9–23.7	100–125	2.7–14.4	2–7.3	150–450
2 weeks	13.4–19.8	88–110	1.5–5.4	2.8–9.1	170–500
2 months	9.4–13.0	84–98	0.7–4.8	3.3–10.3	210–650
6 months	10.0–13.0	73–84	1–6	3.3–11.5	210–560
1 year	10.1–13.0	70–82	1–8	3.4–10.5	200–550
2–6 years	11.5–13.8	72–87	1.5–8.5	1.8–8.4	210–490
6–12 years	11.1–14.7	76–90	1.5–8	1.5–5	170–450
Adult ♂	12.1–16.6	77–92	1.5–6	1.5–4.5	180–430
Adult ♀	12.1–15.1	77–94	1.5–6	1.5–4.5	180–430

Neuts, neutrophils; lymph, lymphocytes and platelets, all  $\times 10^9/L$ .

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### Other haematological variables in childhood

There are important differences in the concentration of various clotting factors during early infancy as described in Tables A3.3 and A3.4 (p.812). Other laboratory investigations where children differ include:

- Reticulocyte counts low in the first 8 weeks of life as neonatal polycythaemia corrects itself.
- HbF comprises 75% of the total Hb at birth, 10% at 5 months, 2% at 1 year, and <1% thereafter.
- Some red cell enzymes (G6PD, PK, hexokinase) have greater activity (150–200% of adult values) in neonatal RBC.
- The LLN for serum ferritin at 1 year (12.5mg/L) is 50% of the LLN at 12 years (25mg/L).
- B<sub>12</sub> and folate levels are around 2  $\times$  higher in infants and younger children than adults.

### Reference

- 1 Lillieyman, J.S. et al. (eds.) (1999). *Pediatric Hematology*, 2e. London: Churchill Livingstone.

## Red cell transfusion and blood component therapy—special considerations in neonates and children

Babies in Special Care Baby Units are now amongst the most intensively transfused of our hospital patients:

- To replace blood losses of investigative sampling.
- To alleviate anaemia of prematurity.

*Note:*

- Hb estimation alone is an inadequate assessment.
- Hb reduction with symptoms, e.g. failure to thrive, is needed to justify transfusion.
- Generally, neonatal Hb <10.5g/dL + symptoms—transfuse; if neonate requiring O<sub>2</sub> support, aim for Hb 13.0g/dL.

### Source of blood

Directed donations from 'walking donors' (including donations from relatives) cannot be regarded as safe as microbiologically screened volunteer donor blood—therefore not recommended.

### Small-volume transfusions

QUAD 'pedipacks' (SAGM blood) ensure that 4 transfusions possible from a single donor and so ↓ donor exposure in infant needing multiple transfusions.

### Pre-transfusion testing

Maternal and neonatal samples should be taken and tested as follows:

#### *Maternal samples*

- ABO and Rh group.
- Antibody screen.

#### *Infant samples*

- ABO and Rh group.
- DAT.
- Antibody screen (if maternal sample unavailable).

*Note:* provided no atypical antibodies are present in maternal or infant serum and the DAT on the infant's cells is -ve, a conventional cross-match is unnecessary. Small-volume replacement transfusions can be given repeatedly during the first 4 months of life without further serological testing. Transfusion centres may specifically designate a supply of low anti-A, B titre group O Rh (D) -ve blood for use in neonatal transfusions.

►After the first 4 months, compatibility testing should conform to requirements for adults.

### Exchange transfusions

- To prevent kernicterus caused by rapidly rising bilirubin.
- Most commonly needed in haemolytic disease of the newborn.
- Plasma-reduced red cells (Hct 0.50–0.60).

- For small-volume transfusions, age of red cells does not matter. For exchange transfusions within 5d of collection ( $[K^+]$  levels rise in older blood).
- Transfusion should not take  $>5h/U$  due to risk of bacterial proliferation.
- Volumes of 5mL/kg/h usually safe.

#### Special hazards

- GvHD: in congenitally immunodeficient neonates immunocompetent donor T lymphocytes can cause GvHD—rare.
- Need to irradiate all blood products in these children. Also irradiate if 1st-degree relatives used as donors.
- CMV infection: particular risk in low birth weight babies, or immunocompromised children undergoing transplantation. CMV seronegative donations should be used. Alternatively use (modern) leucodepletion filter to reduce risk.
- Hypocalcaemia—rare now, due to change of additive.
- Citrate toxicity, also rare nowadays due to improvements in additive.
- Rebound hypoglycaemia, induced by high glucose levels of blood transfusion anticoagulants.
- Thrombocytopenia—dilution, DIC.
- Volume overload.
- Haemolytic transfusion reactions in necrotizing enterocolitis. Thought to be due to the 'T' antigen on baby's RBCs becoming exposed due to action of bacterial toxin entering the blood from diseased gut. Anti 'T' is present in almost all donor plasma.

#### Use of 4.5% albumin

Use controversial, but may be helpful after large-volume paracentesis, as fluid replacement in therapeutic plasma exchange, or in nephrotic syndrome resistant to diuretics. There are better products for resuscitation and volume expansion. Should *not* be used in nutritional protein deficiency or chronic hypoalbuminaemia (e.g. cirrhosis or protein-losing enteropathy). Risk of infection transmission minimal but not zero.

#### Use of immunoglobulin

IV polyvalent immunoglobulin widely used as replacement therapy in immunodeficiencies, for Kawasaki disease to prevent the formation of coronary microaneurysms, and also as non-specific agent for reticuloendothelial blockade in immune cytopenias, chiefly (and usually unnecessarily) in childhood ITP. Can get immunoglobulin with particularly high titre against RSV, HZV, and hepatitis B. Usually this is for intramuscular use only and should not be given IV due to risk of complement activation. IVIg has transmitted hepatitis C in the past due to poor virus inactivation procedures, so should not be used in trivial conditions.

### Use of FFP

Available in aliquots of 50mL. Must be ABO and Rh compatible. Infused via filter. Main indication—DIC. No need for CMV screening, or irradiation. Dose: 10–15mL/kg. Check PT and APTT. Repeat as necessary. May need cryoprecipitate also (➔ Massive blood transfusion, p.658; Cryoprecipitate, p.771), if evidence of ↓ Fgn (<1.0g/L). Both contain untreated plasma, so potential infection risk, though FFP should be virus-inactivated in future. For this reason methylene blue treated FFP now recommended for children <16 years.

### Use of platelets

- Thrombocytopenia more hazardous in neonates, so prophylactic transfusion if count  $<30 \times 10^9/L$ .
- Reserve for children with marrow failure and counts  $<10 \times 10^9/L$  otherwise:
  - Only use in *immune* thrombocytopenia for life-threatening bleeding.
  - Then use massive 'swamping' dose to overwhelm antibody.
  - 1 dose (1 paediatric platelet concentrate) contained in ~50mL 'fresh' plasma, available either from apheresis or buffy coat derived.
  - Check increment 1h later if no clinical response.
  - Care with volume overload (give 10–20mL/kg).
  - Must be administered within 2h of receipt on ward.
  - Irradiate for immunosuppressed children.
  - Refractoriness can arise due to alloimmune antibodies.
  - For children <16 years use platelets collected by apheresis from single donor.

### Use of granulocytes

- Severely infected neonates may develop profound neutropenia.
- Usually respond to antibiotic therapy.
- Granulocyte transfusions very rarely given because of lack of effect, risk of CMV and toxoplasmosis, and respiratory distress syndrome.
- Blood products now routinely leucodepleted to reduce risk of CMV transmission.
- Granulocytes may be useful in patients with prolonged pancytopenia (e.g. in patients with aplastic anaemia or post-BMT) unresponsive to broad-spectrum IV antibiotics.



## Polycythaemia in newborn and childhood

As in adults, polycythaemia in children may be relative or absolute (➔ Polycythaemia vera (PV), p.264). The condition is usually 2° and most commonly seen as a clinical problem in neonates or older children with congenital cyanotic heart disease or high-affinity abnormal haemoglobins. 1° polycythaemia is very unusual in childhood; benign familial erythrocytosis is a very rare autosomal dominant self-limiting condition of unknown aetiology.

### Pathophysiology

Polycythaemia is physiological in the neonatal period with ↑ Hct (range 42–60% in cord blood) persisting in the first few days of life. Pathological polycythaemia is defined in the neonate as Hct >65% (Hb >22.0g/dL), is uncommon (<5% of all births) and usually due to hypertransfusion or hypoxia.

### Causes of polycythaemia in the newborn

- Relative (or 'pseudo'): dehydration, reduced plasma volume.
- Hypertransfusion: delayed cord clamping, maternofetal, twin to twin.
- Hypoxia: placental insufficiency, intrauterine growth retardation.
- Endocrine: congenital adrenal hyperplasia, thyrotoxicosis.
- Maternal disease; toxæmia of pregnancy, DM, heart disease, drugs, e.g. propranolol.
- Other miscellaneous conditions such as Down syndrome.

### Clinical features

- Hyperviscosity may give rise to vomiting, poor feeding, hypotonia, hypoglycaemia, lethargy, irritability, and tremulousness.
- On examination—plethora, cyanosis, jaundice, hepatomegaly.
- Complications include intracranial haemorrhage, respiratory distress, cardiac failure, necrotizing enterocolitis, and neonatal thrombosis.

### Diagnosis

- Clinical presentation may suggest the diagnosis, e.g. anaemic twin.
- FBC (free-flowing venous sample) ↑ neonatal Hct >65%.
- Hypoglycaemia, hypocalcaemia, unconjugated hyperbilirubinaemia.
- Hb studies for excess HbA—?maternal haemorrhage.
- Radiology: CXR shows ↑ vascularity, infiltrates, cardiomegaly.

### Management

- Supportive—IV fluids, close observation for complications.
- Exchange transfusion—partial with FFP/albumin to ↓ Hct <60%. (See Box 12.1.)



**Box 12.1**

$$\text{Vol. of exchange (mL)} = \frac{\text{Blood vol.} \times (\text{observed} - \text{desired Hct})}{\text{Observed Hct}}$$

**Treatment**

As required for associated abnormalities.

**Outcome**

Provided the condition is identified early and appropriate measures taken to reduce the hyperviscosity, the outcome should be good.

## Neonatal anaemia

Intrauterine conditions require a state of polycythaemia. Congenital anaemia is present with cord blood Hb <14.0g/dL in term babies. In the healthy infant, Hb drops rapidly after birth (↻ Table A3.3, p.812) and by end of the neonatal period (4 weeks in a term baby), the mean Hb may be as low as 10.0g/dL. With ↑ RBC destruction, there is a concomitant ↑ in serum bilirubin. Thus complex changes occur in this period making distinction between physiology and pathology difficult.

### Pathophysiology

In normal full-term babies, red cell production ↓ in the first 2–3 months of life. RBC survival shortens; reticulocytes and EPO production ↓; Fe, folate and vitamin B<sub>12</sub> stores are normal. Anaemia in the neonate may be due either to *impaired production of RBCs* or to ↑ *destruction or loss* (Table 12.2).

Blood loss during delivery is common, in ~1% severe enough to produce anaemia. Infection is also a significant cause of anaemia; ↑ haematological disorders are rare. Anaemia in premature infant is almost invariably present, induced, and multifactorial. Jaundice arises in 90% healthy infants making interpretation of a raised bilirubin (↻ Haemolytic disease of the newborn, p.578) a critical piece of the jigsaw when investigating an anaemic neonate.

### Clinical features

History may make the diagnosis. Check events at time of delivery, past obstetric history, maternal and family history. Non-specific symptoms—lethargy, reluctance to feed, failure to thrive—all may indicate anaemia. Clinical features may be helpful in picking up inherited disorders such as Fanconi anaemia.

### Laboratory tests

- FBC and reticulocytes.
- MCV and RBC morphology, bilirubin, and Kleihauer (on mother).
- Special tests may be necessary if cause not apparent (e.g. red cell enzyme defects, inherited BM failure syndromes).
- Glutathione peroxidase deficiency due to acquired selenium deficiency can present transiently with anaemia in neonates.
- Interpret as follows:
  - Reticulocyte count normally very low in first 6 weeks of life.
  - ↑ haemolysis, blood loss.
  - +ve Kleihauer suggests fetomaternal bleed (quantitate amount).
  - Bilirubin ↑ (unconjugated).
  - Check DAT: +ve in immune haemolytic anaemia (except ABO HDN).
  - –ve in other haemolytic anaemias, including ABO HDN.
  - Bilirubin ↑ (conjugated/mixed look for hepatobiliary obstruction/dysfunction).
- Blood film:
  - Note: ↑ polychromasia, occasional NRBC, poikilocytes and spherocytes days 1–4 in healthy babies.

- RBC morphology may suggest congenital spherocytosis, other RBC membrane disorders or HDN.
- ↓ MCV— $\alpha$  thalassaemia syndromes.
- ↑ WBC—reactive, congenital leukaemia.
- Neutropenia—sepsis, marrow failure.

**Table 12.2** Causes of neonatal anaemia**Impaired production    ↑ destruction of RBCs  
of RBCs**

Anaemia of prematurity	Overt or concealed haemorrhage; repeated venepuncture	
Infection		
$\alpha$ thalassaemia	<b>Haemolytic anaemia</b>	
DBA	<i>Non-immune</i>	<i>Immune</i>
Fanconi anaemia	TORCH infection	Rh/ABOHDN
CDA	Congenital RBC abn.	Maternal autoimmune haemolytic anaemia
	Drugs, MAHA	

CDA, congenital dyserythropoietic anaemia; DBA, Diamond–Blackfan anaemia; MAHA, microangiopathic haemolytic anaemia; Rh/ABO HDN, rhesus/ABO haemolytic disease of the newborn; TORCH, toxoplasmosis, rubella, CMV, herpes simplex.

## Anaemia of prematurity

Anaemia is an almost invariable finding in the premature infant. By week 3–4 of life the Hb may be as low as 7.0g/dL in untreated infants. In a study of very low birth weight infants (750–1499g) 75% required blood transfusion. A number of factors are causal.

### Pathogenesis

- RBC production and survival are ↓.
- EPO production very low in the first few weeks.
- Iatrogenic from repeated blood sampling (depletes the RBC mass and Fe stores—by 4 weeks the premature baby may have had its total blood volume removed).

### Clinical features

The ↑ O<sub>2</sub> needs and metabolic demand of the premature baby makes them less able to tolerate ↓ Hb. Over 50% infants <30 weeks' gestation develop tachycardia, tachypnoea, feeding difficulties and ↓ activity when anaemic. High HbF level and ↑ O<sub>2</sub> affinity exaggerates the hypoxia.

### Treatment

- Delayed cord clamping ↑ Fe stores.
- Close control of blood sampling is important.
- Transfusion indications will vary in neonatal units—decide on clinical grounds, particularly if ventilated. The following are guidelines only:
  - Prems <2 week with Hb <14.0g/dL, Hct <40%.
  - Prems >2 week, Hb <11.0g/dL, Hct <32%.
- A rising reticulocyte count in some centres is used as a sign to withhold transfusion. Some studies have shown better weight gain in transfused infants (not confirmed by others).
- Fe supplementation (2mg/kg/d PO) after first 2 weeks and until Fe sufficient.
- EPO—controversial. It can be effective but its indiscriminate use is not encouraged. Large randomized trials have shown it to have an effect, but its expense makes cost-effectiveness a concern and modern neonatal practice has already reduced the need for transfusion making it less necessary. Its best use is probably reserved for transfusion avoidance in infants weighing <1000g. A suitable regimen would be 200–250U/kg SC × 3/week between day 3 and week 6.

### Natural history

Despite the growing safety of blood and its ready availability and convenient packaging to reduce donor exposure, blood transfusion carries definite risks and is to be avoided. It is likely that in affluent societies the use of EPO will increase, despite its limited effect.



## Haemolytic anaemia in the neonate

Normal red cell life span in term infants is <80d, in pre-term infants <50d. Red cell 'cull' in 1st month with jaundice is physiological. Pathological haemolysis, when present, is most commonly due to iso-immune HDN 2° to fetomaternal blood group incompatibility in Caucasian populations. In other ethnic groups G6PD deficiency and congenital infection are major causes.

### Pathophysiology

Physiological haemolysis occurs soon after birth and there is a marked drop in the Hb and RBC count in the 1st weeks of life. Neonatal RBCs are more susceptible to oxidative stress and there is altered RBC enzyme activity compared to adult RBC and reticulocytes. So pathological haemolysis occurs in the neonatal period more than at any other time.

### Causes of haemolytic anaemia

Haemolysis may be due to intrinsic defects of the RBC, usually congenital, or to acquired extracorpuscular factors which may be immune or non-immune.

### Clinical features

Pathological jaundice may be clinically obvious at birth or within 24h (distinguishing it from the common physiological anaemia which occurs >48h after birth, ↻ Haemolytic disease of the newborn, p.578).

- Anaemia may be severe depending on cause.
- Infections are common cause of hyperbilirubinaemia (↻ Haemolytic disease of the newborn, p.578 with specific clinical findings. *In utero* infections (TORCH) do not usually cause severe jaundice cf. post-natal bacterial sepsis where jaundice may be striking and associated with MAHA.
- Splenomegaly at birth indicates a prenatal event; when noted later it may be 2° to splenic clearance of damaged RBC and is non-specific.
- Kernicterus is the major complication of neonatal hyperbilirubinaemia.
- Family history and drug history may be informative.

### Laboratory diagnosis of haemolysis

- ↑ unconjugated bilirubin with anaemia is hallmark of haemolytic anaemia.
- ↑ reticulocytes (haptoglobins are unreliable in the newborn).
- Blood film—may show RBC abnormalities, e.g. spherocytes, elliptocytes, fragmented cells.
- DAT, if +ve indicates immune haemolysis; -ve does not rule this out—especially consider ABO HDN.
- Heinz body test—+ve in drug-induced haemolysis, G6PD deficiency and occasionally other enzyme disorders.
- Intravascular haemolysis—look for haemoglobinuria/haemoglobinuria.



## Congenital red cell defects

### Pathophysiology

The neonate is uniquely disadvantaged when it comes to handling pathological haemolysis because of hepatic immaturity and altered enzyme activity. Thus congenital defects of the RBC commonly present in the newborn except for defects involving the  $\beta$  globin chain (e.g. SCD,  $\beta$  thalassaemia) which become clinically manifest several weeks after birth but can be diagnosed *in utero*, or in the neonatal period if suspected. HS is the commonest congenital haemolytic anaemia in Caucasian populations; half present in the neonatal period. Worldwide, G6PD deficiency occurs in 3% population; neonatal presentation is common in Mediterranean and Canton peoples.  $\alpha$  thalassaemia is incompatible with life causing hydrops fetalis (all 4  $\alpha$  globin genes deleted), or may present as HbH disease (3 of the 4  $\alpha$  globin genes deleted) with mild-to-moderate haemolysis in neonate.

### Hereditary RBC defects in neonatal haemolytic anaemia

- Membrane defects:
  - Hereditary spherocytosis, hereditary elliptocytosis and variants, e.g. stomatocytosis and pyropoikilocytosis (Afro-Americans).
- Haemoglobin defects:
  - $\alpha\delta\beta$  thalassaemia.
  - Unstable haemoglobins (e.g. HbKöln, HbZurich).
- Enzyme defects:
  - Glycolytic pathway; pyruvate kinase and other enzyme deficiencies.
  - Hexose monophosphate shunt; G6PD deficiency, other enzymes.

### Clinical features

Congenital haemolytic anaemia with mild or moderate unconjugated hyperbilirubinaemia. No gross excess of bile pigments in urine (old collective name for these diseases was 'acholuric jaundice'),  $\pm$  hepatosplenomegaly. A +ve family history is common but not invariable.

### Laboratory investigation

May be difficult to diagnose in neonate, especially if post-transfusion. Often have to wait until clinically stable some weeks/months later.

- Exclude acquired disorders, and immune lysis (DAT).
- RBC morphology is one key to diagnosis and further investigation, but spherocytes not specific for HS.
- Further tests for suspected:
  - Membrane defect (osmotic fragility, autohaemolysis, dye binding, membrane chemistry).
  - Hb defect; Hb electrophoresis, HbF, HbA<sub>2</sub> measurement.
  - Enzyme defect; Heinz body prep, screening tests for specific enzymes, G6PD, PK.
- Heinz body test +ve:
  - Drug or chemical induced in neonate without hereditary defect.
  - Enzyme deficiency: G6PD commonest but consider others.
  - Unstable Hb.
  - $\alpha$  thalassaemia.



**Outcome**

Given supportive management including transfusion if necessary, most problems due to congenital red cell defects other than haemoglobinopathies will improve during the 1st weeks and months of life as the infant matures.

Where the haemolysis is likely to be chronic folic supplementation should be commenced.

## Acquired red cell defects

These may be either immune or non-immune. Main cause of the latter will be underlying infection either acquired *in utero* or in the days following delivery. Drug-induced haemolysis in the newborn is rare.

### Pathophysiology

Neonatal RBCs have ↑ sensitivity to oxidative stress due to altered enzyme activity, and are more liable to be destroyed by altered physical conditions, mechanical factors, toxins, and drugs than adult RBC. Infection acquired *in utero* postnatally is a common cause of mild-to-moderate haemolytic anaemia. The mechanism is multifactorial, and includes ↑ reticuloendothelial activity and microangiopathic damage. Drug-induced haemolysis and Heinz body formation is occasionally noted as a transient phenomenon in normal neonatal RBCs as a result of chemical or drug toxicity but is much more often seen when there is an underlying RBC defect such as G6PD deficiency.

### Acquired RBC defects causing congenital haemolytic anaemia

- Infection:
  - Congenital TORCH infections also rare but serious are malaria (can cause stillbirth) and syphilis.
  - Post-natal, either viral or (more commonly) bacterial.
- Microangiopathy—2° to severe infections ± DIC, also Kasabach–Merritt syndrome (giant haemangioma with haemolysis and thrombocytopenia).
- Drug or chemically induced.
- Infantile pyknocytosis.
- (Rare) metabolic disease such as Wilson disease, galactosaemia.

### Clinical features

**Congenital infections:** all rare with adequate antenatal care. Most infants with herpes simplex will be symptomatic with DIC and hepatic dysfunction—haemolysis is not a major finding. Haemolytic anaemia is also mild in CMV and rubella infections but 50% infants with toxoplasmosis will be anaemic (may be severe).

**Postnatal infections:** viral, bacterial and protozoal (congenital malaria can be delayed for some days or weeks or it can be acquired early in life). Anaemia can be severe and associated with DIC.

**MAHA** 2° to toxic damage to the endothelium is rare in the neonate outside the context of DIC and sepsis, and is seen in burns and (classically) in the Kasabach–Merritt syndrome—a visible or covert giant haemangioma with haemolysis, RBC damage and profound thrombocytopenia.

*Drugs/chemical exposure* is more likely to cause Heinz body haemolysis in premature babies or those with G6PD deficiency. Incriminating agents include sulfonamides, chloramphenicol, mothballs, aniline dyes, maternal intake of diuretics, and, in the past, water-soluble vitamin K analogues. Vitamin E (a potent antioxidant) has a number of RBC stabilizing activities. Now rare due to dietary supplements, deficiency used to be seen occasionally in premature infants, following O<sub>2</sub> therapy and diets rich in polyunsaturated fatty acids. Clinical findings included haemolysis, pretibial oedema, and CNS signs. Acanthocytosis/pyknocytosis of the RBC is characteristic, and this may have been the main cause of infantile pyknocytosis.

*Infantile pyknocytosis*: indicates the diagnostic feature of this uncommon acquired disorder. Haemolysis with ↑ numbers (>6–50%) of pyknocytes (irregularly contracted RBCs with multiple projections) in the peripheral blood. Cause unknown, though may be associated with vitamin E deficiency. Anaemia may be severe and present at birth, and is most striking at ~3 weeks (Hb <5g/dL reported). Exchange transfusion occasionally required but the condition spontaneously remits by ~3 months. Its self-limiting nature and ill-understood pathology means that it may not be a distinct clinical entity.

*Metabolic disorders*—rare.

### Laboratory investigation

Criteria set out earlier in this topic will establish the diagnosis of haemolytic anaemia. A +ve DAT points to an immune process—probably Rh/ABO disease. If non-immune, further tests to look for a congenital abnormality.

### The diagnosis may be made by:

- Peripheral blood findings.
- Heinz body +ve—?chemical/drug-induced haemolysis.
- RBC enzyme screen to exclude G6PD or PK deficiency.
- Hb electrophoresis to exclude Hb defects.

Often no definitive cause is found and the HA will be presumed 2° to underlying systemic illness.

### Management

1. General supportive measures for hyperbilirubinaemia (→ Hyperbilirubinaemia, p.582).
2. Treatment of specific conditions:
  - Haemolytic disease of the newborn—→ Haemolytic disease of the newborn, p.578.
  - Neonatal infection as appropriate.
  - Exchange transfusion almost never needed.

### Outlook

Prognosis is that of the underlying condition. Anaemia will usually respond as the underlying condition is brought under control.

## Haemolytic disease of the newborn

Arises when there is blood group incompatibility between mother and fetus. Maternal Abs produced against fetal RBC antigens cross the placenta and destroy fetal RBCs. Most commonly caused by the Rh (D) antigen, but maternal passive immunization with Rh (D) immunoglobulin (anti-D) introduced in the late 1960s transformed the outlook. Despite this, HDN due to anti-D and other red cell antibodies (e.g. anti-c, anti-Kell) still remains an important cause of fetal morbidity.

### Pathogenesis

Placental transfer of fetal cells → maternal circulation is maximal at delivery; the condition does not usually present in the firstborn (*Note*: ABO incompatibility is an exception). Previous maternal transfusion, abortion, amniocentesis, chorionic villus sampling (CVS), or obstetric manipulations can cause antibody formation. Maternal IgG crosses placenta, reacts with Ag +ve fetal RBCs.

### Rh HDN

Classically presents as jaundice in first 24h of life.

- *Mild HDN*: may go unnoticed and presents as persistent hyperbilirubinaemia or late anaemia weeks after birth.
- *Severe HDN*: may result in a macerated fetus, fresh stillbirth, or severely anaemic, grossly oedematous infant (hydrops fetalis) with hepato-splenomegaly 2° to compensatory extramedullary haemopoiesis *in utero*.
- *Kernicterus*: neurological damage 2° to bilirubin deposition in the brain, depends on a number of factors including the unconjugated bilirubin level, maturity of the baby, the use of interacting drugs.

### Diagnosis

Rh HDN is the commonest cause of neonatal immune haemolysis; routine antenatal screening should identify most cases prior to delivery allowing appropriate action.

*In suspected case at delivery cord blood* is tested for:

- ABO and Rh (D) group.
- Presence of antibody on fetal RBCs by DAT.
- Hb and blood film (spherocytes, ↑ polychromasia, NRBCs).
- Serum bilirubin (↑).

*Maternal blood* is tested for:

- ABO and Rh (D) group.
- Serum antibodies against fetal cells (by indirect antiglobulin test, IAGT).
- Antibody titre.
- Kleihauer test—detects and quantitates fetal RBCs in maternal circulation.

*Diagnostic findings include*:

- DAT +ve haemolytic anaemia ± spherocytosis in the infant.
- Maternal anti-D, or other anti Rh antibody—the next most common to produce severe disease is anti-c.

## ABO HDN

Theoretically should occur more frequently since ~1 in 4/5 babies and mothers are ABO incompatible. Usually occurs in group O mothers who may have high titres of naturally occurring IgG anti-A or B, and with a boost to this during pregnancy haemolysis can occur.

### *Clinical features*

1st pregnancies are not exempt, but condition is usually mild. Presentation is later than with Rh HDN (2–4d, but may be weeks after birth).

### *Diagnosis*

May be difficult—DAT commonly and puzzlingly –ve, but offending antibody can be eluted from infant RBC.

### *Antibody studies*

Maternal high titre anti-A/B almost always in a group O mother cord-blood/infant's serum—an inappropriate antibody (e.g. anti-A in a group A baby).

## Other blood group antibodies

Can occasionally produce severe HDN; anti-Kell in particular. Serological testing will establish diagnosis. Maternal autoimmune haemolytic anaemia may produce a similar picture to alloimmune HDN where the auto-anti-body cross-reacts with the infant RBC. Usually the diagnosis will have been made antenatally. Severity in infant varies depending on maternal condition.

## Management—prevention

Routine antenatal maternal ABO and Rh D grouping and antibody testing carried out at booking visit (~12–16 weeks).

- *If antibody +ve* repeat at intervals to check the antibody titre.
- *If antibody –ve and Rh D –ve* repeat at ~28 weeks' gestation. Establish if paternal red cells heterozygous, homozygous, or –ve for the specific Ag.

## Anti-D prophylaxis

- 250IU IM during pregnancy to Rh D –ve women without antibodies to cover any intrauterine manoeuvre or miscarriage.
- After delivery, a standard dose of 500IU anti-D within 72h unless baby is known to be Rh (D) –ve.
- If Kleihauer test result shows a bleed of >4mL give further anti-D.

## Treatment of affected fetus—before delivery

Treatment depends on past obstetric history, the nature and titre of the antibody, and paternal expression of the antigen. The fetal genotype can be established by CVS, fetal blood sampling, and PCR.

*Examination of the amniotic fluid* to assess the degree of hyperbilirubinaemia is indicated with a poor past history (exchange transfusion or stillbirth in previous baby) and high titre (1:8–1:64) of an antibody likely to cause severe HDN (e.g. anti-D, anti-c, or anti-Kell). The amniotic fluid bilirubin at optical density 450nm dictates treatment according to the Liley chart (Fig. 12.1), which estimates the blood concentration.

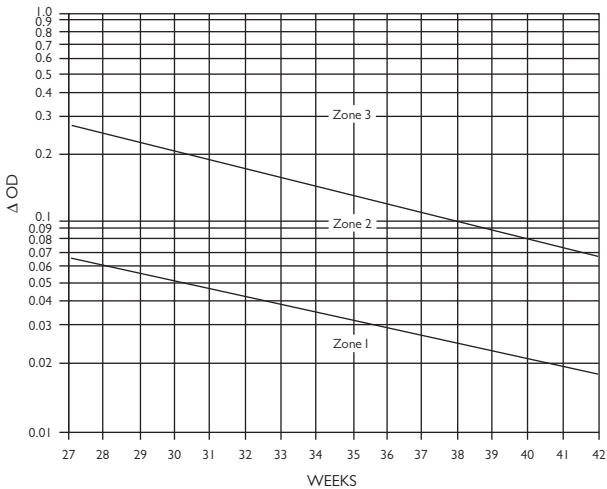
*Options include* intrauterine transfusion (IUT) if fetus is not deemed mature enough for delivery (check lung maturity on phospholipid levels) or induction of labour if it is. Intensive maternal plasmapheresis may be useful to reduce antibody titre. Advances in management of very premature babies are such that nowadays IUT rarely performed.

### After delivery

- Full paediatric support—metabolic, nutritional, and respiratory.
- If unconjugated hyperbilirubinaemia not a problem, treat anaemia (Note: cord blood Hb <14.0g/dL) by simple transfusion.
- Exchange transfusion with Rh (D) –ve and (if possible) group specific blood for:
  - A severely affected hydropic anaemic baby.
  - Hyperbilirubinaemia (bilirubin level at or near 340mmol/L (20mg/dL) or rapidly rising level, e.g. >20mmol/h) in first few days of life.
  - Signs suggesting kernicterus—exchange transfusion may have to be repeated.
- Phototherapy to reduce the bilirubin level.
- Follow-up required—late anaemia can be severe particularly if exchange transfusion has not been carried out.

### Outcome

With modern techniques, the outlook is good even for severely affected infants.



**Fig. 12.1** Liley chart.

The amount of bilirubin can be quantitated by spectrophotometrically measuring absorbance at 450nm wavelength in a specimen of amniotic fluid that has been shielded from light. The results ( $\Delta 450$ ) are plotted on a 'Liley' curve, which is divided into 3 zones.

A result in **zone I** indicates mild or no disease. Fetuses in zone I are usually followed with amniocentesis every 3 weeks. A result in zone II indicates intermediate disease. Fetuses in low zone II are usually followed by amniocentesis every 1–2 weeks. A result above the middle of zone II may require transfusion or urgent delivery.

## Hyperbilirubinaemia

Bilirubin results from the breakdown of haem, mostly Hb haem. It may be unconjugated, water insoluble, pre-hepatic (a mark of excessive RBC breakdown) or conjugated, soluble, post-hepatic ( $\uparrow$  in cholestasis). Unconjugated bilirubin  $\uparrow$  in most neonates and is usually physiological; conjugated hyperbilirubinaemia, on the other hand, is almost always pathological.

### Pathophysiology

Physiological jaundice is defined as a temporary inefficient excretion of bilirubin which results in jaundice in full-term infants between days 2–8 of life. Occurs in ~90% of healthy neonates. Hepatic immaturity and  $\uparrow$  RBC breakdown overloads the neonate's ability to handle the bilirubin which is mainly unconjugated. The bilirubin is rarely  $>100$ micromol/L, though between 2–5d occasionally can be  $>200$  in a term baby or 250 in a healthy pre-term. Levels much above this need investigation. Reaches a maximum by day 3–6 and usually  $\downarrow$  to normal by day 10. In premature neonates it may take longer to settle. HDN due to blood group incompatibility accounts for ~10% cases of hyperbilirubinaemia and about 75% of those requiring exchange transfusion.

### Causes

See Table 12.3.

**Table 12.3** Causes of hyperbilirubinaemia

Unconjugated	Conjugated
Physiological jaundice	Mechanical obstruction
Haemolytic anaemia	Bile duct abnormalities (e.g. atresia, cysts)
Haematoma	Hepatocellular disorders
Polycythaemia	Hepatitis
Biochemical defects	

### Clinical features

- **Note:** *clinical jaundice in the first 24h of life is always pathological.*
- Presenting after this, the jaundice may/may not be pathological—usually not. Inadequate food/fluid intake with dehydration can aggravate the physiological bilirubin. A higher concentration is acceptable for the full-term breast-fed baby (serum bilirubin 240micromol/L) than bottle-fed baby (190micromol/L).
- Jaundice in an active healthy infant is likely to be physiological.
- In a sick infant the underlying cause of the jaundice may be clinically evident—e.g. infection, anaemia, shock, asphyxia, haemorrhage (may be occult).
- Physical examination—hepatosplenomegaly is pathological.
- Maternal history (drugs, known condition) and family history may help.



### Laboratory investigations

- FBC, reticulocyte count, and film—?haemolytic anaemia.
- Serum bilirubin—?conjugated or unconjugated:
  - Unconjugated hyperbilirubinaemia:
    - DAT, maternal and neonatal blood group serology.
    - Infection evaluation including TORCH.
    - Thyroid function.
    - Reducing substances.
  - Conjugated hyperbilirubinaemia:
    - Abdominal USS, bile pigments in stool, liver pathology.
- Further investigation as determined by results and clinical picture.

### Management

- General—adequate hydration, nutrition, other supportive measures.
- Treat underlying cause—antibiotics, metabolic disturbances.
- Haemolytic anaemia—blood transfusion.
- Specific phototherapy (light source with wavelength of 400–500nm) effective in treating most causes of unconjugated hyperbilirubinaemia. *Note:* contraindicated in conjugated hyperbilirubinaemia.
- Exchange transfusion—the indications are complex. Main indication is severe haemolytic anaemia and is used in full-term infants when the bilirubin is  $>340$ micromol/L and at a lower concentration in premature infants.
- Hyperbilirubinaemia due to mechanical obstruction may need surgery.

### Outcome

In most infants hyperbilirubinaemia resolves by 2 weeks. When pathology has been excluded the commonest cause of prolonged hyperbilirubinaemia persisting beyond this period is breastfeeding. In 20% healthy breast-fed infants the bilirubin is still significantly  $\uparrow$  at day 21. Kernicterus is not a complication but the condition causes concern before it spontaneously remits.

## Neonatal haemostasis

Neonates can develop bruising and/or purpura due to defects in platelets, coagulation factors, or both. Coagulation tests should be interpreted with caution because in the term infant the concentration of vitamin K-dependent proteins (II, VII, XI, and X together with protein C and protein S) are 50% of normal adult values, contact factors (XI, XII, PK, and HMWK) are 30–50% of adult concentrations, and all are lower in pre-terms. Factors VIII, V, and vWF are normal. Thrombin inhibitors antithrombin (AT, previously called antithrombin III) and HCII are ↓ but  $\alpha_2$ -M is ↑. Platelet count is the same as adults in both term and pre-term infants. There are technical problems in obtaining uncontaminated (heparin from catheters or IV lines) and adequate venous samples from neonates, causing *in vitro* inhibition or pre-test activation of clotting factors or dilution due to short sampling and thus excess anticoagulant. All can give spurious results.

### Pathophysiology

Haemostatic and fibrinolytic system in neonates is immature. Sepsis, liver disease, necrotizing colitis and RDS can precipitate DIC easily. Thrombocytopenia can be caused by DIC and also immune mechanisms or marrow failure. (See Table 12.4.)

### Clinical features

- Petechiae indicate problems with small vessels or platelets, bruises can be due to platelet deficiencies and/or coagulation disorders.
- Oozing from multiple venepuncture sites in sick infants usually indicates generalized haemostatic failure and DIC.
- Haemorrhagic disease of the newborn due to functional vitamin K deficiency presents in 3 forms with bruising, purpura, and GI bleeding in otherwise well babies; early (within 24h) usually due to maternal drugs such as warfarin; classical (d2–5) in babies who have not been given adequate vitamin K prophylaxis and who have been breast-fed; and late, a variant of the classical form (i.e. insufficient vitamin K, breast-fed) arising at 2–8 weeks and with a higher morbidity and ↑ incidence of ICH.
- Thrombosis usually catheter related, can be rarely associated with AT III deficiency or homozygous protein C and protein S deficiency (neonatal purpura fulminans—a life-threatening condition with widespread peripheral gangrene).
- Haemophilia and other coagulant deficiencies can cause large haematomas but rarely cause trouble in the neonatal period except factor XIII lack—typically presents with bleeding from the umbilical stump.
- In well babies, petechiae and bruises with thrombocytopenia suggests immune basis—antibody usually from mother (alloimmune or autoimmune). Rarely, infants under a month can develop endogenous ITP.
- Clear symptoms of thrombocytopenia with normal platelet count suggests major functional defect—Glanzmann's.
- Marrow failure due to infiltration, aplasia.

**Table 12.4** Disorders causing bleeding in neonates

Inherited (rare)	Acquired (common)
• Haemophilia	• DIC (sepsis, necrotizing colitis, hypoxia, RDS)
• von Willebrand disease (only type 3)	• Vitamin K deficiency
• Other inherited factor deficiencies	• Liver disease
• Inherited thrombotic disorders	• Acquired thrombotic disorders
• Glanzmann thrombasthenia	• Neonatal alloimmune thrombocytopenia
• Inherited BM failure syndromes	• Maternally derived ITP
	• Endogenous ITP
	• Aplasia
	• Leukaemia

## Neonatal alloimmune thrombocytopenia

Occurs when mothers form alloimmune antibodies against fetal platelet-specific antigens that their own platelets lack. These antibodies react with fetal platelets *in utero* causing thrombocytopenia which can be severe, and in some cases life-threatening in late pregnancy and early life. A more serious condition with greater morbidity than thrombocytopenia due to maternal autoantibodies against platelets—i.e. where the mother has ITP.

### Pathophysiology

In >90% cases the mother will be HPA-1a (old term = PLA1) –ve with anti-HPA-1a antibodies against the HPA-1a +ve fetus; only 2% population are HPA-1a –ve (i.e. homozygous HPA-1b). Incidence of NAIT is 1/1000 pregnancies and accounts for 10–20% cases of neonatal thrombocytopenia. Other antigens may be involved, e.g. HPA-5 (Br) and HPA-3 (Bak) are the commonest.

### Clinical features

- Commonly presents in first-born infant and recurs in 85–90%.
- Maternal platelet count normal with no past history of ITP.
- Bleeding manifestations in 10–20% evident within the 1st few days of life, e.g. umbilical haemorrhage, petechiae, ecchymosis, internal haemorrhage, intracranial haemorrhage (ICH).
- Baby's platelet count ↑ to normal over the next 2–3 weeks as the antibody is cleared.
- Haemorrhage *in utero* with fatal ICH in ~1% cases.

See Table 12.5 for laboratory diagnosis.

### Management

#### Of bleeding neonate

Transfuse platelets –ve for Ag (usually HPA-1a –ve); use random donor platelets in an emergency. Maternal platelets (*irradiated*) are a good source. Repeat platelet transfusion PRN. IVIg as for ITP can be used in exceptional cases (response within days). Close observation (ICH is potentially lethal—screen using USS).

#### Of subsequent pregnancies

Cordocentesis *in utero* at ~24 weeks; take 1–3mL blood for platelet count and phenotype. If affected, treatment needs to be started immediately.

### Options

1. *In utero* CMV –ve compatible platelet transfusions at 2–4-weekly intervals (depending on severity and history). Invasive and technically demanding. Keep platelet count  $>50 \times 10^9/L$  (platelets ↓ rapidly so frequent follow-up mandatory).
2. Maternal administration of IVIg (1g/kg) weekly from ~24 weeks onwards: check fetal platelet count ~4 weeks later and again near term; response variable—around 75% respond—transfuse platelets if non-responsive. Check cord blood at birth and treat as necessary.

## Outcome

With aggressive treatment the outcome is good, death *in utero* and ICH occur rarely. A history of a previous ICH correlates with severe thrombocytopenia in subsequent pregnancies.

**Table 12.5** Laboratory diagnosis

Baby	Parents
<ul style="list-style-type: none"> <li>● Severe thrombocytopenia platelets <math>&lt;20 \times 10^9/L</math> in 50%</li> <li>● BM has megakaryocytes ++ (not usually necessary)</li> </ul>	<ul style="list-style-type: none"> <li>● Mother's platelet count normal</li> <li>● Serology:               <ul style="list-style-type: none"> <li>• Mother's platelets usually HPA-1a -ve</li> <li>• Rarer Ab include anti-HPA-3, HPA-5b, HPA-4 (Yuk/Pen)</li> <li>• Mother's serum contains anti-platelet antibody</li> <li>• (Note: antibody titre cannot predict degree of thrombocytopenia in fetus in subsequent pregnancies)</li> </ul> </li> <li>● Father's platelets carry offending antigen</li> </ul>

## Congenital dyserythropoietic anaemias

A rare group of inherited lifelong anaemias with morphologically abnormal marrow erythroblasts and ineffective erythropoiesis. 3 clinically distinguishable types are recognized where inheritance may be recessive (types I and II) or dominant (type III), see Table 12.6. A number of families have been described that share some features but do not fit with the typical patterns.

### Pathophysiology

Ineffective erythropoiesis (cell death within the BM); RBC survival in PB is not much reduced (III). Abnormal serological and haemolytic characteristics (type II CDA) and membrane abnormalities are described but as yet no defining shared defect in all cases of CDA.

### Clinical features

- Age of presentation variable; but usually in older children (>10 years). Can rarely present as neonatal jaundice and anaemia.
- Anaemia—in type I, Hb 8.0–12.0g/dL; type II anaemia may be more severe, patient may be transfusion dependent. Type III (rare) anaemia is mild/moderate.
- Jaundice (2° to intramedullary RBC destruction).
- Gallstones.
- Splenomegaly common.

### Laboratory diagnosis

- Peripheral blood—normocytic/macrocytic RBC with anisopoikilocytosis.
- WBC and platelets usually ↔; reticulocytes slightly ↑.
- BM appearance—striking, showing ↑ cellularity with excess abnormal erythroblasts.
- Type II shows +ve acidified serum test.
- ↑ serum ferritin due to ↑ Fe absorption; haemosiderosis can occur without transfusion dependence. Type III very occasionally Fe deficient due to intravascular haemolysis and haemosiderinuria.
- Genetic analysis for *CDAN1* and *SEC23B*.

### Differential diagnosis

- CDA variants (CDA types IV to VII)—not all CDA falls neatly into 3 subtypes on BM findings, serology, or clinical features.
- PNH—acidified serum test is +ve with heterologous and autologous serum. In HEMPAS +ve with heterologous serum only.
- Other megaloblastic/dyserythropoietic anaemias—including vitamin B<sub>12</sub> and folate deficiency.
- 1°/acquired sideroblastic anaemia.
- Erythroleukaemia (M6 AML).

### Treatment

- Mostly unnecessary.
- Avoid blood transfusion if possible (Fe overload)—Fe chelation as necessary.
- Splenectomy not curative but may decrease transfusion requirements.
- Type I may respond to high-dose interferon alfa; not recommended as routine therapy.

### Natural history

Severity of CDA varies considerably and many patients have good QoL with no therapy. Haemosiderosis is a long-term complication which may impact on survival.

**Table 12.6** Types of congenital dyserythropoietic anaemias

Type	Bone marrow	Blood findings	Inheritance
I	Megaloblastic + intra-nuclear chromatin bridges	Macrocytic RBC	Recessive <sup>b</sup>
II (HEMPAS) <sup>a</sup>	Bi/multinuclearity with peripheral arrangement of the endoplasmic reticulum	Normocytic RBCs pluripolar mitosis. Lysis in acidified serum (not autologous serum)	Recessive
III	Giant erythroblasts with multinuclearity	Macrocytic	Dominant

<sup>a</sup> Hereditary erythroblast multinuclearity with +ve acidified serum test; commonest form, found in ~66% cases. <sup>b</sup> The *CDAN1* gene is mutated in majority of type 1 patients. The *SEC23B* gene is mutated in type II patients.

## Congenital red cell aplasia

First described by Josephs in 1936 and subsequently by Diamond and Blackfan in 1938. Has come to be known as Diamond–Blackfan anaemia (DBA). Incidence now estimated to be 4–7/million live births.

### Pathophysiology

DBA is a heterogeneous disorder with evidence for at least 3 genetic subtypes. Approximately 60% of DBA patients will have a heterozygous mutation in 1 of 10 ribosomal genes (*RPS7*, *RPS10*, *RPS17*, *RPS19*, *RPS24*, *RPS26*, *RPL5*, *RPL11*, *RPL26*, *RPL35A*). These genes encode components important in ribosome biogenesis. DBA therefore appears to be constitutional disorder arising from disruption of a key housekeeping pathway. This is associated with pleiotropic effects in many systems but the maximal impact is on erythropoiesis resulting in a deficiency of red cells.

### Clinical features

- Usually presents in the 1st year of life: in 25% at birth and 90% <6 months of age. Rarely presents >1 year.
- Mildly affected individuals may rarely be detected as older children or adults during family studies.
- Associated physical anomalies in 50%; abnormal facies with abnormal eyes, webbed neck, malformed (including triphalangeal) thumbs, other skeletal abnormalities, short stature, congenital heart lesions, renal defects.
- Anaemia usually severe and child commonly transfusion dependent.
- Susceptibility to infection is not ↑.
- Hepatosplenomegaly absent.
- Family history is +ve in only 10–20% cases; most are sporadic.
- ↑ risk of AML in long survivors; ~5% in biggest series reported to date.

### Laboratory diagnosis

- ↓ Hb, ↓ reticulocytes, ↑ MCV (> ↔ for age), WBC and platelets not ↓.
- Red cells—↔ morphology to red cell anisocytosis/macrocytosis, have ↑ i antigen positivity, ↑ ADA activity.
- ↑ HbF, ↑ EPO, ↑ serum Fe/ferritin.
- BM findings—usually absent erythroid precursors; other cell lines ↔.
- No evidence of parvovirus infection.
- Radiological investigation to define other congenital defects.
- Genetic diagnosis possible in 60% of DBA patients (*RPS19* gene).

### Differential diagnosis

- TEC (↻ Transient erythroblastopenia of childhood, p.592; later presentation, transient, no other defects).
- Drugs, malnutrition, infection.
- Haemolytic anaemias in hypoplastic phase, with parvovirus B19, delayed recovery in HDN.
- Megaloblastic anaemia in aplastic phase.



### Treatment

- Prednisolone 1mg/kg PO in divided doses, slowly ↓ over weeks; 70% respond well. Titrate to lowest dose to maintain Hb >7g/dL. Many achieve this on almost homeopathic doses despite true dependence. Around 10% need high-dose maintenance and have trouble with side effects. 30% steroid resistant.
- Transfusion dependency usual in those who cannot be maintained on very low-dose steroids. Need chelation to prevent Fe overload. Use CMV -ve leucocyte-depleted packed RBC.
- Splenectomy not helpful (unless hypersplenism).
- BM transplantation worth considering for transfusion dependents with suitable sibling donor; risk stratification as for severe thalassaemia (Fe overload). Complicated decision due to chance of spontaneous remission even after years of transfusion dependency.

### Natural history

Spontaneous remission in 10–20% (even after several years). Median survival estimated at 30–50 years, though data patchy. Death due to haemosiderosis, complications of steroid therapy, or evolution of AML or aplastic anaemia. BMT may offer better outlook. DBA Registries are now prospectively gathering new data.

## Acquired red cell aplasia

Isolated failure of erythropoiesis. Most commonly transient—either due to parvovirus B19 infection or transient erythroblastopenia of childhood (TEC). Acquired pure red cell aplasia (PRCA) seen in adults with or without thymoma and probably autoimmune in nature (➔ Aplastic anaemia, p.102) virtually unknown in childhood, though very occasionally seen in adolescents.

### Parvovirus B19 infection

#### Clinical features

- Causes transient reticulocytopenia and (occasionally) neutropenia and thrombocytopenia in otherwise healthy individuals.
- In children with ↑ red cell turnover for any reason (compensated haemolysis, ineffective erythropoiesis) or those with reduced red cell production (marrow suppression or hypoplasia) can produce dramatic falls in Hb ('aplastic crisis').
- Can affect any age.
- Self-limiting as infection subsides following antibody response, 7–10d.
- In immunosuppressed children (e.g. chemotherapy, HIV) anaemia can occasionally become chronic with persisting viraemia.

### Pathogenesis

Parvovirus shows tropism for red cells through the P antigen, and is cytotoxic for erythroid progenitor cells at the colony forming stage (CFU-E) *in vitro*.

### Transient erythroblastopenia of childhood

#### Pathogenesis

Serum and cellular inhibitors of erythropoiesis and defective BM response to stimulating cytokines have been demonstrated. The condition may be idiopathic or associated with viral infection. It is uncommon but not excessively rare and there may be many subclinical cases where a blood count is not done.

#### Clinical features

- Boys and girls equally affected: age range 6 months–5 years; most commonly around 2 years.
- Typically a previously well young child presents with symptoms and signs of anaemia, sometimes but not invariably following an infection. Onset is insidious and the child becomes listless and pale—or just pale.
- Associated infections are usually viral (EBV, mumps), preceding the onset of TEC by some weeks.
- Fever is rare.
- Pallor may be striking.
- No lymphadenopathy or hepatosplenomegaly.
- No physical abnormalities.

#### Laboratory diagnosis

- Normocytic, normochromic anaemia which may be severe (Hb <5g/dL).

- Reticulocytes absent unless in early recovery phase; WBC and platelets usually ↔.
- Blood film shows no abnormality other than anaemia.
- Biochemical profile ↔.
- BM shows normocellular picture with absent erythroid precursors. ↔ Fe content.
- No karyotypic abnormalities.
- Exclude parvovirus infection (⊖ Parvovirus B19 infection, p.592).
- No other investigation is of diagnostic help.

#### *Differential diagnosis*

- Exclude acute blood loss and anaemia of chronic disease.
- Exclude common ALL.
- Diamond–Blackfan anaemia (⊖ Congenital red cell aplasia, p.590). Usually presents within the first 6 months of life and other abnormalities (skeletal malformation, short stature, abnormal facies) are commonly present.
- Parvovirus infection (⊖ Parvovirus B19 infection, p.592)

#### *Treatment*

Blood transfusion should be avoided but may be necessary if symptomatic.

#### *Natural history*

Spontaneously remits (if not then diagnosis probably wrong) commonly within 4–8 weeks but may be up to 6 months. Relapse is rare. There are no long-term sequelae or associations.

## Fanconi anaemia

First described by Fanconi in 1927, Fanconi anaemia (FA) is a clinically heterogeneous disorder usually presenting in childhood with the common feature being slowly progressive marrow failure affecting all 3 cell lines (RBC, WBC, and megakaryocytes) and manifest by peripheral blood pancytopenia and eventual marrow aplasia.

It is usually an autosomal recessive disorder with several different germline genetic mutations involving at least 16 genes (*FANCA*, *FANCB*, *FANCC*, *FANCD1*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCI*, *FANCL*, *FANCM*, *FANCN*, *FANCO*, *FANCP*, and *FANQQ*). One FA subtype (FAB) is X-linked. The products of the FA genes are components of the 'FA-BRCA pathway' important in the maintenance of genomic stability of the cell.

### Pathophysiology of FA

FA affects all cells of the body and the cellular phenotype is characterized by ↑ chromosomal breakage, hypersensitivity to DNA cross-linking agents such as diepoxybutane (DEB) and mitomycin C (MMC), hypersensitivity to oxygen, ↑ apoptosis, and accelerated telomere shortening. The ↑ chromosomal fragility is characteristic and used as a diagnostic test. Apart from progressive marrow failure, 70% of FA patients show somatic abnormalities, chiefly involving the skeleton. 90% develop marrow failure and survivors show an ↑ risk of developing leukaemia, chiefly AML. Rarely, FA can present as AML. There is also an ↑ risk of liver tumours and squamous cell carcinomas.

### Clinical features

- Autosomal recessive inheritance usually, rarely X-linked: in 10–20% the parents are related.
- Phenotypic expression of the disease varies widely, though often similar in any given kindred. Most commonly presents as insidious evolution of pancytopenia, presenting in mid-childhood with a median age of presentation of 9 years. Cell lines affected asynchronously; isolated thrombocytopenia may be first manifestation, lasting 2–3 years before other cytopenias occur.
- 10% present in adolescence or adult life, 4% present in early infancy (<1 year).
- Disorders of skin pigmentation common (60%)—café-au-lait spots, hypo- and hyperpigmentation.
- Short stature in 60%, microcephaly, and delayed development in >20%.
- Congenital abnormalities can affect almost any system—skeletal defects common, >50% in the upper limb especially thumb, spine, ribs.
- Characteristic facies described—elfin-like, with tapering jaw line.

### Diagnosis

#### Laboratory findings

- Pancytopenia and hypoplastic marrow—patchy cellularity.
- BM may also show dyserythropoietic morphology.
- Anaemia varies in its severity and may be macrocytic (MCV 100–120fL).

- Excessive chromosomal breaks/rearrangements in culture of peripheral blood lymphocytes stressed with clastogens (DEB or MMC) is the defining abnormality, and can be used on fetal cell culture for antenatal diagnosis.
- Direct probing for mutations in the FA genes that have been identified and characterized permits molecular diagnosis in >95% of patients, but is complex, slow, and is not routinely available.
- Further investigation for systemic congenital abnormalities is indicated.

#### Differential diagnosis

- Acquired aplastic anaemia (↔ Aplastic anaemia, p.102).
- Other congenital or inherited childhood marrow failure syndromes—  
↔ see Rare congenital marrow failure syndromes, p.596.
- Bloom syndrome—clinically like FA with similar congenital defects, spontaneous chromosomal breaks, and a predisposition to leukaemia but without pancytopenia, or BM hypoplasia. Autosomal recessive. Characteristically have photosensitivity and telangiectatic facial erythema. Due to genetic mutations in the *RECQL3* helicase gene.

### Treatment

#### General

- Supportive—blood transfusion with Fe chelation as required.
- Treatment of associated congenital anomalies where necessary.

#### Specific

- Androgens (oxymetholone 0.5–5mg/kg/d). Most patients respond to treatment but may eventually become refractory.
- Haemopoietic growth factors may offer temporary relief from neutropenia and anaemia.
- BMT is potentially curative, but FA patients hypersensitive to conditioning agents cyclophosphamide and radiation. Using low doses, matched sibling grafts give 80% actuarial survival at 2 years; unrelated donor results less good but improving using fludarabine based protocols. Early survivors showed ↑ risk of tumours, especially head and neck.
- Much interest in gene therapy. Early trials have occurred, but no major therapeutic success. Theoretically should be possible to transduce patient stem cells from those with known mutations with the appropriate wild type FA gene, and for these stem cells to have a natural growth advantage over FA cells. So far responses have been disappointing and short-lived.

### Outcome

Median survival of conventional treatment responders who do not undergo BMT is ~25 years. Non-responders have a median survival of ~12 years. Death most commonly due to marrow failure, but 10–20% will develop MDS or AML after a median period of observation of 13 years.

## Rare congenital marrow failure syndromes

### Amegakaryocytic thrombocytopenia (congenital amegakaryocytic thrombocytopenia—CAMT)

Presents in infancy (usually at birth or within 2 months) with profoundly ↓ platelets and associated physical signs (petechiae and bruising). Around 40% of affected children also have other congenital abnormalities—chiefly neurological or cardiac. Developmental delay is common. The marrow completely lacks megakaryocytes and the disorder evolves to severe aplastic anaemia in around 50% of sufferers, usually in the 1st few years of life. Has none of the unstable DNA features of Fanconi anaemia (↻ see Fanconi anaemia, p.594). Also quite distinct from the syndrome of thrombocytopenia with absent radius (TAR syndrome) since it is a trilineage problem with a much greater mortality. Usually sporadic, but familial cases occur and disorder thought to be inherited. In a subgroup of CAMT patients mutations in the gene encoding for the thrombopoietin receptor (*MPL*) have been identified.

#### Outlook

Without BMT, mortality from bleeding, infection or (occasionally) progression to leukaemia near 100%.

### Amegakaryocytic thrombocytopenia with absent radii (TAR syndrome)

- Usually diagnosed at birth because of lower arm deformity due to bilateral radial aplasia.
- No hyperpigmentation.
- Isolated thrombocytopenia with other cell lines ↔.
- BM lacks megakaryocytes; has adequate WBC/RBC precursors.
- No chromosomal breaks in cell culture.
- Autosomal recessive; the gene mutated is *RBM8A*.
- Thrombopoietin ↑; platelets gradually increase as child grows.
- Bleeding problems greatest in infancy.
- Supportive therapy only needed.

#### Outlook

Usually good, with problems receding as childhood proceeds. Occasional patients continue to have problems with ↓ platelets. No ↑ malignancy.

### Dyskeratosis congenita (DC)

- Inherited multi-system disorder including mucocutaneous and haematopoietic and other abnormalities.
- Clinical triad of abnormal skin pigmentation, leucoplakia of the mucous membranes, dystrophic nails and in 80% patients BM failure develops, usually in the 2nd decade.
- X-linked recessive, autosomal dominant and autosomal recessive subtypes recognized. 10 DC genes (*DKC1*, *TERC*, *TERT*, *TINF2*, *NHP2*, *NOP10*, *C16orf57*, *TCAB1*, *CTC1*, and *RTEL1*) identified. The DC genes have an important role in the maintenance of telomeres.

- Some patients with AA and MDS have *TERC* and *TERT* mutations.
- Chromosome fragility on challenge with DEB or mitomycin C normal—important to distinguish DC from FA (➔ see Fanconi anaemia, p.594).
- Despite this DC is a chromosome instability disorder due to defective telomere maintenance. Results of BMT for aplastic anaemia in DC patients poor, perhaps because of this.
- Anecdotal reports of good response to haemopoietic growth factors. Majority of patients also have a trilineage haemopoietic response to oxymetholone.

### Outlook

10% develop cancers before the age of 40 years—mostly epithelial, but also MDS/AML. Life expectancy depends on development of aplasia or malignancy. 30% survive to middle age. Results of BMT improving with low-intensity fludarabine-based protocols.

### Kostmann syndrome (congenital neutropenia)

- Autosomal recessive, due to biallelic mutations in *HAX1*.
- Severe Kostmann syndrome with neutrophils  $<0.2 \times 10^9/L$ .
- Marrow shows maturation arrest at promyelocyte/myelocyte stage.
- High risk of severe infection in untreated state.
- Not due to germline mutation in G-CSF receptor gene, though abnormal receptor may be present in myeloid precursors. Some cases attributed to mutations in neutrophil elastase (*ELA2*).
- 90% will respond to pharmacological doses of G-CSF and continue to do so for years.
- Up to 10% develop AML/MDS—role of G-CSF not clear, but probably complication of disease revealed by longer survival.

### Diagnosis

Distinguish from cyclical neutropenia (by observation); benign congenital neutropenia (by WBC), and reticular dysgenesis, a severe inherited immunodeficiency with congenital lack of all white cells, including lymphocytes.

### Outlook

Good provided response to G-CSF satisfactory and maintained. Non-responders may need BMT.

### Shwachman–Diamond syndrome

- Congenital exocrine pancreatic insufficiency; chronic diarrhoea, malabsorption, and growth failure associated with neutropenia and skeletal abnormalities.
- BM failure—usually leads to neutropenia but can evolve to pancytopenia.
- BM morphology varies—may be dysplastic/hypo-/aplastic.
- Autosomal recessive, majority of patients have mutations in the *SBDS* gene on 7q11.
- Psychomotor delay common.

### Diagnosis

- Exclude cystic fibrosis (by normal sweat test).
- Exclude FA (by normal chromosome fragility).

- Pearson syndrome clinically similar with severe pancreatic insufficiency but with anaemia rather than neutropenia and marrow shows ring sideroblasts and vacuolization of red and white cell precursors.
- Genetic diagnosis now possible in majority of patients.

#### Treatment

- Supportive with pancreatic enzymes, G-CSF, and antibiotics.
- Greatly ↑ risk (up to 25%) of progression to MDS/leukaemia (AML > ALL).
- Limited experience with BMT for aplasia/leukaemia; may be ↑ risk of cardiotoxicity—ventricular fibrosis seen at autopsy in 2/5 patients who died post BMT.

#### Outlook

Depends on development of severe aplasia or leukaemia; long survivors few if so. Pancreatic insufficiency improves as childhood progresses.

### Seckel syndrome—bird-headed dwarfism

- Rare autosomal recessive disorder with (proportionate) very short stature and mental deficiency.
- Facial features fancifully described as bird-like.
- The *ATR* (ataxia-telangiectasia and Rad3-related protein) gene is mutated in Seckel patients.
- Progression to aplastic anaemia common, clinically similar to FA (↻ see Fanconi anaemia, p.594) but chromosome fragility usually has different features to FA.

### Infantile osteopetrosis

- Pancytopenia can arise in this autosomal recessive disorder where the marrow cavity is obliterated with cortical bone due to a functional deficiency of osteoclasts.
- It is a 1° marrow failure in the sense that there is no marrow, but is a failure of the microenvironment rather than haemopoietic stem cells.

#### Outlook

Poor due to cranial compression, and children usually die in early childhood unless successful allogeneic BMT can replace normal osteoclast function.





## Neutropenia in childhood

Apart from 1° marrow failure due to aplastic anaemia, or other marrow failure syndromes (➡ Aplastic anaemia, p.102), or marrow suppression due to antineoplastic, immunosuppressive, or antiviral chemotherapy, neutropenia can also arise as an immune phenomenon, a cytokine mediated problem or due to cyclic or non-cyclic disturbances of the homeostasis of neutrophil production.

### Pathophysiology

- Commonest cause of a clinically important low neutrophil count in children ( $<0.5 \times 10^9/L$ ) is myelosuppression due to drugs.
- 1° marrow stem cell failure either involving all cell lines or granulopoiesis alone is rare.
- Neutropenia can also be due to less serious inherited deficiencies of neutrophil production where neutropenia can be variable or cyclical and the problem seems to be one of production control rather than 1° stem cell failure.
- Probably due to cytokine disturbances, several microbial infections can cause paradoxical neutropenia, particularly in neonates but also in older children (Table 12.7).
- Autoimmune causes of neutropenia can arise in infancy or later in childhood.
- Isoimmune neutropenia in the newborn—due to maternal antineutrophil antibodies and analogous to HDN.

### Homeostatic disorders

#### *Cyclic neutropenia*

Rare. Neutropenia arising every 21d lasting 3–6d. Counts may fall  $<0.2 \times 10^9/L$ . Associated with episodes of fever, malaise, mucous membrane ulcers, lymphadenopathy. Serious infection can arise and deaths. May improve after puberty. Usually +ve family history when problem encountered in childhood; due to mutations in neutrophil elastase (*ELA2*) gene. Treatment supportive with antibiotics. G-CSF may be useful.

#### *Chronic benign neutropenia*

More common. Persistent rather than cyclical, though often variable without clear periodicity. Neutrophil count usually  $>0.5 \times 10^9/L$ , so clinically mild or silent. May have autosomal dominant or autosomal recessive family history. Variable severity, often mild with few problems. To be distinguished from severe congenital neutropenia (➡ Kostmann syndrome, p.597) by milder clinical course and (usually) later presentation. Some of these patients will have mutations in *ELA2*. Therapy not usually necessary.

**Table 12.7** Infections causing neutropenia

Viruses	Bacteria	Others
RSV	Tuberculosis	Rickettsiae
Malaria	Typhoid/paratyphoid	
Influenza	<i>E. coli</i> (neonates)	
Measles		
Varicella		
HIV		

### Autoimmune neutropenia (AIN)

Infant form of isolated autoimmune neutropenia occurs within 1st year of life; demonstrable autoantibodies. Not familial; girls > boys. Self-limiting and usually relatively benign. Therapy supportive. Steroids not usually needed and can increase infection risk. IVIg has been used.

#### Older children may get

- Isolated AI neutropenia.
- Neutropenia as part of Evans syndrome (↻ Immune thrombocytopenia, p.490).
- Neutropenia as part of multisystem AI disease—lupus erythematosus.
- Immune neutropenia following allogeneic BMT.
- Felty syndrome (rheumatoid arthritis with splenomegaly and hypersplenic cytopenias may also be associated with neutrophil autoantibodies.
- All are potentially more serious and complicated than the infant form and may require immunosuppression as well as supportive therapy.

### Isoimmune neutropenia of the newborn

- Maternal antibodies to fetal neutrophil antigens cross the placenta and give rise to neutropenia in the newborn child.
- Most commonly antibodies directed at neutrophil-specific antigens NA1 and NA2. (Maternal HLA antibodies do not cause trouble because antigens expressed on many tissues so quickly absorbed.)
- Estimated incidence up to 3% of newborns, so may be more common than generally appreciated; perhaps because usually clinically mild and neutropenia thought to be acquired due to drugs/infection.
- Condition resolves by 2 months as antibody disappears.
- Severely affected babies may show recurrent staphylococcal skin infections. Therapy supportive. Need for exchange transfusion very rare.
- Diagnosis based on serology.

### Prognosis of neutropenia

Whatever the cause, severe neutropenia ( $<0.2 \times 10^9/L$ ) is serious and incompatible with long survival if prolonged. It requires careful expert management.

## Disorders of neutrophil function

### Acquired

Mild defects arise in many clinical situations; following some infections, associated with drugs (steroids, chemotherapy) and systemic disease (malnutrition, diabetes mellitus, rheumatoid arthritis, CRF, sickle cell anaemia)—here the underlying condition will dominate the clinical picture.

### Congenital

Inherited defects rare but several important and disabling syndromes occur in children.

### Pathophysiology

Neutrophils produced in BM are released into circulation where they survive for only a few hours. Fundamental role is to kill bacteria. Do this by moving to site of infection drawn there (chemotaxis) by interaction of bacteria with complement and Ig (opsonization) and engulf them (phagocytosis). Killing is accomplished by  $H_2O_2$  generation, release of lysosomal enzymes, neutrophil degranulation (respiratory burst). Several enzyme systems are involved (MPO, cytochrome system, HMP shunt). In the neonate neutrophil function is defective ( $\downarrow$  chemotaxis, phagocytosis, motility) particularly if premature, jaundiced. Killing is normal.

### Classification

Disorders of all aspects of neutrophil function are described and there is no consensus as to how best to classify them. All are rare. In several of the best described conditions multiple defects are present (Table 12.8).

**Table 12.8** Classification

$\downarrow$ Chemotaxis	$\downarrow$ Opsonization	$\downarrow$ Killing
Lazy leucocyte syndrome	Complement $C_3$ deficiency	Chronic granulomatous disease
Hyper IgE syndrome		MPO deficiency
Chediak–Higashi syndrome		
$\downarrow$ Specific neutrophil granules		

### Clinical features

All congenital syndromes are rare and diagnosis of the specific defect difficult. Few haematological/immunological labs are set up to perform the required range of tests. Specialist referral for diagnosis and treatment is indicated and may be able to alter the otherwise grim prognosis in many of these conditions.

#### *Lazy leucocyte syndrome*

Leucocyte adhesion deficiency (LAD) due to mutations in the gene encoding the  $\beta$ -subunit of the  $\beta_2$ -integrins (*LAD1*) or defective glycosylation of ligands on leucocytes recognized by selectin family (*LAD2*). Rare. Autosomal recessive.  $\uparrow$  Recurrent infections often in oral cavity, delayed wound healing.

*Lab findings:* ↑ neutrophil count, ↔ BM, abnormal chemotaxis on Rebeck skin window test. The condition is relatively mild. Treatment is of specific infections with the need for prophylaxis in some patients.

#### **Hyperimmunoglobulin E syndrome**

Also known as Job syndrome (Old Testament) because of recurrent staphylococcal abscesses. Autosomal recessive inheritance, associated with atopic dermatitis and other autoimmune phenomena. Bacterial/fungal infection, chronic dermatitis.

*Lab findings:* ↑ IgE, ↑ eosinophils.

#### **Complement deficiency**

Autosomal recessive inheritance of C<sub>3</sub> deficiency, homozygotes have severe recurrent bacterial infection, particularly encapsulated organisms.

#### **Chronic granulomatous disease (CGD)**

Though rare, commonest life-threatening inherited neutrophil functional defect. >1 disorder. Most are sex linked, boys affected 7 × more frequently than girls, but 3 autosomal mutations recognized. Presents in early life but also in adults; carriers asymptomatic. Multiple skin and visceral abscesses, systemic infection (pneumonia, osteitis, etc.)—bacterial/fungal, lymphadenopathy, hepatosplenomegaly. Lab features: nitro blue tetrazolium (NBT) test (an index of defective respiratory burst) +ve. Specific mutational analysis now possible for earlier and prenatal diagnosis. Outlook better than it used to be. Improved by aggressive antibiotic policy and IFN-γ. BMT little used due to improving outlook with conservative/prophylactic treatment. Early results poor. Prospect of gene therapy attractive with some early progress.

#### **Chediak–Higashi syndrome**

Rare autosomal recessive disorder due to mutations in lysosomal trafficking regulator, *CHS1/LYST*, gene. Multiple defects. Partial oculocutaneous albinism, recurrent infection, lymphadenopathy, peripheral neuropathy and cerebellar ataxia. A fatal accelerated phase occurs in ~85%, usually in the 2nd decade, with lymphocytic infiltration of liver/spleen/nodes/BM, pancytopenia.

#### *Lab findings*

- ↓ Hb, ↓ neutrophil count, ↓ platelet count.
- Characteristic giant greenish grey refractile granules in neutrophils (also lymph inclusions).
- Treatment is supportive. High-dose ascorbic acid may help some patients. Anecdotal reports of successful BMT.

#### **Myeloperoxidase deficiency**

Autosomal recessive. Commonest of neutrophil dysfunction conditions (1:2000) but also least serious. Often asymptomatic. Manifest in diabetics with recurrent infections—commonly *Candida albicans*. Good prognosis.

#### *Lab findings*

- ↑ neutrophil/monocyte peroxidase on histochemical analysis.
- Automated cell counters using MPO activity to count neutrophils may show spurious neutropenia.

## Childhood immune (idiopathic) thrombocytopenic purpura

ITP occurring in children differs from the adult form of the disease (➔ Immune thrombocytopenia, p.490) in 2 ways—first, most cases are of abrupt onset and rapidly self-limiting, and secondly those that progress to chronicity have a lower morbidity and mortality.

### Epidemiology

Incidence of ITP in children overall around 4–5 per 100,000 per year. 10–20% become chronic—i.e. last >6 months.

### Pathophysiology

Thrombocytopenia mediated by antibodies opsonizing platelets that are then destroyed by the reticuloendothelial system. Antibodies can be part of immune complexes non-specifically attached to platelet Fc receptors (as in typical acute childhood ITP) or true autoantibodies usually targeted at glycoproteins IIb/IIIa and Ib (as found in up to 75% of chronic childhood ITP and commonly in adults).

### Clinical features

- Onset of bruising ± petechiae abrupt (80–90%) or insidious (10–20%).
- May have gingival and oral mucosal bleeding or epistaxis.
- Life-threatening bleeding extremely rare.
- Child otherwise perfectly well.
- May have history of recent infection; specific (rubella, varicella) or non-specific (URTI).
- Can follow vaccination.

### Laboratory diagnosis

- Isolated thrombocytopenia; blood count otherwise ↔.
- Marrow shows abundant megakaryocytes and is otherwise ↔ (not necessary to investigate unless clinical course or presenting features unusual).
- Platelet antibody studies difficult to perform and not necessary in most cases since they do not alter management.
- Exclude EBV infection (infectious mononucleosis).
- Exclude multisystem autoimmune disease (ACL, ANA, +ve DAT test—not necessary unless disease becomes chronic).
- Exclude underlying immunodeficiency syndrome (HIV, Wiskott–Aldrich).

### Differential diagnosis

- Congenital or familial thrombocytopenias.
- Leukaemia or aplastic anaemia.
- Other rare thrombocytopenias (e.g. type IIB vWD).
- Multisystem autoimmune disease—Evans syndrome (AIHA + immune thrombocytopenia), systemic lupus erythematosus.
- Immune thrombocytopenia associated with immunodeficiency due to other disease—HIV infection, Hodgkin disease, Wiskott–Aldrich syndrome.

## Management

### *Newly presenting*

Seldom require urgent therapy though this is frequently given, either polyvalent IVIg 0.8g/kg (single dose) or prednisolone 1mg/kg for 7d. Never justified in the absence of obvious bleeding since neither therapy without risk. Simple observation for spontaneous recovery should be preferred.

### *Chronic*

No therapy is needed based on platelet count alone. Absence of symptoms and signs is sufficient. Excessive restriction of activities is seldom justified. Open access to expert help and advice provides reassurance to families and teachers. If therapy required to control symptoms (recurrent nosebleeds, menorrhagia) try local measures or hormonal control. Regular IVIg or steroids seldom effective and may produce more problems than untreated disease. For the most difficult cases (very rare) splenectomy still worth considering, though post-splenectomy mortality may be greater than that of untreated ITP. Newer therapies include danazol and rituximab, though experience still anecdotal and long-term risks not yet evaluated.

### *Life-threatening haemorrhage or other emergency*

Risk of life-threatening haemorrhage very small ( $<1/1000$  in 1st week after diagnosis) though is a function of a platelet count  $<10\text{--}20 \times 10^9/\text{L}$  and the time exposed to this. Risk consequently rises in rare children with chronic unremitting severe disease for  $>1\text{--}2$  years for whom more adventurous therapy (splenectomy, rituximab) can be contemplated, though risk still small and those of treatment may be higher. If a large intracranial (ICH) or other catastrophic bleed occurs, this can be dealt with by simultaneous massive platelet transfusion, IVIg, IV methylprednisolone and (if the diagnosis is beyond doubt) emergency splenectomy. Mortality of major ICH  $<50\%$  given active therapy.

## Outlook

Most children with ITP recover irrespective of therapy, usually within weeks or occasionally within months. Even 75% of those whose problem persists for  $>6$  months eventually spontaneously remit, sometimes several years later. It is very rare for children to carry ITP into adult life and beyond, and the occasional individuals who do are unlikely to be much troubled by it or to develop autoimmune disease of other systems.

## Further reading

Guidelines for the investigation and management of idiopathic thrombocytopenic purpura in adults, children and in pregnancy (2003). *Br J Haematol*, **120**, 574–96.

## Haemolytic uraemic syndrome

Characterized by a triad of *microangiopathic haemolytic anaemia (MAHA)*, *renal failure*, and *thrombocytopenia*. More common in children than adults and of 2 types; the more common epidemic form and the less common sporadic form closely related to thrombotic thrombocytopenic purpura (TTP)—a disease primarily of adults that also rarely occurs in children. TTP has the same triad of signs with two others—fever and neurological disturbances (➔ Thrombotic thrombocytopenic purpura, p.538)

### Pathogenesis

HUS usually occurs in outbreaks and in 90% is due to *Escherichia coli* 0157 and other verocytotoxin-producing *E. coli*. Food sources of the infection include uncooked/undercooked meats, hamburgers, and poor food hygiene. The verocytotoxin causes endothelial damage, particularly of the renal endothelium, leading to the formation of fibrin-rich microthrombi and MAHA. Familial haemolytic uraemic syndrome (5–10% of cases) have a deficiency or dysfunction of complement factor H, which results in excessive C3 activation.

### Clinical features

- Young children (<4 years) are especially prone to the disease.
- Acute onset with a history of abdominal pain and bloody diarrhoea.
- Onset of ↓ urine output heralding renal failure occurs days later.
- In ~10% onset is non-epidemic and insidious—can be associated with chemotherapy/TBI.
- Other symptoms: anaemia (may be severe), jaundice, bruising, bleeding.
- Absence of fever and neurologic signs distinguish it from TTP.

### Laboratory findings

- MAHA may be severe.
- Film shows fragmented RBCs/schistocytes/spherocytes.
- Thrombocytopenia.
- Coagulation tests: PT/APTT—usually ↔; Fgn and F/XDPs also ↔; reduced large vWF multimers.
- Proteinuria and haematuria.
- Biochemical evidence of renal failure.
- Stool culture may be +ve for *E. coli*.

### Differential diagnosis

- TTP (➔ Thrombotic thrombocytopenic purpura, p.538).
- Other causes of MAHA and renal failure.

### Complications

- ARF → CRF rare but more likely in older children and those with sporadic insidious onset HUS.
- Microvascular thrombosis and infarction of other organs.

### Treatment

- Renal failure—fluid restriction, correct electrolyte imbalance.
- If anuria persists >24h—dialysis as necessary.
- Blood transfusion for anaemia.



- Platelet transfusion rarely needed—may ↑ thrombotic risk.
- Severe persistent disease may require plasmapheresis as for TTP (→ Thrombotic thrombocytopenic purpura, p.538).
- Specific treatment: none of proven value. In familial form replacement of factor H with FFP may be helpful.

### Outcome

Epidemic HUS has a good prognosis, patients usually recover and it rarely recurs. CRF does occur occasionally. Insidious onset HUS has a poorer prognosis. Overall mortality ~5%.

## Childhood cancer and malignant blood disorders

### Epidemiology

In Europe 1 child in 600 develops cancer before the age of 15. Annual incidence 1:10,000; ~1200 new cases/year in UK. Pattern of childhood cancers is very different from that seen in adults: carcinomas are rarely seen. Overall, childhood cancer is slightly more common in boys than girls.

- **Leukaemias** account for about 35% of the total: 80% are some type of ALL, 15% some type of AML. 5% CMLs, (adult and juvenile types) or myelodysplastic syndromes. CLL does not occur in children.
- **Brain tumours** are commonest solid malignancies, 25% of the total. Different tumour types from adults; commonest medulloblastoma in posterior fossa.
- **Embryonal tumours** 15%, seen almost exclusively in children. Include neuroblastoma, nephroblastoma (Wilms tumour), rhabdomyosarcoma, hepatoblastoma.
- **Bone tumours** 5% osteosarcoma, Ewing sarcoma.
- **Lymphomas** 9%—NHL and Hodgkin disease. NHL in children closely related biologically to ALL; low-grade disease very rare.
- Remainder of cases are mainly germ cell and primitive neuroectodermal tumours (PNETs), including retinoblastomas.

### Aetiology

- Childhood cancer generally represents aberrant growth and development. Defective repair and renewal may also be important in some cancers.
- Growths arising in infancy are mostly congenital and the genetic mutations concerned arise *in utero*. Causes of such mutations and those arising later in childhood largely unknown.
- Although isolated cases are attributable to high-dose radiation (e.g. thyroid cancer in Chernobyl survivors), there is no convincing link to levels of background radiation or electromagnetic fields.
- Population mixing studies have suggested that patterns of exposure to infection may contribute to some cases of ALL in the peak years of incidence (2–6 years).
- Childhood cancer rarely familial, but study of retinoblastoma (rare tumour that is commonly familial) has led to better understanding of tumour suppressor genes; germline mutation in 1 allele of RB gene in affected families only gives rise to tumours in cells where there is an acquired mutation in the other, healthy, wild type allele (the 'two hit' hypothesis).
- Cancer arising in older children may still be due to intrauterine event as concordance studies in identical twins with ALL have shown identical genetic mutations in malignant cells some years after birth. This suggests twin → twin transfer of potentially malignant clone through shared circulation.

### **Haematological effects of non-haemic tumours (for leukaemias, lymphomas, and MDS see appropriate sections)**

- Marrow infiltration may be evident at the time of presentation (most commonly neuroblastoma, less commonly Ewing sarcoma, rhabdomyosarcoma).
- Associated with anaemia, occasionally other cytopenias. Blood count hardly ever  $\leftrightarrow$  if marrow involved.
- Peripheral blood may show leucoerythroblastic picture, but not as commonly in adult cancers metastasizing to BM.
- Non-haemic tumours appear on cytomorphology as poorly differentiated fragile blast cells, often in sheets or clumps (unlike leukaemic blasts).
- Marrow infiltration may arise as a late event in terminally progressive disease in other tumours, including CNS malignancies, PNETS, and germ cell tumours.

### **Investigations in suspected childhood cancer**

#### *Haematology*

- FBC and film. Leukaemia usually reflected in the blood count:  $\uparrow$  or  $\downarrow$  WBC,  $\pm$  thrombocytopenia and anaemia. Blasts often present. In a small percentage, blood count entirely  $\leftrightarrow$ . With other malignancies there may be signs of marrow infiltration, anaemia, or no abnormalities at all.
- BM aspirate and trephine if blood count abnormal. In children generally done under GA. Bilateral samples needed in the staging of neuroblastoma.

#### *Biochemistry*

- Full biochemical profile.
- Urinary catecholamines for neuroblastoma (easy test to do in unexplained bone pain).
- Tumour markers:  $\alpha$ -FP,  $\beta$ HCG, in hepatoblastoma or germ cell tumours.

#### *Radiology*

- CXR for mediastinal mass (mandatory pre-anaesthetic).
- Abdominal USS.
- CT/MRI scan of 1<sup>o</sup> lesion. CT chest/abdomen may be required for staging. In young children sedation/general anaesthetic usually needed for CT/MRI scans.

#### *Histology*

Solid tumours need adequate biopsy material for diagnosis taken under general anaesthetic.

#### *Genetics*

Fresh tumour material from all childhood cancers should be sent for cytogenetic and molecular genetic studies. Information from these is increasingly being used in risk-stratifying therapy and in predicting outcome.

### Specialist centres for treatment and investigation

Children with suspected malignancy should be referred to a specialist centre for investigation and initial treatment. Thereafter, shared care may be carried out nearer to home at a local hospital. Most children across the country receive treatment as part of a national trial or protocol. This is co-ordinated by the United Kingdom Children's Cancer Study Group and similar groups in other countries, and the success of such trials and studies is one of the reasons for the improved outlook for childhood cancer. Overall long-term survival is ~60%.

### Late effects

It is estimated that soon 1:1000 adults will be survivors of childhood cancer. Long-term follow-up clinics are needed to monitor growth, fertility, side effects from drugs (e.g. cardiotoxicity), and psychological well-being. There is an ↑ risk of further malignancy developing which varies according to both the 1<sup>o</sup> diagnosis and treatment used.



## Childhood lymphoblastic leukaemia

Lymphoblastic leukaemia ('acute' is superfluous, but disease widely known as ALL) is a group of clonal malignancies all arising in developing lymphocytes. There is more overlap with lymphomas than in adults. Commonest malignant disease in childhood (35% of all cancers). Incidence 4–5/100,000 children per year with a peak incidence between 2–6 years of age.

### Aetiology

Many cases thought to be due to antenatal mutations in developing B-cell clones; majority of cases B-cell derived, occur between 2–6 years, and may be abnormal response to infection where exposure to pathogens delayed or precipitated by population mixing. Evidence implicating background ionizing or electromagnetic radiation now discredited. Cause of T-ALL unknown.

### Pathophysiology

- ~80% childhood ALLs arise in developing B lymphocytes; ~20% in developing T cells. Acquired genetic mutations found in the various subtypes are growing in number as the molecular biology of leukaemia unravels.
- In early B cells commonest mutation is a fusion between the *TEL* gene at 12p13 and the *AML1* gene at 21q22—arises in 20% overall; other common mutations are  $t(1;19)(q23;p13.3)$ —8% overall,  $t(9;22)(q34;q11.2)$  *BCR-ABL* (Ph chromosome); 5% overall.
- ~30% have 'high hyperdiploidy' (>50 chromosomes per cell) with or without translocations; 7% show hypodiploidy.
- Infants (<18 months) frequently have a mutation of the *MLL* gene on chromosome 11 involving a range of fusion partners; most commonly AF4 on chromosome 4.
- These changes mark biologically different types of precursor B-derived ALL in terms of clinical features and response to treatment.
- 1–2% of ALLs have features of mature B cells and a mutation where the *MYC* gene is translocated adjacent to the Ig heavy chain locus— $t(8;14)$ . Also called Burkitt-type as the cell biology is similar to that of Burkitt lymphoma.
- T-ALL shows greater genetic diversity than B-derived disease but 12 recurring cytogenetic abnormalities now defined and under study. Clinical importance yet to be defined.
- ALL also classified by immunophenotyping using antibody cell markers for various differentiation antigens designated clusters of differentiation (CD), numbered according to their order of discovery. Immunophenotypes so defined include early pre-B (60%), pre-B (20%), transitional pre-B (1%), B-ALL (1%) and T-ALL (18%).

### Clinical features

- Commonly presents insidiously in 3 ways, separately or combined.
- Signs of marrow failure—often anaemia predominates, with extreme pallor and listlessness (60%); also bruising/petechiae (40%).

- Hepatosplenomegaly and lymphadenopathy ('lymphomatous' features) 10–20%.
- Bone pain mimicking irritable hip(s) or juvenile rheumatoid 10–20%.

### Laboratory features

- Usually pancytopenia with circulating blast cells indicates diagnosis.
- Confirmed by BM examination and classified by immunophenotyping and cytogenetic/molecular genetic analysis. Classification based on expression profiling might become available in the future.
- In sick children with very high blast cell counts classification studies can be carried out on peripheral blood, but marrow always preferred.
- Peripheral blood count may show cytopenias without obvious blasts (aleukaemic picture) when differential diagnosis is aplastic anaemia.
- Kidney/liver function usually normal, but ALLs with high blast counts and rapid cell turnover may have tumour-lysis organ dysfunction before therapy (urate nephropathy with ↑ urea, creatinine, and ↓ urine output).

### Treatment of newly diagnosed disease

- All modern protocols have common elements of remission induction (RI), consolidation/intensification (C/I), CNS directed treatment, and continuing (maintenance) therapy with or without delayed intensification (DI).
- RI drugs include dexamethasone, vincristine, and asparaginase.
- C/I and DI drugs include anthracyclines, cytarabine, cyclophosphamide asparaginase, and thioguanine.
- CNS therapy is intrathecal cytarabine and MTX (radiotherapy now reserved for those with active CNS infiltration only).
- Maintenance therapy is a 2–3 year schedule of daily thiopurine (6-mercaptopurine) and weekly oral methotrexate.
- ALL is the only human malignancy that requires such a drawn-out chemotherapy module, immunosuppressive rather than antineoplastic.
- B-ALL is an exception; it does not respond to conventional ALL therapy, does not need maintenance and is treated on a 6 month intensive lymphoma schedule (➔ Childhood lymphomas, p.616).
- Treatment is usually risk-directed based on biological features of the disease with more intensive schedules reserved for those with adverse prognostic factors (➔ see Prognostic factors, p.614).
- BMT rarely used as 1st-line therapy.

### Outlook

98% overall will remit on modern therapy, 75–80% overall will become long-term disease-free survivors—figures vary according to prognostic factors (➔ see Prognostic factors, p.614). >95% long-term survivors in low-risk disease.

### Treatment of relapse

- Some 20–25% of children will relapse; either on treatment or after its completion.
- Outlook depends on length of 1st remission; very bleak if relapse on treatment.

- Salvage therapy more successful if relapse >2 years off therapy.
- Relapsed T-ALL more difficult to treat successfully than other types.
- Treatment includes intensive chemotherapy with the addition of podophyllins, anthracycline analogues, and fludarabine.
- BMT reserved for those who show slow-to-clear residual disease by PCR amplification of unique disease-specific Ig or TCR gene rearrangements, those who relapse on treatment and those with relapsed T-ALL.

### Prognostic factors

- Several features of ALL predict response to current standard therapies and are used to stratify treatment. Infants <1 year have a poor outlook, and older children >10 years do less well than those 1–10 years.
- High presenting WBC ( $>100 \times 10^9/L$ ) in boys marks high risk, as do some genetic features (*MLL* gene rearrangements, *BCR-ABL* fusion genes, hypodiploidy).
- All children with T-ALL, and all with slow disease clearance during the 1st few days of therapy, are excluded from being classified as low risk.
- Low risk B-precursor ALL is that which shows none of these features.
- Minimal residual disease (MRD) monitoring by molecular methods is being incorporated into clinical trials to assist prognostic stratification.

### Late effects of therapy

With ↑ long survivors, late effects of therapy are becoming more important. Most morbidity seen after TBI given for BMT.

#### Problems include:

- Growth failure due to CNS damage from radiation.
- Intellectual deficit due to CNS damage from radiation.
- Precocious puberty (girls > boys) after cranial radiation.
- Infertility (boys > girls) not dependent on radiation.
- Obesity (girls > boys) not dependent on radiation.
- 7–10-fold ↑ risk of brain tumours not dependent on radiation.





## Childhood lymphomas

Lymphomas account for around 8–10% of all childhood cancers (this equates to around 1 per 100,000 children per year). Around 30–35% are Hodgkin disease, the rest NHL.

### Hodgkin disease

#### *Clinical features*

Uncommon <5 years; incidence increases during the early teenage years. The disease is biologically the same as that of adults and the histological classification is identical (↔ see Hodgkin lymphoma ((HL, Hodgkin disease), p.214 for more details), though mixed cellularity disease may be more common in the young. Staging is also similar to adults (↔ Table 5.9, p.217), but overall children and adolescents have a greater proportion of low-stage disease (I and II). Stage IV accounts for <10% of childhood cases.

#### *Treatment and outcome*

Treatment is so successful that most efforts are currently directed at reducing toxicity and late effects. Radiotherapy for stage I disease is being attenuated, and some therapists have abandoned it as 1st-line treatment and rely on chemotherapy alone. Chemotherapy regimens in turn are evolving (to avoid or minimize alkylating agents and their effect on fertility and anthracyclines with their potential for cardiotoxicity), but at present the traditional drugs are still being used with or without involved field radiotherapy, particularly in stage III or IV disease.

The outlook for even stage IV disease is good, given the best current regimen of chemotherapy and involved field radiotherapy, and >80% should achieve long-term EFS.

### NHL

Childhood NHL are a heterogeneous group of tumours quite different from those seen in adults. Virtually all are disseminated, high-grade diffuse malignancies of immature B or T lymphocytes, and many are closely related to subtypes of ALL that occur in this age group.

Classification of NHL has always been confusing, but the Revised European American Lymphoma system is currently preferred. This maps disease in children into 6 categories:

#### **1. Burkitt lymphoma, Burkitt-like lymphoma, high-grade B-cell disease**

Different manifestations of biologically very closely related diseases and pathologically indistinguishable from B-ALL. ~45% of the total. Characteristic 'starry sky' histological pattern and deeply basophilic blasts on Romanowsky stains with prominent vacuoles (FAB L3 features). Associated with chromosomal translocation involving *MYC* locus on chromosome 8 and Ig heavy chain gene on 14 (or less commonly with a  $\kappa$  or  $\gamma$  light chain gene on 2 or 22) with resultant dysregulation of *MYC* gene transcription; the *MYC* product functions as a transcription factor.

**2. Precursor B-lymphoblastic lymphoma**

Indistinguishable pathologically from common ALL. ~5% of the total. Commonly presents as a solitary SC swelling, typically on the scalp.

**3. Precursor T-lymphoblastic lymphoma**

Indistinguishable pathologically from T-ALL. ~20% of the total. 66% have mediastinal involvement. Marrow involvement common in advanced disease; so may be classified as T-ALL rather than stage IV NHL.

**4. Diffuse large B-cell lymphoma**

Including 1° sclerosing mediastinal form; no leukaemic counterpart, accounts for ~3–4% of the total. Chiefly abdominal. Occasionally mediastinal. Has some features of Burkitt but no *MYC* gene mutation.

**5. Peripheral T-cell lymphoma unspecified**

No leukaemic counterpart. Skin involvement. Retrospective review shows most so classified to be type 6 (large cell anaplastic, see category 6). Poorly defined entity hardly ever seen in children.

**6. Large cell anaplastic, T, or null cell type**

No leukaemic counterpart. More frequently recognized, and complex biological features gradually becoming better understood. ~15% of the total. Used to be diagnosed as peripheral T-cell lymphomas or 'malignant histiocytosis'. Biological hallmarks are Ki-1 (CD30)+, also t(2;5).

Around 9% childhood NHLs defy classification and <1% adult type follicular lymphomas will occasionally arise in older children.

**St Jude staging system for childhood NHL**

A staging system for childhood NHL has been developed (Table 12.9), though therapy is increasingly being directed more by the biology of the disease rather than its anatomical distribution or extent. Staging affects prognosis only within given tumour type.

**Table 12.9** St Jude staging system for childhood NHL

Stage I	Single tumour (extranodal or single nodal anatomic area), excluding mediastinum or abdomen
Stage II	Single extranodal tumour with regional node involvement = 2 nodal areas on the same side of the diaphragm
	2 single extranodal tumours ± regional node involvement on same side of the diaphragm
	1° GIT tumour, usually ileocaecal, ± involvement of associated mesenteric nodes
Stage III	2 extranodal tumours on opposite sides of the diaphragm = 2 nodal areas above and below the diaphragm
	Presence of 1 intrathoracic tumour (mediastinal, pleural or thymic)
	Presence of extensive 1° intra-abdominal disease
	Presence of paraspinal or epidural tumours, regardless of other sites
Stage IV	Any of the above with initial CNS and/or BM involvement

### Treatment

- **Burkitt lymphoma/B-ALL:** short 6-month course of pulsed intensive high-dose therapy (vincristine, steroids, MTX, cyclophosphamide, anthracyclines, and etoposide) including CNS treatment. No maintenance treatment needed.
- **B-precursor lymphoblastic:** if isolated to one site, 6-month programme of ALL-type therapy may suffice, else treat as common ALL with extended maintenance (↻ Lymphoblastic leukaemia, p.612).
- **T-precursor lymphoblastic:** treated as T-ALL (↻ see Treatment of newly diagnosed disease, p.613).
- **Diffuse large B-cell lymphoma:** treated as Burkitt lymphoma (↻ p.206).
- **Large cell anaplastic Ki-1+:** skin, CNS and mediastinal involvement and splenomegaly are adverse features. Best therapy undefined but usually treated with short, intensive Burkitt-like regimens. EFS is around ~75% (high-risk cases ~60%).

### General points on therapy/outlook

- Surgery usually indicated for the complete resection of a localized abdominal 1° tumour when possible.
- Low-dose involved field radiotherapy indicated for airway or spinal cord compression. Mediastinal irradiation for persistent local disease.
- Given best current therapy, the outlook for most patients is good with around 80% EFS for childhood lymphomas overall.



## Childhood acute myeloid leukaemia

AML in children accounts for ~15% of all malignant blood disorders, with around 80–90 new cases arising in the UK each year. Unlike ALL, the disease is classified on morphological grounds using the FAB classification (Table 12.10), as is AML in adults (↔ see Table 4.1, p.127). The frequency of the different subtypes differs in children, however. M6 AML is very rare whereas M7 (megakaryocyte derived AML) is more common—especially in children with Down syndrome.

**Table 12.10** Proportion of children with *de novo* AML by FAB type

	M0	M1	M2	M3	M4	M5	M6	M7
% of total	2	18	29	8	16	17	2	8

### Pathophysiology

AML is a clonal neoplasm arising from developing blood cells affecting all haemopoietic cell lines, most commonly granulocyte or monocyte precursors but also occasionally involving immature erythroblasts or megakaryocytes. Apart from 1°, *de novo* disease for which no cause can be identified, some cases of AML are due to chemotherapy given for other diseases (2° AML), and some arise in children with predisposing syndromes where the risk is greatly ↑ and where specific subtypes of AML may develop.

- 2° AML caused by topoisomerase II inhibitors (e.g. etoposide) has a latency of 1–3 years and is of FAB type M4/M5 with a characteristic *MLL* gene mutation.
- 2° AML caused by alkylating agents (e.g. cyclophosphamide) has a latency of 4–6 years, a myelodysplastic phase, and loss or deletions of chromosomes 5, 7, or both.
- Down syndrome children are 20 × more likely to develop leukaemia than normal children; infants are more likely to develop M7 AML, older Down children develop ALL.
- Other conditions predisposing to AML in children are Fanconi anaemia and Bloom syndrome (↔ Fanconi anaemia, p.594), dyskeratosis congenita, Kostmann syndrome and Shwachman–Diamond syndrome (↔ Rare congenital marrow failure syndromes, p.596), Diamond–Blackfan anaemia (↔ Congenital red cell aplasia, p.590), and neurofibromatosis.

Apart from the specific changes in 2° AMLs, clonal chromosome abnormalities are found in blasts from ~90% of those with *de novo* disease. 2/3 of these are non-random, and many are associated with characteristic clinical and biological features.

**Table 12.11** Commonest genetic mutations in childhood AML

Abnormality	Involved genes	FAB type	Frequency	Clinical features
t(8;21)	<i>ETO; AML1</i>	M2	10–15%	Extramedullary chloromas Good outlook
t(15;17)	<i>PML; RARA</i>	M3	5–10%	Coagulopathy Responds to retinoids
Inv16(p13q32)	<i>MYH11; CBFβ</i>	M4eo	7–10%	Extramedullary deposits Good outlook
t(9;11)	<i>AF9; MLL</i>	M4/M5	7–10%	Infants, CNS disease Poor outlook
t(1;22)	<i>N/K</i>	M7	2–3%	2° myelofibrosis Down syndrome

### Laboratory features

- Peripheral blood shows a variety of abnormalities—usually pancytopenia with circulating blasts.
- WBC seldom  $>50 \times 10^9/L$  though can occasionally be very high with symptoms of leucostasis—deafness, confusion, and impaired consciousness.
- BM usually shows heavy overgrowth of blasts with different morphology depending on FAB type. Auer rods (abnormal elongated 1° granules seen on Romanowsky stains in cytoplasm of malignant myeloblasts) are diagnostic of AML and are not found in health. Seen in all types of AML except M6 and M7, most common in M1/2/3, particularly M3.
- Cytochemistry may help; non-specific esterase +ve in M4/M5 AML, not other types and myeloperoxidase positivity can help distinguish between poorly differentiated AML and ALL. M7 AML may develop extensive marrow fibrosis making aspiration difficult; trephine histology needed.
- Genetic abnormalities common in blast cells (see Table 12.11).
- Immunophenotyping less important than in ALL, though essential for immediate diagnosis of M7 AML which has no distinguishing morphological or cytochemical features. CD antigens expressed vary according to FAB type; CD33 strongly +ve in all, CD13 in M2/3; CD4 in M4/M5 and CD41/61 in M7. M6 disease expresses glycophorin A.

### Clinical features

Children with advanced AML are commonly sicker than those with ALL. They can present with bleeding, haemostatic failure, and/or septicaemia as manifestations of marrow failure and profound neutropenia. Extramedullary chloromas (solid deposits of malignant cells) arise in around 10% of cases. They may precede marrow failure (or even detectable marrow infiltration). They can arise internally around the spine or spinal cord, causing pressure symptoms and mimicking non-haemic solid tumours. They are more common in AML with t(8;21). Peri-orbital chloromas are also not unusual in infants with M4/M5 AML.

### Treatment and outlook

Outcome of therapy for childhood AML has shown a dramatic improvement over the last 15 years. From a dismal outlook in the 1970s through around 30% EFS in the 1980s we have now achieved >50% EFS at the turn of the 21st century. This has been due to increasingly intensive chemotherapy and parallel improvements in supportive treatment for the secondary marrow failure it produces.

The principle of treatment is to ablate marrow with chemotherapy to the point that endogenous recovery occurs within 4–6 weeks and to repeat the process with different drug combinations 4 or 5 times, giving a total treatment time of around 6 months. Results using this approach have improved for children in the best risk groups to the point where allogeneic BMT is no longer considered the consolidation treatment of choice even if a matched donor is available.

- Drugs used in remission induction include daunorubicin, etoposide, cytarabine, and mitoxantrone (mitozantrone).
- Drugs used in post induction and consolidation treatment include amsacrine, high-dose cytarabine, L-asparaginase, etoposide, and mitoxantrone (mitozantrone).
- Good risk patients are those with t(8;21), t(15;17), and inv(16)—together accounting for around 20–25% overall; standard risk patients are those without good risk genetic changes but that respond well and remit after 1 course of chemotherapy (65% overall), and poor risk are those without good genetics who have residual disease at the start of course 2 of treatment (around 10% of the total).
- Long-term EFS for good risk children is around 75–80%, for standard risk around 60–65%, and for poor risk around 15%.
- Allogeneic BMT as consolidation therapy of 1st remission is reserved for children in standard and poor risk groups. It is also used as a salvage strategy for good risk patients who relapse.
- The role of autologous stem cell rescue following myeloablative conditioning in children with AML has not been established.
- The need for skilled supportive therapy confines AML therapy to specialist units.





## Childhood myelodysplastic syndromes and chronic leukaemias

Myelodysplastic syndromes of childhood present a different spectrum of disease from that seen in adults. All are rare. The FAB classification of adult MDS (➔ p.230) has been translated to paediatric disease, but sits uncomfortably and is of limited use clinically or in understanding the complex biology of this diverse group of clonal disorders of marrow function. The proportion that map to the various adult categories is shown in Table 12.12.

- Many children with MDS have a monocytosis which results in their being classified as CMML, also the clinical features and outlook for childhood CMML are quite different.
- RARS is virtually never seen in children.

**Table 12.12** Paediatric modification of WHO classification of myelodysplastic disorders

Category	Diseases
Myelodysplastic/ myeloproliferative disease	Juvenile myelomonocytic leukaemia (JMML)
Down syndrome disease	Transient abnormal myelopoiesis Myeloid leukaemia of DS
Myelodysplastic syndrome	Refractory cytopenia (blood blasts <2%, marrow blasts <5%)
	Refractory anaemia with excess blasts (blood blasts <19%, marrow blasts 5–19%)
	Refractory anaemia with excess blasts in transformation (blood or marrow blasts 20–29%)

### Individual disorders

#### Refractory anaemia

Children with a clonal genetic marker in the marrow who present with refractory anaemia usually progress to RAEB and AML. Those without such a marker probably do not have MDS but some other cause of erythropoietic failure.

#### RAEB and RAEB-t

Many of the RAEB syndromes in children arise in those with pre-existing disease like Down syndrome, trisomy 8, neurofibromatosis type 1, Fanconi anaemia, dyskeratosis congenita, Kostmann syndrome, Diamond–Blackfan anaemia, and Shwachman–Diamond syndrome (➔ see Childhood acute myeloid leukaemia, p.620). All these diseases predispose to leukaemia, and RAEB is merely part of the evolution of AML. A substantial proportion of childhood MDS in the RA or RAEB category is also induced by previous chemotherapy as a prodrome to 2° AML. In

other words all paediatric cases of RAEB/RAEB-t are best regarded as AML and treated as such if the diagnosis is not in any doubt.

- Down syndrome children have a particular predisposition to develop M7 (megakaryoblastic) AML in the first few years of life. This is commonly preceded by a RAEB prodrome where the marrow is hard to aspirate through 2° sclerosis. The decision when to start therapy is difficult, but the overall outlook is potentially good with EFS >50%.

#### *Transient abnormal myelopoiesis (TAM)*

Down children also have a predisposition to develop a transient blast cell overgrowth in infancy that looks like frank leukaemia with blasts in the peripheral blood. It is completely self-limiting within days or weeks and is not associated with marrow failure, arising alongside normal haemopoiesis. It is important that chemotherapy is withheld in what is regarded as a temporary stem cell instability. Rarely the problem can arise in non-Down children, where trisomy 21 is found in the BM only. TAM and M7 in Down syndrome patients has been recently shown to be associated with acquired mutations in *GATA-1*.

#### *Juvenile chronic myelomonocytic leukaemia (JCMML)*

Originally called juvenile chronic myeloid leukaemia to distinguish it from adult type chronic myeloid leukaemia (➔ CML, p.148), this pernicious disease still has a high mortality. It is now recognized to be a clonal disorder, with all marrow cell lines involved. It has several distinctive clinical and haematological features.

- Stigmata of fetal erythropoiesis; high HbF, ↑ red cell i antigen expression and carbonic anhydrase activity, ↑ MCV.
- Modest ↑ WBC; average 30–40 × 10<sup>9</sup>/L, with evident monocytosis, blasts 5–10%, and occasionally a basophilia.
- Marrow appearances unremarkable; modest ↑ blasts.
- Thrombocytopenia; sometimes profound.
- Skin rashes; butterfly distribution on face.
- Increasing hepatosplenomegaly.
- Associated with neurofibromatosis type 1. May be present in >10% of cases.
- Poor outlook with progression associated with wasting, fever, infections, bleeding, and pulmonary infiltrations.

#### *Monosomy 7 syndrome*

Conventional cytogenetic analysis of the marrow in JCMML shows no abnormality in the classic syndrome, though there is a subvariety (or similar condition that may nevertheless be biologically distinct) where monosomy 7 is found. Whether this is a different disorder is not clear. Apart from the different genetics, monosomy 7 syndrome and JCMML have several features in common. However, monosomy 7 children may have:

- A longer prodrome with RA or RAEB and no monocytosis.
- They may respond to AML chemotherapy (JCMML responds poorly and seldom remits).
- They may remain stable for years without therapy.
- They respond better to BMT (JCMML achieve <40% EFS even with BMT).

*Adult-type chronic myeloid (granulocytic) leukaemia (ATCML)*

(↻ see Adult CML p.148.) More common than JCMML, though still rare, ATCML arises in around 1 in 500,000 children per year (20 in whole of UK). Tends to affect older children (60% >6 years) though it has been reported in a 3-month-old infant.

- Associated with Ph chromosome and t(9;22) *BCR-ABL* fusion gene in all haemopoietic cells exactly as seen in adults.
- Natural history exactly the same as the disease in adults with chronic phase and eventual progression to accelerated acute phase.
- Only curative therapy is allogeneic BMT, but impressive remissions of so far unknown length are now being achieved with novel tyrosine kinase inhibitor imatinib. For patients who lack a compatible SCT donor this is becoming 1st-line therapy in place of interferon alfa or hydroxycarbamide.



## Histiocytic syndromes

Monocytes are formed in the marrow and move through the peripheral blood into the tissues where they become histiocytes, either in the mononuclear phagocytic system (MPS) or the dendritic cell system (DCS). MPS cells are antigen processing, are predominantly phagocytic, and include many organ-specific cells such as Kupffer cells and pulmonary alveolar macrophages. DCS cells include tissue-based Langerhan cells (LC) which are antigen presenting. There is a variety of syndromes where histiocytes proliferate and malfunction and some of these carry a high mortality. A few are clonal neoplasms but most are produced by cytokine disturbances. In 1991 a new classification of histiocytic syndromes was set out as shown in Table 12.13.

**Table 12.13** Histiocytic syndromes

<b>Class I</b>	<p><i>Disorders of dendritic cells</i></p> <ul style="list-style-type: none"> <li>● Langerhan cell histiocytosis (LCH) (previously known as histiocytosis X)</li> </ul>
<b>Class II</b>	<p><i>Disorders of macrophages</i></p> <ul style="list-style-type: none"> <li>● Haemophagocytic syndromes</li> <li>● Haemophagocytic lymphohistiocytosis (HLH):               <ul style="list-style-type: none"> <li>• 1° (genetic)</li> <li>• 2° (to infection or malignant disease)</li> </ul> </li> <li>● Sinus histiocytosis with massive lymphadenopathy</li> <li>● Histiocytic necrotising lymphadenitis</li> </ul>
<b>Class III</b>	<ul style="list-style-type: none"> <li>● Malignant histiocytic disorders:               <ul style="list-style-type: none"> <li>• Malignant histiocytosis</li> <li>• Monocytic leukaemias</li> </ul> </li> </ul>

### Class I: Langerhan cell histiocytosis (LCH)

Cellular destructive tissue infiltration with LC. These are well-differentiated large cells (15–25microm) with an indented nucleus and inconspicuous nucleolus; they are not phagocytic. Other reactive cells (granulocytes, eosinophils, macrophages) are often present. Diagnostic criteria of LC include the presence of Burbeck granules on electron microscopy and immunochemical positivity for CD1A. The aetiology of LCH remains unclear. Despite some evidence of clonality (not itself evidence of malignancy), no genetic mutations have been identified and the disorder is not regarded as a form of cancer.

#### *Clinical features*

LCH is primarily a disease of the very young with a peak incidence of 1–3 years. It can present in a variety of ways, from a small bone lesion heavily admixed with eosinophils (eosinophilic granuloma), through multiple lytic bone lesions, exophthalmos, and diabetes insipidus (Hand–Schuller–Christian disease) to multiple tissue infiltration involving skin, liver, lung, bone, and BM (Letterer–Siwe disease). The eponymous terms are no longer used for what is now regarded as a common pathology and the overarching term LCH is preferred. This is staged on

the basis of the number of organ systems showing infiltration, and virtually any can be involved. The skin rash of LCH is characteristically in skin folds and scaly with red/brown papules. It may be mistaken for nappy rash. Systemic symptoms including fever and weight loss are common in advanced disease. It can be staged as follows:

- *Stage A* Involvement of bones  $\pm$  local nodes and adjacent soft tissue.
- *Stage B* Skin  $\pm$  mucous membranes involvement,  $\pm$  related nodes.
- *Stage C* Soft tissue involvement—not stage A, B or D.
- *Stage D* Multisystem disease with combinations of A, B, C.

### Diagnosis

Based on tissue biopsy. Skeletal survey to define extent of disease; also bone scan, MRI. Urine osmolality studies for diabetes insipidus. BM aspirate and biopsy if anaemic or other cytopenias present.

### Treatment

Local curettage of any isolated lesion, with or without intralesional steroids. Stable and symptomless disease can be simply observed for spontaneous resolution. Options for widespread disease include steroids and chemotherapy—rarely radiotherapy. Indications for chemotherapy include organ dysfunction and/or disease progression/recurrence. Drugs commonly used include steroids, vinblastine, or etoposide, singly or combined.

### Outcome

Generally good, but widespread organ involvement with dysfunction and progression indicates a poor prognosis. Overall mortality 15–20%. Long-term sequelae include pulmonary/liver fibrosis, diabetes insipidus, growth failure. Risk of malignant disease  $\uparrow$ , chiefly leukaemias and lymphomas.

## Class II: macrophage functional disorders—haemophagocytic syndromes

### 1°(genetic)

1° haemophagocytic lymphohistiocytosis (HLH) is an autosomal recessively inherited disease of infants and young children (>50% <1 year) also known as familial erythrophagocytic lymphohistiocytosis (FEL) due to the striking degree of marrow red cell phagocytosis. Genetic HLH is a heterogeneous disorder with at least 4 genetic subtypes (FHLH1-4). 4 genes (Perforin, *PRF1*, on 10q21; *Munc13-4* on 17q25; *Syntaxin 11* on 6q24; *STXBP2*) have been identified and account for ~50% of patients. Defects in these genes lead to cytokine dysregulation (high concentrations of IL-1 and 2, GM-CSF, and TNF) and 'activated' T cells and histiocytes prompting haemophagocytosis. CNS involvement is common. Laboratory investigation shows peripheral blood cytopenias, hypertriglyceridaemia, and hypofibrinogenaemia. Histopathology shows histiocyte/lymphocyte infiltration and haemophagocytosis in BM, nodes and spleen. Genetic testing possible in ~ 50%.

*Treatment and outcome*

The use of ALG and CSA in the 'HLH' protocol enables the disease to be brought under control. If this is followed by a SCT survival can be good. Otherwise the disease is usually rapidly fatal.

2°

- Clinical and laboratory picture is similar to 1° (genetic) HLH.
- Distinction between the 2 may be difficult.
- Affects more older patients, often immunocompromised.
- Commonly associated with underlying viral/bacterial infection when called infection-associated haemophagocytic syndrome (IAHS).
- Triggered by a wide variety of infections including (especially) EBV and malaria. Also associated with some malignancies (usually involving T cells) and lipid infusions.

*Treatment and outcome*

Good survival rates if underlying infection easily treatable. Otherwise has high mortality.

**Class III: malignant histiocytosis**

Monocyte-derived acute leukaemias account for 10% of AMLs arising in children. What used to be called 'malignant histiocytosis' with hepatosplenomegaly, fever, wasting, and pancytopenia and tissue infiltration with large monocytoid cells is now recognized as a lymphocyte-derived lymphoma (large cell anaplastic, CD30+, ↻ 1° p.199). It is doubtful whether true histiocyte-derived malignancies other than AML occur in children.





## Haematological effects of systemic disease in children

Non-haematological disease in children can produce a variety of haematological effects specific to the disease, to childhood, or both. Some of the more striking examples are listed here.

- *Wilson disease*: genetic defect in copper metabolism that occasionally presents as a brisk non-immune haemolytic anaemia without specific features. More commonly presents with liver dysfunction, neurological symptoms, or renal disease.
- *Cyanotic congenital heart disease*: commonly associated with mild thrombocytopenia for ill-understood reasons.
- *Mast cell disease*: abnormal accumulations of mast cells in the skin or internal organs. Mast cell leukaemia does not occur in children. Commonest manifestation is urticaria pigmentosa in infants. Bullous or urticarial lesions eventually become infiltrated with mast cells. Marrow involvement rare. The cells produce histamine and cause itching. Condition resolves by adulthood.
- *Juvenile rheumatoid arthritis*: classically the anaemia of chronic inflammatory disease—a defective marrow response to anaemia in a variety of chronic inflammatory disorders primarily due to cytokine mediated inhibition of erythropoiesis rather than deficiency of EPO. True Fe deficiency also occurs due to NSAID therapy. Neutropenia may arise, either immune mediated or due to hypersplenism.
- *Systemic lupus erythematosus*: commonly associated with immune cytopenias (all cell lines) and anticardiolipin antibodies. May also produce marrow hypoplasia.
- *Epstein–Barr virus infection*: infects CD21 +ve B lymphocytes and other tissues including nasopharyngeal epithelium. 1<sup>o</sup> infection in the immunocompetent may be asymptomatic in the early years of childhood but in adolescence produces the syndrome of infectious mononucleosis ('glandular fever') associated with a striking atypical lymphocytosis. Occasionally there are associated self-limiting immune cytopenias—especially thrombocytopenia. The majority of adults harbour the latent virus in B cells. EBV in some cellular immune deficiency states (such as X-linked LPD also known as Purtilo's or Duncan syndrome) can produce a fatal infection with uncontrolled lymphoproliferation and infection-associated haemophagocytosis (➔ HLH, p.629).
- *Parvovirus B19 infection*: causes 'fifth disease' in infants and young children with the characteristic 'slapped cheek' rash on the face. More importantly shows tropism for marrow erythroblasts and causes temporary red cell hypoplasia. This causes very low Hb concentrations in children with chronic haemolysis (e.g. sickle cell disease). Persistent viraemia can arise in the immunosuppressed and can cause transfusion dependency.
- *TORCH infections*: a miscellaneous group of congenital infections—toxoplasmosis, rubella, cytomegalovirus, herpes simplex and syphilis. All can cause neonatal anaemia and thrombocytopenia.

- *Leishmaniasis*: the Mediterranean type of visceral leishmaniasis primarily affects young children under 5 years. Infected sand flies transmit parasites that develop in macrophages and the child presents often several weeks or months later with fever and progressive pancytopenia and hepatosplenomegaly. Fatal if untreated but responds well to pentavalent antimony or amphotericin B.
- *Hookworms (ankylostoma)*: are a common cause of Fe deficiency in tropical underdeveloped countries. Infestations may be heavy with each worm consuming up to 0.2mL blood per day.
- *Tapeworms*: a rare cause of vitamin B<sub>12</sub> deficiency in societies that eat raw fish—Baltic states, Japan, and Scandinavia, due to infestation by *Diphyllobothrium latum*.
- *Kawasaki disease*: an acute multisystem disease of young children, presumed to be infective but no organism has so far been identified. Presents with conjunctivitis, rashes, reddening of the mucous membranes, hands and feet with desquamation and lymphadenopathy. Coronary artery aneurysms develop in ~20%; fatal in 3%. Haematological manifestations include anaemia (normochromic normocytic), neutrophilia, and a striking 2° thrombocytosis that may linger after the acute phase has passed. Treatment is with aspirin and high-dose IVIg.

## Nutritional disorders

### *Fe deficiency*

Occurs in apparently healthy children (cf. adults where main cause is blood loss). Linked to rapid growth and poor intake the first 2 years of life and again at adolescence. Cows' milk is a poor source of iron. Cereals inhibit its absorption. Premature infants run out of Fe by 2 months of age.

### *Protein-calorie malnutrition*

Covers adequate calories with protein lack (kwashiorkor) and simple calorie lack (marasmus)—or both. Chiefly in undeveloped countries, but also occasionally in vegan families, with GI disease or other chronic illness. Concomitant Fe or folate deficiency may be present. Normochromic normocytic anaemia is usual.

### *Scurvy*

Occasionally seen in infants due to poor intake with fruit juices being boiled. Pseudoparalysis due to painful legs. Petechial, periorbital, or subdural haemorrhages can arise. Bleeding tendency due to loss of vascular integrity with collagen deficiency.

## Poisons

### *Lead poisoning*

Inhibits haem synthesis and the activity of pyrimidine-5'-nucleotidase, causing hypochromic anaemia with basophilic stippling of red cells and ring sideroblasts in the BM. Also causes abdominal pain. Commonest in toddlers eating flakes of old lead-based paint.

### *Sodium chlorate*

A common weedkiller, also a powerful oxidizing substance causing acute intravascular haemolysis and renal failure if ingested.

### *Nitrates, aniline dyes, nitrobenzene, and azo compounds*

Can all cause methaemoglobinaemia. If >20% metHb formed, exchange transfusion may be needed.

## Storage disorders

### *Gaucher disease*

Inherited (autosomal recessive) disorder resulting in deficiency of the enzyme glucocerebrosidase ( $\beta$ -glucosidase). Most common form has accumulated glycolipid in macrophages of spleen, liver, and BM. May be diagnosed at any time during life depending on severity. Some cases are not identified until adulthood. Rarer type 2 disease has severe progressive neurological deterioration from birth, usually fatal within 1 year due to glycolipid accumulation in the CNS. Diagnosis by assay of deficient enzyme, but characteristic  $\odot$  see Chapter 18, Rare diseases. Gaucher cells evident in BM (laden macrophages with appearance of crumpled tissue paper).

### *Niemann–Pick (N-P) disease*

Though rare, commonest cause of foamy macrophages in marrow of affected patients. Caused by the inherited deficiency of sphingomyelinase resulting in accumulation of sphingomyelin. 4 types of N-P disease have been defined: type A (classic N-P disease) presents in the 1st year of life with developmental delay, neurodegeneration, and death within 3 years; type B with visceral rather than CNS involvement also presents in infancy and is also progressive and fatal but spares the CNS; type C presents later in childhood but then shows neurodegeneration with death in the 2nd or 3rd decade; and type D patients simply store sphingomyelin in viscera without ill health and live to adulthood.

### *Marrow and blood cells in storage disorders* $\odot$ see Chapter 18, Rare diseases

The foamy macrophages seen in the marrow of N-P patients vary between types and are not in any way diagnostic or specific. Foamy macrophages are also seen in several other storage disorders and a variety of other clinical circumstances. Pseudo-Gaucher cells are seen in chronic granulocytic leukaemia, thalassaemia, and some atypical mycobacterial infections. Several storage disorders produce vacuolation of peripheral blood leucocytes, particularly lymphocytes, and this is also a non-specific finding. Diagnosis always rests on the appropriate enzyme assay.

# Haematological emergencies

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## Septic shock/neutropenic fever

- ▶▶ One of the commonest haemato-oncological emergencies.
- May be defined as the presence of symptoms or signs of infection in a patient with an absolute neutrophil count of  $<1.0 \times 10^9/L$ . In practice, the neutrophil count is often  $<0.1 \times 10^9/L$ .
- Similar clinical picture also seen in neutrophil function disorders such as MDS despite  $\leftrightarrow$  neutrophil numbers.
- Beware: can occur without pyrexia, especially patients on steroids.

### Immediate action

- ▶▶ Urgent clinical assessment.
- Follow ALS guidelines if cardiorespiratory arrest (rare).
- More commonly, clinical picture is more like cardiovascular shock  $\pm$  respiratory embarrassment viz: tachycardia, hypotension, peripheral vasodilatation, and tachypnoea. Occurs with both Gram +ve (now more common with indwelling central catheters) and Gram -ve organisms (less common but more fulminant).
- Immediate rapid infusion of albumin 4.5% or Gelofusine to restore BP.
- Insert central catheter if not *in situ* and monitor CVP.
- Start O<sub>2</sub> by face mask if pulse oximetry shows saturations  $<95\%$  (common) and consider arterial blood gas measurement—care with platelet counts  $<20 \times 10^9/L$ —manual pressure over puncture site for 30min.
- Perform full septic screen (➡ see Guidelines for use of IV antibiotics, p.682).
- Give the 1st dose of 1st-line antibiotics immediately, e.g. ureidopenicillin and loading dose aminoglycoside (ceftazidime or ciprofloxacin if pre-existing renal impairment). Follow established protocols.
- If the event occurs while patient on 1st-line antibiotics, vancomycin/ciprofloxacin or vancomycin/meropenem are suitable alternatives.
- Commence full ITU-type monitoring chart.
- Monitor urine output with urinary catheter if necessary—if renal shutdown has already occurred, give single bolus of IV furosemide. If no response, start renal dose dopamine.
- If BP not restored with colloid despite  $\uparrow$  CVP, consider inotropes.
- If O<sub>2</sub> saturations remain  $\downarrow$  despite 60% O<sub>2</sub> delivered by rebreathing mask, consider ventilation.
- Alert ITU giving details of current status.

### Subsequent actions

- Discuss with senior colleague.
- Amend antibiotics according to culture results or to suit likely source if cultures -ve (➡ see Treatment of neutropenic shock when source unknown, p.684 and Treatment of neutropenic shock when source known/suspected, p.686).
- Check aminoglycoside trough levels after loading dose and before 2nd dose as renal impairment may determine reducing or withholding next dose. Consider switch to non-nephrotoxic cover, e.g. ceftazidime/ciprofloxacin.

- Continue antibiotics for 7–10d minimum and usually until neutrophil recovery.
- If cultures show central line to be source of sepsis, remove immediately if patient not responding.

## Acute transfusion reactions

The differential diagnosis of severe, life-threatening transfusion reaction includes acute haemolytic reactions (usually due to ABO incompatible transfusion), severe allergic reaction/anaphylaxis, bacterial transfusion-transmitted infection, anaphylaxis, transfusion-related acute lung injury (TRALI), and transfusion-associated circulatory overload (TACO). The British Committee for Standards in Haematology (BCSH) has produced a guideline for the investigation and management of acute transfusion reactions (see Fig. 13.1).

**Acute haemolytic reaction** is most commonly due to human error with transfusion of ABO-incompatible red cells which react with the patient's anti-A or anti-B antibodies. Activation of complement causes rapid destruction of the transfused red cells in the circulation (intravascular haemolysis) and triggers the release of inflammatory cytokines. The patient quickly becomes shocked and develops acute renal failure and disseminated intravascular coagulation (DIC). See Table 13.1.

**Transfusion of a blood component contaminated by bacteria** is an uncommon but potentially fatal event seen in particular with platelet components which are stored at 22–24°C rather than red cells which are stored refrigerated. Implicated organisms include Gram -ve bacteria, including *Pseudomonas*, *Yersinia*, and *Flavobacterium*.

**Bacterial screening** of platelets is now carried out throughout the UK which has reduced the risk of such events. The transfusion of a contaminated pack can produce rapid onset of fever (usually >2°C above baseline), rigors, and hypotension, which may be initially indistinguishable from an acute haemolytic reaction or severe allergic reaction. Stop transfusion and give IV fluids. See → p.639 for investigation and management. Blood cultures should be taken from the patient and the blood unit should be sent back to the blood service for microbiological culture with withdrawal of any linked components from the same donation. Start patient on broad-spectrum antibiotics.

**Severe allergic or anaphylactic reaction** can result in severe hypotension or shock associated with wheeze (bronchospasm), stridor, or swelling of face, limbs, or mucous membranes (angio-oedema) and urticaria most commonly reported with plasma-rich components such as platelets or FFP but can occur with red cells. This requires immediate intervention to ensure the airway is patent and the administration of adrenaline (epinephrine) according to the 2008 UK Resuscitation guidelines.

**Transfusion-related acute lung injury (TRALI)** is caused by antibodies in the donor blood reacting with the patient's neutrophils sequestered in the lungs causing leakage of plasma into the alveolar spaces. Most cases present within 2h of transfusion (maximum 6h) with severe dyspnoea and cough with frothy pink sputum, often associated with hypotension and occasionally fever and rigors. Chest x-ray shows bilateral nodular shadowing. TRALI is often confused with acute heart failure due to circulatory overload. Treatment is supportive, with high concentration oxygen therapy and ventilatory support and steroid therapy is not effective.



**Transfusion-associated circulatory overload (TACO)** presents with acute or worsening pulmonary oedema within 6h of transfusion with respiratory distress, tachycardia, raised blood pressure and evidence of positive fluid balance. TACO is a significant cause of morbidity and mortality due to blood transfusion. The Serious Hazards of Transfusion (SHOT) reporting scheme received 71 reports of TACO in 2011; contributing to death in two patients and responsible for 24 cases of major morbidity.

Elderly patients are at particular risk and many have predisposing medical conditions such as heart failure, renal impairment, hypoalbuminaemia, and fluid overload. Treatment includes stopping the transfusion and administering oxygen and diuretic therapy with careful monitoring and specialist support if required. The risk of TACO is reduced by careful pre-transfusion assessment of predisposing factors, prescription of appropriate volume and flow rate, and adequate monitoring during the procedure.

### Clinical approach to severe acute transfusion reactions

All patients should be transfused in clinical areas where they can be directly observed to allow prompt detection and immediate management of acute reactions. If presumed transfusion reaction the transfusion must be stopped with immediate assessment.

- Stop and disconnect the blood pack and giving-set immediately (but *do not* discard).
- Maintain venous access with physiological saline, commence resuscitation.
- Take samples for FBC, renal and liver function tests, blood cultures, coagulation screen, repeat compatibility testing, DAT, LDH, and assessment of urine for haemoglobin.
- Check identification details between the patient, their ID identity band, and the blood component.
- Consider key/additional features.
- Fever and shock without anaphylaxis: ?ABO mismatched transfusion; ? bacterial sepsis.
- Dyspnoea without shock: ?TRALI vs TACO—check O<sub>2</sub> sats or blood gases and CXR.
- Dyspnoea/stridor with shock: ?severe allergic reaction or anaphylaxis.
- Seek early support and advice from critical care and haematology teams.

### Less severe acute transfusion reactions

Include febrile non-haemolytic transfusion reactions (FNHTR) characterized by fever, sometimes accompanied by shivering and myalgia. These are much less common since leucodepleted blood components were introduced. Mild FNHTR (pyrexia 38°C but <2°C rise from baseline) can often be managed with an antipyretic such as paracetamol. The patient should be monitored closely in case these are early signs of a more severe ATR.

In the case of moderate FNHTR (pyrexia  $>2^{\circ}\text{C}$  from baseline or  $>39^{\circ}\text{C}$  or rigors and/or myalgia) consider the possibility of a haemolytic or bacterial reaction—stop the transfusion, assess, and investigate as above.

Mild allergic reactions result in pruritus and urticarial rash with no change in vital signs. They are most common in patients receiving plasma-rich components such as FFP or platelets. These symptoms often improve with an antihistamine, e.g. chlorpheniramine. The patient must, however, be monitored closely for the development of a more severe reaction.

Haemovigilance relates to procedures around monitoring and reporting adverse events in relation to blood transfusion. The Blood Safety and Quality Regulations mandate that such adverse incidents must be reported via the Serious Adverse Blood Reactions and Events online system to MHRA. This also includes online reporting to the SHOT reporting scheme.

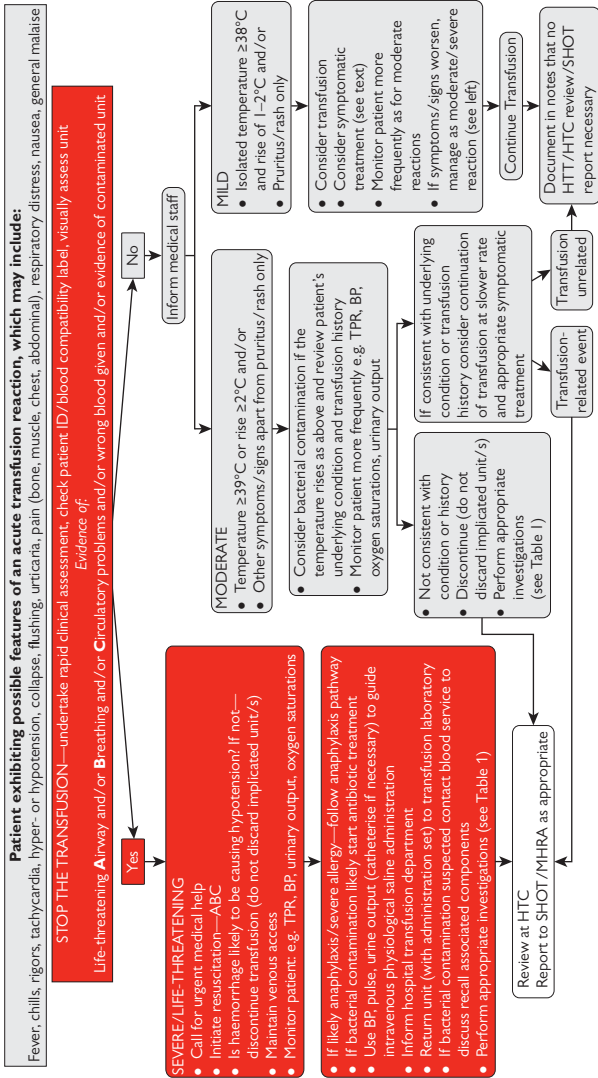
Discuss all suspected transfusion reactions with a member of your Hospital Transfusion Team who can advise about investigation and management together with reporting to haemovigilance schemes.

**Table 13.1** Acute haemolytic transfusion reaction

Symptoms	Signs
<ul style="list-style-type: none"> <li>● Patient restless/agitated</li> <li>● Flushing</li> <li>● Anxiety</li> <li>● Chills</li> <li>● Nausea and vomiting</li> <li>● Pain at venepuncture site</li> <li>● Abdominal, flank, or chest pain</li> <li>● Diarrhoea</li> </ul>	<ul style="list-style-type: none"> <li>● Fever</li> <li>● Hypotension</li> <li>● Oozing from wounds or venepuncture sites</li> <li>● Haemoglobinaemia</li> <li>● Haemoglobinuria</li> </ul>

### Further reading

Tinagete, H. (2012). Guideline on the investigation and management of acute transfusion reactions. Prepared by the BCSH Blood Transfusion Task Force. *Br J Haematol*, **159**, 143–53.



**Fig 13.1** Acute transfusion reactions. Reproduced from Tingate, H. et al. Guideline on the investigation and management of acute transfusion reactions, *Br J Haem*, **159**(2), 143–53 (26 Aug 2012), with permission from Wiley Blackwell.

## Delayed transfusion reaction

Occurs in patients immunized through previous pregnancies or transfusions. Antibody weak (so not detected at pretransfusion stage). 2° immune response occurs—antibody titre ↑.

Certain patient groups such as those with sickle cell anaemia are more prone to form red cell antibodies with risk of delayed haemolytic transfusion reaction. Close communication with the haematology team and the transfusion laboratory is essential to ensure appropriate care of these patients.

### Symptoms/signs

- Occur 7–10d after blood transfusion.
- Fever, anaemia, and jaundice.
- ± haemoglobinuria.

### Management

- Check DAT and repeat compatibility tests.
- Seek haematology advice and if needed in complex cases specialist advice from the Red Cell Immunology Team at the National Blood Service regarding further transfusion.

## Post-transfusion purpura

Profound thrombocytopenia occurring 5–10d after blood or platelet transfusion. Usually due to high titre of anti-HPA-1a antibody in HPA-1a –ve patient. Now very rarely seen.

### Features

- Rare.
- Multiparous ♀ most commonly (previous pregnancies or transfusions).
- Caused by platelet-specific alloantibodies (usually anti-HPA-1a).
- Platelets ↓↓ with associated bleeding/bruising—may be severe and even life-threatening.

### Management

- IVIg if bleeding.
- If platelet transfusion needed, use random donor platelets (no evidence that HPA-1a negative platelets superior).

## Hypercalcaemia

### Clinical symptoms

- General: weakness, lassitude, weight loss.
- Mental changes: impaired concentration, drowsiness, personality change and coma.
- GIT: anorexia, nausea, vomiting, abdominal pain (peptic ulceration and pancreatitis are rare complications).
- Genitourinary: polyuria, polydipsia.

### Clinical effects

- Cardiovascular: ↓ QT interval on ECG, cardiac arrhythmias and hypertension.
- Renal: dehydration, renal failure, and renal calculi.

### Haematological causes

- Myeloma.
- High-grade lymphoma.
- Adult T-cell leukaemia/lymphoma (ATLL).
- Acute lymphoblastic leukaemia.

Hypercalcaemia occurs in other clinical situations including metastatic carcinoma of breast, prostate, and lung. Theories for occurrence in haematological malignancy include ↑ bone resorption mediated by osteoclasts under the influence of locally or systemically released cytokines such as PTH-related peptide, TGF, TNF- $\alpha$ , M-CSF, interleukins, and prostaglandins. ↑ intestinal absorption of  $\text{Ca}^{2+}$  due to ↑ 1,25-hydroxycholecalciferol.

↔ range for plasma [ $\text{Ca}^{2+}$ ] 2.12–2.65mmol/L. 40% of plasma  $\text{Ca}^{2+}$  is bound to albumin. Most laboratories measure the total plasma  $\text{Ca}^{2+}$  although only unbound  $\text{Ca}^{2+}$  is physiologically active. For accurate measurement of plasma or serum  $\text{Ca}^{2+}$  blood sampling should be taken from an uncuffed arm, i.e. without the use of a tourniquet. (See Table 13.2.)

**Table 13.2** Correct for albumin

Albumin <40g/L	Corrected calcium = ( $\text{Ca}^{2+}$ ) + 0.02 [40 – (albumin)]
Albumin >45g/L	Corrected calcium = ( $\text{Ca}^{2+}$ ) – 0.02 [(albumin) – 45]

### Management

- ▶ An emergency if  $\text{Ca}^{2+}$  >3.0mmol/L.
- Rehydrate with normal saline 4–6L/24h.
- Beware fluid overload—use loop diuretics and CVP monitoring if necessary.
- Stop thiazide diuretics and consider regular loop diuretics.
- Give bisphosphonates, e.g. disodium pamidronate 60–90mg IV stat (↻ see Table 13.3) or zoledronic acid 4mg IV in 100mL Normal saline over at least 15min.
- Treat underlying malignancy.
- Consider dialysis if complicating factors (CCF, advanced renal failure).

- Other therapeutic options:
  - Calcitonin (salmon) 200IU 8-hourly.
  - Corticosteroids (e.g. prednisolone 60mg/d PO).
  - ↑ 25mcg/kg IV 3-weekly.
  - Plicamycin.

**Table 13.3** Treatment of hypercalcaemia with disodium pamidronate

Serum Ca <sup>2+</sup> (mmol/L)	Pamidronate (mg)
Up to 3.0	15–30
3.0–3.5	30–60
3.5–4.0	60–90
>4.0	90

Infuse slowly (see BNF).

Response often takes 3–5d.

## Hyperviscosity

Common haematological emergency. Defined as increase in whole blood viscosity as a result of an increase in either red cells, white cells, or plasma components, usually Ig.

### Commonest situations arise as a result of:

- ↑ in red cell volume in PRV.
- High blast cell numbers in peripheral blood, e.g. AML or ALL at presentation.
- Presence of monoclonal Ig, e.g. Waldenström macroglobulinaemia (IgM).

### Clinical features—polycythaemia (e.g. PRV)

Lethargy, itching, headaches, hypertension, plethora, arterial thromboses viz: MI, CVA, and visual loss (central retinal artery occlusion).

#### ►Emergency treatment

Isovolaemic venesection. Remove 500mL blood volume from large-bore vein (antecubital usually) with simultaneous replacement into another vein of 500mL 0.9% saline. Repeat daily until PCV <0.45.

### Clinical features—high WBC (e.g. AML)

Dyspnoea and cough (pulmonary leucostasis); confusion, ↓ conscious level, isolated cranial nerve palsies (cerebral leucostasis), visual loss (retinal haemorrhage or CRVT).

#### ►Emergency treatment

- Unless machine leucapheresis can be obtained immediately, venesect 500mL blood from large-bore vein and replace isovolaemically with packed red cells if Hb <7.0g/dL—otherwise replace with 0.9% saline to avoid ↑ whole blood viscosity.
- Arrange leucapheresis on cell separator machine. Use white cell interface program to apheresis with replacement fluids depending on Hb as above. 2h is usually maximum tolerated.
- Initiate tumour lysis prophylactic protocol (➡ see Tumour lysis syndrome, p.690) in preparation for chemotherapy.
- Start chemotherapy as soon as criteria allow (high urine volume of pH >8 and allopurinol commenced). This is crucial as leucapheresis in this situation is only a holding manoeuvre while the patient is prepared for chemotherapy.
- Continue leucapheresis daily until leucostasis symptoms resolved or until WBC <50 × 10<sup>9</sup>/L.

### Hypergammaglobulinaemia (e.g. Waldenström macroglobulinaemia)

Lethargy, headaches, memory loss, confusion, vertigo, visual disturbances from cerebral vessel sludging—rarely MI, CVA.

#### ►Emergency treatment

- Unless immediate access to plasma exchange machine available, venesect 500mL blood from large bore vein with isovolaemic replacement with 0.9% saline unless Hb <7.0g/dL when use packed red cells.



- Arrange plasmapheresis on a cell separator machine using plasma exchange program. Aim for 1–1.5 blood volume exchange (usually 2.5–4.0L) starting at lower end of range initially. Repeat daily until symptoms resolved.
- Maintenance plasma exchanges at 3–6 weekly intervals may be sufficient treatment for some forms of Waldenström macroglobulinaemia. However, if hyperviscosity due to IgA myeloma or occasionally IgG myeloma, chemotherapy will need to be initiated.

### Note

Diseases in which the abnormal Ig shows activity at lower temperature, e.g. cold antibodies associated with CHAD (➔ see Cold haemagglutinin disease, p.100) require maintenance of plasmapheresis inlet and outlet venous lines and all infusional fluids at 37°C. Polyclonal ↑ in Ig (e.g. some forms of cryoglobulinaemia) can also rarely cause hyperviscosity symptoms. Management is as described for monoclonal immunoglobulins (➔ Cryoglobulinaemia, p.363).

## Disseminated intravascular coagulation

Pathological process characterized by generalized intravascular activation of the haemostatic mechanism producing widespread fibrin formation, resultant activation of fibrinolysis, and consumption of platelets/coagulation factors (esp. I, II, V). Usually the result of serious underlying disease but may itself become life-threatening (through haemorrhage or thrombosis). Mortality in severe DIC may exceed >80%. Haemorrhage usually the dominant feature and is the result of excessive fibrinolysis, depletion of coagulation factors and platelets, and inhibition of fibrin polymerization by FDPs. Wide range of disorders may precipitate DIC.

### Pathophysiology—DIC may be initiated by:

- Exposure of blood to tissue factor (e.g. after trauma).
- Endothelial cell damage (e.g. by endotoxin or cytokines).
- Release of proteolytic enzymes into the blood (e.g. pancreas, snake venom).
- Infusion or release of activated clotting factors (factor IX concentrate).
- Massive thrombosis.
- Severe hypoxia and acidosis.

### Causes of DIC

*Tissue damage (release of tissue factor):* e.g. trauma (esp. brain or crush injury), thermal injury (burns, hyperthermia, hypothermia), surgery, shock, asphyxia/hypoxia, ischaemia/infarction, rhabdomyolysis, fat embolism.

*Complications of pregnancy (release of tissue factor):* e.g. amniotic fluid embolism, abruptio placentae, eclampsia and pre-eclampsia, retained dead fetus, uterine rupture, septic abortion, hydatidiform mole.

*Neoplasia (release of tissue factor, TNF, proteases):* e.g. solid tumours, leukaemias (esp. acute promyelocytic).

*Infection (endotoxin release, endothelial cell damage):* e.g. Gram -ve bacteria (e.g. meningococcus), Gram +ve bacteria (e.g. pneumococcus), anaerobes, *M. tuberculosis*, toxic shock syndrome, viruses (e.g. Lassa fever), protozoa (e.g. malaria), fungi (e.g. candidiasis), Rocky Mountain spotted fever.

*Vascular disorders (abnormal endothelium, platelet activation):* e.g. giant haemangioma (Kasabach–Merritt syndrome), vascular tumours, aortic aneurysm, vascular surgery, cardiac bypass surgery, malignant hypertension, pulmonary embolism, acute MI, stroke, subarachnoid haemorrhage.

*Immunological (complement activation, release of tissue factor):* anaphylaxis, acute haemolytic transfusion reaction, heparin-associated thrombocytopenia, renal allograft rejection, acute vasculitis, drug reactions (quinine).

*Proteolytic activation of coagulation factors:* e.g. pancreatitis, snake venom, insect bites.

*Neonatal disorders:* e.g. infection, aspiration syndromes, small-for-dates infant, respiratory distress syndrome, purpura fulminans.

**Other disorders:** e.g. fulminant hepatic failure, cirrhosis, Reye's syndrome, acute fatty liver of pregnancy, ARDS, therapy with fibrinolytic agents, therapy with factor IX concentrates, massive transfusion, acute intravascular haemolysis familial ATIII deficiency, homozygous protein C or S deficiency.

### Clinical features

Clinical features may be masked by those of the disorder which precipitated it and rarely is the cause of DIC obscure. DIC should be considered in the management of any seriously ill patient (see Table 13.4). The specific features of DIC are:

- Ecchymoses, petechiae, oozing from venepuncture sites, and post-op bleeding.
- Renal dysfunction, ARDS, cerebral dysfunction, and skin necrosis due to microthrombi.
- MAHA.

**Table 13.4** Clinical features of DIC

Acute (uncompensated) DIC	Rapid and extensive activation of coagulation, fibrinolysis, or both, with depletion of procoagulant factors and inhibitors and significant haemorrhage
Chronic (compensated) DIC	Slow consumption of factors with $\leftrightarrow$ or $\uparrow$ levels; often asymptomatic or associated with thrombosis

### Laboratory features

The following investigations are useful in establishing the diagnosis of DIC though the extent to which any single test may be abnormal reflects the underlying cause of DIC.

- D-dimers—more specific and convenient than FDP titre (performed on plasma sample). Significant  $\uparrow$  of D-dimers plus depletion of coagulation factors  $\pm$  platelets is necessary for diagnosis of DIC.
- PT—less sensitive, usually  $\uparrow$  in moderately severe DIC but may be  $\leftrightarrow$  in chronic DIC.
- APTT—less useful. May be normal or even  $<\leftrightarrow$ , particularly in chronic DIC.
- Fibrinogen (Fgn)— $\downarrow$  or falling Fgn levels are characteristic of many causes of DIC in the presence of D-dimers. Greatest falls are seen with tissue factor release.
- Platelet count— $\downarrow$  or falling platelet counts are characteristic of acute DIC, most notably in association with infective causes.
- Blood film may show evidence of fragmentation (schistocytes) though the absence of this finding does not exclude the diagnosis of DIC.
- Antithrombin levels are frequently  $\downarrow$  in DIC and degree of reduction in plasma antithrombin and plasminogen may reflect severity.
- Factor assays rarely necessary or helpful. In severe DIC levels of most factors are reduced with the exception of FVIIIc and Vwf which may be  $\uparrow$  due to release from endothelial cell storage sites.

**Management of DIC**

- Identify and, if possible, remove the precipitating cause.
- Supportive therapy as required (e.g. volume replacement for shock).
- Replacement therapy if bleeding: platelet transfusion if platelets  $<50 \times 10^9/L$ , cryoprecipitate to replace Fgn, and FFP to replace other factors FFP 12–15mL/kg, 2 pools cryoprecipitate (contains 5 units per pool) if fibrinogen  $<1.5\text{gm/dL}$ .
- Monitor response with platelet count, PT, Fgn, and D-dimers.
- Heparin (IVI 5–10IU/kg/h) for DIC associated with APML, carcinoma, skin necrosis, purpura fulminans, microthrombosis affecting skin, kidney, bowel, and large vessel thrombosis.
- ATIII concentrate in intractable shock or fulminant hepatic necrosis.
- Protein C concentrate in acquired purpura fulminans or severe neonatal DIC.

## Overdosage of thrombolytic therapy

- Large doses of any thrombolytic agent (streptokinase, urokinase, TPA) will cause 1° fibrinolysis by proteolytic destruction of circulating Fgn and consumption of plasminogen and its major inhibitor  $\alpha$ 2-antiplasmin.
- Overdosage is associated with high risk of severe haemorrhage particularly at recent venepuncture sites or surgical wounds; intracranial haemorrhage occurs in 0.5–1% of patients treated with thrombolytic therapy.
- Superficial bleeding at venepuncture site may be managed with local pressure and the infusion continued.
- Bleeding at other sites or where pressure cannot be applied necessitates cessation of thrombolytic therapy ( $t_{1/2} < 30\text{min}$ ) and determination of the thrombin time (if used to monitor thrombolytic therapy) or Fgn level. If strongly indicated and bleeding minimal or stopped the infusion may be restarted at 50% the initial dose when the thrombin time has returned to the lower end of the therapeutic range ( $1.5 \times$  baseline).

### Treatment of serious bleeding after thrombolytic therapy

- Stop thrombolytic infusion immediately.
- Discontinue any simultaneous heparin infusion and any antiplatelet agents.
- Apply pressure to bleeding sites, ensure good venous access, and commence volume expansion.
- Check Fgn and APTT.
- Transfuse 2 pools cryoprecipitate (contains 5 units per pool), consider use of fibrinogen concentrate).
- Monitor Fgn, repeat cryoprecipitate to maintain Fgn  $> 1.0\text{g/L}$ .
- If still bleeding, transfuse 2–4U FFP.
- If bleeding time  $> 9\text{min}$ , transfuse 10U platelets.
- If bleeding time  $< 9\text{min}$ , commence tranexamic acid.

## Heparin overdose

The most serious complication of heparin overdose is haemorrhage. The therapeutic range using the APTT is 1.5–2.5 × average ↔ control. The plasma  $t_{1/2}$  following IV administration is 1–2h. The  $t_{1/2}$  after SC administration is considerably longer.

### Management guidelines—APTT > therapeutic range

↻ See Table 13.5.

**Table 13.5** Management guidelines

Without haemorrhage	<b>Continuous IV infusion</b> Stop infusion, if markedly elevated, recheck after 0.5–1h; restart at lower dose when APTT in therapeutic range
	<b>Intermittent SC heparin</b> Reduce dose recheck 6h after administration
With haemorrhage	<b>Continuous IV infusion</b> Stop infusion; if bleeding continues, administer protamine sulfate by slow IV injection (1mg neutralizes 100IU heparin, max. dose 40mg/injection)
	<b>Intermittent SC heparin</b> If protamine is required, administer 50% of calculated neutralization dose 1h after heparin administration and 25% after 2h



## Heparin-induced thrombocytopenia

Uncommon but sometimes life-threatening condition due to immune complex-mediated thrombocytopenia in patients treated with heparin. Early recognition reduces morbidity and mortality.

### Incidence

Estimated incidence 1–3% of patients receiving heparin for  $\geq 1$  week. Occurs both with full dose regimens and 'minidose' regimens (5000 IU bd) or low doses used for 'flushing' IV lines. Less common with LMWH.

### Pathogenesis

IgG antibodies formed in response to heparin therapy form immune complexes with heparin and PF4, bind to platelet Fc receptors, trigger aggregation and cause thrombocytopenia. Thrombin activation causes vascular thrombosis and microthrombi cause microvascular occlusion.

### Clinical features

- HIT causes a fall in the platelet count  $\sim 8$  d (4–14 d) after a patient's 1st exposure to heparin but may occur within 1–3 d in a patient who has recently had prior exposure to heparin.
- Platelet count generally falls to  $\sim 60 \times 10^9/L$  but may fall to  $< 20 \times 10^9/L$ .
- Venous and arterial thromboses occur in up to 15%.
- Bleeding is rare.
- Microvascular occlusion may cause progressive gangrene extending proximally from the extremities and necessitating amputation. In patients with HIT (thrombocytopenia and thrombosis) limb amputation is required in  $\sim 10\%$  and mortality approaches 20%.

HIT should be suspected in any patient on heparin in whom the platelet count falls to  $< 100 \times 10^9/L$  or drops by  $\geq 30$ –40% or develops a new thromboembolic event 5–10 d after ongoing heparin therapy.

▶▶ Heparin should be discontinued immediately and confirmatory investigations undertaken.

### Diagnostic test

ELISA using PF4 to detect antibodies to heparin-low molecular weight protein complex; may miss 5–10% of cases with antibodies to other proteins and up to 50% false +ves after CABG.

### Management

- Discontinue heparin (platelet count normally recovers in 2–5 d).
- Substitute alternative anticoagulation where necessary and to prevent further thromboembolic events:
- Recombinant hirudin:
  - Thrombin inhibitor; anticoagulant effect lasts  $\sim 40$  min.
  - Slow IV bolus followed by IVI.
  - Dose determined by body weight and renal function (see product literature).
  - Monitor 4 h after IV bolus dose using APTT or ecarin clotting time (ECT); target range 1.5–2.5 mean n APTT; reduce target to 1.5 if



concomitant warfarin therapy and discontinue hirudin when INR  $\geq 2.0$ .

- Adverse effects bleeding (esp. with warfarin), anaemia, haematoma, fever, and abnormal LFTs.
  - Argatroban:
    - Thrombin inhibitor;  $t_{1/2}$  ~45min.
    - Initiate IV infusion at dose of 2mcg/kg/min.
    - Check APTT at 2h and adjust dose for APTT 1.5–3 baseline (max. 100sec).
    - $\downarrow$  dose by 75% if hepatic insufficiency.
    - Side effect—bleeding.
  - Danaparoid:
    - A heparinoid with low level cross-reactivity with HIT antibodies.
    - IV bolus dose by weight (see product literature) followed by decremental infusion schedule and maintenance infusion.
    - Monitor by factor Xa inhibition assay 4h after dose (target range 0.5–0.8U/mL).
    - Prolonged  $t_{1/2}$  of 25h.
    - Side effect—bleeding.
- ▶▶ LMWHs frequently cross-react with HIT antibodies and are not recommended.

## Warfarin overdosage

Haemorrhage is a potentially serious complication of anticoagulant therapy and may occur with an INR in the therapeutic range if there are local predisposing factors, e.g. peptic ulceration or recent surgery, or if NSAIDs are given concurrently.

### Management guidelines

See Table 13.6.

**Table 13.6** Management guidelines for warfarin overdose

INR	Action
>7.0	Without haemorrhage: stop warfarin and consider a single 5–10mg oral dose of vitamin K if high bleeding risk; review INR daily
4.5–7.0	Without haemorrhage: stop warfarin and review INR in 2d
>4.5	<i>With severe life-threatening haemorrhage:</i> give factor IX concentrate (50U/kg) single 2–5mg IV dose of vitamin K
>4.5	<i>With less severe haemorrhage:</i> e.g. haematuria or epistaxis, withhold warfarin for = 1d and consider a single 0.5–2mg IV dose of vitamin K
2.0–4.5	With haemorrhage: investigate the possibility of an underlying local cause; reduce warfarin dose if necessary; give FFP/factor IX concentrate only if haemorrhage is serious or life threatening

### Vitamin K administration to patients on warfarin therapy

Effect of vitamin K is delayed several hours even with IV administration. Doses >2 mg cause unpredictable and prolonged resistance to oral anti-coagulants and should be avoided in most circumstances where prolonged warfarin therapy is necessary. Particular care must be taken in patients with prosthetic cardiac valves who may require heparin therapy for several weeks to achieve adequate anticoagulation if a large dose of vitamin K has been administered.



## Massive blood transfusion

Hospitals must have local major haemorrhage protocols that need to be adapted for specific clinical areas. All medical, nursing, laboratory, and support staff should know where to find the major blood loss protocol in relevant areas and be familiar with the contents, supported by training and regular drills.

While there are arbitrary definitions of massive blood loss, e.g. loss of 1 blood volume within a 24h period, 50% blood volume loss within 3h, loss of 150mL/min, these may be difficult to apply in the acute situation. There is much focus on practice recommendations for transfusion support of bleeding in trauma patients but specific attention is also needed on the management of patients with bleeding in other clinical settings such as gastrointestinal, obstetrical, or surgical haemorrhage.

Good communication is essential between all teams involved in the management of patients. The switchboard can play a key role in initial alert of key members followed by contact of further teams as needed such as the senior clinician depending on clinical area, anaesthetist/ITU team, senior nurse/midwife, Transfusion Laboratory and other laboratories (Haematology & Coagulation, Biochemistry), clinical haematologist on call, Radiology including interventional radiology, porter/other support staff.

There should be designated Team Leader co-ordinating management who should also nominate a specific member of the team to communicate with the laboratory staff throughout the incident.

Hospitals must have a strategy to ensure that red cells and components are readily available in life-threatening bleeding. The patient must have a correctly labelled blood sample for pre transfusion testing before Group O emergency blood is administered. Group-specific blood should be made as soon as possible to conserve Group O RhD negative stocks.

FFP used early may pre-empt the development of significant coagulopathy. While traditional wisdom recommends use of FFP with the aim of maintaining the prothrombin time (PT) and activated partial thromboplastin time (APTT) to be at a ratio of  $<1.5 \times$  normal, in practice there are often significant delays in the availability of laboratory-based coagulation testing. An empirical approach pending further clinical studies includes the administration of FFP early in the resuscitation process at a dose of 15–20mL/kg (pragmatically 4 units) and before coagulation investigation results are known (although baseline tests should have been taken). Further treatment should then be guided by results of laboratory based (e.g. PT/APTT) or if available near patient tests of coagulation (e.g. TEG ROTEM).

Fibrinogen supplementation should be given if fibrinogen levels fall below 1.5g/L tested using the Clauss method or if available as guided by TEG/ROTEM. Cryoprecipitate is given at a standard dose of two 5-unit pools. Fibrinogen concentrate is currently not licensed in UK but is used extensively in Europe as an alternative to cryoprecipitate at a dose of 3–4g.

One unit of platelets should be administered when the platelet count falls below  $75 \times 10^9/L$ , (or  $100 \times 10^9/L$  in complex trauma especially with head injury. If a patient is still actively bleeding aim to keep greater than  $75 \times 10^9/L$  otherwise aim to keep platelets  $>50 \times 10^9/L$ ).

Adult trauma patients with or at risk of major haemorrhage, in whom antifibrinolytics are not contraindicated, should be given tranexamic acid as soon as possible after injury, in a dose of 1g over 10min followed by a maintenance infusion of 1g over 8h. The use of tranexamic acid should be considered in non-traumatic major bleeding where there is no contraindication.<sup>1</sup>

### Investigations

- Baseline tests:
  - Haematocrit.
  - Platelet count.
  - Fgn.
  - PT.
  - APTT ratio.
  - Group.
  - Screen.
- Frequent reassessment of tests to monitor effect of, and need for, further replacement therapy.

### Management

➔ See Table 13.7.

**Table 13.7** Management guidelines

Haematocrit	<0.30	Transfuse red cells
Platelet count	< $75 \times 10^9/L$	Transfuse platelets
Fgn	<1.5g/L	Transfuse cryoprecipitate
PT $\pm$ APTT ratio	>1.5 $\times$ control	Transfuse FFP

### Red cell transfusion

- Immediate access to Emergency Group O blood if severe haemorrhage (♀ of childbearing age should receive O Rh (D) neg blood).
- Switch to Group specific blood as soon as patient's blood group known.
- Crossmatched blood needed only if patient has positive antibody screen—but even in patients with red cell antibodies lifesaving transfusion should not be delayed—communication with the hospital transfusion laboratory is essential; also seek urgent clinical haematology advice.
- Uncrossmatched group O Rh (D) -ve blood may be transfused in the emergency situation until group-specific blood can be made available; group O Rh (D) +ve red cells may be given to ♂ and older ♀ if necessary.

### Platelet transfusion

- Check that platelets available in the transfusion laboratory; may need to be ordered from the blood service.
- If possible give ABO compatible platelets .
- If have to give D positive platelets to a D-negative ♀ of child bearing potential then may need to give anti-D immunoglobulin—seek haematology advice.

### FFP

- Takes up to 30min to thaw; dose required 12–15mL/kg.
- Use immediately for optimum replacement of coagulation factors.
- Each unit contains ~200–280mL plasma and 0.7–1.0IU/mL activity of each coagulation factor.
- ABO group compatible FFP should be administered—no crossmatch is required.
- If large volumes infused, serum  $[Ca^{2+}]$  should be monitored to exclude hypocalcaemia due to citrate toxicity.

### Cryoprecipitate

- Precipitate formed when FFP is thawed at 4°C; resuspended in 10–15mL plasma and refrozen at –18°C; takes up to 305min to thaw and pool.
- ABO compatible group if possible as with FFP.
- Contains 80–100IU FVIII, 100–250mg Fgn, 50–60mg fibronectin, and 40–70% of the original vWF.
- Should be used immediately for optimum replacement of Fgn and factor VIII.
- The adult dose is 2 pools of cryoprecipitate (each pool contains 5 units). Give when fibrinogen <1.5g/L.

### Reference

1. CRASH-2 Trial Collaborators (2010). Effects of tranexamic acid on death, vascular occlusive events, and blood transfusion in trauma patients with significant haemorrhage (CRASH-2): a randomised, placebo-controlled trial. *Lancet*, **376**, 23–32.



## Paraparesis/spinal collapse

May be due to tumour in the cord, spinal dura, or meninges or by extension of a vertebral tumour into the spinal canal with compression of the cord or as a result of vertebral collapse.

Spinal cord compression from vertebral collapse in a haematological patient is most commonly due to myeloma (in up to 20% of patients) but may occur in a patient with HL (3–8%) or occasionally NHL. Spinal cord involvement by leukaemia is most common in AML, less so in ALL and CGL, and least common in CLL.

Onset of paraplegia may be preceded for days or weeks by paraesthesia but in some patients the onset of paraplegia may follow initial symptoms by only a few hours.

Symptoms suggestive of spinal cord compression require urgent assessment by CT or MRI and referral to a neurosurgical unit for assessment for surgical decompression. Where this is not possible, early radiotherapy may provide symptomatic improvement. However, if treatment is delayed until paraparesis has developed, this often proves to be irreversible despite surgery and/or radiotherapy.



## Leucostasis

Term is applied both to organ damage due to 'sludging' of leucocytes in the capillaries of a patient with high circulating blast count and to the lodging and growth of leukaemic blasts, usually in AML, in the vascular tree eroding the vessel wall and producing tumours and haemorrhage.

### Features

- More common in AML and blast crisis of CML.
- Leucostatic tumours are associated with an exponential increase in blasts in the peripheral blood and, prior to the development of effective chemotherapy, haemorrhage from intracerebral tumours was not an uncommon cause of death.
- Pulmonary or cerebral leucostasis are serious complications which may occur in patients who present with a blast count  $>50 \times 10^9/L$ .
- Leucocyte thrombi may cause plugging of pulmonary or cerebral capillaries. Vascular rupture and tissue infiltration may occur.
- Less common manifestations are priapism and vascular insufficiency.
- Pulmonary leucostasis causes progressive dyspnoea of sudden onset associated with fever, tachypnoea, hypoxaemia, diffuse crepitations, and a diffuse interstitial infiltrate on CXR.
- Pulmonary haemorrhage and haemoptysis may occur. More common with monocytic leukaemias and the microgranular variant of acute promyelocytic leukaemia. Differentiation from bacterial or fungal pneumonia may be difficult.
- Cerebral leucostasis may cause a variety of neurological abnormalities.
- Anaemia may protect a patient with marked leucocytosis from the effects of  $\uparrow$  whole blood viscosity. Transfusion of RBCs to correct anaemia prior to chemotherapy may initiate leucostasis.

### Management

Urgent leucapheresis is required for a patient with marked leucostasis ( $>200 \times 10^9/L$ ) or in any patient in whom leucostasis is suspected. Chemotherapy may be commenced concomitantly to further reduce the leucocyte count but may be associated with a high incidence of pulmonary and CNS haemorrhage.

## Thrombotic thrombocytopenic purpura

### Definition

Fulminant disease of unknown aetiology characterized by ↑ platelet aggregation and occlusion of arterioles and capillaries of the microcirculation. Considerable overlap in pathophysiology and clinical features with HUS—fundamental abnormality may be identical.

### Incidence

Rare, ~1 in 500,000 per year. ♀:♂ = 2:1. HUS much commoner in children, TTP commoner in adults—peak age incidence is 40 years, and 90% of cases <60 years old. There is some case clustering.

### Clinical features

- Classical description is of a pentad of features:
  - 1. Microangiopathic haemolytic anaemia.
  - 2. Severe thrombocytopenia.
  - 3. Neurological involvement.
  - 4. Renal impairment.
  - 5. Fever.

In practice, few patients have the ‘full Monty’. 50–70% have renal abnormalities (cf. nearly all with HUS) and they are less severe. Neurological involvement is more prevalent in TTP than HUS. 40% of TTP patients have fever.

- *Haemolysis*—severe and intravascular causing jaundice.
- *Thrombocytopenia*—severe, mucosal haemorrhage likely and intracranial haemorrhage may be fatal.
- *Neurological*—from mild depression and confusion → visual defects, coma and status epilepticus.
- *Renal*—haematuria, proteinuria, oliguria and ↑ urea and creatinine. HUS>TTP.
- *Fever*—very variable, weakness and nausea common.
- Other disease features: serious venous thromboses at unusual sites (e.g. sagittal sinus—microthrombi in the brain seen on MRI scan). Abdominal pain severe enough to mimic an acute abdomen is sometimes seen due to mesenteric ischaemia. Diarrhoea is common, particularly bloody in HUS.

### Diagnosis and investigations

- Made on the clinical features—exclude other diseases, e.g. cerebral lupus, sepsis with DIC.
- FBC shows severe anaemia and thrombocytopenia.
- Blood film usually shows gross fragmentation of red cells, spherocytes, and nucleated red cells with polychromasia.
- Reticulocytes ↑↑.
- LDH ↑↑ (>1000IU/L).
- Clotting screen including Fgn and FDPs usually ↔ (cf. DIC).
- Serum haptoglobin low or absent.
- Urinary haemosiderin +ve.
- Unconjugated bilirubin ↑.

- DAT –ve.
- BM hypercellular.
- U&E show ↑ (HUS>TTP).
- Proteinuria and haematuria.
- Renal biopsy shows microthrombi.
- Stool culture for *Escherichia coli* 0157 +ve in most cases of HUS in children, less often in adult TTP.
- MRI brain scan shows microthrombi and occasional intracranial haemorrhage.
- ► **Lumbar puncture**—do not proceed with LP unless scans clear and there is suspicion of infective meningitis.
- Look for evidence of viral infection. Association of syndrome with HIV, SLE, usage, and the 3rd trimester of pregnancy.

### Treatment is a haematological emergency—seek expert help immediately

- 1 Unless antecubital venous access is excellent, insert a large-bore central apheresis catheter (may need blood product support).
- 2 May need ITU level of care and ventilation.
- 3 Initiate plasmapheresis as soon as possible:
  - Exchange 1–1.5 plasma volume daily until clinical improvement. May need 3–4L exchanges. Replacement fluid should be solely FFP. In the event of delayed access to cell separator facilities, start IV infusions of FFP making intravascular space with diuretics if necessary. Once response achieved, ↓ frequency of exchanges gradually. If no response obtained within 1 week, change FFP replacement to *cryosupernatant* (rationale: it lacks HMW multimers of vWF postulated in endothelial damage disease triggers).
- 4 Give RBC as necessary but reserve platelet transfusions for severe mucosal or intracranial bleeding as reports suggest they may worsen the disease.
- 5 Cover for infection with IV broad-spectrum antibiotics including teicoplanin if necessary to preserve the apheresis catheter.
- 6 Start anticonvulsants if fitting.
- 7 Most would start high-dose steroids (prednisolone 2mg/kg/d PO) with gastric protection although evidence is equivocal.
- 8 Aspirin/dipyridamole/heparin may be considered for non-responders.
- 9 Refractory patients (~10%) should be considered for IV vincristine.
- 10 Still refractory patients may achieve remission with splenectomy.
- 11 Response to treatment may be dramatic, e.g. ventilated, comatose patient watching TV in the afternoon after plasma exchange in the morning!

### Prognosis

- 90% respond to plasma exchange with FFP replacement.
- ~30% will relapse. Most respond again to further plasma exchange but leaves 15% who become chronic relapsers.
- Role of prophylaxis for chronic relapsers unclear. Intermittent FFP infusions or continuous low-dose aspirin may help individual cases.

## Sickle crisis

### Management

- ▶ Early and effective treatment of crises essential (hospital).
- ▶ Rest patient and start IV fluids and O<sub>2</sub> (patients often dehydrated through poor oral intake of fluid + excessive loss if fever).
- ▶ Start empirical antibiotic therapy (e.g. cephalosporin) if infection is suspected whilst culture results (blood, urine or sputum) are awaited.
- ▶ Analgesia usually required—e.g. IV opiates (diamorphine/morphine) especially when patients are first admitted to hospital. Switch later to oral medication after the initial crisis abates.
- ▶ Consider exchange blood transfusion (if neurological symptoms, stroke or visceral damage). Aim to ↓ HbS to <30%.
- ▶ Exchange transfusion if PaO<sub>2</sub> <60mm on air (▶ chest syndrome).
- ▶ α-adrenergic stimulators for priapism.
- ▶ Seek advice of senior haematology staff.
- ▶ Consider regular blood transfusion if crises frequent or anaemia is severe or patient has had CVA/abnormal brain scan.
- ▶ Top-up transfusion if Hb <4.5g/dL (hunt for cause).

Transfusion and splenectomy may be lifesaving in children with splenic sequestration.

### Management of acute chest syndrome

- O<sub>2</sub> supplementation.
- Antibiotics (include cover for community-acquired organisms and atypicals).
- Monitor fluid balance aiming for euvoalaemia.
- Implement pain management, avoid splinting and oversedation.
- Bronchodilator therapy.
- Red cell transfusion if respiratory compromise or clinical deterioration.

### Further reading

📄 [www.bcshguidelines.com/pdf/SICKLE.V4\\_0802.pdf](http://www.bcshguidelines.com/pdf/SICKLE.V4_0802.pdf)

# Supportive care

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## Quality of life

In managing any disease problem a key objective is to improve the quality of a patient's survival as well as its duration. Part of the clinical decision-making process takes into account QoL in judging the most appropriate treatment. QoL is also evaluated as an outcome in clinical trials parallel to conventional measures such as survival.

Defining QoL precisely is not easy; it has been described as a measure of the difference at a particular time point between the hopes and expectations of the individual and that individual's present experiences. QoL is multifaceted and can only be assessed by the individual since it takes into account many aspects of that individual's life and their current perception of what, for them, is good QoL in their specific circumstances.

A clear distinction exists between performance scores (e.g. Karnofsky or WHO) which record functional status and assess independence; these are assessed by the physician according to pre-set criteria. They have erroneously been considered to be surrogate markers of QoL.

Patient QoL as assessed by the treating physician has been shown to be unreliable in an oncological setting.

There is no single determinant of good QoL. A number of qualities which go to make up QoL are capable of assessment; these include: *ability to carry on normal physical activities, ability to work, to engage in normal social activities, a sense of general well-being, and a perception of health.*

Several validated instruments now exist to measure QoL; these mainly involve questionnaires completed by the patient. They are simple to complete and involve 'yes' or 'no' answers to specific questions, answers on a linear analogue scale or the use of 4- or 7-point Likert scales.

### Available QoL instruments include:

- Functional Living Index–Cancer (FLIC).<sup>1</sup>
- Cancer Assessment of Cancer Therapy (FACT).<sup>2</sup>
- European Organisation for Research and Treatment in Cancer (EORTC) Quality of Life Questionnaire C-30 (QLQ C30).<sup>3</sup>

Data from validated QoL questionnaires are now accepted as a requirement and a clinical end point in many major clinical trials, especially in malignancies, particularly those where survival differences are minimal between contrasting therapy approaches. Where survivals are minimally affected it is essential to focus on treatments which will offer the best QoL.

## References

- <sup>1</sup> Schipper, H. *et al.* (1984). Measuring the quality of life of cancer patients: the Functional Living Index–Cancer: development and validation. *J Clin Oncol*, **2**, 472–83.
- <sup>2</sup> Cella, D.F. *et al.* (1993). The Functional Assessment of Cancer Therapy scale: development and validation of the general measure. *J Clin Oncol*, **11**, 570–9.
- <sup>3</sup> Aaronson, N.K. *et al.* (1993). The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. *J Natl Cancer Inst*, **85**, 365–76.



## Pain management

Pain is a clinical problem in diverse haematological disorders, notably in sickle cell disease, haemophilia, and myeloma. Acknowledgement of the need to manage pain effectively is an essential part of successful patient care and management in clinical haematology.

Pain may be local or generalized. More than one type of pain may be present and causes may be multifactorial. It is most important to listen to the patient and give them the chance to talk about their pain(s). Not only will this help determine an appropriate therapeutic strategy, the act of listening and allowing the patient to talk about their pains and associated anxieties is part of the pain management process.

Engaging the patient in 'measuring' their pain is often helpful; it enables specific goals to be set and provides a means to assess the effectiveness of the analgesic strategy. A variety of scales have been employed, with simple numerical values ('pain score out of ten') being the most common. These strategies must be tailored to the individual patient's comprehension—often young children are being assessed.

Basic to the control of pain is to manage and remove the pathological process causing pain, wherever this is possible. Analgesia must be part of an integrated care plan which takes this into account.

Analgesic requirements should be recorded regularly as these form a valuable 'semi-quantitative' end point of pain measurement. Reduction in requirements, for example, is an indicator that attempts to remove or control the underlying cause are succeeding.

Managing pain successfully involves patient and family/carer participation, a collaborative multidisciplinary approach in most categories of haematological disorder-related pain; medication should aim to provide continuous pain relief wherever possible with a minimum of drug-related side effects. Anticipating pain with the use of regular medications rather than a 'PRN' basis can be key to analgesic success. Treatment strategies are based on the WHO pain ladder, simple analgesics being used initially, in combination with opiates of ↑ strength if pain is not relieved (Table 14.1).

**Table 14.1** Analgesics

<b>Simple non-opioid analgesics</b>	Paracetamol: 1g 4–6-hourly, oral as tablets or liquid; suppositories available
	No contraindication in liver disease; useful in mild-to-moderate pain
<b>Anti-inflammatory drugs</b>	Ibuprofen 800mg or diclofenac 75–100mg bd as slow release formulations can be synergistic with other analgesics; combined formulations of diclofenac with misoprostol may reduce risks of gastric irritation bleeding, or used with gastric acid suppressants; useful in combination with paracetamol or weak opioids
	Caution should be exercised in renal impairment (notably in myeloma)



**Table 14.1** Analgesics (*continued*)

<b>Weak opioids</b>	Codeine 30–60mg usually combined with paracetamol 1g as co-codamol 30/500 tablets; usual dosage is 2 tablets 6-hourly or dihydrocodeine 30–60mg up to 4-hourly provide effective analgesia for moderate pain
	Confusion, drowsiness may be associated with initial usage in some. Weak (and strong) opioids cause constipation; usually requires simple laxatives
<b>Strong opioids</b>	Morphine available as liquid or tablets commencing at 5–10mg and given 4-hourly is treatment of choice in severe pain
	Once daily requirements are established patients can be 'converted' to 12-hourly slow release morphine preparations
	Breakthrough pain can be treated with additional doses of 5–10mg morphine. Diamorphine preferred for parenteral usage. Highly soluble and suitable for use in a syringe driver for continuous administration or as a 4-hourly injection. Can be administered as a nasal spray in children. Accumulation can occur with renal impairment
<b>Alternatives to opioids</b>	Tramadol may be given orally
	Fentanyl given as slow release transdermal patches may be a valuable alternative to slow release morphine for moderate-to-severe chronic pain. It is also available in 'lollipop' and sublingual tablets form for rapid relief
	Oxycodone is a newer synthetic opiate which has been successfully employed as an alternative to morphine where analgesia is inadequate or adverse effects intolerable

For chronic pain give analgesia PO regularly, wherever possible.

- Pain control is very specific to the individual patient, there is no 'correct' formula other than the combination of measures which alleviate the pain.
- The clinician should work 'upwards' or 'downwards' through the levels of available analgesics to achieve control.
- Constipation due to analgesics should be managed appropriately.
- Nausea or vomiting may occur in up to 50% patients with strong opiates; anti-emetics should be given to avoid nausea or vomiting.
- Additional general measures include:
  - Radiotherapy for localized cancer pain and lytic lesions in myeloma.
  - Physical methods, e.g. TENS or consideration of nerve root block.
  - Surgery, especially in myeloma where stabilizing fractures and pinning will relieve pain and allow mobility.
  - Encouraging/allowing patients to utilize 'alternative' approaches. including relaxation techniques, aromatherapy, hypnosis, etc.

- Additional drug therapy:
  - Antidepressants, e.g. amitriptyline may help in neuropathic pain.
  - Anticonvulsants, e.g. carbamazepine and gabapentin may be helpful in neuropathic pains especially in post-herpetic neuralgia.
  - Corticosteroids, particularly dexamethasone, to relieve leukaemic bone pain in late-stage disease.
  - Very rarely can spinal anaesthesia be used in acute pain problems 2° to myelomatous disease. This can be a bridge to a more definitive solution.

Many hospitals also run specific pain clinics and have palliative care or pain control teams who can review in-patients and offer advice. The support and expertise available should be enlisted particularly for difficult problems with persistent localized pain, e.g. post-herpetic neuralgia. For long-term painful conditions it is essential to work with medical and nursing colleagues in Primary Care and in Palliative Care so that the patient receives appropriate support in hospital as well as in the community setting. Physiotherapy care can also be appropriate, particularly with chronic joint damage (e.g. in haemophilic arthropathy), and orthopaedic referral is often useful.

The severe acute pain in sickle crises often requires high and frequent doses of strong opiates. Health professionals unused to caring for patients with these conditions are sometimes cautious about use of such high doses and fear that patients may become addicted. Situations can arise whereby the patients are not receiving appropriate care because of such fears. These can be generally resolved by good communication and education. Electronic note keeping and databases can help in communication. Psychological dependence on opiates is rare, and may be associated with difficult psychosocial circumstances which makes care for such patients challenging.

## Psychological support

Many haematological disorders are long-term conditions; the specific diagnosis can be seen to 'label' the patient as different or ill and therefore will exert a profound influence on their life and that of their immediate family or carers. Patients (and their families) experience and demonstrate a number of reactions to their diagnosis, the clinical haematologist needs to have an awareness of this and respond accordingly.

### Reactions to serious diagnosis include:

- Numbness.
- Denial.
- Anger.
- Guilt.
- Depression.
- Loneliness.

Ultimately most patients come to acceptance of their condition; carers/partners will also go through a similar range of reactions. The clinician needs to be aware of the way in which news of a diagnosis is likely to affect a patient and their family/carers and respond appropriately. In the first instance this will often involve the need to impart the diagnosis, what it means, and what needs to be done clinically. There is no 'right way' to impart bad or difficult news. It is very important to make and take time to tell the patient of the diagnosis. Wherever possible this should be done in a quiet, private setting. The presence of an experienced Clinical Nurse Specialist is advised. Numbness at learning of a serious diagnosis often means that very little is taken in initially other than the diagnostic label. The various reactions just listed may subsequently emerge during the time the patient comes to accept the diagnosis, what it means, and what is to be done clinically. Literature can be very useful to convey disease information.

Within the haematological team there should be support available to the patient and family/carers which can provide them with practical information about the disease and its management. Simply knowing there is a sympathetic ear may be all that is required in the way of support; however, for some patients and families/carers more specialized support may be needed, e.g. availability of formal counselling or access to psychological or psychiatric support.

Children may experience a variety of haematological diseases requiring admission to hospital. They will have ongoing needs with regards to schooling and exams. Specialist professionals such as play therapists can help families through frightening and confusing experiences.

Use can be made of local or national patient support groups; knowledge of others in similar predicaments can help diffuse anger and loneliness. Support groups can also be a valuable resource in providing information and experience which patients and families/carers find helpful.

*The most effective psychological support for haematological patients is to see them as individuals and not 'diseases'.*

*Clinical nurse specialists play an absolutely vital role and must where possible be involved in the patient's journey.*

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# Protocols and procedures

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## Note

Please check local protocols and guidelines since these may differ to those outlined in this handbook. Local guidelines should be followed.

## Acute leukaemia: investigations

- FBC, blood film, reticulocytes.
- ESR & CRP.
- Serum B<sub>12</sub>, red cell folate, and ferritin.
- Blood group, antibody screen, and DAT/DCT.
- Coagulation screen, PT, APTT, fibrinogen.
- BM aspirate for morphology, cytogenetics, immunophenotype (type peripheral blood if relevant) plus additional samples if required by clinical trials.
- BM trephine biopsy recommended at diagnosis.

### Biochemistry

- U&E, LFTs.
- Ca<sup>2+</sup>, phosphate, random glucose.
- LDH.

### Virology

- Hepatitis & HIV serology.
- CMV IgG and IgM.

### Immunology

- Serum immunoglobulins.
- Autoantibody screen profile.
- HLA type—class 1 always in case HLA matched platelets are required, class 1 and 2 if allogeneic transplant indicated.

### Bacteriology

- Baseline blood cultures.
- Throat swab.
- MSU.
- Stool for fungal culture.
- Swabs for MRSA.

### Cardiology

- ECG.
- Echocardiogram: if in cardiac failure, infective endocarditis, older patient, or suspected/significant cardiac history.

### Radiology

- CXR.
- Other imaging as guided by clinical picture (chloromas).

### Other

- If any evidence of severe dental caries or gum disease refer patient for dental assessment.
- Consider semen storage, or egg harvesting pre-chemo.



## Platelet reactions and refractoriness

Reactions to platelet transfusion are common and range from mild temperatures to rigors. The development of an urticarial rash is also frequently seen. Most reactions are 2° to plasma-proteins. When a transfusion reaction develops, the following steps should be taken:

- Stop the transfusion.
- Give 10mg chlorphenamine IV and 1g of paracetamol PO.
- Cover future transfusions with chlorphenamine and paracetamol 30min pre-transfusion.
- Hydrocortisone 100mg IV stat may be (sparingly) used for refractory reactions but not routinely recommended.
- Pethidine is a suitable alternative for severe reactions/rigors and is almost invariably effective. Give 25mg IV stat with repeat dose if necessary.
- The possibility of generation of HLA and platelet-specific antibodies should also be considered.

### Platelet refractoriness

More than 2/3 of patients receiving multiple transfusions with random platelets develop anti-HLA antibodies. Refractoriness is defined as failure of 2 consecutive transfusions to give corrected increment of  $>7.5 \times 10^9/L$  1h after platelet transfusion in absence of fever, infection, severe bleeding, splenomegaly, or DIC. See Box 15.1 for calculating platelet increment.

The most common physical mechanism is of platelet circulatory half-life reduction caused by concurrent sepsis or coagulopathy, e.g. DIC. Immunological causes include induction of anti-HLA antibodies due to allo-sensitization from previous transfusions or generation of anti-platelet antibodies such as in ITP. Investigation should be considered if platelet transfusions fail to maintain a platelet count  $>10 \times 10^9/L$  at all times.

### Proceed as follows

- Aim for group-specific platelet transfusions.
- Check FBC pre-platelet infusion, 1h and 12h post-infusion to assess the rate of platelet count reduction. Failure to show a rise of platelet count by at least  $10 \times 10^9/L$  at 1h or any rise after 12h post-infusion merits further testing.
- Samples should be sent to a blood transfusion centre for HLA-antibody screening (10mL EDTA samples and 20mL serum).
- The patient's own HLA type should be checked.
- If negative for HLA-antibodies, HPA-antibodies should be tested for.
- If HLA or HPA antibodies are identified, the provision of HLA or platelet antigen matched platelet products may improve the platelet transfusion responsiveness.
- In emergencies random platelets can and should be used if no HLA-matched platelets are available.



**Box 15.1 Calculating platelet increment  $[(P_1 - P_0) SA]/n$** 

$P_0$  = platelet count pre-transfusion ( $\times 10^9/L$ )

$P_1$  = platelet count post-transfusion ( $\times 10^9/L$ )

SA = surface area

n = number of units of platelets transfused

Corrected increment 60min after transfusion  $>7.5 \times 10^9/L$  indicates successful platelet transfusion ( $P_1 - P_0$ ).

## Prophylactic regimen for neutropenic patients

Infective risk is related to the severity and duration of neutropenia. Higher risk is associated with concurrent immunological defects, e.g. hypogammaglobulinaemia in myeloma, T-cell defects, e.g. in HIV, additional immunosuppressive agents, e.g. ciclosporin post-transplant, and older patients. Principal risk is from bacterial organisms but fungi and viruses, especially herpes (HSV, HZV) as well as CMV are also seen in prolonged neutropenia. Generally prophylaxis is only recommended for patients with prolonged phases of neutropenia.

### Typical protocols include:

- **Isolation procedures:** isolation rooms with +ve pressure filtered air. Strict hand washing by all patient contacts is the only isolation measure of universally proven benefit. Others include visitor restriction, gloves, aprons, gowns, masks, and full reverse barrier nursing.
- **Drinks:** avoid mains tap water. Avoid unpasteurized milk and freshly squeezed fruit juice.
- **Food:** avoid cream, ice-cream, soft, blue, or ripened cheeses, live yoghurt, raw eggs or derived foods, e.g. mayonnaise and soufflés, cold chicken, meat paté, raw fish/shellfish, unpeeled fresh vegetables/salads, unpeeled fruit, uncooked herbs and spices. These are general guidelines, a realistic approach with common sense should prevail.
- **General mouthcare:** antiseptic mouthwash (e.g. chlorhexidine gluconate) 4-hourly, swish and spit. For generalized ulceration use 0.9% saline mouthwash hourly, swish and spit. Oral antifungal prophylaxis could be with nystatin suspension 1mL 4-hourly swish and spit or swallow.
- **Antibacterial prophylaxis:** aim to alter flora and prevent exogenous colonization. Principal agents: ciprofloxacin and co-trimoxazole. Prophylactic ciprofloxacin during prolonged periods of neutropenia has shown a reduction on morbidity and in some studies mortality and is recommended. Local guidelines should be followed.
- **Antifungal prophylaxis:** a systemic azole compound is most routinely used. Fluconazole is inferior to itraconazole liquid. Posaconazole has been shown to be superior to both and is becoming more commonly used. Local guidelines should be followed.
- **Antiviral prophylaxis:** aciclovir is the most useful drug at preventing viral infection (mainly herpes reactivation). Generally 400mg BD is recommended throughout prolonged phases of neutropenia. Local guidelines should be followed.
- **Additional prophylaxis for specific situations:** history of, or radiological evidence of, tuberculosis (TB). Consideration should be given to standard anti-TB prophylaxis, e.g. isoniazid for 6 months particularly if prolonged neutropenia is expected. Splenectomized patient— at ↑ risk from encapsulated organisms particularly *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis*. Use phenoxymethylpenicillin 500mg od PO or erythromycin 250 mg bd PO if penicillin allergic as prophylaxis switching to high dose amoxicillin/cefotaxime if febrile.



## Guidelines for use of IV antibiotics in neutropenic patients

### Urgent/immediate action required if neutropenic patient develops:

- Single fever spike  $>38^{\circ}\text{C}$ .
- 2 fever spikes  $>37.5^{\circ}\text{C}$  1h apart.
- Symptoms or signs of sepsis or infection without fever (e.g. hypotension and tachycardia).

### Assessment

- Search for localizing symptoms or signs of infection.
- Full clinical examination noting BP, pulse, RR, and oxygen saturations. Examination including oral examination, chest, perineum, line sites, skin, and fundi.
- $\text{O}_2$  saturation (pulse oximetry).
- FBC, U&E, creatinine, LFTs, CRP, PT, APTT, fibrinogen.
- Perform a septic screen:
  - Blood cultures (10mL per bottle optimizes organism recovery) if central line present, take paired peripheral and central samples.
  - Single further blood culture set if non-response at 48–72h or condition changes.
  - Repeat cultures every 24h if ongoing fever.
- Swab relevant sites: wounds, central line exit site, throat.
- Sputum culture.
- MSU.
- Faeces if symptomatic incl. *C. difficile* toxin.
- Viral serology if clinically relevant.
- CXR.
- Other imaging as relevant, consider sinus CT.
- Consider bronchoalveolar lavage if chest infiltrates present.
- Consider risk of invasive fungal infection: HRCT chest if high risk and unresponsive to antibiotics.

### Empirical treatment

Start IV broad-spectrum antibiotics to provide adequate cover. Stop prophylactic ciprofloxacin. Local protocols are based on local experience and epidemiology of infections and organism. Follow local protocol where available. One possible protocol is shown here:

**1st line:** piperacillin-tazobactam 4.5g tds plus gentamicin 5–7mg/kg OD. If patient has history of penicillin allergy use ceftazidime 2g tds instead of piperacillin-tazobactam.

If suspected line infection (exit site inflammation, symptoms typically after line-use) add vancomycin 1g bd and consider line removal.

If there are signs of perianal sepsis, mucositis or intra-abdominal infection or if *C. difficile* is suspected add metronidazole 500mg tds IV/oral.

- Patients on od gentamicin should have pre-dose level checked 24h after 1st dose then twice weekly if satisfactory.

- Patients on vancomycin should have predose levels checked immediately before 3rd or 4th dose (2nd if renal impairment) then twice weekly if satisfactory.

### Reassess at 48h

*If no response to antibiotics and –ve blood cultures:*

- Consider switching to 2nd-line antibiotics, e.g. meropenem 1g tds ± vancomycin (if central line *in situ*).
- Consider line removal.
- Further imaging as clinically indicated.

### Reassess after further 48h

*If no response to antibiotics and –ve blood cultures:*

*If high risk for invasive fungal infection:*

Consider starting antifungal therapy if clinically suggestive or signs of invasive fungal infection on imaging (e.g. liposomal amphotericin 3–5mg/kg/d, including a 1mg test-dose). Stop prophylactic antifungal. Request HRCT and consider bronchoscopy with bronchio-alveolar lavage (BAL).

*If low risk for invasive fungal infection:*

Consider switching antibiotics after microbiology advice.

### Reassess after further 48–72h

- Further management empirical and individual. Discuss with microbiology. Sometimes switching antifungal agent might be indicated.

### Duration of therapy

If fever responds and cultures are –ve, continue anti-infective treatment until afebrile for 48h or minimum 5d course. If still neutropenic restart prophylactic anti-infectives. Antifungal therapy should be continued until complete response and neutrophil regeneration; no fixed duration can be recommended; oral voriconazole or posaconazole can facilitate early discharge during this period. Wherever possible switching to oral agents as soon as clinically possible is indicated.

### +ve cultures

Antibiotic therapy may be changed on the basis of +ve cultures and sensitivities at any time.

## Treatment of neutropenic sepsis: source unknown

▶▶ One of the most common haemato-oncological emergencies.


- May be defined as the presence of symptoms or signs of infection in a patient with an absolute neutrophil count of  $<1.0 \times 10^9/L$ . In practice, the neutrophil count is often  $<0.1 \times 10^9/L$ .
- Similar clinical picture also seen in neutrophil function disorders such as MDS despite  $\leftrightarrow$  neutrophil numbers.
- Can occur without pyrexia, especially patients on steroids.
- In neutropenic patients all new symptoms must be taken seriously and assessed for possible underlying infective causes. Known neutropenic patients or patients at risk of neutropenia must have easy and instant access to acute care/hospital admission.

### Immediate action

▶▶ Urgent clinical assessment. See  Septic shock, p.636 for further details; summary:

- Fluid resuscitation.
- Insert central catheter if not *in situ* and monitor CVP.
- Start O<sub>2</sub> by face mask if pulse oximetry shows saturations  $<95\%$ .
- Perform full septic screen.
- Give 1st dose of 1st-line antibiotics immediately. Follow established protocols.
- Monitor fluid balance chart/urine output.
- If BP not restored with appropriate fluid resuscitation, consideration should be given to inotropes.
- If O<sub>2</sub> saturations remain low despite 60% O<sub>2</sub> delivered by rebreathing mask, consider (non-invasive) ventilation.
- Discuss with senior colleague and alert ITU.

### Subsequent actions

- Amend antibiotics according to culture results.
- Follow neutropenic sepsis protocol as outlined on  p.680.
- Continue antibiotics for a minimum of 5d and often until neutrophil recovery.
- If cultures show central line to be source of sepsis, remove immediately if patient not responding.
- Consider central line removal at presentation if lines suspected source.



## Treatment of neutropenic sepsis: source known/suspected

### Central indwelling catheters

Very common. Organisms usually *Staph. epidermidis* but can be other *Staph.* spp. and even Gram -ve organisms. Line might appear fine, but there may be erythema/exudate around entry or exit sites of line, tenderness/erythema over SC tunnel or discomfort over line track. Suspect line if rigors/symptoms in period after line use/flush. Blood cultures must be taken from each lumen and peripherally. Add vancomycin 1g bd IV to neutropenic protocol. If no response or clinical deterioration, remove line immediately.

### Perianal or peridental

Common sites of infection in neutropenic patients. Perianal lesions may become secondarily infected if skin broken. Add metronidazole 500mg IV tds to standard therapy. Painful SC abscesses may form and may require surgical incision. Gum disease and localized tooth infections/abscesses are frequently seen. Arrange OPG and dental review as surgical intervention may be required in non-responders. If possible, best delayed until neutrophil recovery in most cases—then do electively before next course of chemotherapy.

### Atypical organisms

#### Risk group

- Patients with HIV infection, HCL, or post-SCT are at high risk.
- *Mycoplasma* and other atypicals are commonly found and usually community acquired (except *Legionella*); typically occur shortly after return to hospital and in patients with chronic lung disease.
- Follow local protocols, e.g. clarithromycin.

### *Pneumocystis jirovecii* pneumonia

#### Risk group

Lymphoid malignancy on long-term treatment esp. ALL, steroid usage, prolonged neutropenia/immune-suppression, purine analogues, e.g. fludarabine and cladribine.

#### Treatment

High-dose co-trimoxazole IV initially—watch renal function and adjust dose to creatinine. Consider giving short pulse of steroid 0.5mg/kg at start of treatment. At-risk patients should remain on long-term prophylaxis until chemo finished and lymphocyte count is  $>1.0$  and/or absolute CD4 lymphocyte count  $>500 \times 10^6/L$ . Use co-trimoxazole 480mg bd on Monday, Wednesday, and Friday only, provided neutrophil count maintained  $>1.0 \times 10^9/L$ . Otherwise, or in case of allergy, use nebulized pentamidine 300mg every 3–4 weeks with preceding nebulized salbutamol 2.5mg.



## Fungal

### Risk group

Prolonged severe neutropenia, chronic steroid and antibiotic usage, GvHD. *Aspergillus* and other moulds increasingly common with intensive chemotherapy protocols and post-stem cell transplant.

### Treatment

- Follow local protocols. AmBisome® 3–5 mg/kg frequently used as 1st line. Treatment duration decided on an individual basis, but usually at least until neutrophil recovery.
- Voriconazole should be used in suspected/proven fungal CNS infections.
- Caspofungin can be used as an alternative for 1st-line treatment of invasive fungal infections.
- Switching to oral agents (posaconazole or voriconazole) should be considered as soon as clinically feasible.

## Viral: CMV

### Risk group

Allogeneic SCT where donor or recipient is CMV +ve. Disease usually due to reactivation rather than *de novo* infection. Apart from pneumonitis, may cause graft suppression, gastritis, oesophagitis, weight loss, hepatitis, retinitis, haemorrhagic cystitis, colitis, and vertigo.

### Treatment

Ganciclovir or foscarnet. Consider switching agent if lack of response. Monitor by CMV-PCR in PB.

## Viral: HSV/HZV

### Risk group

SCT patients and patients on prolonged treatment of lymphoid malignancy. Rare causes of lung disease.

### Treatment

High-dose IV aciclovir 5–10mg/kg tds IV for minimum 10d.

## Viral: RSV

### Risk group

Post-SCT recipients.

### Treatment

Consider ribavirin nebulizers.

## TB and atypical mycobacteria

### Risk group

Prolonged T-cell immunosuppression e.g. chronic steroid or ciclosporin therapy, chronic GvHD, previous history and/or treatment for TB, uncontrolled or newly-diagnosed HIV.

### Treatment

Often difficult to diagnose; empirical treatment required with standard triple therapy.

## Prophylaxis for patients treated with purine analogues

The purine analogues fludarabine and cladribine used in standard lymphoproliferative protocols induce neutropenia in all cases. Nadir ~14d post-treatment initiation and neutrophil counts may fall to zero for several days or even weeks. They are associated with the usual neutropenic infections.

In addition, purine analogues have a particular property of inhibition of CD4 helper lymphocyte subsets within weeks of initiation of therapy (nadir at 3 months) and may last for >1 year following cessation of therapy. This profound CD4 function inhibition predisposes to fungal infection, as well as a higher incidence of HZV infection and *P. jirovecii*. Reduced lymphocyte function also predisposes to transfusion associated GvHD by passerger lymphocytes in donor blood transfusions.

### **The following preventive measures are recommended:**

#### *Recommended*

- Irradiation of all cellular blood products (2500cGy) from d1 of initiation of therapy and continued until at least 2 years post-treatment.
- *P. jirovecii* prophylaxis from start of therapy—usually co-trimoxazole 480mg bd Mondays, Wednesdays, and Fridays. In patients who are allergic or already severely neutropenic, co-trimoxazole may be substituted by pentamidine nebulizers 300mg 4-weekly with 2.5mg of salbutamol nebulizer pre-treatment. *P. jirovecii* prophylaxis should continue for 3 months after the end of treatment.
- HZV prophylaxis—aciclovir 400mg qds if considered. Most physicians will not wish to have patients continuously on this dosage throughout treatment and for a year post-treatment so suggest: counsel patients about the risk of shingles and advise to contact the hospital immediately if shingles suspected. Aciclovir 400mg BD can be used, but dose below optimum recommended dose.

#### *Optional*

- Antibacterial prophylaxis; consider use of ciprofloxacin 250mg bd PO from d7 → d21 of each course. Not standard practice.
- Antifungal prophylaxis: follow local protocol. Fluconazole, itraconazole (liquid), and posaconazole used.



## Tumour lysis syndrome

Potentially life-threatening metabolic derangement resulting from treatment-induced or spontaneous tumour necrosis causing renal, cardiac, or neurological complications. Usually occurs in rapidly proliferating, highly chemosensitive neoplasms with high tumour load: leukaemias with high WBC counts (mainly ALL or AML; rarely CML, CLL, PLL, or ATLL) and high-grade NHL (particularly Burkitt lymphoma or DLBCL). May occur before or up to 5d (usually 24–72h) after initiation of chemotherapy.

### Pathophysiology and clinical features

Rapid lysis of large numbers of tumour cells releases intracellular electrolytes and metabolites into the circulation causing numerous metabolic abnormalities to develop rapidly:

- *Hyperuricaemia* due to metabolism of nucleic acid purines; (solubility ↓ by high acidity); may cause arthralgia and renal colic.
- *Hyperkalaemia* due to rapid cell lysis; often earliest sign of TLS; aggravated by renal failure; may cause paraesthesia, muscle weakness, and arrhythmias.
- *Hyperphosphataemia* due to rapid cell lysis; precipitates calcium phosphate in tissues.
- *Hypocalcaemia* 2° to hyperphosphataemia; may cause paraesthesia, tetany, carpo-pedal spasm, altered mental state, seizures, and arrhythmias.
- *Acute kidney injury* predisposed by volume depletion and pre-existing dysfunction; due to uric acid nephropathy, acute nephrocalcinosis, and precipitation of xanthine; oliguria/anuria leads to volume overload and pulmonary oedema; uraemia causes malaise, lethargy, nausea, anorexia, pruritus, and pericarditis; may require dialysis; usually reversible with prompt therapy.

### Principles of management

- Identify high-risk patients, initiate preventative measures prior to chemotherapy, and monitor for clinical and laboratory features of TLS.
- Detect features of TLS promptly and initiate supportive therapy early.
- Raise/increase staff awareness of possible TLS.

### Prevention and management

- Monitor weight, strict fluid balance, renal function, serum K<sup>+</sup>, phosphate, Ca<sup>2+</sup>, uric acid, and ECG. Twice daily for first 48h in patients with high clinical likelihood of TLS. Monitor parameters at least 2× daily in patients with TLS.
- Ensure aggressive IV hydration:
  - Aim for urine output >100mL/h (>3L/d) from prior to chemotherapy and in high-risk patients, until 48–72h after initiation of chemotherapy.
  - Furosemide (20mg IV) may be given cautiously to maintain adequate diuresis in well-hydrated patients; may be used to treat hyperkalaemia or fluid overload but may cause uric acid or Ca<sup>2+</sup> deposition in dehydrated patients; no proven benefit in initial treatment of TLS and not routinely given.

- Prevent hyperuricaemia: allopurinol and rasburicase not given together.
  - *Allopurinol*: xanthine oxidase inhibitor; prevents uric acid formation.
  - Dose: 300–600mg/d PO for prophylaxis if normal renal function (100mg/d if creatine >100mmol/L).
  - Side effects: rash, xanthine urolithiasis; reduce dose in renal impairment or mercaptopurine, 6-thioguanine or azathioprine therapy.
  - *Rasburicase*: recombinant urate oxidase; converts uric acid to water-soluble metabolites without increasing excretion of xanthine and other purine metabolites; very rapidly reduces uric acid levels and simplifies management of high-risk patients.
  - Dose 200mcg/kg daily IVI over 30min for up to 5–7d; recommended in all BL, high count leukaemia and as rescue treatment in hyperuricaemia plus rapidly rising creatinine, oliguria, phosphate  $\geq 2\text{mmol/L}$  or  $\text{K}^+ \geq 5.5\text{mmol/L}$ .
  - Side effects: fever, nausea, vomiting; less common: haemolysis, allergic reactions or anaphylaxis; contraindicated in G6PD deficiency and pregnancy.
- Alkalinization of urine:
  - Not routinely done; requires close monitoring.
  - Administer  $\text{NaHCO}_3$  PO (3g every 2h) or IV through central line to  $\uparrow$  urinary pH to 7.0 and maximize uric acid solubility.
  - Risk of more severe symptoms or hypocalcaemia and  $\uparrow$   $\text{CaPO}_4$  precipitation in tubules. Requires close monitoring of urinary pH (test all urine passed), serum bicarbonate, and uric acid; withdraw IV sodium bicarbonate when serum bicarbonate  $>30\text{mmol/L}$ , urinary pH  $>7.5$  or serum uric acid normalized.
- Control of electrolytes:
  - Hyperkalaemia: treat aggressively:
    - Restrict dietary  $\text{K}^+$  intake and eliminate  $\text{K}^+$  from IV fluids.
    - Use  $\text{K}^+$  wasting diuretics with caution.
    - $\text{K}^+ >5\text{mmol/L}$ , start  $\text{Ca}^{2+}$  resonium 15g PO qds and  $\uparrow$  hydration; recheck  $\text{K}^+$  after 2h.
    - $\text{K}^+ >6\text{mmol/L}$ , check ECG; commence IVI of 50mL 50% dextrose with 20 units of soluble insulin over 1h.
    - ECG changes or  $\text{K}^+ >6.5\text{mmol/L}$ , give 10mL  $\text{Ca}^{2+}$  gluconate 10% or  $\text{CaCl}_2$  10% IV cardioprotection.
  - Hyperphosphataemia:
    - Commence oral phosphate binding agent.
    - Infuse 50mL 50% dextrose with 20 units of soluble insulin IV over 1h.
- Dialysis:
  - Seek renal and critical care consultations early if initial measures fail to control electrolyte abnormalities or renal failure. Dialysis indicated if persistent hyperkalaemia ( $>6\text{mmol/L}$ ) or hyperphosphataemia ( $>3\text{mmol/L}$ ) despite treatment, fluid overload, rising urea or creatinine, hyperuricaemia ( $>0.6\text{mmol/L}$ ), or symptomatic hypocalcaemia.

## Administration of chemotherapy

Cytotoxic chemotherapeutic drugs may cause serious harm if not prescribed, dispensed, and administered with great care. Drugs should be prescribed, dispensed, and administered by an experienced multidisciplinary team with shared clear information on:

- The fitness of the patient to receive chemotherapy: clinical and based on blood tests (recent FBC and biochemistry).
- Appropriate protocol and chemotherapeutic regimen for the patient. All treatment should be discussed in a multidisciplinary setting.
- Prescribed drugs and individualized dosage for the patient's surface area or weight. Different calculations for BSA exist: most chemotherapy is now prescribed through an electronic prescribing system, where the BSA is calculated; smart phones apps also used.
- Safe cumulative maximum doses (e.g. anthracyclines) with safeguarding mechanism to monitor this.
- Appropriate supportive treatment required, e.g. allopurinol, antiemetic prophylaxis, anti-infective prophylaxis, and hydration. Different for each schedule.

Chemotherapy for IV administration should be reformulated carefully in accordance with the manufacturer's instructions by an experienced pharmacist using a class B laminar airflow hood or isolator. Care should be taken to ensure that the drug is administered within the expiry time after it has been reformulated in the form chosen.

Many cytotoxic drugs are best administered as a slow IVI in glucose or 0.9% saline over 30min–2h. Vesicants, (e.g. mitoxantrone, daunorubicin) should be administered as a slow IV 'push'. However this should only be administered through the side access port of a freely flowing infusion of 0.9% saline or dextrose and should never be injected directly into a peripheral vein.

NB Following a 2008 UK National Patient Safety Agency alert, vinca-alkaloids must be administered as a short IV infusion (not in syringe or via IV 'push') to avoid the risk of inadvertent intrathecal administration.

If the patient does not have an indwelling IV catheter (central line, Hickman line, or other), an IV cannula of adequate bore should be inserted into a vein to permit a freely flowing 0.9% saline infusion to be commenced. The site chosen should be one where the cannula can be easily inserted and observed, can be fastened securely, and will not be subject to movement during drug administration. The veins of the forearm are the most suitable for this purpose followed by those on the dorsum of the hand. The antecubital fossae and other sites close to joints are best avoided. The risk of extravasation is ↑ by the use of a cannula which has not been inserted recently and by the use of steel (butterfly) cannulae.

A slow 'push' injection should be administered carefully into the side access port on the IV line with continuous observation of the drip chamber to ensure that the infusion is continuing to run during injection of the cytotoxic drug. The patient should be asked whether any untoward sensations are being experienced at the site of the infusion and the site

should be carefully observed to ensure that no extravasation is occurring. Patency of the IV site should be verified regularly throughout the procedure.

The administration of potentially extravasable chemotherapy, site of cannulation, condition of the site, and any symptoms associated with administration should be clearly documented in the patient's notes.

### **Prevention of extravasation**

- Use a peripherally inserted central catheter line or other central line for slow infusion of high-risk cytotoxics.
- Administer cytotoxics through a recently sited cannula located where it cannot be dislodged, e.g. the forearm rather than near joints.
- Administer vesicants by slow IV push into the side arm port of a fast-running IV infusion of compatible solution.
- Administer the most vesicant drug first.
- Assess a peripheral site continuously for signs of redness or swelling.
- Verify the patency of the IV site prior to vesicant infusion and regularly throughout. If in doubt, stop and investigate. Resite if not satisfactory.
- Advise the patient to report any symptoms of pain, burning, or discomfort at the infusion site.
- Never hurry. Administer cytotoxics slowly to allow dilution and assessment of the infusion site.

## Antiemetics for chemotherapy

### Classification of antiemetics

**Dopamine antagonists:** block D<sub>2</sub> receptors in the chemoreceptor trigger zone (CTZ). Examples are metoclopramide and domperidone—both have additional effects on enhancing gastric emptying. Side effects with metoclopramide, include extrapyramidal reactions and occasionally oculogyric crisis (younger female patients at most risk therefore use domperidone).

**Phenothiazines:** examples are prochlorperazine and cyclizine—particular benefit in opioid-induced nausea. Side effects include anticholinergic effects and drowsiness.

**Benzodiazepine:** lorazepam most commonly used. Advantages are long t<sub>1/2</sub> and additional anxiolytic effect. Side effects include drowsiness.

**5-HT<sub>3</sub> antagonists:** block 5-HT<sub>3</sub> receptors in the CTZ. Examples include ondansetron and granisetron. Side effects include headaches, bowel disturbance, and rashes.

**Cannabinoids:** nabilone is the major drug. Side effects include depersonalization experiences.

**Steroids:** examples are dexamethasone and prednisolone. Side effects include predisposition to fungal infection, hypertension, irritability and sleeplessness, gastric erosions and, with chronic use, diabetes and osteoporosis.

**NK1 receptor antagonists:** e.g. aprepitant, used in addition to dexamethasone and 5-HT<sub>3</sub> antagonist for highly emetic chemotherapy, e.g. high-dose cisplatin.

### Emesis with chemotherapy

Categorized as anticipatory, acute, or delayed.

**Anticipatory:** occurs days to hours in advance of chemotherapy. Psychogenic in origin, it occurs in patients with previous bad experiences of nausea and vomiting and almost unknown prior to 1st dose. May be largely prevented by ensuring a positive experience with 1st dose by use of prophylactic antiemetics.

**Acute:** occurs <24h, sometimes within minutes of IV chemotherapy or within hours of oral chemotherapy. Generally responds best to antiemetics.

**Delayed:** occurs >24h after the end of a chemotherapy course, up to 6–7d. The most difficult form to treat; requires continuation of antiemetics throughout post-chemo period and even the newer agents such as the 5-HT<sub>3</sub> receptor antagonists are relatively ineffective.

**Antiemetics:** may be used singly or in combination and should be administered regularly, prophylactically and orally. Optimal control in the early phase is essential to prevent nausea and vomiting in the late phase. IV antiemetics are only required if patient is unable to take oral drugs. Choice of antiemetic is determined largely by patient preferences and



degree of emetic potential of the chemotherapy regimen used, classed as high, moderate high, moderately low, and low.

**Risk factors:** female sex, age <30 years, history of sickness in pregnancy or with travel, prior chemotherapy-induced nausea/vomiting, anxiety. Other factors: radiotherapy, other medications, metabolic disorders, constipation, GI obstruction. If  $\geq 3$  factors, consider additional antiemetic therapy from start of chemotherapy.

### Grading of nausea and vomiting

- Grade 1: able to eat; vomiting: 1 episode/24h.
- Grade 2: oral intake significantly ↓; vomiting: 2–5 episodes/24h.
- Grade 3: no significant intake, requiring IV fluids; vomiting  $\geq 6$  episodes/24h.
- Grade 4: requiring TPN, or physiological consequences requiring ITU; haemodynamic collapse.

### High emetic risk

Cisplatin  $\geq 50\text{mg}/\text{m}^2$ , high-dose cyclophosphamide  $>1.5\text{g}/\text{m}^2$ , carmustine  $>250\text{mg}/\text{m}^2$ , dacarbazine, chlormethine (mustine), TBI.

### Moderately high emetic risk

Cisplatin  $<50\text{mg}/\text{m}^2$ , daunorubicin, doxorubicin  $\geq 60\text{mg}/\text{m}^2$ , idarubicin, cytarabine  $\geq 1\text{g}/\text{m}^2$ , ifosfamide, carboplatin, carmustine  $\leq 250\text{mg}/\text{m}^2$ , lomustine.

### Moderately low emetic risk

Doxorubicin  $<60\text{mg}/\text{m}^2$ , mitoxantrone, gemcitabine, etoposide, methotrexate (MTX)  $50\text{--}250\text{mg}/\text{m}^2$ , oral cyclophosphamide, oral procarbazine.

### Low emetic risk

Examples include oral chlorambucil, oral melphalan, oral busulfan, oral hydroxycarbamide, oral 6-mercaptopurine, vinca-alkaloids, MTX  $<50\text{mg}/\text{m}^2$ , fludarabine, cladribine (2-CDA), bleomycin, asparaginase and most steroid-containing protocols.

### Antiemesis of high and moderately high emetic risk

#### FOLLOW LOCAL GUIDELINES

- *Acute:* immediately before chemotherapy: ondansetron 8mg IV plus dexamethasone 8mg IV plus ondansetron 8mg PO ~12h after IV ondansetron dose.
- *Delayed:* dexamethasone 2mg tds for 3d, starting the morning after chemotherapy plus domperidone 10–20mg PO tds regularly for 3d, then PRN.
- *Chemotherapy containing cisplatin  $>50\text{--}70\text{mg}/\text{m}^2$ :* as above plus 20–60min before chemotherapy: aprepitant 125mg PO in acute phase and aprepitant 80mg PO d2 and d3.

- *2nd-line; acute:* as for 1st-line, but substitute the single oral dose of ondansetron with ondansetron 8mg PO + 8h and + 16h after IV ondansetron. Consider need for lorazepam pre-chemotherapy if any anticipatory nausea.
- *2nd-line; delayed:* substitute the single oral dose of ondansetron with: ondansetron 8mg PO every 12h × 3 doses, starting 12h after IV ondansetron. Increase the duration of dexamethasone up to 5–7d. Alternative options include levomepromazine or cyclizine as a substitute or in addition to domperidone.
- *2nd-line for grade 3 or 4 nausea/vomiting:* consider aprepitant. Aprepitant 125mg PO 20–60min before chemotherapy starts, followed by aprepitant 80mg PO od on the morning of d2 and d3.

### **Antiemesis of moderately low emetic risk**

- *Acute:* metoclopramide 10mg IV (or domperidone 20mg PO tds) plus dexamethasone 8mg IV.
- *Delayed:* dexamethasone 2mg tds for 3d starting the morning after chemotherapy plus domperidone 10–20mg PO tds regularly for 3d, then PRN.
- *2nd-line:* ondansetron and dexamethasone in acute phase. Consider all other alternatives (levomepromazine, cyclizine in addition to domperidone).

### **Antiemesis of low emetic risk**

- *Acute:* no routine prophylaxis required for most regimens. Consider metoclopramide 1–20mg PO tds/PRN or domperidone 20mg PO tds/PRN.
- *Delayed:* consider domperidone 20mg PO tds/PRN if required.
- *2nd-line:* dexamethasone 8mg PO/IV prior to chemotherapy plus domperidone 20mg PO tds/PRN) for 3–4d.

### **Other situations**

- If severe emesis occurred requiring hospital visit/admission (grade 3 or 4), consider aprepitant.
- Some schedules have many days of chemotherapy and the antiemetic doses of steroids (dexamethasone) might be preferred to be avoided (to avoid steroid side effects, infections). Consider alternative antiemetics in this case.



## Intrathecal chemotherapy

Administration of IT chemotherapy has to be controlled by strict (national) guidelines to prevent administration of the wrong chemotherapy agent intrathecally. This has happened more than once in the past with catastrophic effects. The guidelines used must require all medical staff, nursing staff, and pharmacists involved in the procedure to undergo training and annual refresher courses to remain eligible to make, administer, and support the administration of IT chemotherapy; this requires strict compliance with all grades of staff who may be involved in IT therapy, checking procedures and administration of IT therapy. IV chemotherapy due as part of the same cycle of treatment must be given on a separate day; IT chemotherapy must be administered in a designated area.

### Indications

- Given for both prophylaxis and treatment of CNS disease.
- May be used in addition to other CNS disease strategies such as high-dose IV MTX or craniospinal irradiation.
- CNS involvement is detected by biopsy, presence of blasts on CSF cytospin and/or abnormalities on MRI or CT.
- The only drugs used intrathecally are:
  - Methotrexate.
  - Cytarabine (Ara-C).
  - Hydrocortisone.
  - All have strict upper dosage limits; follow the protocol.

Never use any other cytotoxic drugs for intrathecal injection; fatal consequences may ensue. Keep local anaesthetic separate from the drugs to be given intrathecally as syringes could get mixed up.

### Common protocols

- CNS prophylaxis for high-grade NHL: MTX 10–12.5 mg 4–6 injections at weekly intervals or every 3 weeks coinciding with cycles of treatment
- ALL: CNS prophylaxis should strictly follow treatment protocols.
- CNS prophylaxis for AML: not routinely given anymore as prophylaxis.
- CNS treatment for acute leukaemias: triple IT regimens used (MTX 12.5–15mg, cytarabine 30–50mg and hydrocortisone 15mg). Usually given twice weekly until CSF clear of blasts then weekly to a maximum of 6 total courses. Consider using folinic acid rescue.

## Technique

- Platelet count should be checked prior to procedure. Generally Platelets  $>50 \times 10^9/L$  is recommended but local guidelines may vary.
- Standard contraindications to lumbar puncture apply; patient might need imaging or fundoscopy prior to procedure. Alternatives will be needed if contraindicated.
- Cytotoxics should be made up freshly in smallest possible volume in a sterile pharmacy.
- Consider GA only for children. No sedation for adults as patients should be involved in final checking procedure.
- Fine-bore needles should be used to avoid CSF leakage and post LP headache.
- Aim to remove the same volume of CSF as you are injecting intrathecally (may be several mL if giving triple chemotherapy).
- Take samples for CSF cytospin to determine blast cell concentration, microbiology for MC&S, biochemistry for protein and glucose.
- Check syringe cytotoxic dose carefully with another person before connecting.
- Connect syringe and aspirate gently to confirm position in CSF. Inject slowly, drawing back at intervals to reconfirm position. Disconnect syringe and connect other syringes in turn if giving 'triple' ITs.
- In triple IT, the hydrocortisone should be given last.
- Follow standard post-LP precautions. Flat bedrest 30–60min and adequate fluid intake. Document procedure in notes.
- Repeated IT chemotherapy carries risk of CSF leakage and post-LP headache. Manometry pre-injection may help assess whether less CSF should be withdrawn pre-injection.
- Symptoms of MTX-induced neurotoxicity occur in a low number of patients. Presents with features of meningo-encephalitis. Aetiology is unknown. Treat with short pulse of high-dose steroids. Do not give further IT MTX to these patients.

## Management of extravasation

Inappropriate or accidental administration of chemotherapy into SC tissue rather than into the IV compartment. Causes pain, erythema, and inflammation which may lead to sloughing of the skin and severe tissue necrosis. Appropriate early treatment can prevent the most serious consequences of extravasation. All chemotherapy units should have a protocol with which all staff administering chemotherapy are familiar. A checked/regularly updated extravasation kit for the management of extravasation giving first aid instructions and further directions should be readily available.

The risk of tissue damage relates to the drug's ability to bind to DNA, to kill replicating cells, to cause tissue or vascular dilatation and its pH, osmolarity, concentration, volume and formulation components, e.g. alcohol, polyethylene glycol.

Cytotoxic drugs are classified into 5 groups by their potential to cause serious necrosis when extravasated.

### *Group 1: neutrals*

Alentuzumab; asparaginase; bevacizumab; bortezomib; bleomycin; cetuximab; cladribine; cyclophosphamide; cytarabine; eribulin; fludauridine; gemcitabine; ifosfamide; ipilimumab; melphalan; pemetrexed; pentostatin; rituximab; thiotepa; beta-interferons; trastuzumab.

### *Group 2: inflammitants*

Raltitrexed; fluorouracil; methotrexate.

### *Group 3: irritants (cause local inflammation, pain, and necrosis)*

Bendamustine; carboplatin; etoposide; irinotecan; dexrazoxane.

### *Group 4: exfoliants*

Cisplatin; liposomal daunorubicin; docetaxel; liposomal doxorubicin; mitoxantrone; oxaliplatin; topotecan.

### *Group 5: vesicants*

Amsacrine; carmustine; dacarbazine; dactinomycin; daunorubicin; doxorubicin; epirubicin; idarubicin; mitomycin; paclitaxel; streptozocin; treosulfan; vinblastine; vincristine; vindesine; vinorelbine; vinflunine.

## Symptoms and signs

- Burning, stinging, or pain at the injection site.
- Induration, swelling, venous discolouration/erythema at the site.
- No blood return from the cannula.
- Reduced flow rate.
- ↑ resistance to administration.
- Severe phlebitis and/or local hypersensitivity.
- Local risk factors, e.g. difficult cannulation and one patient symptom.

### General treatment guidelines

- Stop the injection/infusion immediately.
- Disconnect the infusion/syringe.
- Withdraw as much of the drug as possible (via existing cannula or central venous access device).
- Mark area of skin with indelible pen and if possible take a photograph of the area as soon as possible.
- If appropriate, remove the peripheral cannula. Leave central lines in place.
- Establish whether a hot pack or cold pack should be used; for neutral drugs neither is required.
- In case of a large volume (depending on size and age of patient as well as the drug involved) extravasation of a vesicant or exfoliant, urgent saline flush-out of the extravasation area should be always be considered (best within 2h; no later than 24h after extravasation). For anthracycline extravasation, the flush-out technique should not be used in combination with dexrazoxane.
- Cytotoxics requiring *warm* pack: inject 1500IU hyaluronidase (in 1mL WFI) via pin-cushion SC injections in 0.1–0.2mL volumes around the site; apply a warm pack to aid the absorption of hyaluronidase (warm pack to remain *in situ* for 2–4h).
- Cytotoxics requiring *cold* pack, either:
  - Cold pack + hydrocortisone cream. Apply cold pack for 15–20min 3–4 times a day for up to 3d. Apply hydrocortisone 1% cream tds, as long as redness persists. **Or**
  - Cold pack + DMSO (98% dimethyl sulfoxide): apply within 10–25min of the extravasation occurring. Contact with normal skin should be minimized. Once DMSO dries, apply 1% hydrocortisone cream and 30min of cold compression. Repeat process every 2h for 24h then reduce frequency. **Or**
  - Consider dexrazoxane if extravasation of doxorubicin, idarubicin, epirubicin or daunorubicin occurs of  $\geq 5\text{mL}$  peripherally or any volume centrally; do *not* use DMSO in conjunction. Dexrazoxane is a DNA topoisomerase II drug that protects against tissue damage with certain anthracyclines. Side effects include nausea (very common) and neutropenia/thrombocytopenia (common).
- Elevate the limb if possible.
- Manage the situation symptomatically: analgesia as required.
- Appropriate follow-up should be organized.
- In some cases review by plastic surgery team recommended.
- Document the incident in the patient's notes.

## Specific procedures after extravasation of cytotoxics commonly used in haematology

See Table 15.1.

**Table 15.1** Immediate treatment required per cytotoxic drug.

Drug	Cold/warm pack	Treatment
Amsacrine	Cold	Hydrocortisone cream
Bendamustine	Cold	Hydrocortisone cream
Bleomycin	None	No antidote
Bortezomib	None	No antidote
Carbazitaxel	Warm	Hyaluronidase
Carboplatin	Cold	Hydrocortisone cream
Carmustine	Cold	Hydrocortisone cream
Cisplatin	Cold	Hydrocortisone cream
Cladribine	None	No antidote
Cyclophosphamide	None	No antidote
Cytarabine	None	No antidote
Dacarbazine	Cold	Hydrocortisone cream
Dactinomycin	Cold	DMSO
Daunorubicin	Cold	DMSO, consider dexrazoxane
Docetaxel	Warm	Hyaluronidase
Doxorubicin	Cold	DMSO, consider dexrazoxane
Epirubicin	Cold	DMSO, consider dexrazoxane
Eribulin	None	No antidote
Etoposide	Cold	Hydrocortisone cream
Fludarabine	None	No antidote
Fluorouracil	Cold	Hydrocortisone cream
Gemcitabine	None	No antidote
Idarubicin	Cold	DMSO, consider dexrazoxane
Ifosfamide	None	No antidote
Irinotecan	Cold	Hydrocortisone cream
Liposomal daunorubicin	Cold	DMSO (for 10–14d)
Liposomal doxorubicin	Cold	DMSO (for 10–14d)
Melphalan	None	No antidote
Methotrexate	Cold	Hydrocortisone cream

(continued)



**Table 15.1** (continued) Immediate treatment required per cytotoxic drug.

Mitomycin	Cold	DMSO
Mitoxantrone	Cold	DMSO
Oxaliplatin	Warm	Hyaluronidase
Paclitaxel	Warm	Hyaluronidase
Pemetrexed	None	No antidote
Pentostatin	None	No antidote
Raltitrexed	Cold	Hydrocortisone cream
Streptozocin	Cold	Hydrocortisone cream
Topotecan	Cold	Hydrocortisone cream
Treosulfan	Cold	Hydrocortisone cream
Vinblastine	Warm	Hyaluronidase
Vincristine	Warm	Hyaluronidase
Vindesine	Warm	Hyaluronidase
Vinflunine	Warm	Hyaluronidase
Vinorelbine	Warm	Hyaluronidase

### Further reading

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## Anticoagulation therapy: heparin

For acute thrombosis DVT/PE start with heparin and warfarin simultaneously. Essential to confirm diagnosis—but start treatment whilst awaiting results of investigations. When warfarin stable—stop heparin.

### Heparin

Main advantage over oral anticoagulation is immediate anticoagulant effect and short  $t_{1/2}$ . Two main products: standard UFH, a mixture of polysaccharide chains, mean MW 15,000,  $t_{1/2}$  1.0–1.5h, and LMWH, fragments of UFH (mean MW 5000) with longer  $t_{1/2}$  (3–6h) and greater bioavailability. LMWH has significant advantages: 1 daily SC injection, no monitoring, no dose adjustment, low risk of HIT. Heparins act by potentiating coagulation inhibitor antithrombin resulting in antithrombin and anti-Xa activity. Both UFH and LMWH depend on renal clearance.

### Therapeutic anticoagulation

*LMWH*: given SC od on basis of weight (see individual products for dosage). Usually continued for 4–7d until warfarin effect, INR >2.0.

*Standard IV UFH*: initial IV bolus 5000IU in 0.9% saline given over 30min (lower loading dose for small adult/child). Follow with 15–25IU/kg/h using a solution of 25,000IU heparin in 50mL 0.9% saline (= 500IU/mL) and a motorized pump, e.g. for 80kg adult dose is  $80 \times 25 = 2000\text{IU/h}$ . Monitor IVI with APTT ratio, aim for ratio of 1.5–2.5, check 6h after starting treatment. Adjust dose as shown in Table 15.2.

Check APTT ratio 10h after dose change; daily thereafter. Use fresh venous sample—do not take from line. Continue heparin until INR in therapeutic range for warfarin—takes ~5d; massive ileo-femoral thrombosis and severe PE may require 7–10d of heparin.

### Contraindications

Caution if renal, hepatic impairment, recent surgery, known bleeding diathesis, severe hypertension.

### Immediate complications of therapy

- Bleeding occurs even when APTT ratio within the therapeutic range but risk ↑ with ↑ APTT ratio. Treatment: stop heparin until APTT ratio <2.5. In life-threatening bleeding use protamine sulfate: 1mg/100IU of heparin given in preceding hour.
- Thrombocytopenia—mild ↓ platelets common early in heparin therapy; not significant. Severe thrombocytopenia less common (HITT); occurs 6–10d after therapy begun; may be associated thrombosis. Stop heparin. Give alternative antithrombin drug such as lepirudin or danaparoid. Do not start warfarin until thrombocytopenia resolved.

### Prophylactic anticoagulation

LMWH now used in preference to UFH. LMWH given at low dose SC od. Recommended dose usually greater for orthopaedic surgery than general surgery. Continue until patient discharged and mobile.

- Moderate/high-risk patients—LMWH given 2h pre-op and once daily (☞ see *BNF* for dosage). UFH SC 5000IU 2h before surgery and bd until patient is mobile.
- Medical patients are also at risk of VTE and should be assessed for risk and considered for LMWH prophylaxis.

### Conclusions

The ↑ convenience and proven efficacy means that for most clinical situations LMWH will now be preferred to UFH.

**Table 15.2** Heparin infusion adjustment

APTR	>5.0	4.1–5.0	3.1–4.0	2.5–3.0	TARGET (1.5–2.5)	<1.2	10.5–2.5
Dose	Stop <sup>a</sup> ↓500U/h	↓300U/h	↓100U/h	↓50U/h	No change	↑400U/h	↑↑200U/h

<sup>a</sup> Nil for 0.5–1.0h; check APTT ratio.

**Doses and dose adjustments (UFH) should follow local guidelines.**

### Further reading

Weitz, J.I. (1997). Low-molecular-weight heparins. *N Engl J Med*, **337**, 688–98.

## Oral anticoagulation with VKA

Warfarin is the drug of choice; few side effects, well tolerated. A vitamin K antagonist (VKA), it takes ~72h to be effective; stable state takes 5–7d.  $t_{1/2}$  ~35h. Circulates mainly bound to albumin; free warfarin is active. Many drugs ↑ warfarin effect by displacing it from albumin. Monitored by PT using the international normalized ratio (INR).

### Administration

Given daily. Usually given with heparin on d1. If massive thrombosis, delay warfarin for 2–3d. Standard adult regimen = 10mg/d for 2d. Load with caution using reduced dose if liver disease, interacting drugs, patient >80 years. Check INR <1.4 before loading. Check INR daily for 1st 4d<sup>1</sup> on d3, ~16h after 2nd dose, and adjust as follows.

Target INR usually 2.5 except for mechanical heart valves in mitral position when target is 3.0 or 3.5.

### Complications

Haemorrhage. Easy bruising common within therapeutic range—is patient on aspirin? Rate of major bleeds ~2.7/100 treatment years, ↑ age ↑ INR. Rare side effects—alopecia, warfarin-induced skin necrosis, hypersensitivity, purple toe syndrome.

See ↻ Warfarin overdose, p.656 for details.

### Asymptomatic patient

- INR >5.0: stop warfarin and reduce dose by at least 25%. Check INR within 1 week.
- INR >8.0: consider oral vitamin K 1–5mg.

### Symptomatic patient

Moderate bleeding, INR 5.0–8.0, give vitamin K 1mg slowly IV. INR >8.0: give vitamin K 1mg and FFP or factor concentrate. Severe bleeding: vitamin K 5mg IV, and concentrate containing factors II, VII, IX, and X (e.g. Beriplex). Observe in hospital. Vitamin K reverses over-anticoagulation in 24h. Look for causes of over-anticoagulation, e.g. heart failure, alcohol, drugs.

### Reference

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### Further reading

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## Oral anticoagulation with NOAC

Dabigatran etexilate is an oral prodrug which is hydrolysed in the liver to the direct thrombin inhibitor dabigatran. Doses recommended for clinical use are 150 mg od, 220mg od, 110 mg bd, and 150mg bd. Peak plasma levels are reached 2–3h after ingestion. Dabigatran is 80% renally excreted with a  $t_{1/2}$  of ~13h with a GFR of >80 mL/min and 18h with a GFR of 30–50 mL/min. There is a dose-dependent effect of dabigatran on laboratory clotting tests.

Rivaroxaban is an oral direct inhibitor of factor Xa. Doses recommended for clinical use are 10mg od and 20mg od (15mg bd for first 3 weeks of treatment of DVT). Peak plasma levels are reached 2–3h after ingestion. Rivaroxaban is 33% renally excreted and in patients with normal renal function has a  $t_{1/2}$  of 9h. There is a dose-dependent effect of rivaroxaban on laboratory clotting tests.

For both drugs peak plasma concentrations are in the range of 100–400ng/mL. Trough concentrations are in the range of 20–150ng/mL.

Urgent assessment of the degree of anticoagulation may be required:

- Before surgery or invasive procedure when a patient has taken a drug in the previous 24h (or longer if CrCl <50mL/min).
- When a patient is bleeding.
- When a patient has taken an overdose.
- When a patient has developed renal failure.
- When a patient has thrombosis on treatment (to assess whether there is failure of therapy or lack of adherence).
- The APTT using most reagents can be used for urgent determination of the relative intensity of anticoagulation due to dabigatran. The APTT cannot be used to determine the drug level. A normal TT means the level of dabigatran must be very low.
- With an appropriate reagent the PT (or APTT) can be used for the urgent determination of the relative intensity of anticoagulation due to rivaroxaban. The PT is usually more sensitive. It cannot be used to determine the drug level.

## Management of needlestick injuries

Every doctor dealing with high-risk patients is concerned to prevent exposure to blood and body fluids, particularly a needlestick injury. The UK DoH published guidance on post-exposure prophylaxis (PEP) for HIV in 2008.<sup>1</sup> Your hospital/GP surgery should have a policy for the prevention and management of contamination incidents—check this out. Hospitals should have a 24 h service providing advice and treatment.

### Risk to healthcare workers

2 types of injury—*sharps injury* where intact skin is breached by sharp object contaminated with blood/blood-stained body fluids or unfixed tissue, and *contamination injury* where blood/blood-stained body fluid comes into contact with mucous membranes or non-intact skin. HBV and HIV are the 2 major concerns. All healthcare workers should be vaccinated against HBV. Risk of contracting HIV from percutaneous exposure to HIV-infected blood is ~0.3%. The amount of blood injected and a high viral load in the patient's blood ↑ the risk.

### General guidelines

#### Prevention

All healthcare workers must adopt universal precautions when handling blood/blood stained fluids—wear gloves, avoid blood spillage, use decontamination procedures if spillage occurs, label high-risk specimens, care with needles (do not resheath), disposal in burn bins, etc.

#### Immediate action in event of exposure

- Encourage bleeding and/or wash under running water.
- Contact Occupational Health/Emergency departments for help.
- Establish patient status re: blood-borne viruses.
- Take blood from patient/test for viruses (with consent).
- Take blood from needlestick victim and store. Check HBV immunity/ later tests if necessary.


### Treatment

- Decision to treat will be made by an experienced medical staff member. Treatment recommended for 'all healthcare workers exposed to high-risk body fluids or tissues known to be, or strongly suspected to be, infected with HIV through percutaneous exposure, mucous membrane exposure or through exposure of broken skin'. Zidovudine alone given as soon as possible ↓ risk of seroconversion by 80% but failures are well described. Prophylaxis with triple therapy now recommended. Treat for 4 weeks as soon as possible with:
  - Zidovudine 200mg tds/250mg bd + lamivudine 150mg bd + indinavir 800mg tds. A 'starter pack' should be available in an accessible place at all times.
- Known exposure to hepatitis B:
  - No immunity—give HepB Ig 500mg IM; vaccinate immediately.
  - Known immunity with HepB Ab >100IU/L in past 2 years—no action.
  - Immunity—HepB Ab status not known—give booster dose.

## Reference

1. Department of Health (2008). *HIV post-exposure prophylaxis: guidance from the UK Chief Medical Officers' Expert Advisory Group on AIDS*. J<sup>R</sup> [www.dhsspsni.gov.uk/hss-md-34-2008-attachment-1.pdf](http://www.dhsspsni.gov.uk/hss-md-34-2008-attachment-1.pdf)

## Chemotherapy protocols

The schedules discussed on  pp.711–743 are the most frequently used chemotherapy regimens. Where available, local guidelines should be followed. Recommendations on dose reduction, count recovery, and G-CSF use are also aimed at being guidance; local advice and guidance should be followed. Different schedules and combinations are in use with geographical differences.

### Protocols covered in the following topics

- ABVD
- BEACOPP/escalated BEACOPP
- BEAM/LEAM
- Bortezomib/dexamethasone
- CHOP 21 and CHOP14
- ChIVPP
- CODOX-M/IVAC  $\pm$  R
- CTD/CTDa
- DHAP  $\pm$  R
- ESHAP  $\pm$  R
- FC  $\pm$  R
- ICE  $\pm$  R
- Lenalidomide/dexamethasone
- MPT
- Mini-BEAM  $\pm$  R
- Nordic schedule (R-maxi-CHOP and R-high-dose cytarabine for MCL)
- PMitCEBO
- R-Bendamustine
- R-CHOP
- R-CVP and CVP
- Rituximab: monotherapy and maintenance therapy
- Stanford V.



# ABVD

## Indication

HL (1st line).

## Schedule

28d cycle (Table 15.3).

**Table 15.3** ABVD

Days	Drug	Dose	Route
1 & 15	Doxorubicin	25mg/m <sup>2</sup>	IV bolus
1 & 15	Bleomycin <sup>a</sup>	10,000IU/m <sup>2</sup>	IV in 500mL 0.9% saline
1 & 15	Vinblastine	6mg/m <sup>2</sup> IV (max. 10mg)	IVI in 50mL 0.9%
1 & 15	Dacarbazine	375mg/m <sup>2</sup>	IV in 250mL 0.9% saline over at least 60min. Saline over 5–10min

<sup>a</sup> Administer 100mg hydrocortisone IV before bleomycin when dexamethasone not used as an antiemetic.

## Administration

- Out-patient regimen.
- Aim to give treatment on time irrespective of neutrophil count. Avoid G-CSF unless clinically indicated.
- Consider sperm banking or egg-harvesting.
- Add allopurinol 300mg/d (100mg/d if creatinine clearance <20mL/min) for 1st cycle.
- Antiemetic therapy for highly emetogenic regimens.
- In patients with hepatic dysfunction, reduce doxorubicin and vinblastine to 50% dose for bilirubin 1.7–2.5 × ULN and reduce to 25% dose for bilirubin 2.5–4 × ULN.
- Number of treatments dependent on staging (2–8 cycles).
- Addition of involved field radiotherapy (IFRT) depending on staging and bulk of disease.
- Main toxicities: myelosuppression; alopecia; mucositis; pulmonary toxicity; cardiomyopathy; peripheral neuropathy; constipation; skin reactions to bleomycin; rigors during bleomycin infusion; vein pain during dacarbazine infusion; ovarian failure; infertility.

## BEACOPP/escalated BEACOPP

### Indication

HL (advanced stage, refractory/relapsed).

### Schedule

21d cycle (Table 15.4).

**Table 15.4** BEACOPP and escalated BEACOPP

Days	Drug	Standard dose	Escalated dose
1	Doxorubicin	25mg/m <sup>2</sup> IV bolus	35mg/m <sup>2</sup> IV bolus
1	Cyclophosphamide	650mg/m <sup>2</sup> IVI	1250mg/m <sup>2</sup> IVI
1–3	Etoposide <sup>a</sup>	100mg/m <sup>2</sup> /d IVI	200mg/m <sup>2</sup> /d IVI
1–7	Procarbazine	100mg/d PO	100mg/d PO
1–14	Prednisolone	40mg/m <sup>2</sup> /d PO	40mg/m <sup>2</sup> /d PO
8	Bleomycin <sup>b</sup>	10,000IU/m <sup>2</sup> IVI	10,000IU/m <sup>2</sup> IVI
8	Vincristine	1.4mg/m <sup>2</sup> IVI in 50mL 0.9% saline (max. 2mg)	1.4mg/m <sup>2</sup> IVI in 50mL 0.9% saline (max. 2mg)
9 until neutrophils >1.0x10 <sup>9</sup> /L	G-CSF	Only if necessary	Standard

<sup>a</sup> Etoposide may be given orally at 200mg/m<sup>2</sup>.

<sup>b</sup> Administer 100mg hydrocortisone IV before bleomycin when dexamethasone not used as an antiemetic.

### Administration

- Out-patient regimen.
- Consider sperm banking in or egg-harvesting.
- Add allopurinol 300mg/d (100mg/d if creatinine clearance <20mL/min) throughout first treatment cycle.
- Antiemetic therapy for highly emetogenic regimens from d1–8.
- Infection prophylaxis with co-trimoxazole and aciclovir recommended.
- Reduce bleomycin and etoposide doses to 75% for creatinine clearance 12–60mL/min and to 50% dose if <12mL/min.
- Reduce doxorubicin, etoposide and vincristine doses to 50% if bilirubin 1–2 × ULN, to 25% if 2–4 × ULN and omit if >4 × ULN.
- Withhold procarbazine if severe myelosuppression develops.
- Discontinue procarbazine if paraesthesia, neuropathy, confusion, stomatitis or diarrhoea; restart at reduced dose after recovery
- Consider G-CSF support after first episode of febrile neutropenia or delay of treatment ≥1 week.
- Repeat treatment if neutrophils ≥1.0 × 10<sup>9</sup>/L and platelets ≥100 × 10<sup>9</sup>/L.
- If it is considered essential to continue or delays of 2–3 weeks fail to achieve adequate recovery, consider dose-reduction.
- Administer every 3 weeks for 6–8 cycles.
- IFRT depending on site and bulk of disease.



## BEAM/LEAM

### Indications

Myeloablative conditioning regimen for autologous SCT in:

- Aggressive NHL with chemosensitive relapse or to consolidate remission in poor prognostic disease.
- HL: refractory or 2nd remission.
- Indolent NHL refractory to 2nd-line therapy.

### Schedule

Single cycle (Table 15.5).

**Table 15.5** BEAM and LEAM

Days	Drug	Dose	Route
-7	Carmustine (BCNU) in BEAM	300mg/m <sup>2</sup>	IVI in 500mL 5% glucose over 1h; avoid storage in PVC container for >24h
	Lomustine (CCNU) in LEAM <sup>a</sup>	200mg/m <sup>2</sup>	
-6 to -3 (inclusive)	Cytarabine	200mg/m <sup>2</sup> bd	IVI in 100mL 0.9% saline over 30 min
-6 to -3 (inclusive)	Etoposide	200mg/m <sup>2</sup> /d	IVI in 1L 0.9% saline over 2h
-2	Melphalan <sup>b</sup>	140mg/m <sup>2</sup>	IVI in 250mL 0.9% saline within 60min of reconstitution
0	Thaw and reinfuse haematopoietic stem cells <sup>c</sup>		

<sup>a</sup> Dose as per LACE protocol.<sup>1</sup>

<sup>b</sup> Ensure excretion of melphalan by aggressive hydration ( $\pm$  furosemide).

<sup>c</sup> Ensure stem cell dose  $\geq 2.0 \times 10^6$ /L CD34+ cells; do not re-infuse stem cells within 24h of melphalan infusion.

## Administration

- In-patient regimen.
- Ensure adequate venous access by inserting a dual lumen tunnelled central venous catheter.
- Severe myelosuppression is expected (neutrophils  $<0.1 \times 10^9/L$  and platelets  $<20 \times 10^9/L$ ).
- Add allopurinol 300mg (100mg if creatinine clearance  $<20\text{mL}/\text{min}$ ) od for 1st week unless remission status confirmed.
- Antiemetic therapy for highly emetogenic regimens.
- Give mouth care (nystatin and chlorhexidine mouthwash) and consider oral systemic antibacterial (e.g. ciprofloxacin).
- Give antifungal prophylaxis until neutrophil recovery  $\geq 1.0 \times 10^9/L$ —refer to local protocol for patients with severe neutropenia.
- Consider  $H_2$  antagonist or PPI.
- G-CSF to start once neutropenic  $<0.5 \times 10^9/L$  until count recovery. Consider use of pegylated G-CSF.
- Consider aciclovir antiviral prophylaxis.
- Consider *Pneumocystis jirovecii* prophylaxis for 6 months after count recovery; refer to local protocol (generally co-trimoxazole 480mg bd tiw).
- Do not use if creatinine clearance is  $<40\text{mL}/\text{min}$ .
- Main toxicities: diarrhoea, mucositis, alopecia, myelosuppression infertility.
- All patients must receive irradiated cellular blood components for at least 12 months post-SCT to prevent transfusion associated GvHD.

## Reference

1. Perz, J.B. et al. (2007). LACE-conditioned autologous stem cell transplantation for relapsed or refractory Hodgkin's lymphoma. *Bone Marrow Transplant*, **39**, 41–7.

## Bortezomib/dexamethasone

### Indications

- Multiple myeloma.
- AL amyloid.

### Schedule 1

21d cycle (Table 15.6).

**Table 15.6** Bortezomib-dexamethasone

Days	Drug	Dose	Route
1, 4, 8, 11	Bortezomib	1.3mg/m <sup>2</sup>	SC
1+2, 4+5, 8+9, 11+12	Dexamethasone	20mg	PO

### Schedule 2 (attenuated)

35d cycle (Table 15.7).

**Table 15.7** Bortezomib-dexamethasone

Days	Drug	Dose	Route
1, 8, 15, 22	Bortezomib	1.3 mg/m <sup>2</sup>	SC
1+2, 8+9, 15+16, 22+23	Dexamethasone	20mg	PO

### Administration

- Out-patient regimens.
- Bortezomib now generally given as SC bolus, but can be given IV.
- Bortezomib containing schedule preferred in patients with renal impairment.
- Schedule 2 preferred for patients >70 years old or patients with comorbidities.
- Weekly cyclophosphamide 500mg PO can be added to both schedules.
- Add allopurinol 300mg od PO (100mg if significant renal impairment) for first cycle.
- Antiemetic therapy for mildly emetogenic regimens.
- Give 4–8 cycles (to maximum response plus 2 cycles).
- Consider a dose reduction of bortezomib if CrCl <20mL/min.
- Hepatic impairment: use bortezomib with caution in mild to moderate hepatic impairment and consider a dose reduction—clearance is mainly via hepatic metabolism. Contraindicated in severe hepatic impairment. If bilirubin >1.5 × ULN, reduce bortezomib to 0.7 mg/m<sup>2</sup> in the 1st treatment cycle. Consider dose escalation to 1.0 mg/m<sup>2</sup>, or further dose reduction to 0.5 mg/m<sup>2</sup>, in subsequent cycles based on patient tolerability.

- Main toxicities: myelosuppression (thrombocytopenia common but recovers rapidly; neutropenia usually less severe); postural hypotension; rash; GI toxicity; peripheral neuropathy; exacerbation/development of heart failure; steroid side effects; injection site reactions; haemorrhagic cystitis, if cyclophosphamide included.

## CHOP 21 and CHOP 14

### Indications

- (Aggressive and low-grade) NHL.
- T-cell lymphoma.
- Rituximab may be added to either regimen (R-CHOP) for CD20+ NHL.

### Schedule

21d or 14d cycle (Tables 15.8 and 15.9).

**Table 15.8** CHOP 21: 21d cycle

Days	Drug	Dose	Route
1	Cyclophosphamide	750mg/m <sup>2</sup>	IVI in 250mL 0.9% saline over 30min
1	Vincristine	1.4mg/m <sup>2</sup> (max. 2mg <sup>a</sup> )	IVI in 50mL 0.9% saline over 5–10min
1	Doxorubicin	50mg/m <sup>2</sup>	IV bolus
1–5	Prednisolone	100 mg	PO in a.m. with food

<sup>a</sup> Cap vincristine at 1mg for patients aged >70 years

**Table 15.9** CHOP 14: 14d cycle

Days	Drug	Dose	Route
1	Cyclophosphamide	750mg/m <sup>2</sup>	IVI in 250mL 0.9% saline over 30min
1	Vincristine	1.4mg/m <sup>2</sup> (max. 2mg <sup>a</sup> )	IVI in 50mL 0.9% saline over 5–10min
1	Doxorubicin	50mg/m <sup>2</sup>	IV bolus
1–5	Prednisolone	100mg	PO in a.m. with food
4–13	G-CSF: filgrastim or lenograstim	5mcg/kg/d or 263mcg/d <sup>b</sup>	SC from d5 for 5–7d

<sup>a</sup> Cap vincristine at 1mg for patients aged >70 years

<sup>b</sup> G-CSF dose dependent on weight. Use 368mcg/d in patients >80kg



## Administration

- Out-patient regimen.
- Consider sperm banking or egg-harvesting.
- Add allopurinol 300mg/d (100mg if creatinine clearance  $<20\text{mL}/\text{min}$ ) throughout 1st treatment cycle.
- Antiemetic therapy for moderately emetogenic regimens.
- CNS prophylaxis with intrathecal MTX should be administered for certain patients (see Chapter 5). Some guidelines use high IPI (4–5) as indication for IT. For patients with BM, testicular, orbital, paranasal/nasopharyngeal or paraspinal involvement prophylaxis should be considered.
- In patients with renal impairment, reduce cyclophosphamide dose to 75% for creatinine clearance  $10\text{--}50\text{mL}/\text{min}$  and to 50% for clearance  $<10\text{mL}/\text{min}$ .
- In patients with hepatic dysfunction, reduce doxorubicin dose by 50% for bilirubin  $1.5\text{--}3 \times \text{ULN}$  and to 25% for bilirubin  $>3 \times \text{ULN}$ .
- Repeat treatment when neutrophils  $\geq 1.0 \times 10^9/\text{L}$  and platelets  $\geq 100 \times 10^9/\text{L}$ .
- Standard 6 cycles.
- Etoposide or mitoxantrone have sometimes been used to substitute doxorubicin in patients with previous cardiac disease.
- Main toxicities: myelosuppression; alopecia; mucositis; cardiomyopathy; peripheral neuropathy; constipation; haemorrhagic cystitis; ovarian failure; infertility; organ failure.
- Risk of TLS: ensure premedicated with allopurinol or rasburicase and good hydration.
- Consider 1° or 2° G-CSF prophylaxis.

## ChIVPP

### Indication

HL in elderly patients unfit for ABVD or other intensive 1st-line treatment. Possible salvage treatment in relapse.

### Schedule

28d cycle (Table 15.10).

**Table 15.10** ChIVPP

Days	Drug	Dose	Route
1–14	Chlorambucil	6mg/m <sup>2</sup> /d (max. 10mg)	PO
1 & 8	Vinblastine	6mg/m <sup>2</sup> (max. 10mg)	IVI in 50mL 0.9% saline over 5–10min
1–14	Procarbazine	100mg/m <sup>2</sup> /d (max. 150mg)	PO
1–14	Prednisolone	40mg	PO in a.m. with food

### Administration

- Out-patient regimen.
- Consider sperm banking or egg-harvesting.
- Add allopurinol 300mg/d (100mg if creatinine clearance <20mL/min) throughout 1st treatment cycle.
- Antiemetic therapy for moderately emetogenic regimens.
- Consider H<sub>2</sub>-antagonist or PPI.
- Alcohol prohibited with procarbazine; avoid tyramine containing foods.
- In patients with renal failure, consider dose reduction of procarbazine if creatinine >180micromol/L.
- In patients with hepatic dysfunction, reduce vinblastine dose to 50% if bilirubin 26–51micromol/L or AST 60–180IU/L; omit vinblastine if bilirubin >51micromol/L and AST/ALT >180IU/L; consider dose reduction of chlorambucil in severe hepatic dysfunction.
- Repeat treatment cycle when neutrophils >1.0 × 10<sup>9</sup>/L and platelets >100 × 10<sup>9</sup>/L, otherwise delay 1 week.
- Main toxicities: myelosuppression; constipation; peripheral neuropathy; stomatitis; alopecia; steroid side effects; ovarian failure; infertility.



**CODOX-M/IVAC ± R****Indication**

Burkitt lymphoma or Burkitt-like (grey-zone) lymphoma.

**Schedule**

Administer 2 alternating cycles of each regimen, i.e. A/B/A/B. Start each regimen as soon as possible after regeneration of neutrophils to  $>1.0 \times 10^9/L$  and unsupported platelets to  $>75 \times 10^9/L$ . In low-risk patients (low IPI) 3 cycles of CODOX-M can be considered (Tables 15.11 and 15.12).

**Table 15.11** Regimen A: CODOX-M

Days	Drug	Dose	Route
1	Cyclophosphamide	800mg/m <sup>2</sup>	IVI in 500mL 0.9% saline over 30min
2–5		200mg/m <sup>2</sup> /d	IVI in 250mL 0.9% saline over 15min × 4d
1 & 8	Vincristine	1.5mg/m <sup>2</sup> (max. 2mg)	IV bolus
1	Doxorubicin	40mg/m <sup>2</sup>	IVI in 50mL 0.9% Saline over 5–10min
1 & 3	Cytarabine <sup>a</sup>	70mg	IT injection
10	MTX <sup>b</sup>	300mg/m <sup>2</sup>	IVI in 250mL 0.9% saline over 60min
		2700mg/m <sup>2</sup>	IVI in 1L 0.9% saline over 23h
11	Calcium folinate <sup>c</sup>	15mg/m <sup>2</sup>	IV 36 h after <b>start</b> of MTX; repeat 3hrly for 12 h then 15mg/m <sup>2</sup> IV/PO 6hrly until serum MTX level $<5 \times 10^{-8}$ M
13 until neutrophils $>1.0 \times 10^9/L$	G-CSF (filgrastim or lenograstim)	5mcg/kg/d or 263mcg/d*	SC
15	MTX <sup>a</sup>	12mg	IT injection
16	Calcium folinate <sup>a</sup>	15mg	PO 24h after IT MTX

<sup>a</sup> In patients with CNS involvement at diagnosis, intensified CNS therapy is required during the first 2 cycles: cytarabine 70mg IT on d1, 3, & 5; MTX 12.5mg IT on d15 & 17; calcium folinate 15mg on d16 & 18.

<sup>b</sup> MTX should only be given if creatinine clearance  $>50\text{mL}/\text{min}/\text{m}^2$ . UK trials have used a lower dose of MTX than the original published regimen. Reduce MTX dose in patients aged  $>65$  years (d10: 100mg/m<sup>2</sup> IVI over 60min then 900mg/m<sup>2</sup> IVI over 23h). Alkalinize urine to pH  $\geq 7$  with 3L/m<sup>2</sup> IV fluids plus NaHCO<sub>3</sub> from 24h prior to start of MTX until serum MTX level  $<5 \times 10^{-8}$  M (i.e. until calcium folinate no longer required). Check serum MTX levels every 24h from 48h after start of MTX infusion.

<sup>c</sup> If 48h serum MTX level  $>2 \times 10^{-6}$  M (2.0micromols/L) dose of calcium folinate should be doubled. Calcium folinate may be administered PO after 24h of IV administration if patient not vomiting.

\*Consider higher dose of G-CSF if  $>80\text{kg}$  (368 mcg/d).

**Table 15.12** Regimen B: IVAC

Days	Drug	Dose	Route
1–5	Etoposide	60mg/m <sup>2</sup> /d	IVI in 500mL 0.9% saline over 60min
1–5	Mesna <sup>a</sup>	300mg/m <sup>2</sup> /d	IV bolus or IVI over 15min immediately before ifosfamide
		1500mg/m <sup>2</sup> /d	IVI mixed with ifosfamide in 500mL 0.9% saline over 60min
		900mg/m <sup>2</sup> /d	IVI in 500mL 0.9% saline over 12h
1–5	Ifosfamide <sup>b</sup>	1500mg/m <sup>2</sup> /d	IVI over 60min mixed with Mesna in 500mL 0.9% saline
1 & 2	Cytarabine <sup>c</sup>	2g/m <sup>2</sup> every 12h (4 doses)	IVI in 1L 0.9% saline over 3h
5	MTX <sup>d</sup>	12mg	IT injection
6	Calcium folinate <sup>d</sup>	15mg	PO 24h after IT MTX
7 until neutrophils >1.0 × 10 <sup>9</sup> /L	G-CSF (filgrastim or lenograstim)	5mcg/kg/d or 263mcg/d <sup>e</sup>	SC

<sup>a</sup> In patients aged >65 years, reduce mesna doses to 200mg/m<sup>2</sup> IVI over 15min, 100mg/m<sup>2</sup> over 60min with ifosfamide & 600mg/m<sup>2</sup> over 12h.

<sup>b</sup> In patients aged >65 years, reduce ifosfamide dose to 1000mg/m<sup>2</sup> IVI over 60min.

<sup>c</sup> Add prednisolone 0.5% eye drops tds for 5–7d during & after cytarabine. In patients aged >65 years, reduce cytarabine dose to 1g/m<sup>2</sup> IVI over 3h every 12h (total 4 doses).

<sup>d</sup> In patients with CNS involvement at diagnosis, intensified CNS therapy is required during the first two cycles: MTX 12.5mg IT on d5; calcium folinate 15mg PO on d6; cytarabine 70mg IT on d7 & 9.

<sup>e</sup> Consider higher dose of G-CSF if >80kg (368 mcg/d).

**Administration**

- Very intensive in-patient regimen; insert tunnelled central venous catheter if possible.
- Discuss risk of infertility and consider sperm storage. Often no time for egg harvesting due to clinical urgency of treatment.
- Check creatinine clearance before high-dose MTX.
- High risk of TLS; start IV hydration with  $\geq 4.5$  L/m<sup>2</sup>/d with furosemide PRN to maintain urine output.
- Commence rasburicase (0.2mg/kg for 5–7d) or allopurinol 300mg/d.
- Consider antibiotic and antiviral prophylaxis.
- Antifungal prophylaxis.
- Prophylactic PPI, omeprazole 20mg od or equivalent.
- CODOX-M highly emetogenic on d1, use 5-HT<sub>3</sub> antagonist plus metoclopramide 10mg qds or domperidone 20mg qds for 2d or cyclizine 50mg tds IV/ SC continuous infusion 150mg/d.
- IVAC d1–5 very highly emetogenic, use 5-HT<sub>3</sub> antagonist plus dexamethasone 8mg bd plus metoclopramide or domperidone or cyclizine.
- Main toxicities: myelosuppression; alopecia; mucositis; cardiomyopathy; peripheral neuropathy; constipation; organ failure; ovarian failure; infertility.
- Some centres add rituximab 375mg/m<sup>2</sup> to each cycle.



## CTD and CTDa

### Indications

- Multiple myeloma.
- AL amyloid.

### CTD schedule

21d cycle (Table 15.13).

**Table 15.13** CTD

Days	Drug	Dose	Route
1, 8, 15	Cyclophosphamide	500mg/d	PO
1–21	Thalidomide	50mg/d for 3 weeks then 100mg/d for 3 weeks then 200mg/d	PO
1–4 & 15–18	Dexamethasone <sup>a</sup>	40mg/d	PO

<sup>a</sup> Reduce d1–4 only from cycle 4 onwards.

### CTDa (attenuated) schedule

28d cycle (Table 15.14).

**Table 15.14** CTDa

Days	Drug	Dose	Route
1, 8, 15, 22	Cyclophosphamide	500mg/d	PO
1–21	Thalidomide	50mg/d for 4 weeks increasing by 50mg/d every 4 weeks to 200mg/d	PO
1–4 & 15–18	Dexamethasone <sup>a</sup>	20mg/d (alternative: 20–40mg weekly on d1, 8, 15, 22)	PO

<sup>a</sup> Reduce d1–4 only from cycle 4 onwards.

### Administration

- Out-patient regimens.
- CTDa in elderly patients with co-morbidities.
- Women of childbearing potential must have negative pregnancy test within 24h before starting thalidomide;
- All patients should be enrolled in a pregnancy-prevention programme.
- Add allopurinol 300mg od PO (100mg if significant renal impairment) for 1st cycle.
- Consider VTE prophylaxis: add aspirin (75mg/d) for patients at standard risk of thrombosis; add prophylactic dose LMWH for high-risk patients.
- Antiemetic therapy for mildly emetogenic regimens.
- Commence H<sub>2</sub> antagonist or PPI.



- Consider regular laxative.
- Do not give cyclophosphamide if serum creatinine >300micromol/L after rehydration.
- Consider G-CSF if treatment delays are prolonged or frequent.
- Omit thalidomide for 1 cycle if grade 3/4 constipation, neuropathy, fatigue, sedation, rash, tremor, or oedema; reintroduce at 50mg/d.
- Repeat for 4–6 cycles.
- Main toxicities: myelosuppression; haemorrhagic cystitis; teratogenicity; sedation (take thalidomide at bedtime); dry skin or rash; peripheral neuropathy; dizziness; bradycardia and syncope; alopecia (mild); constipation (often requiring laxatives); ↑ risk of thromboembolic events; steroid side effects.

## DHAP ± R

### Indications

- Salvage chemotherapy for relapsed/refractory NHL and HL.
- ± followed by mobilization of peripheral blood stem cells.

### Schedule

21–28d cycle depending on count recovery (Table 15.15).

**Table 15.15** DHAP±R

Days	Drug	Dose	Route
–1	Rituximab	375mg/m <sup>2</sup>	IVI in 500mL 0.9% saline over 3–5h
1–4	Dexamethasone	40mg/d	PO in the morning with food
1	Cisplatin	<sup>a</sup> 100mg/m <sup>2</sup>	IVI in 500mL 0.9% saline over 1h
2	Cytarabine	<sup>a</sup> 2g/m <sup>2</sup> ×2	IVI in 1L 0.9% saline over 3h twice 12h apart

<sup>a</sup> Cap surface area at 2m<sup>2</sup>.

### Administration

- In-patient regimen.
- Ensure adequate venous access by inserting a dual-lumen tunnelled central venous catheter.
- Discuss risk of infertility and consider sperm storage.
- Main toxicities: severe myelosuppression; alopecia; mucositis; cardiomyopathy; peripheral neuropathy; constipation; haemorrhagic cystitis; ovarian failure; infertility; conjunctivitis/iritis.
- Allopurinol 300mg od PO (100mg if renal impairment) for 1st cycle.
- Consider aciclovir prophylaxis.
- Antiemetic therapy for highly emetogenic regimens.
- Aggressive pre- and post-hydration including potassium/magnesium supplementation is required with cisplatin.
- Prednisolone 0.5% eye-drops qds until 5d after completion of chemotherapy.
- Standard antimicrobial prophylaxis as dictated by local policy to cover duration of severe neutropenia.
- G-CSF daily starting day +5 optional to shorten neutropenia and necessary to mobilize peripheral blood stem cells.
- Reduce cisplatin to 75% dose if creatinine clearance 45–60mL/min, 50% dose if creatinine clearance 30–45mL/min; do not give if creatinine clearance <30mL/min. Creatinine clearance should be assessed before each course of treatment.
- Cytarabine should be used with caution in severe renal impairment; consider reducing dose of cytarabine if hepatic impairment.
- Delay next cycle for 1 week if neutrophils <1.0 × 10<sup>9</sup>/L or platelets <100 × 10<sup>9</sup>/L.

- Patients with HL and those in whom stem cell collection is planned within 2 weeks must receive irradiated cellular blood components to prevent transfusion-associated GvHD.
- 2–6 cycles in total but usually consolidated with high-dose therapy and autologous stem cell transplant in responding patients <65 years of age.

## ESHAP ± R

### Indications

- Treatment of refractory/relapsed aggressive NHL and HL.
- ± Mobilization of peripheral blood stem cells.

### Schedule

21–28d cycle as soon as neutrophils  $>1.0 \times 10^9/L$  and platelets (unsupported)  $>100 \times 10^9/L$  (Table 15.16).

**Table 15.16** ESHAP±R

Days	Drug	Dose	Route
0	Rituximab	375mg/m <sup>2</sup>	IVI in 500mL 0.9% saline over 3–5h
1	Cytarabine	<sup>a</sup> 2g/m <sup>2</sup>	IVI in 500mL 0.9% saline over 2h
1–4	Etoposide	40mg/m <sup>2</sup> /d	IVI in 250mL 0.9% saline over 60min
1–4	Cisplatin	25mg/m <sup>2</sup> /d	IVI in 1L 0.9% saline over 24h
1–5	Methylprednisolone	500mg/d	IVI in 100mL 0.9% saline over 30min

<sup>a</sup> Consider 1g/m<sup>2</sup> if  $>70$  years old

### Administration

- In-patient regimen.
- Ensure adequate venous access by inserting a dual-lumen tunnelled central venous catheter.
- Severe myelosuppression (neutrophils  $<0.1 \times 10^9/L$  and platelets  $<20 \times 10^9/L$ ) is expected.
- Add allopurinol 300mg (100mg if creatinine clearance  $<20\text{mL}/\text{min}$ ) od for 1st cycle.
- Discuss risk of infertility and consider sperm storage.
- Antiemetic therapy for highly emetogenic regimens.
- Aggressive pre- and post-hydration including potassium/magnesium supplementation required with cisplatin.
- Prednisolone 0.5% eye-drops qds until 5d after completion of chemotherapy.
- Mouth care (nystatin and chlorhexidine mouthwash).
- Antibacterial and antifungal prophylaxis until neutrophil recovery  $\geq 1.0 \times 10^9/L$ .
- Consider H<sub>2</sub> antagonist or PPI.
- Consider starting G-CSF on d7 either to shorten neutropenia or to facilitate peripheral blood stem cell collection around d16.
- Reduce cisplatin to 50% dose if creatinine clearance 40–60mL/min; do not give if creatinine clearance  $<40\text{mL}/\text{min}$ .
- Reduce cytarabine to 50% dose and omit etoposide if serum bilirubin  $>50\text{micromol}/L$ .
- Creatinine clearance should be assessed before each course of treatment.

- Patients with HL and those in whom stem cell collection is planned within 2 weeks must receive irradiated cellular blood components to prevent transfusion-associated GvHD.
- 2–6 cycles in total but usually consolidated with high-dose therapy and autologous stem cell transplant in responding patients <65 years of age.
- Main toxicities: myelosuppression; neuropathy; ototoxicity; nephrotoxicity; cytarabine syndrome, including conjunctivitis; mucositis; alopecia; steroid side effects; ovarian failure; infertility.

**FC ± R****Indications**

- CLL.
- NHL (including LPC-lymphoma, WM, MCL).

**Schedule**

28d cycle (Table 15.17).

**Table 15.17** FC ± R

Days	Drug	Dose	Route
1	Rituximab	<sup>a</sup> 375 mg/m <sup>2</sup>	IVI in 500mL 0.9% saline over 3–5h
1–5	Fludarabine	<sup>b</sup> 24 mg/m <sup>2</sup> /d	PO
1–5	Cyclophosphamide	<sup>b</sup> 150 mg/m <sup>2</sup> /d	PO

<sup>a</sup> In CLL: rituximab 375 mg/m<sup>2</sup> cycle 1 given fractionated over 2d. Rituximab dose 500 mg/m<sup>2</sup> from cycle 2.

<sup>b</sup> Alternative schedule IV: cyclophosphamide 250mg/m<sup>2</sup>/d d1–3 and fludarabine 25mg/m<sup>2</sup>/d d1–3.

**Administration**

- Out-patient regimen.
- Allopurinol 300mg od PO (100mg if significant renal impairment) for 1st cycle.
- Oral systemic *Pneumocystis jirovecii* prophylaxis according to local protocol (generally co-trimoxazole 480mg bd 3d per week) throughout treatment and for 8 weeks after completion.
- Consider aciclovir prophylaxis.
- Antiemetic therapy for moderately emetogenic regimens.
- Reduce to 50% doses if renal impairment (creatinine clearance 30–60mL/min); do not give if creatinine clearance <30mL/min.
- Delay next cycle for 1 week if neutrophils <1 × 10<sup>9</sup>/L or platelets <75 × 10<sup>9</sup>/L.
- All cellular blood components should be irradiated for 1 year after therapy to prevent transfusion associated GvHD.
- Administer 6 cycles.
- In LPC-lymphoma and WM rituximab is omitted from cycle 1 due to a possible flare-effect (rise in paraprotein).
- Discuss risk of infertility and consider sperm storage.
- Consider G-CSF as 1<sup>o</sup> or 2<sup>o</sup> prophylaxis.
- Main toxicities: severe cytokine release syndrome (usually occurs within 1–2h of the 1st rituximab infusion) of fever, headache, rigors, flushing, nausea, rash, URTI symptoms; tumour lysis syndrome; myelosuppression; alopecia; opportunistic infections; GI upset, chiefly diarrhoea, autoimmune haemolytic anaemia; infertility; ovarian failure; haemorrhagic cystitis.

## ICE ± R

### Indications

- Treatment of refractory/relapsed aggressive NHL and HL.
- Mobilization of peripheral blood stem cells.

### Schedule

14d cycle (Table 15.18).

**Table 15.18** ICE ± R

Days	Drug	Dose	Route
1	Rituximab <sup>a</sup>	375mg/m <sup>2</sup>	IVI in 500mL 0.9% saline over 3–5h
1–3	Etoposide	100mg/m <sup>2</sup> /d	IVI in 500mL–1L 0.9% saline over 30–60mins
2	Ifosfamide	5g/m <sup>2</sup>	IV continuous infusion in 1L 0.9% saline over 24h
2	Mesna (mixed with ifosfamide)	5g/m <sup>2</sup>	IV continuous infusion over 24h
2	Carboplatin <sup>b</sup>	AUC 5 (capped at 800mg)	IVI in 250mL 0.9% saline over 1h
7–14	G-CSF <sup>c</sup> (filgrastim or lenograstim)	5mcg/kg/d or 263mcg/d <sup>d</sup>	SC

<sup>a</sup> An initial dose of rituximab is administered 48h before initiation of cycle 1 (d2); patients completing 3 cycles receive 4 doses of rituximab.

<sup>b</sup> Calculate creatinine clearance (CrCl).

<sup>c</sup> Double G-CSF dose on final cycle and continue until end of stem cell collection.

<sup>d</sup> Higher dose G-CSF if >80kg: 368mcg/d.

### Administration

- Intensive in-patient regimen; insert tunnelled central venous catheter if possible.
- Discuss risk of infertility and consider sperm storage.
- Allopurinol 300mg od PO (100mg if significant renal impairment) for 1st cycle.
- Consider antibacterial infection prophylaxis with ciprofloxacin.
- Consider oral antifungal prophylaxis.
- Consider aciclovir prophylaxis.
- Antiemetic therapy for highly emetogenic regimens.
- Delay next cycle if neutrophils <1.0 × 10<sup>9</sup>/L or platelets <50 × 10<sup>9</sup>/L at d15.
- Patients who achieve CR or PR with ICE ± R should proceed to autologous stem cell transplantation.
- Main toxicities: myelosuppression; alopecia; mucositis; cardiomyopathy; peripheral neuropathy; constipation; haemorrhagic cystitis; ovarian failure; organ failure; infertility.

## Lenalidomide/dexamethasone

### Indications

- Multiple myeloma.
- AL amyloid.

### Schedule 1

28d cycle (Table 15.19).

**Table 15.19** Lenalidomide-dexamethasone

Days	Drug	Dose	Route
1–21	Lenalidomide	25mg	PO
1–4 <sup>a</sup>	Dexamethasone	20–40mg	PO

<sup>a</sup> Additional 4d pulses may be given, e.g. on d8–11 ± d15–18, where rapid disease reduction is needed at the start of the course.

### Administration

- Out-patient regimens.
- Weekly cyclophosphamide 500mg PO can be added.
- Add allopurinol 300mg od PO (100mg if significant renal impairment) for 1st cycle.
- Antiemetic therapy not generally required.
- Commence H<sub>2</sub> antagonist or PPI.
- Treatment is continued for as long as response is maintained. Consider stopping dexamethasone after 6 cycles.
- If neutrophils  $<0.5 \times 10^9/L$  post treatment interrupt treatment. Once recovered to  $\geq 0.5 \times 10^9/L$ , re-start treatment at lower dosing.
- Dose reduction in renal impairment: CrCl 30–49mL/min lenalidomide dose 10mg OD; CrCl  $<30$  (not requiring dialysis) dose 15mg every other day; CrCl  $<30$  (requiring dialysis) dose 5mg od (taken after dialysis on dialysis days).
- Main toxicities: teratogenicity; myelosuppression; muscle cramps; constipation or diarrhoea; rash; ↑ risk of thromboembolic events; haemorrhagic cystitis, if cyclophosphamide included.



## MPT

### Indications

- Multiple myeloma.

### Schedule

28d cycle (Table 15.20).

**Table 15.20** Lenalidomide-dexamethasone

Days	Drug	Dose	Route
1–7	Melphalan <sup>a</sup>	4mg/m <sup>2</sup>	PO
1–7	Prednisolone	40mg/m <sup>2</sup>	PO
1–28	Thalidomide	50mg increased to 100 mg after cycle 1.	PO

<sup>a</sup> Consider dose reduction or reduce number of days in elderly patients or patients with comorbidities.

### Administration

- Out-patient regimens.
- Schedule for older patients.
- Add allopurinol 300mg od PO (100mg if significant renal impairment) for 1st cycle.
- Women of childbearing potential must have negative pregnancy test within 24h before starting thalidomide.
- All patients should be enrolled in a pregnancy prevention programme.
- Consider VTE prophylaxis: add aspirin (75mg/d) for patients at standard risk of thrombosis; add prophylactic dose LMWH for high-risk patients.
- Antiemetic therapy for mildly emetogenic regimens.
- 6 cycles.
- Main toxicities: myelosuppression; teratogenicity; dry skin or rash; sedation; peripheral neuropathy; constipation (often requiring laxatives); dizziness; bradycardia and syncope; ↑ risk of thromboembolic events; steroid side effects; ovarian failure; infertility.

## Mini-BEAM ± R

### Indications

- Salvage therapy of refractory/relapsed NHL and HL.
- Mobilization of peripheral blood stem cells.

### Schedule

1–2 cycles (Table 15.21).

**Table 15.21** Mini-BEAM±R

Days	Drug	Dose	Route
0	Rituximab	375mg/m <sup>2</sup>	IVI in 500mL 0.9% saline over 3–5h
1	Carmustine	60mg/m <sup>2</sup>	IVI in 250mL 5% dextrose over 1h; avoid storage in PVC container for >24h
2–5	Cytarabine	100mg/m <sup>2</sup> bd	IVI in 100mL 0.9% saline over 30min
2–5	Etoposide	75mg/m <sup>2</sup>	IVI in 500mL 0.9% saline over 1h
6	Melphalan <sup>a,b</sup>	30mg/m <sup>2</sup>	IV bolus in 100mL 0.9% saline within 30min reconstitution

<sup>a</sup> Ensure adequate diuresis is achieved before administering melphalan.

<sup>b</sup> Ensure that melphalan is administered on a weekday; Mondays or Tuesdays provide optimal timing for stem cell collection.

### Administration

- In-patient regimen.
- Ensure adequate venous access by inserting a dual-lumen tunnelled central venous catheter.
- Severe myelosuppression (neutrophils  $<0.1 \times 10^9/L$  and platelets  $<20 \times 10^9/L$ ) is expected.
- Add allopurinol 300mg (100mg if creatinine clearance  $<20\text{mL}/\text{min}$ ) od for 1st cycle.
- Antiemetic therapy for moderately emetogenic regimens.
- Mouth care.
- Consider antibacterial and antifungal prophylaxis.
- Consider H<sub>2</sub> antagonist or PPI.
- Consider starting G-CSF on d9 either to shorten neutropenia or to facilitate peripheral blood stem cell collection around d18.
- Note: do not use mini-BEAM if creatinine clearance  $\leq 40\text{mL}/\text{min}$ .
- Patients with HL and those in whom stem cell collection is planned within 2 weeks must receive irradiated cellular blood components to prevent transfusion-associated GvHD.
- A 2nd course can be given when neutrophils  $>1.0 \times 10^9/L$  and platelets (unsupported)  $>100 \times 10^9/L$ ; generally 4–6 weeks.
- Consolidate in responding patients with high-dose therapy and autologous stem cell transplant.
- Main toxicities: prolonged ( $>7\text{d}$ ) myelosuppression, with risk of infections and haemorrhage; alopecia; mucositis; pulmonary toxicity; ovarian failure; infertility.

## Nordic schedule (R-maxi-CHOP and R-high-dose cytarabine for MCL)

### Indications

MCL (1st line in fit patients; followed by PBSCH).

### Schedule:

6 cycles (alternating R-maxi-CHOP and R-high dose cytarabine) (Tables 15.22 and 15.23).

**Table 15.22** R-maxi-CHOP (cycles 1, 3, 5)

Day	Drug	Dose	Route
1	Rituximab	375mg/m <sup>2</sup> <sup>a</sup>	IV
1	Cyclophosphamide	1200mg/m <sup>2</sup>	IV
1	Doxorubicin	75mg/m <sup>2</sup>	IV
1	Vincristine	1.4mg/m <sup>2</sup> (max. 2 mg)	IV
1	Prednisolone	100mg	Oral

<sup>a</sup> Omit rituximab cycle 1. Give with cycle 3 & 5.

**Table 15.23** R-High-dose cytarabine (cycle 2, 4, 6)

Day	Drug	Dose	Route
1	Rituximab	375mg/m <sup>2</sup> <sup>a</sup>	IV
1+2	Cytarabine	3000mg/m <sup>2</sup> twice daily (4 doses total) Consider 2000mg/m <sup>2</sup> if >60 years	IV

<sup>a</sup> Additional rituximab cycle 6 d9.

### Administration

- In-patient regimen.
- Dual-lumen tunnelled central venous catheter required.
- Allopurinol 300mg (100mg if creatinine clearance <20mL/min) od for 1st cycle.
- Antiemetic therapy for highly emetogenic regimen for all cycles.
- Consider antibacterial and antifungal prophylaxis.
- Consider H<sub>2</sub> antagonist or PPI.
- Consider starting G-CSF on d5 to shorten neutropenia.
- Dose reduction for renal/hepatic impairment as per to local guidelines.
- Main toxicities: TLS; prolonged myelosuppression, with risk of infections and haemorrhage; alopecia; mucositis; cardiomyopathy; peripheral neuropathy; constipation; haemorrhagic cystitis; cytarabine syndrome (includes fever, myalgia, bone pain, rash and conjunctivitis); severe cytokine release syndrome; ovarian failure; infertility.

## PMitCEBO

### Indications

Salvage NHL, particularly >60 yrs old.

### Schedule

2-weekly for 6–8 cycles (Table 15.24).

**Table 15.24** PMitCEBO

Day	Drug	Dose	Route
1	Mitoxantrone	7mg/m <sup>2</sup>	IV
1	Cyclophosphamide	300mg/m <sup>2</sup>	IV
1	Etoposide	150mg/m <sup>2</sup>	IV
8	Vincristine	1.4mg/m <sup>2</sup> (max. 2mg)	IV
8	Bleomycin	10,000IU/m <sup>2</sup>	IV
	Prednisolone	50mg daily week 1-4 then alternate days	PO

### Administration

- In-patient regimen.
- Discuss infertility.
- Ensure adequate venous access by inserting a dual-lumen tunnelled central venous catheter.
- Add allopurinol 300mg (100mg if creatinine clearance <20mL/min) od for 1st cycle.
- Antiemetic therapy for highly emetogenic regimen for d1 and for moderately emetogenic regimens d8.
- Consider antibacterial and antifungal prophylaxis.
- Consider H<sub>2</sub> antagonist or PPI.
- Consider starting G-CSF on d9 to shorten neutropenia.
- Main toxicities: myelosuppression; alopecia; mucositis; cardiomyopathy; peripheral neuropathy; constipation; skin reactions to bleomycin; pulmonary toxicity; steroid side effects; rigors with bleomycin; ovarian failure; infertility.

## R-Bendamustine

### Indications

- NHL.
- CLL.

### NHL schedule

28d cycle (Table 15.25).

**Table 15.25** R-Bendamustine

Days	Drug	Dose	Route
1	Rituximab	375mg/m <sup>2</sup>	IV
1, 2	Bendamustine	90–120mg/m <sup>2</sup>	IV

### CLL schedule

28d cycle (Table 15.26).

**Table 15.26** R-Bendamustine

Days	Drug	Dose	Route
1	Rituximab	375mg/m <sup>2</sup> fractionated in cycle 1 over 2d, then 500mg/m <sup>2</sup> on d1 from cycle 2	IV
1, 2	Bendamustine	70–100mg/m <sup>2</sup>	IV

### Administration

- Out-patient regimens.
- Add allopurinol 300mg od PO (100mg if significant renal impairment) for 1st cycle.
- Antiemetic therapy for highly emetogenic regimens.
- Commence H<sub>2</sub> antagonist or PPI.
- No dose reduction required if CrCl > 10mL/min. Liver impairment: bilirubin (micromol/L) < 21: 100% dose; 21–51: 70% dose; > 51: no data available.
- Main toxicities: severe cytokine release syndrome (usually occurs within 1–2h of the 1st rituximab infusion); myelosuppression; hypersensitivity reactions to bendamustine (e.g. rash, urticaria); alopecia; TLS (ensure pre-medicated with allo-purinol and good hydration); ovarian failure; infertility.

## R-CHOP

### Indication

B-cell NHL (standard treatment).

### Schedule

21d or 14d cycle (Table 15.27).

**Table 15.27** R-CHOP

Day	Drug	Dose	Route
1	Rituximab	375mg/m <sup>2</sup>	IVI in 500mL 0.9% saline over 3–5h
1	Cyclophosphamide	750mg/m <sup>2</sup>	IVI in 250mL 0.9% saline over 30min
1	Vincristine	1.4mg/m <sup>2</sup> (max. 2mg <sup>a</sup> )	IV bolus
1	Doxorubicin	50mg/m <sup>2</sup>	IV bolus
1–5	Prednisolone	100mg	PO in a.m. with food

<sup>a</sup> Max. 1mg for patients >70 years.

### Administration

- Out-patient regimen.
- Consider sperm banking or egg-harvesting.
- Add allopurinol 300mg/d (100mg if creatinine clearance <20mL/min) throughout 1st treatment cycle.
- Antiemetic therapy for moderately emetogenic regimens.
- CNS prophylaxis with intrathecal MTX should be administered for certain patients (see Chapter 5). Some guidelines use high IPI (4–5) as indication for IT. For patients with BM, testicular, orbital, paranasal/nasopharyngeal or paraspinal involvement prophylaxis should be considered.
- In patients with renal impairment, reduce cyclophosphamide dose to 75% for creatinine clearance 10–50mL/min and to 50% for clearance <10mL/min.
- In patients with hepatic dysfunction, reduce doxorubicin dose by 50% for bilirubin 1.5–3 × ULN and to 25% for bilirubin >3 × ULN.
- Repeat treatment when neutrophils  $\geq 1.0 \times 10^9/L$  and platelets  $\geq 100 \times 10^9/L$ .
- Standard 6 cycles.
- Main toxicities: myelosuppression; alopecia; mucositis; cardiomyopathy; peripheral neuropathy; constipation; haemorrhagic cystitis; ovarian failure; infertility; organ failure.
- Risk of TLS: ensure pre-medicated with allopurinol or rasburicase and good hydration.

## R-CVP and CVP

### Indications

- Low-grade NHL.
- CLL.

### Schedule

21d cycle (Table 15.28).

**Table 15.28** R-CVP & CVP

Day	Drug	Dose	Route
1	Rituximab	375mg/m <sup>2</sup>	IVI in 500mL 0.9% saline over 3–5h
1	Cyclophosphamide	750mg/m <sup>2</sup>	IV bolus
1	Vincristine	1.4mg/m <sup>2</sup> (max. 2mg) <sup>a</sup>	IVI in 50mL 0.9% saline over 5–10min
1–5	Prednisolone	100mg/d	PO in a.m. with food

<sup>a</sup> Consider maximum 1mg vincristine in patients >70 years.

### Administration

- Out-patient regimen.
- Add allopurinol 300mg/d (100mg/d if creatinine clearance <20mL/min) for 1st cycle.
- Antiemetic therapy for moderately emetogenic regimens.
- Repeat treatment when neutrophils >1.0 × 10<sup>9</sup>/L and platelets ≥100 × 10<sup>9</sup>/L; reduce cyclophosphamide to 75% dose if neutrophils 1.0–1.49 × 10<sup>9</sup>/L and platelets ≥100 × 10<sup>9</sup>/L and reduce to 50% dose if neutrophils 0.5–1.0 × 10<sup>9</sup>/L and/or platelets 50–100 × 10<sup>9</sup>/L; omit cyclophosphamide if neutrophils <0.5 × 10<sup>9</sup>/L and/or platelets <50 × 10<sup>9</sup>/L.
- 6–8 cycles.
- Main toxicities: severe cytokine release syndrome; myelosuppression; alopecia; mucositis; peripheral neuropathy; constipation; haemorrhagic cystitis; ovarian failure; infertility.
- TLS rare.

## Rituximab: monotherapy and maintenance therapy

### Indications

- CD20+ NHL and LPD.
- Used as monotherapy, in combination with chemotherapy and as maintenance.

### Monotherapy schedule for FL

Single 28d cycle (Table 15.29).

**Table 15.29** Rituximab monotherapy

Days	Drug	Dose	Route
1, 8, 15, 22	Rituximab	375mg/m <sup>2</sup>	IVI in 500mL 0.9% saline over 3–5h

### Maintenance schedules for FL

(Tables 15.30 and 15.31).

**Table 15.30** Rituximab maintenance for FL in first response

Months	Drug	Dose	Route
2-monthly for 2 years (12 infusions)	Rituximab	375mg/m <sup>2</sup>	IVI in 500mL 0.9% saline over 3–5h

**Table 15.31** Rituximab maintenance for FL in second response

Months	Drug	Dose	Route
3-monthly for 2 years (8 infusions)	Rituximab	375mg/m <sup>2</sup>	IVI in 500mL 0.9% saline over 3–5h

### Administration

- In combination regimens rituximab may be given 1–2d before chemotherapy if logistically required.
- Monitor closely for cytokine release syndrome: fever, chills, rigors within first 2h usually.
- Less common side effects include: flushing, angioedema, nausea, urticaria/rash, fatigue, headache, throat irritation, rhinitis, vomiting, tumour pain and features of TLS, bronchospasm, hypotension.
- Interrupt rituximab infusion if severe dyspnoea, bronchospasm, or hypoxia.
- Care should be taken in patients with high WCC with the 1st infusion of rituximab and fractionation of the 1st dose should be considered.
- Maintenance in FL and MCL recommended.



## Stanford V

Largely replaced by ABVD.

### Indications

- HL.
- Schedule: 12 weeks of chemotherapy comprising 3 cycles of 28d followed 2–4 weeks later by IFRT in patients with disease >5cm and if appropriate/indicated (Table 15.32).

**Table 15.32** Stanford V

Days	Drug	Dose	Route
1 & 15	Doxorubicin	25mg/m <sup>2</sup> <sup>a</sup>	IV bolus
1 & 15	Vinblastine <sup>b</sup>	6mg/m <sup>2</sup> <sup>a</sup>	IVI in 50mL 0.9% saline over 5–10min
1	Chlormethine (mustine)	500mg/m <sup>2</sup> <sup>a</sup>	IV bolus
1–28 <sup>c</sup>	Prednisolone	40mg/m <sup>2</sup> on alternate days <sup>a</sup>	PO in a.m. with food
8 & 22	Vincristine <sup>d</sup>	1.4mg/m <sup>2</sup> (max. 2mg) <sup>a</sup>	IVI in 50mL 0.9% saline over 5–10min
8 & 22	Bleomycin <sup>e</sup>	5000 units/m <sup>2</sup>	IVI in 250mL 0.9% saline over 1h
15 & 16	Etoposide	60mg/m <sup>2</sup> /d <sup>a</sup>	IVI in 250mL 0.9% saline over 45min

<sup>a</sup> Cap surface area at 2m<sup>2</sup> for calculating dose.

<sup>b</sup> Reduce vinblastine dose to 4mg/m<sup>2</sup> after 2nd cycle for patients >50.

<sup>c</sup> Taper prednisolone dose by 10mg every other day during weeks 10–12.

<sup>d</sup> Reduce vincristine dose to 1mg/m<sup>2</sup> after 2nd cycle for patients >50.

<sup>e</sup> Administer hydrocortisone 100mg IV before infusion of bleomycin unless dexamethasone used as antiemesis therapy.

### Administration

- Intensive regimen with severe myelosuppression.
- Discuss risk of infertility and consider sperm storage or egg-harvesting.
- Consider IV hydration for patients with bulk disease.
- Allopurinol 300mg od PO (100mg if creatinine clearance <20mL/min) for first 4 weeks.
- Antiemetic therapy for highly emetogenic regimens.
- Commence H<sub>2</sub> antagonist or PPI.
- Oral systemic *Pneumocystis jirovecii* prophylaxis according to local protocol recommended (generally co-trimoxazole 480mg bd 3d per week) throughout treatment and for 8 weeks after completion.
- Antifungal and antiviral prophylaxis.
- Consider regular laxatives.
- In patients with renal impairment, when creatinine clearance <60mL/min administer 80% mustine dose, 85% etoposide dose and 75% bleomycin dose; <30mL/min omit mustine, administer 75% etoposide dose <60mL/min.

- In patients with hepatic dysfunction, if bilirubin  $1-2 \times \text{ULN}$  reduce doxorubicin, vinblastine, vincristine and etoposide doses to 50%;  $2-4 \times \text{ULN}$  reduce doses to 25%;  $>4 \times \text{ULN}$  omit doxorubicin, vinblastine, and etoposide.
- If neutrophils  $0.5-1.0 \times 10^9/\text{L}$ , administer 65% doses of doxorubicin, mustine, vincristine and etoposide but 100% dose of other drugs; if  $<0.5 \times 10^9/\text{L}$  delay doxorubicin, mustine, vincristine and etoposide by 1 week but no delay or dose reduction of other drugs.
- If dose reduction or delay is required, administer G-CSF SC on d3-7 and d17-21 of each cycle.
- No dose reductions or delay if platelets  $>10 \times 10^9/\text{L}$ ; if  $<10 \times 10^9/\text{L}$ , transfuse platelets to maintain count  $>10 \times 10^9/\text{L}$ .

# Haematological investigations

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## Full blood count

Rapid analysis by the latest-generation automated blood counters using either forward-angle light scatter or impedance analysis provides enumeration of leucocytes, erythrocytes, and platelets and quantification of haemoglobin and MCV, plus derived values for haematocrit, MCH, and MCHC, red cell distribution width (a measure of cell size scatter), mean platelet volume and platelet distribution width, and a 5-parameter differential leucocyte count. The counter also flags samples which require direct morphological assessment by examination of a blood film.

**Sample:** peripheral blood EDTA; the sample should be analysed in the laboratory within 4h.

## Blood film

Morphological assessment of red cells, leucocytes, and platelets should be performed by an experienced individual of all samples in which the FBC has revealed any result significantly outside the ↔ range, samples in which a flag has been indicated by the automated counter and if clinically indicated. A manual differential leucocyte count may be performed and may differ from that produced by the automated counter most notably in patients with haematological disease affecting the leucocytes.

**Sample:** peripheral blood EDTA; the sample should be analysed in the laboratory within 4h. May be made directly from drop of blood or EDTA sample, air-dried, and fixed.

## Plasma viscosity

This test is a sensitive but non-specific index of plasma protein changes which result from inflammation or tissue damage. The plasma viscosity is unchanged by haematocrit variations and delay in analysis up to 24h and is therefore more reliable than the ESR. It is not affected by sex but is affected by age, exercise, and pregnancy.

**Sample:** peripheral blood EDTA; the sample should be analysed in the laboratory within 24h.

## ESR

This test is a sensitive but non-specific index of plasma protein changes which result from inflammation or tissue damage. The ESR is affected by haematocrit variations, red cell abnormalities (e.g. poikilocytosis, sickle cells), and delay in analysis and is therefore less reliable than measurement of the plasma viscosity. The ESR is affected by age, sex, menstrual cycle, pregnancy, and drugs (e.g. OCP, steroids).

**Sample:** peripheral blood EDTA; the sample should be analysed in the laboratory within 4h.

## Haematinic assays

Measurement of the serum B<sub>12</sub> and red cell folate are necessary in the investigation of macrocytic anaemia, and serum ferritin in the investigation of microcytic anaemia, in order to assess body stores of the relevant haematinic(s). Serum folate levels are an unreliable measurement of body stores of folate. The serum ferritin may be elevated as an acute phase protein in patients with underlying neoplasia or inflammatory disease (e.g. rheumatoid arthritis) and may give an erroneously ↔ level in an Fe deficient patient.

**Sample:** clotted blood sample and peripheral blood EDTA.

## Haemoglobin electrophoresis

This test is performed in the diagnosis of abnormal haemoglobin production (haemoglobinopathies or thalassaemia). It is usually performed on cellulose acetate at alkaline pH (8.9) but may be performed on citrate agar gel at acid pH (6.0) to detect certain haemoglobins more clearly. Haemoglobin electrophoresis has been largely replaced by HPLC analysis.

**Sample:** peripheral blood EDTA.

## Haptoglobin

The serum haptoglobin should be measured in patients with suspected haemolysis (extravascular or intravascular) and is frequently reduced in patients with haemolysis. Low concentrations of haptoglobin may be found in hepatocellular disease. It should generally be accompanied by estimation of the serum methaemalbumin, free plasma haemoglobin, and urinary haemosiderin.

**Sample:** clotted blood.

## Schumm's test

This spectrophotometric test for methaemalbumin (which has a distinctive absorption band at 558nm) should be measured in patients with suspected intravascular haemolysis and may be abnormal in patients with significant extravascular (generally splenic) haemolysis. It should generally be accompanied by estimation of the serum haptoglobin level, free plasma haemoglobin, and urinary haemosiderin.

**Sample:** heparinized blood or clotted blood.

## Kleihauer test

The Kleihauer test which exploits the resistance of fetal red cells to acid elution should be performed on all Rh(D) -ve women who deliver a Rh(D) +ve infant. Fetal cells appear as darkly staining cells against a background of ghosts. An estimate of the required dose of anti-D can be made from the number of fetal cells in a low power field.

**Sample:** maternal peripheral blood EDTA.

## Reticulocytes

### Definition

- Immature RBCs formed in marrow and found in normal peripheral blood.
- Represent an intermediate maturation stage in marrow between the nucleated red cell and the mature red cell.
- No nucleus but retain some nucleic acid.

### Detection and measurement

- Demonstrated by staining with supravital dye for the nucleic acid.
- Appear on blood film as larger than mature RBCs with fine lacy blue staining strands or dots.
- Some modern automated blood counters using laser technology can measure levels of reticulocytes directly.
- Usually expressed as a % of total red cells, e.g. 5%, though absolute numbers can be derived from this and total red cell count.

### Causes of ↑ reticulocyte counts

*Marrow stimulation due to:*

- Bleeding.
- Haemolysis.
- Response to oral Fe therapy.
- Infection.
- Inflammation.
- Polycythaemia (any cause).
- MPDs.
- Marrow recovery following chemotherapy or radiotherapy.
- EPO administration.

### Causes of ↓ reticulocyte counts

*Marrow infiltration due to:*

- Leukaemia.
- Myeloma.
- Lymphoma.
- Other malignancy.

*Marrow underactivity (hypoplasia) due to:*

- Fe, folate, or B<sub>12</sub> deficiency. *Note:* return of reticulocytes is earliest sign of response to replacement therapy.
- Immediately post-chemotherapy or radiotherapy.
- Autoimmune disease especially rheumatoid arthritis.
- Malnutrition.
- Uraemia.
- Drugs.
- Aplastic anaemia (➔ see p.102).
- Red cell aplasia (➔ see p.106).

## Urinary haemosiderin

### Usage

The most widely used and reliable test for detection of chronic intravascular haemolysis.

### Principle

Free Hb is released into the plasma during intravascular haemolysis. The haemoglobin binding proteins become saturated resulting in passage of haem-containing compounds into the urinary tract of which haemosiderin is the most readily detectable.

### Method

- A clean catch sample of urine is obtained from the patient.
- Sample is spun down in a cytocentrifuge to obtain a cytospin preparation of urothelial cells.
- Staining and rinsing with Perl's reagent (Prussian blue) is performed on the glass slides.
- Examine under oil-immersion lens of microscope.
- Haemosiderin stains as blue dots within urothelial cells.
- Ignore all excess stain, staining outside cells or in debris all of which are common.
- True +ve is only when clear detection within urothelial squames is seen.

### Cautions

An Fe-staining +ve control sample should be run alongside test case to ensure stain has worked satisfactorily. Haemosiderinuria may not be detected for up to 72h after the initial onset of intravascular haemolysis so the test may miss haemolysis of very recent onset—repeat test in 3–7d if –ve. Conversely, haemosiderinuria may persist for some time after a haemolytic process has stopped. Repeat in 7d should confirm.

### Causes

See Table 16.1.

**Table 16.1** Causes of haemosiderinuria

Common causes	<ul style="list-style-type: none"> <li>• Red cell enzymopathies, e.g. G6PD and PK deficiency but only during haemolytic episodes</li> <li>• Mycoplasma pneumonia with anti-I cold haemagglutinin</li> <li>• Sepsis</li> <li>• Malaria</li> <li>• Cold haemagglutinin disease TTP/HUS</li> <li>• Severe extravascular haemolysis (may cause intravascular haemolysis)</li> </ul>
Rarer causes	<ul style="list-style-type: none"> <li>• PNH</li> <li>• Prosthetic heart valves</li> <li>• Red cell incompatible transfusion reactions</li> <li>• Unstable haemoglobins</li> <li>• March haemoglobinuria</li> </ul>



## Ham's test

### Usage

Diagnostic test for paroxysmal nocturnal haemoglobinuria (PNH). Now replaced by immunophenotyping methods.

### Principle

- Abnormal sensitivity of RBCs from patients with PNH to the haemolytic action of complement.
- Complement is activated by acidification of patient's serum to pH of 6.2 which induces lysis of PNH red cells but not ↔ controls.

**Specificity:** high—similar reaction is produced only in the rare syndrome HEMPAS (a form of congenital dyserythropoietic anaemia type II) which should be easily distinguished morphologically.

**Sensitivity:** low—as the reaction is crucially dependent on the concentration of magnesium in the serum.

It appears to be a technically difficult test in most laboratories. Patients with only a low % of PNH cells may be missed at an early stage of the disease. Markedly abnormal PNH cells are usually picked up in ~75% of patients. Less abnormal cells are detected in only ~25% of patients.

### Alternative tests

- Sucrose lysis—an alternative method of complement activation is by mixing serum with a low ionic strength solution such as sucrose.
  - *Sensitivity* of this test is high but *specificity* is low—i.e. the opposite of Ham's test.
- Immunophenotypic detection of the deficiency of the PIG transmembrane protein anchors in PNH cells is becoming a more widely used alternative cf. PNH section. Monoclonal antibodies to CD59 or CD55 (DAF) are used in flow cytometric analysis. Major advantage is that test can be performed on neutrophils and platelets in PB which are more numerous than the PNH red cells.

## Immunophenotyping

### Definition

Identification of cell surface proteins by reactivity with monoclonal antibodies of known specificity.

### Uses

- Aids diagnosis and classification of haematological malignancy. Assess cellular clonality.
- Identify prognostic groups.
- Monitor minimal residual disease (MRD).
- The advent of immunophenotyping (or 'flow'), together with cytogenetics, has led to new disease classification systems (such as the WHO Classification of Tumours of the Haematopoietic and Lymphoid Tissues), moving away from historical classifications solely based on morphology.

### Terminology and methodology

Cell surface proteins are denoted according to their cluster differentiation (CD) number. These are allocated after international workshops define individual cell surface proteins by reactivity to monoclonal antibodies. Most cells will express many such proteins and pattern of expression allows cellular characterization.

#### *Monoclonal antibodies (MoAbs)*

MoAbs are derived from single B-lymphocyte cell lines and have identical antigen binding domains (idiotypes). It is easy to generate large quantities of MoAbs for diagnostic use.

- Cell populations from, e.g. PB or BM, or cell suspensions are incubated with a panel of MoAbs, e.g. anti-CD4, anti-CD34 which are directly or indirectly bound to a fluorescent marker antibody, e.g. FITC (fluorescein isothiocyanate).
- The sample is passed through a flow-cytometer, a fluorescence-activated cell sorter (FACS) machine.
- By assessing forward and side light scatter of the laser, the FACS instruments assign cells to a graphical plot by virtue of cell size and granularity.
- Allows subpopulations of cells, e.g. mononuclear cells in blood sample to be selected.
- The reactivity of this cell subpopulation to the MoAb panel can then be determined by fluorescence for each MoAb.

### Common diagnostic profiles

- AML CD13+, CD33+,  $\pm$  CD 34,  $\pm$  CD14 +ve.
- Common ALL CD10 and TdT +ve.
- T-ALL CD3, CD7, TdT +ve.
- B-ALL CD10, CD19, surface Ig +ve.
- CLL CD5, CD19, CD23, weak surface Ig +ve.

### Clonality assessment

Particularly useful in determining whether there is a monoclonal B cell or plasma cell population.

- ► Monoclonal B cells from, e.g. NHL will have surface expression of  $\kappa$  or  $\lambda$  light chains *but not both*.
- ► Polyclonal B cells from, e.g. patient with infectious mononucleosis will have both  $\kappa$  and  $\lambda$  expression.

## Cytogenetics

Acquired somatic chromosomal abnormalities are common in haematological malignancies. Determination of patterns of cytogenetic abnormalities is known as karyotyping. See Table 16.2 for examples.

### Uses

- Aid diagnosis and classification of haematological malignancy.
- Assess clonality.
- Identify prognostic groups.
- Monitor MRD.
- Determine engraftment and chimerism post-allogeneic transplant.

### Terminology

- Normal somatic cell has 46 chromosomes; 22 pairs plus XX or XY.
- Numbered 1–22 in decreasing size order.
- 2 arms meet at centromere—short arm denoted p, long arm denoted q.
- Usually only visible during condensation at metaphase.
- Stimulants and cell culture used—colchicine to arrest cells in metaphase.
- Stained to identify regions and bands, e.g. p1, q3.

### Common abnormalities

- See Table 16.2
- Whole chromosome gain, e.g. trisomy 8 (+8).
- Whole chromosome loss, e.g. monosomy 7 (–7).
- Partial gain, e.g. 9q+ or partial loss, e.g. 5q–.
- Translocation—material repositioned to another chromosome; usually reciprocal, e.g. t(9;22)—the Philadelphia (Ph) translocation.
- Inversion—part of chromosome runs in opposite direction, e.g. inv(16) in M4Eo.
- Many translocations involve point mutations known as oncogenes, e.g. *BCR*, *ras*, *myc*, *bcl-2*.

### Molecular cytogenetics

- Molecular revolution is further refining the specific abnormalities in the genesis of haematological malignancies.
- Techniques such as FISH and PCR can detect tiny amounts of abnormal genes.
- BCR-ABL probes are now used in diagnosis and monitoring of treatment response in CML.
- IgH and T-cell receptor (TCR) genes are useful in determining clonality of suspected B and T cell tumours respectively.
- Specific probes may be used in diagnosis and monitoring of subtypes of AML, e.g. PML-RARA in AML M3.

**Table 16.2** Common karyotypic abnormalities**CML**

t(9;22) Ph chromosome translocation creates *BCR-ABL*.

**AML**

t(8;21) AML M2, involves *AML-ETO* genes—has better prognosis.  
 t(15;17) AML M3 involves *PML-RARA* genes—has better prognosis.  
 inv(16) AML M4Eo—has better prognosis.  
 -5, -7 Complex abnormalities have poor prognosis.

**MDS**

-7, +8, +11 Poor prognosis.  
 5q- syndrome Associated with refractory anaemia and better prognosis.

**MPD**

20q- and +8 Common associations.

**ALL**

t(9;22) Ph translocation, poor prognosis.  
 t(4;11) Poor prognosis.  
 Hyperdiploidy ↑ total chromosome number—good prognosis.  
 Hypodiploidy ↓ total chromosome number—bad prognosis.

**T-ALL**

t(1;14) Involves *tal-1* oncogene.  
 t(8;14)

**B-ALL and Burkitt lymphoma**

t(8;14) Involves *myc* and IgH genes, poor prognosis.

**CLL**

+12, t(11;14)

**ATLL**

14q11

**NHL**

t(14;18) Follicular lymphoma, involves *bcl-2* oncogene.  
 t(11;14) Small cell lymphocytic lymphoma, involves *bcl-1* oncogene.  
 t(8;14) Burkitt lymphoma, involves *myc* and IgH genes.

## Human leucocyte antigen (HLA) typing

HLA system major histocompatibility complex (MHC) is the name given to the highly polymorphic gene cluster region on human chromosome 6 which codes for cell surface proteins involved in immune recognition.

### Box 16.1 The gene complex is subdivided into 2 regions

<b>Class 1</b>	The A, B, and C loci. These proteins are found on most nucleated cells and interact with CD8+ T lymphocytes.
<b>Class 2</b>	Comprising of DR, DP, DQ loci present only on B lymphocytes, monocytes, macrophages and activated T lymphocytes. Interact with CD4+ T lymphocytes.

- Class 1 and 2 genes are closely linked so one set of gene loci is usually inherited from each parent though there is a small amount of crossover. (See Box 16.1.)
- There is ~1:4 chance of 2 siblings being HLA identical.
- There are other histocompatibility loci apart from the HLA system but these appear less important generally except during HLA matched SCT when even differences in these minor systems may cause GvHD.

### Typing methods

Class 1 and 2 antigens were originally defined by serological reactivity with maternal antisera containing pregnancy-induced HLA antibodies. Many problems with technique and too insensitive to detect many polymorphisms. Molecular techniques are increasingly employed such as SSP. Molecular characterization is detecting vast class 2 polymorphism.

### Importance of HLA typing

- Matching donor/recipient pairs for renal, cardiac, and marrow SCT.
- Degree of matching more critical for stem cell than solid organ transplants.
- Sibling HLA matched SCT is now treatment of choice for many malignancies.
- Unrelated donor SCTs are increasingly performed but outcome is poorer due to HLA disparity. As molecular matching advances, improved accuracy will enable closer matches to be found and results should improve.
- Functional tests of donor/recipient compatibility.
- Mixed lymphocyte culture (MLC)—now rarely used.
- Cytotoxic T lymphocyte precursor assays (CTLp)—determine the frequency of CTLs in the donor directed against the recipient—provides an assessment of GvHD occurring.

**HLA-related transfusion issues**

- HLA on WBC and platelets may cause immunization in recipients of blood and platelet transfusions.
- May cause refractoriness and/or febrile reactions to platelet transfusions.
- WBC depletion of products by filtration prevents this.
- Diagnosis of refractoriness confirmed by detection of HLA or platelet specific antibodies in patient's serum.
- Platelet transfusions matched to recipient HLA type may improve increments.

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# Blood transfusion

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## Introduction

Transfusion medicine has changed dramatically with an increasing awareness of the risks involved with blood transfusion and a greater evidence base supporting clinical transfusion practice. It has become apparent that lower thresholds of transfusion provide equal or even improve outcomes for patients. More and more guidelines are available that help guide the appropriate use of blood and components and these have also helped to improve safety of transfusion practice. There are limited resources, as well as a rising demand for specific blood components and an ever more decreasing number of donors.

If there are any questions about the administration of blood, or appropriateness of blood components for individual patients contact a member of your hospital transfusion team (which includes the consultant haematologist responsible for transfusion, specialist transfusion practitioner and transfusion laboratory manager). Specialist transfusion advice from a consultant haematologist in transfusion is also available via the National Blood Service (e.g. NHS Blood and Transplant in England).



## Using the blood transfusion laboratory

### Requests for group & save (blood-grouping) or cross-matching (compatibility testing)

Transfusion samples are taken in an EDTA bottle (6mL) and must be signed by the person taking the sample thus vouching for the identity of the patient. Both sample and request form must be clearly, legibly identified with 3 points of identification for the patient: name, date of birth, and hospital number. In emergencies where the identity and date of birth of the patient are not known, emergency numbers and wristbands must be used.

- In case of any discrepancies between sampling and request form the sample will be discarded.
- Since errors related to having the 'wrong blood in tube' are relatively common with potential risk of ABO mismatched transfusions, the current British Committee for Standards in Haematology (BCSH) guideline (2013) recommend that a 2nd sample should be requested for confirmation of the blood group of any new patient, provided this does not impede the delivery of urgent red cells or components.
- Clinical information including the indication for transfusion or time and date of surgery must be given.
- The majority of laboratories in the UK now use automated ABO and RhD blood grouping and red cell antibody testing with advanced information technology systems for documentation and reporting of results. Red cells that are ABO and RhD compatible can be provided using electronic issue (or 'computer cross-match'), with no further testing needed, provided the patient does not have any antibodies and that there are robust automated systems in place for antibody testing and identification of the patient. If a patient has red cell antibodies, electronic issue should not be used and red cell units negative for the relevant antigen (s) should be cross-matched for transfusion.
- For major elective surgery where the need for transfusion is likely, the transfusion laboratory should receive a group & save sample 7d prior to surgery so appropriate blood can be made available (➔ see Maximum surgical blood ordering schedule, p.778).
- In emergencies, unmatched group O blood must be available for immediate use if major haemorrhage with group O Rh (D) -ve units in particular reserved for women of childbearing age. For all patients a switch to ABO RhD compatible units should be made as soon the blood group is known. Women of childbearing age should also receive K negative units to avoid the risk of forming anti-K antibodies.
- The ABO and RhD antigens are particularly important but there are many other antigens on red cells that may result in formation of antibodies following pregnancy or transfusion, such as Kell, other Rh antigens (c, C, E and e), Duffy antigens (fya, fyb), Kidd antigens (jka, jkb), and these antibodies can cause delayed transfusion reactions or haemolytic disease of the newborn.

### Issue and administration of blood and blood products

All hospitals must have a transfusion policy with clear guidance on correct patient identification and the safe administration of blood and components. Errors carry the potential for major morbidity or fatality. Patients receiving a blood transfusion must wear an identification band, with the minimum patient identifiers including last name, first name, date of birth, and unique patient number. Positive patient identification is essential at all stages of the blood transfusion process including blood sampling, collection of blood from storage and delivery to the clinical area, and administration to the patient.

- The prescription of blood must be made by a registered medical practitioner and the indication for and details of the product's administration must be recorded in the case record.
- Units of blood are issued for an individual patient and labelled accordingly. They must be collected from the transfusion laboratory or a blood fridge with appropriate check of the patient identity and taken to the clinical area where they will be used.
- Before administering, the patient's details and the unit of blood are rechecked by trained nursing staff at the bedside immediately prior to the transfusion. Any discrepancies identified must be referred urgently to the blood bank and the clinician responsible for the patient. Transfusion cannot proceed until any ambiguity about identity has been resolved.
- Blood products must be given within their expiry date.
- Always check for damage to the pack, discoloration, or any other visible abnormality.
- Administration of the red cell unit must commence within 30min after leaving the blood bank and be completed within 4h of commencing infusion.
- If a blood warmer needed, ensure a safe, approved warming procedure is used.
- No drug or other infusion solution should be added to any blood component.
- Blood can only be stored in specially designated, monitored refrigerators.
- Platelets should be used immediately and cannot be kept on the ward and must not be placed in a fridge.
- Administration of all blood components must be recorded in the case notes as well as documented on a special blood transfusion chart (usually part of the IV fluid chart). The number of blood units or blood components given should be entered in the notes.

Each unit transfused must have 'traceability'. So hospitals can trace the fate of each unit of blood/blood component (including name and patient identification). This is as required by the Blood and Safety Qualifications that became UK law in 2005 based on the EU blood directive on blood safety setting standards of quality and safety for the collection, testing, processing, storage, and distribution of human blood and components.

## Transfusion of red blood cells

The Better Blood Transfusion's health service circulars published in 1998, 2002, and 2007 provide strong recommendations for promoting safe transfusion practice within hospitals, with particular emphasis on the appropriate use of blood and components in all clinical areas.

All hospitals must have hospital transfusion committees (HTCs), with multidisciplinary representation. These committees are responsible for overseeing implementation of guidelines, clinical audit, and training of all staff involved in transfusion. The HTC has an essential role within the hospital clinical governance framework and must be accountable to the chief executive.

The hospital transfusion team, which comprises the transfusion nurse specialist, transfusion laboratory manager, and consultant haematologist in transfusion, undertakes various activities on a day-to-day basis to achieve the objectives of the HTC.

Many of the the principles of the Better Blood Transfusion initiatives have now been encompassed in patient blood management, an evidence-based, patient-focused initiative, involving an integrated multidisciplinary team approach, with the aims of optimizing the patient's own blood volume (especially red cell mass), minimizing the patient's blood loss, and optimizing the patient's physiological tolerance of anaemia, thereby reducing unnecessary transfusion.

Valid consent for blood transfusion should be obtained and documented in the patient's clinical record. Specific written consent for transfusion is not needed but patients must be informed of the indication for transfusion together with risks and benefits and alternatives available.

Transfusion of red cells should only be given where there is a clear clinical indication with no alternatives available (see Table 17.1). Haematinic deficiency (iron or B<sub>12</sub> and folate deficiency) should be treated with replacement therapy rather than with transfusion. There has been a trend in favour of lowering the haemoglobin (Hb) threshold used as a 'trigger' for red cell transfusion (e.g. Hb trigger of 7g or 8g/L for most patients with a threshold of 8g or 9g/L used if there is a history of cardiac disease).

The initial assessment should include an evaluation of the patient's age, body weight, and any co-morbidity that can predispose to transfusion-associated circulatory overload, such as cardiac failure, renal impairment, or hypoalbuminaemia, and fluid overload should be considered when prescribing the volume and rate of transfusion.

As a general guide, transfusing 1 unit of red cells gives an Hb increment of 1g/L but only if applied as an approximation for a 70–80kg patient. The use of single-unit transfusions in small, frail adults (or prescription in mL, as for paediatric practice) is recommended.

- Red cells have a shelf life of 35d at 4°C and are supplied as concentrated red cells with a PCV between 0.55 and 0.75. Most units in the UK are supplied in 'optimal additive solution', SAGM, which allows removal of all the plasma for preparation of other blood

components and results in a less viscous product. The volume of a unit of concentrated cells is  $280 \pm 20\text{mL}$ .

- All blood in the UK is leucodepleted at source since 1999.
- There may be additional 'special requirements' for some patients such as those with sickle cell disease, who are at very high risk of forming red cell alloantibodies, which ↑ the risk of delayed haemolytic transfusion reactions. Patients with haemoglobinopathy should therefore receive blood that is matched for the patient's full extended Rh type (c, C, D, Ee) and K type, to prevent their forming antibodies to these highly immunogenic antigens.
- Irradiated blood is needed for immunocompromised patients to reduce the risk of transfusion associated graft vs host disease.
- Leucodepletion of blood components (other than granulocytes) provides adequate CMV risk reduction for most clinical situations, but CMV seronegative red cell and platelet components should be provided for intra-uterine transfusions and for neonates, and for pregnant women requiring repeat elective transfusions during the course of pregnancy.
- Frozen RBCs are available at the National Blood Service. They are expensive to process, store, and handle and have a shorter shelf life after thawing. Clinical usage is restricted to patients with extremely rare blood groups or with highly problematic red cell allo-antibodies.
- In autoimmune haemolytic disorders transfusion can be lifesaving as a short term support pending response to immunosuppression.

**Table 17.1** Indications for red cell transfusion

Blood loss	Acute haemorrhage, especially where >30% blood loss, e.g. following surgery, trauma, childbirth, gastrointestinal bleed
Bone marrow disorders causing anaemia	Myelodysplasia Haematological malignancies (e.g. acute leukaemia, myeloma) BM fibrosis BM infiltration with secondary cancer Aplastic anaemia Chemotherapy/radiotherapy or bone marrow transplantation
Inherited RBC disorders	<ul style="list-style-type: none"> <li>● Homozygous <math>\beta</math>-thalassaemia</li> <li>● Red cell aplasia</li> <li>● Hb SS (some circumstances)</li> </ul>
Acquired RBC disorders	<ul style="list-style-type: none"> <li>● Autoimmune haemolytic anaemia</li> <li>● Selected use in renal failure</li> </ul>
Neonatal and exchange transfusions	<ul style="list-style-type: none"> <li>● Haemolytic disease of the newborn</li> </ul>

**Monitoring**

- Monitoring of the patient involves recording temperature, pulse, O<sub>2</sub>-saturation, and BP before transfusion, every 15min for the 1st hour and hourly until transfusion is finished.
- Adverse events should be meticulously recorded.
- Major reactions require immediate cessation of the transfusion and instigation of a full investigative protocol (➔ see Transfusion reactions, p.638).
- Minor febrile reactions are not uncommon. Review by a registered medical practitioner is advised. Their occurrence should be recorded, simple measures such as slowing the rate of infusion or administration of an antihistamine may deal with the problem; if not, transfusion of the specific unit should be stopped.
- An RBC pack should be given within 30min of removal from the blood bank fridge.
- The target infusion time for an individual unit should be 4h.





## Platelet transfusion

Platelets are given to prevent or treat bleeding associated with thrombocytopenia. They may also be given as prophylaxis against bleeding e.g. in patients undergoing intensive chemotherapy (see Table 17.2). Platelets may be required to cover surgery and dentistry.

An adult therapeutic dose (ATD) or 1 unit of platelets can be produced either by single-donor apheresis or by centrifugation of whole blood followed by separation and pooling of the platelet-rich layer from 4 donations suspended in plasma.

Platelets can be stored for 5d at 20–24°C with constant agitation to maintain optimal platelet function.

Bacterial screening of platelets before release can reduce the risk of bacteriological contamination, with an extension of the shelf life of platelet units to 7d.

Platelet components must not be placed in a refrigerator.

- Guidelines state a general threshold for procedures with regards to a patient's platelet count. A platelet count of  $\geq 50 \times 10^9/L$  is sufficient for minor surgery and procedures such as lumbar punctures, biopsies (including liver biopsy), insertion and removal of central lines.
- For major surgery as well as neurosurgery and eye surgery a platelet count of  $\geq 100 \times 10^9/L$  is recommended.
- Patients can tolerate low platelet counts very well and platelets should not be given if the patient is well with no signs of bleeding and a platelet count  $>10 \times 10^9/L$ .

**Table 17.2** Indications for platelet transfusion

↓ production due to BM failure/infiltration	<ul style="list-style-type: none"> <li>● Acute and chronic leukaemias</li> <li>● Myelodysplasia</li> <li>● Myeloproliferative disorders and myelofibrosis</li> <li>● Marrow infiltration with other malignant tumours</li> <li>● Post-chemotherapy or TBI</li> <li>● Aplastic anaemia</li> </ul>
↑ platelet destruction in peripheral circulation	<ul style="list-style-type: none"> <li>● Hypersplenism 2° splenic infiltration or portal hypertension</li> <li>● Consumptive coagulopathies e.g. DIC</li> <li>● Avoid in TTP</li> <li>● Acute and chronic ITP (in emergencies only)</li> <li>● Alloimmune thrombocytopenias, e.g. PTP and perinatal thrombocytopenia (need to be HPA typed)</li> <li>● Sepsis</li> <li>● Drug induced</li> </ul>
Massive blood transfusion	<ul style="list-style-type: none"> <li>● Platelet count <math>&lt;50 \times 10^9/L</math> anticipated after 1.5–2 x blood volume replacement)</li> <li>● Aim to maintain platelet count <math>&gt;75-10^9/L</math></li> </ul>
Platelet function abnormalities	<ul style="list-style-type: none"> <li>● Antiplatelet drugs and bleeding</li> <li>● Myelodysplasia</li> <li>● Rare congenital disorders, e.g. Bernard–Soulier</li> </ul>

- In certain conditions, such as MDS or ITP, even platelet counts in single figures are acceptable as long as there are no clinical signs of bleeding.
- Where there is high consumption of platelets, i.e. bleeding, infection, or DIC, further doses of platelets might be needed to achieve haemostasis.
- Even in immune causes of thrombocytopenia where routinely platelet transfusions are not indicated, platelet transfusions should be given in the acute event of a life-threatening bleed to try and achieve haemostasis.
- Platelet refractoriness is defined as failure to increment more than  $10 \times 10^9/L$  after 2 separate transfusions of random platelets. It is very important to follow-up further transfusions with increments so the diagnosis can be confirmed and management optimized.
- Both immune and non-immune causes should be considered:
  - *Non-immune*: old low-dose platelets, high consumption (DIC, bleeding), hepatosplenomegaly, drug-induced (amphotericin, AmBisome, vancomycin, heparin, and others).
  - *Immune*: class-I HLA antibodies or HPA-antibodies. HLA-matched should be given if HLA antibodies are suspected.
- Platelets are all leucodepleted at source since 1999.
- Group ABO matched platelets are preferred. Do not give group O platelets to group A, B, or AB patients, unless they have been tested -ve for high titre antibodies (HTO).
- Indications for irradiated and CMV -ve products are the same as for red cell transfusions.
- 'Washed platelets': platelets in additive solution (plasma extracted and additive added) may be needed for patients with severe allergic reactions. They only have a 24h shelf life.

## Fresh frozen plasma

FFP is produced by separation and freezing of plasma at  $-30^{\circ}\text{C}$ . As a vCJD reduction step, single donation units sourced outside the UK, treated with methylene blue to reduce microbial activity, are indicated for all children born after 1996. Solvent-detergent plasma is prepared commercially from pools of 300–5000 plasma donations that have been sourced from non-UK donors and treated with solvent and detergent to reduce the risk of viral transmission. (See Tables 17.3 and 17.4.)

FFP is ABO grouped. Rh (D) grouping is not needed. Irradiation is not required.

**Table 17.3** Composition of FFP

Factor	II	VII	IX	X
Range (U/dL)	53–121	41–140	32–102	61–150
Median	82.5	92.0	61.0	90.5

### Indications for use

- Prothrombin complex concentrate and not FFP should be used for reversal of warfarin anticoagulation (↔ see Warfarin overdose, p.656).
- DIC (↔ see Disseminated intravascular coagulation, p.648).
- Liver disease and biopsy. Routinely done but not evidence based.
- Massive blood transfusion. (↔ See Massive blood transfusion, p.658).
- Isolated coagulation deficiencies where no specific concentrate is readily available.
- Treatment of thrombotic thrombocytopenia purpura/haemolytic uraemic syndrome (TTP/HUS) (↔ see Thrombotic thrombocytopenic purpura, p.664).

### Contraindications for use

- Hypovolaemic shock. Never use FFP as a plasma-expander!
- Plasma-exchange, except in TTP/HUS.

### Instructions for use

- The average volume of 1U is 220–250mL.
- FFP must be given as soon as possible after thawing. If unused it can be stored in the transfusion laboratory fridge at  $4^{\circ}\text{C}$  for 24h.
- Dose 10–15mL/kg body weight.
- Usual dose in an adult = 4U.
- Check PT and APTT before and 5min after infusion to assess response.
- In major haemorrhage further doses may be needed until bleeding is controlled.

**Table 17.4** Half-life of infused coagulation factors in FFP

<12h	Factors V, VII, VIII, and protein C
>12 <24h	Factor IX and protein S
>24 <48h	Factor X
>48h	Fibrinogen, factors XI, XII, XIII, ATIII

### What to give

- FFP ideally should be ABO group specific, but this is not essential. group O FFP should only be given to group O patients, since may contain high-titre anti-A and anti-B antibodies.
- FFP in the UK is collected from ♂ donors to reduce the risk of TRALI.
- Patients born after 1996 should receive FFP from non-UK donors to reduce the risk of transmission of vCJD. This plasma is treated with methylene blue (MB-FFP) a form of pathogen inactivation.
- Octaplas: FFP treated with solvent detergent (SD-FFP). It is virally inactivated FFP (only lipid coated viruses, so not parvovirus B19 and HAV). Octaplas as FFP, is ABO matched (not Rh (D)).

### Cryoprecipitate

#### Indications for use

Cryoprecipitate is prepared by slow thawing of FFP at 4–6°C. The precipitate formed is cryoprecipitate which is then stored at –30°C. It is rich in factors VIII, XIII, fibrinogen, and vWF. It is available as pools of 5 units with 2 pools used for 1 adult dose when needed.

- Additional support for the clotting defects induced by massive transfusion.
  - See Massive blood transfusion, p.658. Hypo/dysfibrinogenaemia in liver disease/transplant to prevent and treat bleeding.
- Should not be used in haemophilia A and vWD since virally inactivated specific concentrates are available.
- Congenital dys/hypofibrinogenaemia—fibrinogen concentrate is licensed and available for this indication in UK.

#### Contraindications

- Should never be given for plasma-expansion.
- Of no proven value as empirical treatment in post-op or uraemic bleeding.

#### Instructions for use

- Kept frozen until required. Use as soon as possible after thawing.
- Shelf life of 4h at room temperature post thawing—should not be placed in a refrigerator.
- ABO compatibility if possible but not essential.
- Dose 1U per 5–10kg body weight (regular adult dose is 10U or 2 pools).
- 1 pool = 5 units of cryoprecipitate.
- Aim to keep fibrinogen >1.5g/L.

### Further reading

Keeling, D. *et al.* (2011) Guidelines on oral anticoagulation with warfarin—fourth edition. *Br J Haematol*, **154**, 311–24.

## Intravenous immunoglobulin

IVIg is used as antibody replacement in 1° and 2° antibody deficiency states, and as immune modulator (Table 17.5).

### Preparations of IVIg

- Contain predominantly IgG (with small amounts of IgA and IgM).
- Prepared from large pool of normal donors, e.g. >1000.
- Contain all subclasses of IgG encountered in normal population.
- In view of the risk of vCJD, non-UK plasma is used for fractionation.

### Mechanisms of action

The mechanism of action is not fully understood: natural anti-idiotypic antibodies suppress antibody production in patient; Fc receptor blockade on macrophages (thereby blocking RE function) and T/B lymphocytes (inhibits autoantibody production); suppression of production of inflammatory mediators (e.g. TNF- $\alpha$ , IL-1) produced by macrophages.

### Administration

Usual dose 1g/kg/d for 2d for immune modulation or 0.4–0.6g/kg/month for 2° immune deficiency (CLL, myeloma, etc.).

Check the patient's observations pre-infusion. With the 1st infusion patient should be monitored closely and observations taken ½ hourly for the first hour. Side effects are more likely at the start of an infusion and in the 1st hour (see pack insert and BNF for details).

### Complications

- Fevers, chills.
- Backache.
- Myalgic symptoms.
- Flushing.
- Nausea  $\pm$  vomiting.
- Severe allergic reactions in IgA-deficient patients (due to small amount of IgA in IVIg preparation).

*Note:* UK Department of Health Guidelines are being developed to promote good IVIg prescribing practice across all specialties that use IVIg. These guidelines have been written in response to the world shortage of IVIg since it was felt that many patients were receiving IVIg where little evidence exists for its beneficial effect.

**Table 17.5** Uses for IVIg

<i>Antibody replacement</i>	
<ul style="list-style-type: none"> <li>● 1° immune deficiency</li> <li>● Transient hypogammaglobulinaemia of infancy</li> <li>● Common variable immune deficiency</li> <li>● Sex-linked hypogammaglobulinaemia</li> <li>● Late-onset hypogammaglobulinaemia</li> <li>● Hypogammaglobulinaemia + thymoma</li> </ul>	<ul style="list-style-type: none"> <li>● 2° immune deficiency</li> <li>● CLL</li> <li>● Non-Hodgkin lymphoma</li> <li>● Multiple myeloma</li> <li>● Post-BMT</li> </ul>
<i>Immune modulation</i>	
<ul style="list-style-type: none"> <li>● Autoimmune disease, ITP</li> <li>● Autoimmune haemolytic anaemia</li> <li>● Autoimmune neutropenia</li> <li>● Red cell aplasia</li> <li>● Coagulation factor inhibitors</li> <li>● Post-transfusion purpura (PTP)</li> <li>● Neonatal platelet alloimmunization</li> <li>● Thrombocytopenia in pregnancy</li> </ul>	<ul style="list-style-type: none"> <li>● Prophylaxis/treatment of CMV in BMT patients</li> <li>● B19-induced red cell aplasia</li> <li>● Haemophagocytic syndromes (viral)</li> </ul>

Data from Department of Health, *Clinical Guidelines for Immunoglobulin Use*, 2nd ed., June, 2011.

## Transfusion transmitted infections

In the UK, all donations are tested for syphilis, hepatitis B, hepatitis C, human T-lymphotropic virus type 1 (HTLV1) and HTLV2 and human immunodeficiency virus (HIV). Data available for 2008–2010, estimating the risk of a potentially infectious unit entering the blood supply in the UK, are shown in Table 17.6. Where indicated by their travel history, donors are also tested for malaria and *Trypanosoma cruzi* antibodies. Other discretionary tests include anti-HBc (e.g. after body piercing). Some donations are also tested for cytomegalovirus (CMV) antibody to help provide CMV-negative blood for particular patient groups. In the UK, the Advisory Committee on the Safety of Blood, Tissues and Organs in the UK (SaBTO) has reviewed evidence available and recommended that leucodepletion of all blood components (other than granulocytes) provides adequate CMV risk reduction for almost clinical situations, but CMV seronegative red cell and platelet components should be provided for intrauterine transfusions and for neonates, and for pregnant women requiring repeat elective transfusions during the course of pregnancy. Bacterial screening of platelets (BACTalert) is now undertaken by some blood services such as NHS Blood and Transplant in England and has reduced the risk of bacterial contamination with potentially fatal consequences due to septic shock post transfusion.

Variant Creutzfeldt-Jakob disease (vCJD): to date, there have been 3 cases in the UK where blood transfusion may have been implicated in transmission of vCJD and 1 case where an abnormal prion was demonstrated at autopsy, in a patient who did not have neurological symptoms but was known to have received blood from a donor who subsequently developed vCJD. A further transmission of vCJD prions was described in February 2009, in a patient with haemophilia who had received batches of factor VIII to which a donor who subsequently developed vCJD had contributed plasma. The patient died of other causes but was found to have evidence of prion accumulation in his spleen. There is no blood test currently available for detecting prions.

The full risk of vCJD in the UK population remains uncertain and accordingly the UK blood services have taken a number of precautionary measures to reduce the potential risk of transmission of prions by blood, plasma and blood products, the latter requiring fractionation of very large volumes of plasma. These include:

- Universal leucodepletion (removal of white cells) of all blood donations since 1998.
- Importation of plasma for countries other than the UK for fractionation to manufacture plasma products.
- Importation of FFP for use in children born after January 1996.
- Exclusion of blood donors who have received a transfusion in the UK since 1980.



**Table 17.6** Transfusion transmitted infection in the UK—approximate risk 2008–2010

Testing introduced	Examples of testing methods used	Approximate risk of infection
1940s	Antibody	
1970 onwards	Surface antigen (HBsAg)	1 in 1 million
1985/2002 onwards	Antibody/nucleic acid testing	1 in 6 million
1991/1999 onwards	Antibody/nucleic acid testing	1 in 72 million

### Transfusion-associated graft versus host disease (TA-GVHD)

Donor T lymphocytes in blood products engraft and clonally expand in the recipient. Activated donor lymphocytes produce cytokines, and cause general inflammation and tissue damage.

- Very rare.
- Related to the number of lymphocytes in donor blood products. Greatly reduced with lymphocyte depletion.
- High-risk patients: defective inherited or acquired recipient cell mediated immunity, HLA matched transfusions or transfusions from relatives, transplant patients, patients who had purine analogues.
- Symptoms within 2–30d: fever, skin rash, diarrhoea, hepatitis, pancytopenia.
- 90% mortality.
- Prevention: leucocyte depleted, irradiated blood products (HLA matched products should ALWAYS be irradiated).

TA-GVHD is invariably fatal. Patients who are considered to be at risk of developing this condition as a result of exposure to donor T lymphocytes must receive irradiated blood products.

## Irradiated blood products

See Box 17.1 for indications.

### **Box 17.1 Indications for irradiated blood-products**

- Allogeneic and autologous BMT or PBSC recipients from 1 month before pre-transplant conditioning until at least 6 months post transplant.
- Allogeneic BM and stem cell donors.
- Hodgkin lymphoma.
- Those who have received purine analogue drugs (fludarabine, cladribine, pentostatin (deoxycoformycin)).
- All donations from HLA matched donors or 1st- or 2nd-degree relatives.
- Children with congenital immunodeficiency states.
- Intra-uterine transfusions and transfusions for infants who have had intra-uterine transfusions.
- Exchange transfusions in neonates.

## Strategies for reducing blood transfusion in surgery

### Preoperative assessment

Patients should be assessed preoperatively with attempts made to optimize the haemoglobin (mainly by correcting Fe deficiency). They should also be assessed for risk of bleeding with an appropriate plan for managing antiplatelet and anticoagulant therapy pre- and postoperatively.

### Intraoperative blood salvage

Routine preoperative autologous donation and storage of blood prior to surgery is no longer recommended in the UK. However intraoperative cell salvage (IOCS) of the patient's own blood should be used depending on the type of surgery (e.g. orthopaedic, cardiac, vascular, urological etc). This technique may be used for almost any surgical procedure (provided no faecal contamination) and is also being used for cancer surgery.

IOCS allows blood lost during surgery to be collected and washed using suction catheters and filtration systems and then re-infused into patients. Its use is ↑ in the UK.

- Preoperative haemodilution: involves reducing the Hb concentration prior to surgery. This reduces blood viscosity and red cell loss (through reduced haematocrit). 2–3U of blood are collected with replacement using crystalloids or colloid solutions.
- Hb is reduced to ~10g/dL and haematocrit to 30% (0.3).
- The collected blood is re-infused at the end of surgery.
- This technique is not commonly used.

### Pharmacological methods of blood saving

Tranexamic acid is an antifibrinolytic which has been shown to improve outcomes in patients with traumatic haemorrhage. It should be given to adult trauma patients as soon as possible after injury, in a dose of 1g over 10min followed by a maintenance infusion of 1g over 8h.

The use of tranexamic acid should also be considered in non-traumatic major bleeding where there is no contraindication.

Aprotinin—no longer widely used due to risk of adverse outcomes.

## Maximum surgical blood ordering schedule (MSBOS)

A system of tailoring blood requirements to particular elective surgical procedures, including—importantly—procedures which do not usually require blood cover.

- The ABO group and Rh (D) type of the patient is determined on duplicate samples, and the serum screened for significant RBC ('atypical') antibodies. If there are no antibodies, the serum is kept available ('saved') for a determined period (usually a week)—this is the 'Group, Screen, and Save' (G&S) procedure.
- If there are no atypical antibodies, and the planned surgery is likely to need perioperative transfusion, the required number of red cell units are matched by routine tests, labelled, and set aside in an accessible refrigerator. Storage conditions must meet certain standards (continuous recording of appropriate temperature, alarms, etc.).
- If more blood is required than anticipated, extra units must be readily available. If the need is urgent, suitable arrangements—such as rapid matching (using the 'saved' serum) and despatch procedures—must enable the timely supply of blood.
- If there are atypical antibodies, which may occur in up to 10% patients, their specificity must be determined and, if clinically significant, sufficient (extra) red cell units lacking the relevant antigen provided and matched by detailed techniques. (These are often referred to as 'phenotyped red cells'.)
- If there is no 'MSBOS' more units must be matched than are usually required for transfusion, in order to give rapid access if extra blood is needed. Matched 'bespoke' blood is therefore unavailable for other patients for the 2–3d set aside.
- A good MSBOS gives better access to blood stocks and enables more efficient use, in particular of O Rh (D) –ve blood. There is no good reason for regarding O Rh (D) –ve blood as a 'universal' donation type. It can be antigenic; and it is a precious resource, being available from <8% of the population.
- The surgical team must be confident in the system, and the blood bank staff committed to 'minimal barriers'. The cross-match:transfusion ratio of a blood bank may well become <2 (i.e. overall <2U matched for every unit transfused) which is an indication of efficient practices. It could even be nearer to 1 than to 2.
- MSBOS schedules will vary between hospitals—depending on demographic factors, general layout, access to the blood bank refrigerators and use of electronic issue, types of surgery, etc.



## **Patients refusing blood transfusion for religious reasons, i.e. Jehovah's Witnesses**

- Christian sect numbering 120,000 in the UK.
- Pose ethical and management difficulties due to their refusal of blood transfusion, derived from a literal interpretation of a number of biblical passages (Acts 15: 28–29).
- Jehovah's Witnesses still die during both elective and emergency surgery due to their beliefs.

### **Elective surgery—discuss**

- Risks of surgery and the specific risk of refusing blood.
- Extent of religious belief. What blood derived products they personally are willing to accept, e.g. albumin, factor concentrates, etc?
- What blood components not acceptable even if life-threatening bleeding, e.g. red cells and may also include platelets, FFP and Cryo.
- Discuss with Jehovah's Witnesses Hospital Liaison Committee.
- Check that the patient has a signed advance directive clearly stating what treatment is or is not acceptable.

If the surgeon agrees to an operation, communication then becomes paramount and the Jehovah's Witness should be referred to both an anaesthetist and haematologist. This should be done as early as possible once the decision is made to proceed with surgery to allow time for any optimization and for further counselling with their family.

### **Preoperative considerations**

- Check FBC and haematinics (iron, B<sub>12</sub>, folate); replace if needed; consider use of IV iron if intolerant of oral iron or if short time period (<2 weeks) prior to surgery.
- If anaemic then consider use of erythropoietin (EPO); discuss with your hospital pharmacy regarding preparation available and dosing; be aware of adverse events including thromboembolic risk and contraindications to EPO.
- Check if patient will accept intraoperative cell salvage and arrange for this to be available.
- ITU bed available.
- Review anticoagulant and antiplatelet therapy and manage accordingly based on the patient's underlying risk factors. Stop NSAIDs.

### **Operative considerations**

#### *Surgeon*

- Consultant input.
- Positioning of patient—to prevent venous congestion.
- Tourniquets if possible.
- Meticulous haemostasis.
- Fibrin glues.

### **Anaesthetist**

- Consultant input.
- Regional blocks.
- Hypotensive anaesthesia.
- Intraoperative cell salvage.
- Pharmacological methods to improve clotting e.g. tranexamic acid.
- Consider use of interventional radiology where appropriate for prevention and treatment of bleeding.

### **Postoperative considerations**

- Observation for re-bleed—HDU, senior surgeon review.
- Optimize HbFe, EPO (B<sub>12</sub> and folate if needed).
- Reduce phlebotomy and use paediatric samples for blood tests.

### **Emergency surgery**

- Check patient has advanced directive.
- Early investigations CT, USS abdomen, pelvis to diagnose source of bleeding.
- Interventional radiology or surgery for control of bleeding (surgical techniques as above).
- Tranexamic acid for prevention and treatment of bleeding.
- Intraoperative cell salvage depending on type of surgery.
- Optimize Hb postoperatively—Iron, EPO (B<sub>12</sub> and folate if needed).

### **Children**

- Communication with parent.
- Judicial intervention may be required—must discuss with consultant responsible for care and refer to hospital guidance for management of children of parents who are Jehovah's Witnesses.

### **Further reading**

Marsh, J.C. and Bevan, D.H. (2002). Haematological care of the Jehovah's Witness patient. *Br J Haematol*, **119**, 25–37.

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## Rare diseases

- Gaucher disease 784
- Niemann–Pick disease 786
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- The porphyrias 790
- Retroperitoneal fibrosis 792
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- Osteopetrosis 794

### Note

The title of this chapter is possibly a misnomer in itself as many conditions in malignant and general haematology discussed in this handbook are exceptionally rare. The 'rare' diseases discussed here are conditions that are not discussed elsewhere in this work and that to a certain extent defy classification or categorization. They are apt to be things that never crossed your worried mind; the kind that blindsides you at 4p.m. on some idle Tuesday.

## Gaucher disease

Gaucher disease is the most common type of sphingolipidosis, a group of genetic lysosomal storage diseases, defined by its dysregulated metabolism of sphingolipids. It is an autosomal recessive inherited disorder that results in the accumulation of cerebrosides in organs and tissues. Cerebrosides deposit mainly in the spleen, liver, kidneys, lungs, brain, and BM.

### Pathophysiology

Defect in glucocerebrosidase (beta-glucosylceramidase), preventing the breakdown of glucocerebrosides. The accumulation happens particularly in macrophages; this leads to the typical Gaucher cell on morphology. About 80 mutations have been described. Different mutations in the enzyme determine the remaining activity of the enzyme. Prenatal diagnosis is available if mutation known.

### Diagnosis:

- Clinical and family history.
- Glucocerebrosidase assay and DNA analysis.
- Sometimes: raised ALP, ACE, and immunoglobulins.
- Bloodfilm/BM: Gaucher cells with typical cytoplasm with a fibrillar pattern ('crinkled paper' morphology) and macrophages filled with lipids.

### Clinical

- Bruising.
- Fatigue.
- Anaemia and thrombocytopenia.
- Hepatosplenomegaly.
- Pingueculae: conjunctival deposits.
- Skeletal disorders and painful bony lesions; 'Erlenmeyer-flask deformity' due to BM expansion and cortical bone resorption.
- Joint swelling and pains.
- Hypersplenism.
- Neurological symptoms: convulsions, hypertonia, and mental retardation in type 2; myoclonus, convulsions, dementia, and ocular muscle apraxia in type 3.
- Lymphadenopathy.
- Skin pigmentation: yellow-brown complexion.

### Classification

The disease is classified into 3 types based on clinical features (CNS involvement, severity, and rate of progression).

**Type 1:** most common and often mild; non-neuronopathic Gaucher disease. Seen in all ages and should not be called 'adult type Gaucher'. Progression of disease is variable. Variable severity and rate of onset of symptoms. Hepatosplenomegaly invariably a symptom; bruising and fatigue common. No CNS involvement. Good prognosis.

**Type 2:** 'acute infantile neuronopathic type': very rare and rapidly progressive. Affects the CNS, spleen, liver, lungs, and bones. Typically

presents in 1st year of life with severe neurological symptoms. Severe neurodegenerative progression. Life-expectancy 2–3 years.

**Type 3:** clinically variable. Can progress rapidly with visceral disease but little CNS involvement, however dementia and parkinsonism in adolescence can occur. Most don't live past the age of 30. High prevalence in the Norrbotten province of northern Sweden.

### Treatment

- Enzyme replacement therapy (ERT) with IV recombinant glucocerebrosidase. No effect in type 2 (neurological symptoms). Hepatosplenomegaly, skeletal abnormalities, and cytopenias can improve. Weekly IV infusion often for life. Velaglucerase alfa and Taliglucerase alfa approved (orphan drug status). Success variable. Oral ERT: miglustat.
- Substrate reduction therapy, if not suitable for ERT.
- BMT/PBSCT: rarely performed. Not for neurological disease.
- Splenectomy: occasionally performed if hypersplenic.
- Supportive medication: antiepileptics and bisphosphonates.
- Role of gene therapy in the future?

### Further reading

- Dahl, N. et al. (1990). Gaucher disease type III (Norrbottnian type) is caused by a single mutation in exon 10 of the glucocerebrosidase gene. *Am J Hum Genet*, **47**, 275–8.
- Grabowski, G.A. (2008). Phenotype, diagnosis, and treatment of Gaucher's disease. *Lancet*, **372**(9645), 1263–71.
- Grabowski, G.A. (2012). Gaucher disease and other storage disorders. *Hematology Am Soc Hematol Educ Program*, **2012**, 13–18.
- Jmoudiak, M. et al. (2005). Gaucher disease: pathological mechanisms and modern management. *Br J Haematol*, **129**, 178–88.
- Schmitz, J. et al. (2007). Therapy of adult Gaucher disease. *Haematologica*, **92**(102), 148–52.

## Niemann–Pick disease

Rare metabolic disorder; type of sphingolipidosis. Autosomal recessive disorder. Often fatal in infants; some patients with milder course live into their teens or early adulthood. Mutations in the *SMPD1* gene (Type A/B) lead to no sphingomyelinase production; mutations in *NPC1* and *-2* (Type C) lead to abnormalities in the lipid-transport proteins. Incidence 1:250,000 (1:40,000 in Ashkenazi Jews). Genetic counselling and pre-natal diagnosis is possible.

### Pathophysiology

Sphingomyelin deficiency leads to accumulation of sphingomyelin in the lysosomes, leading to organ and tissue accumulation and dysfunction.

### Classification

- Type A: classic infantile.
- Type B: visceral type.
- Type C: subacute/juvenile. Most common form.

### Clinical

- Hepatosplenomegaly.
- Lung symptoms.
- CNS: ataxia, dysarthria, dysphagia.
- Mental retardation.
- BM deposition can lead to pancytopenia.

### Diagnosis:

- Blood tests.
- Raised cholesterol often seen.
- BM: foam cells (due to multiple vacuoles) on aspirate: lipid-laden macrophages in the marrow and ‘sea-blue histiocytes’ on pathology.
- Sphingomyelin assay.

### Treatment

- Statins to control cholesterol.
- Supportive measures.
- BMT/PBSCT can be considered in rare cases.
- Enzyme replacement therapy though results poor.

### Further reading

Vanier, M.T. (2013). Niemann–Pick diseases. *Handb Clin Neurol*, **113**, 1717–21.



## Castleman disease

Synonyms: giant or angio-follicular lymph node hyperplasia.

A rare lymphoproliferative disorder which can present with local or widespread disease. It is not considered a malignancy although there is a hyperproliferation of B lymphocytes. Over-secretion of interleukin-6 (Il-6) and HHV-8 (human herpes virus-8) infection are implicated in its aetiology. It is more often seen in HIV patients. The obvious differential diagnosis includes lymphoma and other cancers, as well as reactive causes of lymphadenopathy. Diagnosis is made on excision biopsy; diagnosis can be difficult and repeat biopsy might be required. Castleman disease is also seen in POEMS syndrome and implicated in a significant number of patients with paraneoplastic pemphigus. Unicentric and polycentric Castleman disease have been described.

### Unicentric Castleman disease (UCD)

- Single-site disease, with often only local symptoms.
- Good prognosis.
- Hyaline vascular variant (80%): aetiology unknown; associated symptoms rare; associated diagnoses include paraneoplastic pemphigus and TTP. Treatment with surgery or radiotherapy.
- Plasma cell variant (20%): aetiology HHV-8 related; usually more systemic symptoms and associated cytopenias. Associated diagnoses include amyloidosis and renal insufficiency. Most treated with surgery, but radiotherapy, ganciclovir, chemotherapy, or Il-6 antibody might all be needed.

### Multicentric Castleman disease (MCD)

- Plasma cell variant only.
- Lymphadenopathy at multiple sites. Often unwell.
- Raised ESR/CRP and cytopenias common.
- Aetiology HHV-8 related. Associated diagnoses include POEMS syndrome, HIV infection, Kaposi sarcoma and amyloidosis.
- Treatments include ganciclovir (if HHV-8 positive) and rituximab. Steroids, combination chemotherapy, Il-6 monoclonal antibodies and immunomodulators (thalidomide) have been used with varying success.
- In HIV patients HAART should be commenced or optimized.
- High relapse rates.

### Further reading

Dispenzieri, A. *et al.* (2012) The clinical spectrum of Castleman's disease. *Am J Hematol*, **87**(11), 997–1002.

Ramasamy, K. *et al.* (2012). Rituximab and thalidomide combination therapy for Castleman disease. *Br J Haematol*, **158**(3), 421–3.

Roca, B. (2009). Castleman's disease. A Review. *AIDS Rev*, **11**(1), 3–7.

Talat, N. *et al.* (2011). Castleman's disease: systematic analysis of 416 patients from the literature. *Oncologist*, **16**(9), 1316–24.

Uldrick, T.S. *et al.* (2012). Recent advances in Kaposi sarcoma herpesvirus-associated multicentric Castleman disease. *Curr Opin Oncol*, **24**(5), 495–505.

## Kikuchi disease

Synonyms: Kikuchi–Fujimoto disease (KFD), histiocytic necrotizing lymphadenitis, subacute necrotizing lymphadenitis.

A rare disease affecting young adults typically presenting with cervical lymphadenopathy. Main prevalence in Japan though isolated cases elsewhere reported. Differential diagnosis with malignancies (lymphoma), infective/reactive lymphadenitis (TB, sarcoidosis) and auto-immune disorders. Diagnosis is made on excision biopsy. If the diagnosis is in doubt a repeat biopsy might be required.

### Pathophysiology

Kikuchi disease is caused by a non-specific hyperimmune reaction. Many different aetiologies have been described, including auto-immune disorders (SLE, antiphospholipid syndrome), infective agents (bacterial, viral) and genetic predisposition.

### Symptoms

- Self-limiting disease with most lymphadenopathy resolved by 6 months. Recurrence in 3–5%.
- Fever.
- Cervical lymphadenopathy: often tender.
- Hepatosplenomegaly rare.
- Skin rashes rare.
- Headaches.
- Aseptic meningitis has been described.
- Mortality very rare.

### Management

- Supportive: self-limiting disease.
- NSAIDs and antipyretics.

### Further reading

Veer, V. et al. (2012). Kikuchi–Fujimoto disease: a case report and literature review. *Case Rep Otolaryngol*, **2012**, 497604.

Weiss, L.M. et al. (2013). Benign lymphadenopathies. *Mod Pathol*, **26**(Suppl. 1), S88–96.

## The porphyrias

Rare inherited or acquired group of disorders, seen in all races. Over 200 mutations have been described. If the genetic defect is known, family screening and antenatal screening is possible. The acquired porphyrias are 2° to liver disease, mercury or arsenic poisoning.

### Pathophysiology

Porphyryns are the precursors of haem, essential for the production of haemoglobin. In the porphyrias enzymatic defects lead to an insufficient production of haem and deposition of toxic porphyrin. Each particular enzymatic defect leads to different porphyryns, which depending on their chemical properties deposit in different locations. Some porphyryns induce photosensitivity. The subtype of porphyrias is dependent on what enzyme is deficient. Pathophysiologically porphyrias are divided into hepatic or erythropoietic, based on the site of haem deposition.

### Signs and symptoms

Symptomatically the porphyrias are divided into acute or cutaneous porphyrias. Precipitating factors for acute attacks are stress, infections, barbiturates, some antibiotics (sulfonamides), surgery, and low caloric intake. All toxic intermediates can trigger a haemolytic anaemia.

### Acute porphyrias

- Hepatic porphyria (neurovisceral).
- Affects the nervous system: abdominal pain, vomiting, muscle weakness, neuropathy, arrhythmias, constipation/diarrhoea, seizures and mental disturbances (anxiety, depression, hallucinations). Acute attacks can be fatal.
- ↑ risk of hepatocellular carcinoma.
- Variants: ADP: Ala-dehydratase porphyria; AIP: acute intermittent porphyria; HCP: hereditary coproporphyria; VP: variegate porphyria (mixed porphyria), the only porphyria with both skin lesions and neurological symptoms.
- More common and dominant inheritance (AIP, HCP, and VP).

### Cutaneous porphyrias

- Erythropoietic porphyrias. Symptoms due to complement activation.
- Skin: photosensitivity, blisters, itch, skin necrosis, lichenification, erythema.
- ↑ hair-growth (hypertrichosis).
- No neurological symptoms.
- Variants: CEP: congenital erythropoietic porphyria; PCT: porphyria cutanea tarda; EPP: erythropoietic proto-porphyrin; HEP: hepatoerythropoietic porphyria.

### Diagnosis

- Biochemical analysis of blood, urine or stool sample. Protect samples from light.
- ↑ porphobilinogen (a precursor) in urine: ↑ precursor production through feedback mechanism. Raised in almost all acute porphyrias (not in acquired porphyria).
- Testing should be done on samples during an acute attack otherwise false negatives are common.



### Treatment of acute porphyria

- Avoid precipitating factors: drugs, stress, hormone replacement, infections, surgery, low carbohydrate diet.
- Glucose 10% infusion if severe porphyria attack is suspected.
- Symptom control: pain relief (opiates), antiemetics.
- Neurological and psychiatric problems: treat chronic neuropathic pain, gut dysmotility, chronic pain, and depression.
- Seizures: most antiepileptics can exacerbate acute attacks. Barbiturates must be avoided.
- Treat underlying liver disease (in acquired porphyria).
- Treatment of iron overload.
- Hormone treatment: oral contraceptives can be used to modify hormonal fluctuations if this is triggering attacks.
- Specifically for erythropoietic porphyria: avoid sunlight; chloroquine to increase porphyrin secretion.
- For neurovisceral attacks (acute, hepatic porphyrias): haem supplementation: haematin (US) or haem arginate (UK). Should be given as soon as an attack starts; proven to reduce duration and intensity of attacks. Haem supplementation inhibits ALA-synthase and eliminates the feedback on precursor production. Preventative heme can be given every 10–14 days.
- In CEP, HEP, EPP: reduction of erythropoiesis can help prevent attacks: hydroxycarbamide, venesections, hypertransfusion.

### Further reading

Balwani, M. et al. (2012). The porphyrias: advances in diagnosis and treatment. *Hematology Am Soc Hematol Educ Program*, **2012**, 19–27.

Stein, P. et al. (2013). Best practice guidelines on clinical management of acute attacks of porphyria and their complications. *Ann Clin Biochem*, **50**(Pt 3):217–23.

## Retroperitoneal fibrosis

Synonym: Ormond's disease.

This condition is a descriptive term; a proliferation of fibrous tissue is seen in the retroperitoneum, mainly around the aorta.

### Causes

Many aetiologies have been proposed. There is an association with auto-immune conditions, previous radiotherapy, malignancy, certain drugs (hydralazine, beta-blockers) and infections.

### Symptoms

- Back pain.
- Hydronephrosis and renal impairment.
- Hypertension.
- VTE.
- Peripheral oedema 2° to obstruction.
- Malaise and weight loss.

### Diagnosis

Imaging can be diagnostic on its own (confluent mass surrounding the aorta). If in doubt a biopsy is required.

### Treatment

- Immune suppression has reported responses in 20–50%.
- Surgery might be required.
- Steroids have been used with varying success.

### Further reading

Ormond, J.K. (1965). Idiopathic retroperitoneal fibrosis: a discussion of the etiology. *J Urol*, **94** (4), 385–90.

Vaglio, A. et al. (2006). Retroperitoneal fibrosis. *Lancet*, **367**(9506), 241–51.

van Bommel, E.F. (2002). Retroperitoneal fibrosis. *Neth J Med*, **60**(6), 231–42.

## Langerhans cell histiocytosis

Synonyms: histiocytosis X; dendritic cell histiocytosis; eosinophilic granuloma (older term, a misnomer); Hand–Schüller–Christian disease if multiple lesions; Letterer–Siwe disease if disseminated.

Clonal proliferation of Langerhans cells (myeloid lineage-derived dendritic cells). Generally considered to be a malignancy. Uni- or multifocal disease. The BRAF mutation (activating mutation of a proto-oncogene) is seen in ~60% of cases. Disease most frequently seen in children. Prognosis poor if multiple organs involved and young age. Solitary lesions and limited disease can regress spontaneously.

### Clinical

- Unifocal: solitary lesions (skull, mandible, soft tissue).
- Multifocal unisystem: Hand–Christian–Schüller disease: fever, bone lesions, and diffuse skin lesions (mainly scalp). Triad of diabetes insipidus, exophthalmos, and lytic lesions.
- Multifocal multisystem: Letterer–Siwe disease: disseminated.
- Constitutional symptoms: fever, lethargy, weight loss.
- Lymphadenopathy and pancytopenia due to BM disease.
- Endocrine glands involved: diabetes insipidus most common.
- Rare: lung, GI, or CNS.

### Diagnosis mainly on biopsy

- Eosinophilic micro-abscesses and necrosis. Langerhans cells are seen (oval cells without dendritic processes).
- CD1a+, Langerin (CD207)+, S100+, CD68+, CD163+, lysosyme+, CD45+.
- Haemophagocytosis often seen.
- Langerin induces the formation of Birbeck granules.
- Other: radiology for staging.

### Treatment:

- Solitary lesions: surgery or limited radiotherapy.
- Chemotherapy for systemic disease: schedules including prednisolone, vinca-alkaloids, 6-mercaptopurine and etoposide have been used. Cladribine also has shown activity.

### Further reading

- Donadieu, J. et al. (2013). Medical management of Langerhans cell histiocytosis from diagnosis to treatment. *Expert Opin Pharmacother*, **13**(9), 1309–22.
- Haupt, R. et al. (2013). Langerhans cell histiocytosis (LCH): guidelines for diagnosis, clinical work-up, and treatment for patients till the age of 18 years. *Pediatr Blood Cancer*, **60**(2), 175–84.
- Hirasaki, S. et al. (2012). Multifocal Langerhans cell histiocytosis in an adult. *Intern Med*, **51**(1), 119–20.

## Osteopetrosis

Synonym: marble bone disease; Albers–Schönberg disease.

Rare inherited disorder whereby the osteoclast function is impaired (number of osteoclasts variable). This leads to impaired bone resorption with ongoing bone formation leading to hardened bones. Despite this, patients with osteopetrosis have an ↑ risk of fractures and other bone problems. Aetiology is unknown. Can occur at any age.

### Symptoms

- Impaired growth.
- Bone deformities.
- Pathological fractures.
- Bone expansion leads to BM replacement which leads to extramedullary haematopoiesis: hepatosplenomegaly, pancytopenia.
- Blindness, deafness, and facial nerve palsies due to nerve compression.

### Differential diagnosis

- Hypoparathyroidism.
- Paget's disease: a disease of excessive bone breakdown and formation leading to enlarged and malformed bones.
- Metastatic cancer: prostate, breast.
- Myelofibrosis.
- Multiple myeloma.
- Sickle cell/thalassaemia (extramedullary haematopoiesis).

### Treatment

- BMT/PBSCT is the only possible curative option.
- Vitamin D.
- Interferon has been used with varying results.
- Supportive: analgesia.

# Haematology online

Haematology online *796*



## Haematology online

There are many Internet resources available for haematology, including organizations, journals, atlases, conference proceedings, and news-groups. The main difficulty with Internet resources is that they change so frequently and they are constantly being updated and outdated. It would be impossible to list every known site, and we have provided only URLs for those we think are of most value.

### General websites

#### *Bloodline*

🔗 [www.bloodline.net](http://www.bloodline.net)

#### *BloodMed*

🔗 [www.bloodmed.com](http://www.bloodmed.com)

#### *Breaking Barriers*

🔗 [www.liv.ac.uk/breakingbarriers](http://www.liv.ac.uk/breakingbarriers)

#### *Clinical Leaders of Thrombosis (C.L.O.T.)*

🔗 [www.clotuk.com](http://www.clotuk.com)

#### *Congenital Dyserythropoietic Anaemia Research Initiative Oxford*

🔗 [www.imm.ox.ac.uk/congenital-dyserythropoietic-anaemia-cda](http://www.imm.ox.ac.uk/congenital-dyserythropoietic-anaemia-cda)

#### *Elimination of Leukaemia Fund*

🔗 [www.leukaemiafund.org.uk](http://www.leukaemiafund.org.uk)

#### *Free Medline Searching*

🔗 [www.ncbi.nlm.nih.gov/PubMed/](http://www.ncbi.nlm.nih.gov/PubMed/)

#### *Health News—The Media Medical Agency*

🔗 [www.health-news.co.uk/](http://www.health-news.co.uk/)

#### *International Medical News*

🔗 [www.internationalmedicalnews.com](http://www.internationalmedicalnews.com)

#### *Lymphoma Forum*

🔗 [www.lymphoma.org.uk](http://www.lymphoma.org.uk)

#### *lymphoma-net.org*

🔗 [www.lymphoma-net.org](http://www.lymphoma-net.org)

#### *MPD Voice*

🔗 [www.mpdvoice.org.uk](http://www.mpdvoice.org.uk)

#### *Medical Matrix*

🔗 [www.medmatrix.org/Index.asp](http://www.medmatrix.org/Index.asp)

#### *Meducation*

🔗 [www.meducation.com](http://www.meducation.com)

#### *MRC Molecular Haematology Unit*

🔗 [www.imm.ox.ac.uk/mrc-molecular-haematology-unit](http://www.imm.ox.ac.uk/mrc-molecular-haematology-unit)

#### *Myeloma Forum*

🔗 [www.ukmf.org.uk/guidelines.shtml](http://www.ukmf.org.uk/guidelines.shtml)

**National Library of Medicine**

☞ [www.nlm.nih.gov](http://www.nlm.nih.gov)

**NIH Consensus Conferences**

☞ <http://odp.od.nih.gov/consensus>

**Oncolink**

☞ [www.oncolink.com](http://www.oncolink.com)

**Pharmaceutical Technology**

☞ [www.pharmaceutical-technology.com](http://www.pharmaceutical-technology.com)

**Reuters Health News**

☞ [www.reutershealth.com](http://www.reutershealth.com)

**RD Direct**

☞ [www.rddirect.org.uk](http://www.rddirect.org.uk)

**WebPath**

☞ <http://library.med.utah.edu/WebPath/>

**Atlases****Atlas of Genetics and Cytogenetics in Oncology and Haematology**

☞ <http://atlasgeneticsoncology.org>

**Atlas of Hematology**

☞ <http://www.hematologyatlas.com/>

**Atlas of Hematology (Nagoya)**

☞ <http://pathy.med.nagoya-u.ac.jp/atlas/doc/atlas.html>

**The Crookston Collection, University of Toronto**

☞ [www.thecrookstoncollection.com](http://www.thecrookstoncollection.com)

**Journals and books****ASH Education Book**

☞ [www.asheducationbook.org](http://www.asheducationbook.org)

**Blackwell Publishing**

☞ [www.blackwellpublishing.com](http://www.blackwellpublishing.com)

**Blood**

☞ [www.bloodjournal.org](http://www.bloodjournal.org)

**Blood Book.com**

☞ [www.bloodbook.com](http://www.bloodbook.com)

**Blood Cells, Molecules and Disease**

☞ [www.scripps.edu/bcmd](http://www.scripps.edu/bcmd)

**British Journal of Haematology**

☞ [www.blackwellpublishing.com/journals/bjh](http://www.blackwellpublishing.com/journals/bjh)

**British Medical Journal**

☞ [www.bmj.com](http://www.bmj.com)

**Cancer**

☞ [http://www.onlinelibrary.wiley.com/journal/10.3322/\(ISSN\)1542-4863](http://www.onlinelibrary.wiley.com/journal/10.3322/(ISSN)1542-4863)

*Haematologica*

🔗 [www.haematologica.org](http://www.haematologica.org)

*Hematological Oncology*

🔗 [www.interscience.wiley.com/journal/hon](http://www.interscience.wiley.com/journal/hon)

*Journal of Clinical Investigation*

🔗 [www.jci.org](http://www.jci.org)

*Journal of Clinical Oncology*

🔗 [www.jco.ascopubs.org](http://www.jco.ascopubs.org)

*Lancet*

🔗 [www.thelancet.com](http://www.thelancet.com)

*Leukemia*

🔗 [www.Nature.com/leu](http://www.Nature.com/leu)

*Leukemia and Lymphoma*

🔗 [www.informahealthcare.com/journal/lal](http://www.informahealthcare.com/journal/lal)

*Nature*

🔗 [www.nature.com](http://www.nature.com)

*New England Journal of Medicine*

🔗 [www.nejm.org](http://www.nejm.org)

*Oxford University Press*

🔗 [www.oup.com](http://www.oup.com)

*Science*

🔗 [www.sciencemag.org](http://www.sciencemag.org)

**Societies and organizations***American Association of Blood Banks (AABB)*

🔗 [www.aabb.org](http://www.aabb.org)

*American Association for Cancer Research (AACR)*

🔗 [www.aacr.org](http://www.aacr.org)

*American Cancer Society*

🔗 [www.cancer.org](http://www.cancer.org)

*Association of Clinical Scientists*

🔗 [www.assclinsci.org](http://www.assclinsci.org)

*American Medical Association*

🔗 [www.ama-assn.org](http://www.ama-assn.org)

*American Society of Clinical Oncology*

🔗 [www.asco.org](http://www.asco.org)

*American Society of Hematology*

🔗 [www.hematology.org](http://www.hematology.org)

*American Society of Pediatric Hematology/Oncology (ASPHO)*

🔗 [www.aspho.org](http://www.aspho.org)

*Anticoagulation.org.uk*

🔗 [www.anticoagulation.org.uk](http://www.anticoagulation.org.uk)



*Aplastic Anemia Foundation of America & MDS International Foundation*

📄 [www.aamds.org/](http://www.aamds.org/)

*Association of Pediatric Oncology Nurses (APON)*

📄 [www.apon.org](http://www.apon.org)

*Bloodline*

📄 [www.bloodline.net](http://www.bloodline.net)

*British Blood Transfusion Society (BBTS)*

📄 [www.bbts.org.uk](http://www.bbts.org.uk)

*British Committee for Standards in Haematology*

📄 [www.bcsghguidelines.com](http://www.bcsghguidelines.com)

*British Society of Blood and Marrow Transplantation*

📄 [www.bsbmt.org](http://www.bsbmt.org)

*British Society for Haematology*

📄 [www.b-s-h.org.uk](http://www.b-s-h.org.uk)

*British Society for Haemostasis & Thrombosis (BSHT)*

📄 [www.bsht.bham.ac.uk](http://www.bsht.bham.ac.uk)

*Centers for Disease Control (CDC), Atlanta, USA*

📄 [www.cdc.gov](http://www.cdc.gov)

*European Bone Marrow Transplant Association*

[www.embt.org](http://www.embt.org)

*European Hematology Association*

📄 [www.ehaweb.org](http://www.ehaweb.org)

*European Society of Paediatric Haematology and Immunology*

📄 [www.esphi.eu](http://www.esphi.eu)

*Imperial Cancer Research Fund*

📄 [www.cancerresearchuk.org](http://www.cancerresearchuk.org)

*Institute of Biomedical Science*

📄 [www.ibms.org](http://www.ibms.org)

*International Histiocytosis Organization (Histiocytosis Association of America)*

📄 [www.histio.org](http://www.histio.org)

*International Hospital Federation*

📄 [www.hospitalmanagement.net/](http://www.hospitalmanagement.net/)

*International Myeloma Foundation UK*

📄 [www.myeloma.org.uk](http://www.myeloma.org.uk)

*Leukemia and Lymphoma Society*

📄 [www.leukemia-lymphoma.org](http://www.leukemia-lymphoma.org)

*Leukemia Research Fund*

📄 [www.lrf.org.uk](http://www.lrf.org.uk)

*Leukemia Society of America*

📄 [www.leukemia.org](http://www.leukemia.org)

*Medical Research Council*

🔗 [www.mrc.ac.uk](http://www.mrc.ac.uk)

*National Blood Service*

🔗 [www.blood.co.uk](http://www.blood.co.uk)

*National Cancer Institute*

🔗 [www.cancer.gov](http://www.cancer.gov)

*National Children's Leukemia Foundation*

🔗 [www.leukemiafoundation.org](http://www.leukemiafoundation.org)

*National Guidelines Clearinghouse*

🔗 [www.guidelines.gov](http://www.guidelines.gov)

*National Heart, Lung, and Blood Institute*

🔗 [www.nhlbi.nih.gov](http://www.nhlbi.nih.gov)

*National Institutes of Health*

🔗 [www.nih.gov](http://www.nih.gov)

*NHS Centre for Reviews and Dissemination*

🔗 [www.york.ac.uk/inst/crd](http://www.york.ac.uk/inst/crd)

*NHS Professionals*

🔗 [www.nhsprofessionals.nhs.uk](http://www.nhsprofessionals.nhs.uk)

*Royal College of Pathologists*

🔗 [www.rcpath.org](http://www.rcpath.org)

*Royal College of Physicians*

🔗 [www.rcplondon.ac.uk](http://www.rcplondon.ac.uk)

*Royal Society of Medicine*

🔗 [www.roysocmed.ac.uk](http://www.roysocmed.ac.uk)

*Sickle Cell Information Centre Home*

🔗 [www.scinfo.org](http://www.scinfo.org)

*Society for Hematopathology (Dartmouth College)*

🔗 [www.sh-eahp.org](http://www.sh-eahp.org)

*The Cochrane Library*

🔗 [www.thecochranelibrary.com/view/0/index.html](http://www.thecochranelibrary.com/view/0/index.html)

*UK NEQAS Schemes*

🔗 [www.ukneqas.org.uk](http://www.ukneqas.org.uk)

*UK CLL support association*

🔗 [www.clisupport.org.uk](http://www.clisupport.org.uk)

*Wellcome Trust*

🔗 [www.wellcome.ac.uk](http://www.wellcome.ac.uk)

*World Federation of Hemophilia*

🔗 [www.wfh.org](http://www.wfh.org)

*World Health Organization*

🔗 [www.who.int/en](http://www.who.int/en)

**Guidelines, trials, and evidence-based practice**

*British Committee for Standards in Haematology (BCSH)*

🔗 [www.bcsghguidelines.com](http://www.bcsghguidelines.com)

*Cochrane Library (The)*

🔗 [www.cochrane.org](http://www.cochrane.org)

*Clinical trials: hematology (CenterWatch)*

🔗 [www.centerwatch.com](http://www.centerwatch.com)

*Comprehensive Sickle Cell Center at Grady Health System*

🔗 [www.scinfo.org](http://www.scinfo.org)

*National Center for Biotechnology Information*

🔗 [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)

*National Guideline Clearinghouse (Agency for Health Care Policy and Research (AHCPR))*

🔗 [www.guidelines.gov](http://www.guidelines.gov)

*National Library of Medicine*

🔗 [www.nlm.nih.gov](http://www.nlm.nih.gov)

*Patient-Reported Health Instruments*

🔗 <http://phi.uhce.ox.ac.uk>

*Transfusionguidelines.org.uk*

🔗 [www.transfusionguidelines.org.uk](http://www.transfusionguidelines.org.uk)

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## Charts and nomograms

- Karnofsky performance status [804](#)
- WHO/ECOG performance status [804](#)
- WHO haematological toxicity scale [805](#)
- Body surface area nomogram [806](#)
- Gentamicin dosage nomogram [807](#)
- The Sokal Score for CML prognostic groups [808](#)

## Karnofsky performance status

**Table A2.1** Karnofsky performance status

Normal, no complaints; no evidence of disease	100%
Able to carry on normal activity; minor signs or symptoms of disease	90%
Normal activity with effort; some signs or symptoms of disease	80%
Cares for self; unable to carry on normal activity or to do active work	70%
Requires occasional assistance but is able to care for most of his/her needs	60%
Requires considerable assistance and frequent medical care	50%
Disabled; requires special care and assistance	40%
Severely disabled; hospitalization is indicated although death not imminent	30%
Very sick; hospitalization necessary	20%
Moribund; fatal processes progressing rapidly	10%
Dead	0%

## WHO/ECOG performance status

**Table A2.2** WHO/ECOG performance status

0	Fully active; able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature, e.g. light housework, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair

### Further reading

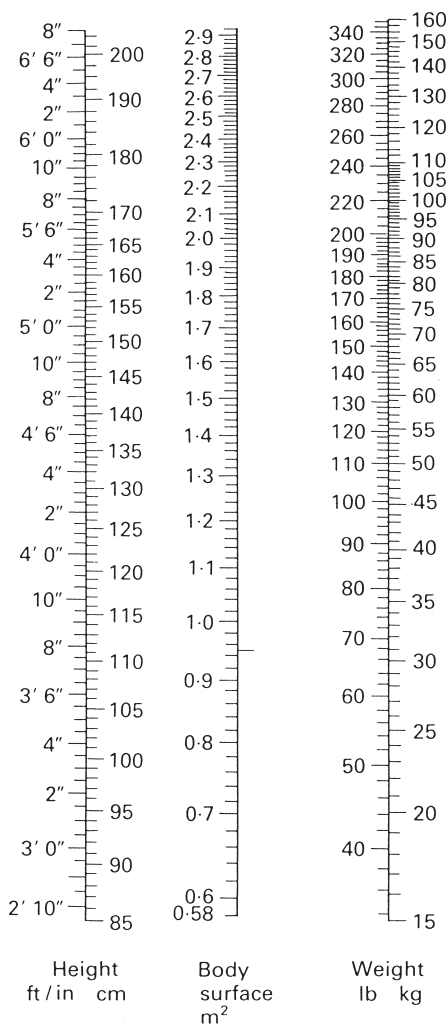
Oken, M.M. et al. (1982). Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol*, **5**, 649.

## WHO haematological toxicity scale

**Table A2.3** WHO haematological toxicity scale

Parameter	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Haemoglobin (g/dL)	≥11.0	9.5–10.9	8.0–9.4	6.5–7.9	<6.5
Leucocytes ( $\times 10^9/L$ )	≥4.0	3.0–3.9	2.0–2.9	1.0–1.9	<1.0
Granulocytes ( $\times 10^9/L$ )	≥2.0	1.5–1.9	1.0–1.4	0.5–0.9	<0.5
Platelets ( $\times 10^9/L$ )	≥100	75–99	50–74	25–49	<25
Haemorrhage	None	Petechiae	Mild blood loss	Gross blood loss	Debilitating blood loss

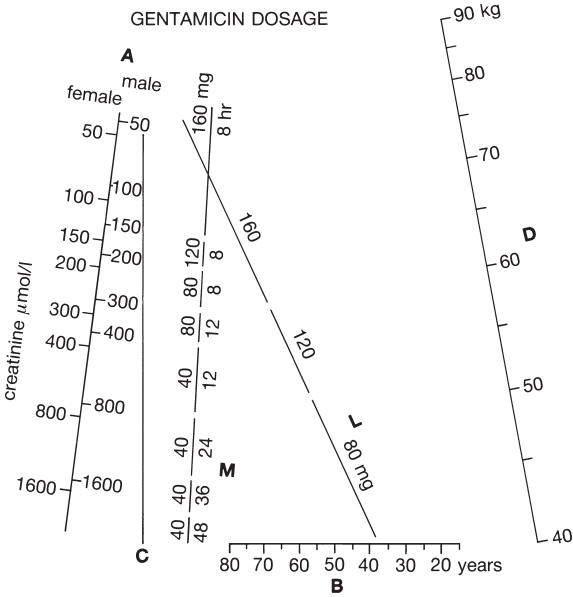
## Body surface area nomogram



**Fig. A2.1** Body surface area nomogram. Reproduced with permission from Ramrakha, P. and Moore, K. (1997). *Oxford Handbook of Acute Medicine*, 2e. Oxford University Press, Oxford.



## Gentamicin dosage nomogram



**Fig. A2.2** Gentamicin dosage nomogram. Reproduced with permission from Longmore, M. et al. (2007). *Oxford Handbook of Clinical Medicine*, 7e. Oxford University Press, Oxford.

## The Sokal Score for CML prognostic groups

**Table A2.4** The Sokal Score for CML prognostic groups

Score	= $\text{Exp}[0.0116 (\text{age}-43.4)]$ + 0.0345 (spleen size[cm]-7.51) + 0.188 ( $[\text{platelets}(10^9/\text{L})/700]^2-0.563$ ) + 0.0887 (blasts[%]-2.1)
Low risk	<0.8
Intermediate risk	= 0.8-1.2
High risk	>1.2

# Normal ranges

Adult normal ranges *810*

Paediatric normal ranges *812*

## Adult normal ranges

**Table A3.1** Haematology

Haemoglobin	130–180g/L	(♂)
	115–165g/L	(♀)
Haematocrit	0.40–0.52	(♂)
	0.36–0.47	(♀)
RCC	$4.5\text{--}6.5 \times 10^{12}/\text{L}$	(♂)
	$3.8\text{--}5.8 \times 10^{12}/\text{L}$	(♀)
MCV	77–95fL	
MCH	27.0–32.0pg	
MCHC	32.0–36.0g/dL	
WBC	$4.0\text{--}11.0 \times 10^9/\text{L}$	
Neutrophils	$2.0\text{--}7.5 \times 10^9/\text{L}$	
Lymphocytes	$1.5\text{--}4.5 \times 10^9/\text{L}$	
Eosinophils	$0.04\text{--}0.4 \times 10^9/\text{L}$	
Basophils	$0.0\text{--}0.1 \times 10^9/\text{L}$	
Monocytes	$0.2\text{--}0.8 \times 10^9/\text{L}$	
Platelets	$150\text{--}400 \times 10^9/\text{L}$	
Reticulocytes	0.5–2.5% (or $50\text{--}100 \times 10^9/\text{L}$ )	
ESR	2–12mm/1st hour (Westergren)	
Red cell mass	25–35mL/kg	(♂)
	20–30mL/kg	(♀)
Serum B <sub>12</sub>	150–700ng/L	
Serum folate	2.0–11.0mcg/L	
Red cell folate	150–700mcg/L	
Serum ferritin	15–300mcg/L (varies with sex and age)	
	14–200mcg/L (premenopausal ♀)	
INR	0.8–1.2	
PT	12.0–14.0sec	
APTT ratio	0.8–1.2	
APTT	26.0–33.5sec	
Fibrinogen	2.0–4.0g/L	
Thrombin time	± 3sec of control	
XDPs	<250mcg/L	
D-dimer	<500ng/mL	
Factors II, V, VII, VIII, IX, X, XI, XII	50–150IU/dL	
RiCoF	45–150IU/dL	
vWF: Ag	50–150IU/dL	
Protein C	80–135U/dL	
Protein S	80–135U/dL	
Antithrombin III	80–120U/dL	
APCR	2.12–4.0	
Bleeding time	3–9min	

**Table A3.2** Biochemistry and immunology

Serum urea	3.0–6.5mmol/L 11.5–16.5g/dL	
Serum creatinine	60–125 micromol/L	
Serum sodium	135–145mmol/L	
Serum potassium	3.5–5.0mmol/v	
Serum albumin	32–50g/L	
Serum bilirubin	<17micromol/L	
Serum alk phos	100–300IU/L	
Serum calcium	2.15–2.55mmol/L	
Serum LDH	200–450IU/L	
Serum phosphate	0.7–1.5mmol/L	
Serum total protein	63–80g/L	
Serum urate	0.18–0.42mmol/L	
Serum $\gamma$ -GT	10–46IU/L	
Serum iron	14–33mcmol/L 11–28mcmol/L	(♂) (♀)
Serum TIBC	45–75mcmol/L	
Serum ALT	5–42IU/L	
Serum AST	5–42IU/L	
Serum free T4	9–24pmol/L	
Serum TSH	0.35–5.5mU/L	
<b>Immunology</b>		
IgG	5.3–16.5g/L	
IgA	0.8–4.0g/L	
IgM	0.5–2.0g/L	
<i>Complement:</i>		
C3	0.89–2.09g/L	
C4	0.12–0.53g/L	
C1 esterase	0.11–0.36g/L	
CH50	80–120%	
C-reactive protein	<6mg/L	
Serum $\beta_2$ -microglobulin	1.2–2.4mg/L	
<i>CSF proteins:</i>		
IgG	0.013–0.035g/L	
Albumin	0.170–0.238g/L	
<i>Urine proteins:</i>		
Total protein	<150mg/24h	
Albumin (24h)	<20mg/24h	

## Paediatric normal ranges

**Table A3.3** Full blood count

Age	Hb (g/L)	MCV (fL)	Neuts	Lymph	Platelets
Birth	149–237	100–125	2.7–14.4	2–7.3	150–450
2 weeks	134–198	88–110	1.5–5.4	2.8–9.1	170–500
2 months	94–130	84–98	0.7–4.8	3.3–10.3	210–650
6 months	100–130	73–84	1–6	3.3–11.5	210–560
1 year	101–130	70–82	1–8	3.4–10.5	200–550
2–6 years	115–138	72–87	1.5–8.5	1.8–8.4	210–490
6–12 years	111–147	76–90	1.5–8	1.5–5	170–450
Adult ♂	121–166	77–92	1.5–6	1.5–4.5	180–430
Adult ♀	121–151	77–94	1.5–6	1.5–4.5	180–430

Neuts, neutrophils; lymph, lymphocytes and platelets (all  $\times 10^9/L$ )

**Table A3.4** Haemostasis

Parameter	Neonate	Adult level
Platelet count	150–400 $\times 10^9/L$	As adult
Prothrombin time	Few sec longer than adult	Up to 1 week
APTT	Up to 25% increase	By 2–9 months
Thrombin time		As adult
Bleeding time	2–10min	As adult
Fibrinogen	2.0–4.0g/L	As adult
<i>Vit K factors:</i>		
Factor II	30–50% adult level	Up to 6 months
Factor VII	30–50% adult level	By 1 month
Factor IX	20–50% adult level	Up to 6 months
Factor X	30–50% adult level	Up to 6 months
Factor V		As adult
Factor VIII	Variable: 50–200% adult level	
vW factor	Usually raised (up to 3 $\times$ adult level)	
Factor XI	20–50% adult level	6–12 months
Factor XII	20–50% adult level	3–6 months
Factor XIII	50–100% adult level	1 month
FDP/XDP	Up to twice adult level	By 7 days
AT	50–80% adult level	6–12 months
Protein C	30–50% adult level	Up to 24 months
Protein S	30–50% adult level	3–6 months
Plasminogen	30–80% adult level	2 weeks

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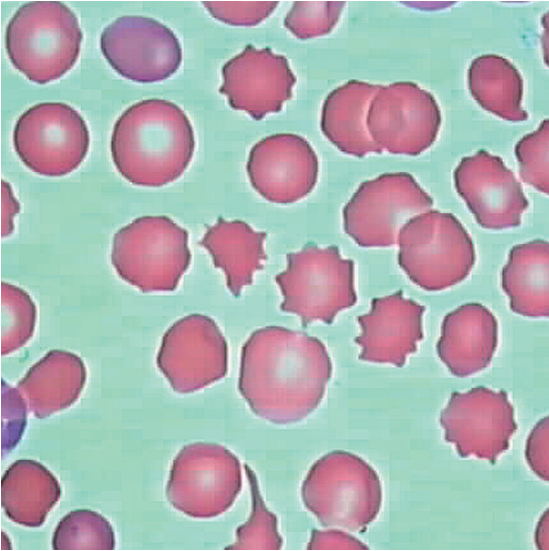
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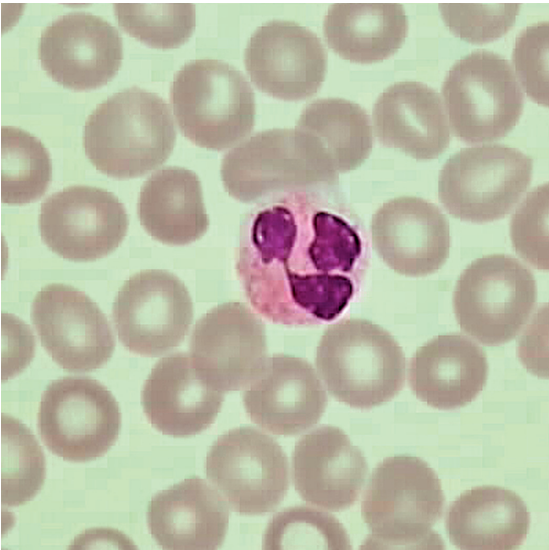


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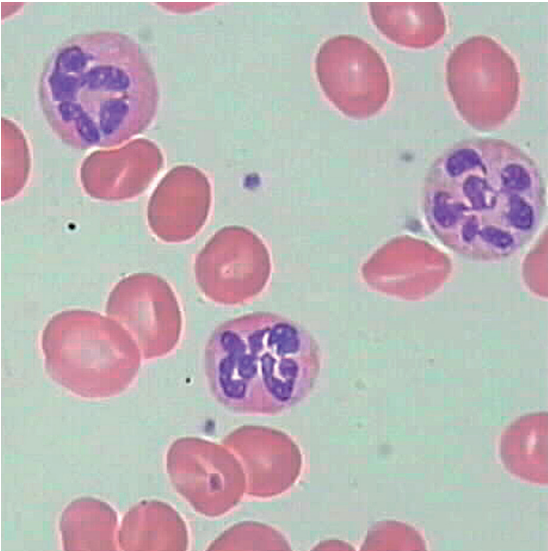
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- X-linked  
 lymphoproliferative disorder 632
- X-linked SCID 547
- Z**
- Zieve's syndrome 41, 82
- zinc protoporphyrin 43



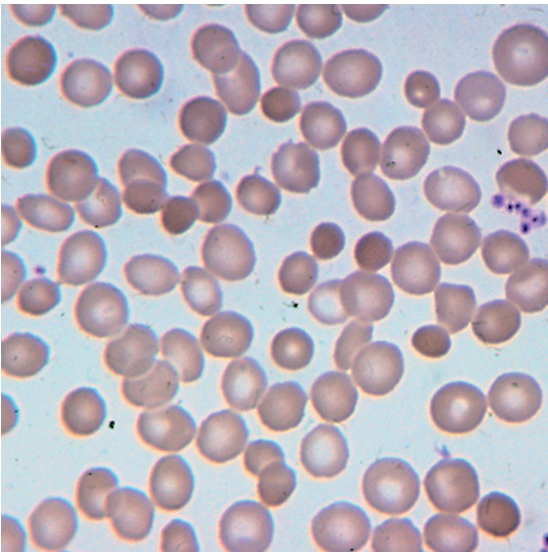
**Fig 2.1** Blood film: chronic renal failure with burr (irregular shaped) cells (see Fig. 2.1 p.35).



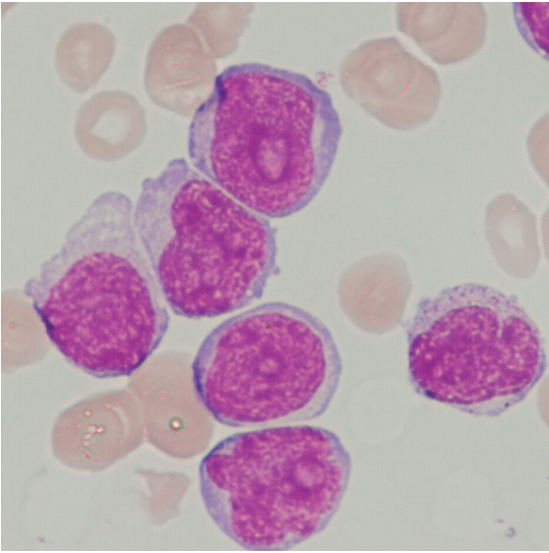
**Fig 2.3** Blood film: normal neutrophil: usually has <5 lobes. This one has 3 lobes (see Fig. 2.3 p.49).



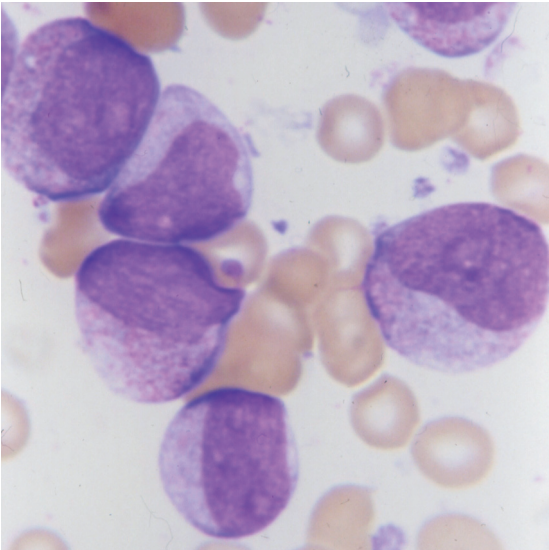
**Fig. 2.4** Hypersegmented neutrophils with 7–8 lobes: found in B<sub>12</sub> or folate deficiency. *Note:* blood films and marrow appearances are identical in B<sub>12</sub> and folate deficiencies (↔ see Fig. 2.4 p.49).



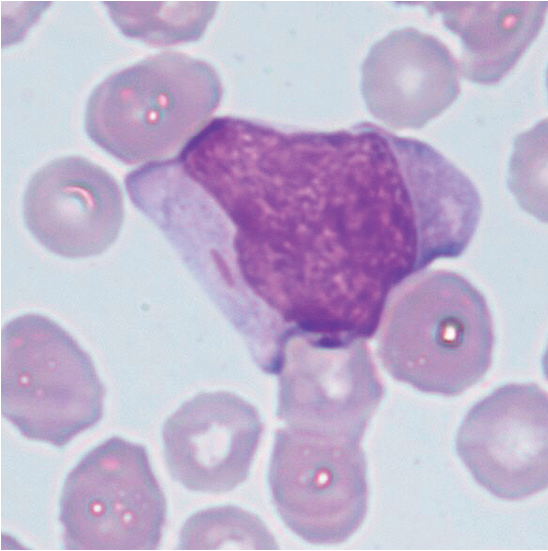
**Fig. 2.11** Blood film in hereditary spherocytosis. *Note:* large numbers of dark spherical red cells (↔ see Fig. 2.11 p.86).



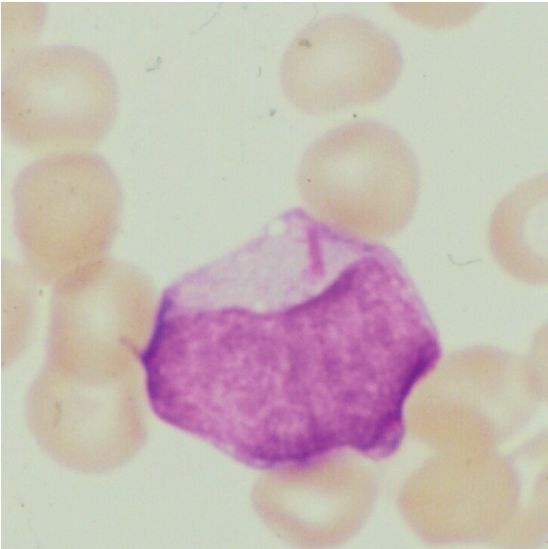
**Fig. 4.1** Bone marrow showing myeloblasts in AML (↻ see Fig. 4.1 p.125).



**Fig. 4.2** Bone marrow showing myeloblasts in AML (↻ see Fig. 4.2 p. 125).

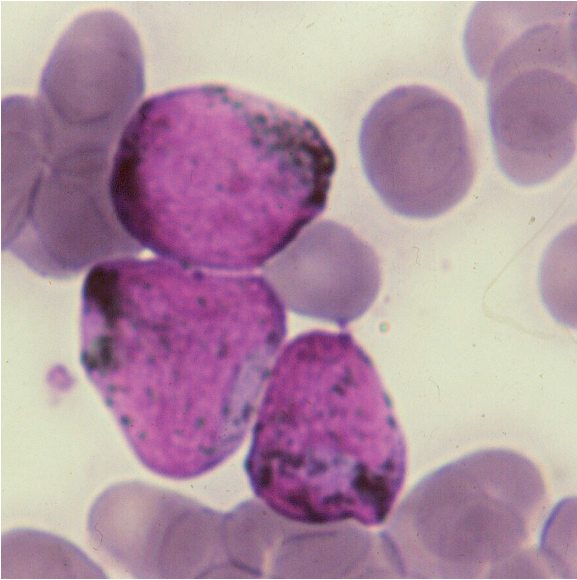


**Fig. 4.3** AML: myeloblast with large Auer rod (left) (↻ see Fig. 4.3 p.125).



**Fig. 4.4** AML: myeloblast with large Auer rod (top of cell) (↻ see Fig. 4.4 p.126).

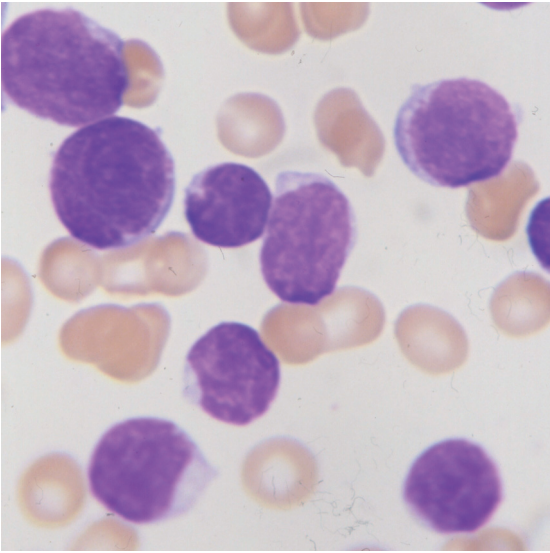




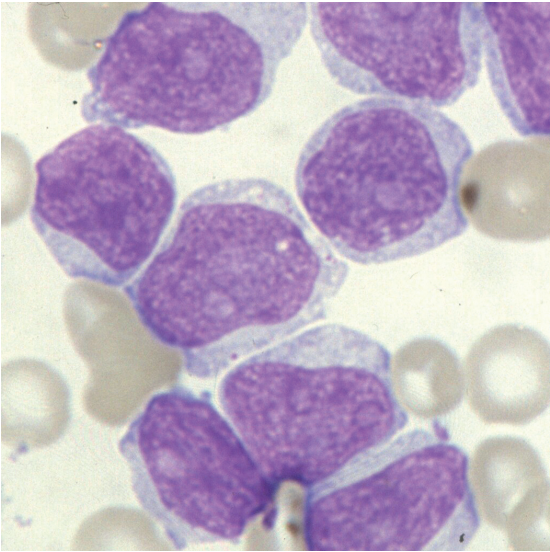
**Fig. 4.5** AML: Sudan Black stain (dark granules clearly seen in myeloblasts) (↻ see Fig. 4.5 p.126).



**Fig. 4.6** Gum hypertrophy in patient with AML (↻ see Fig. 4.6 p.131).

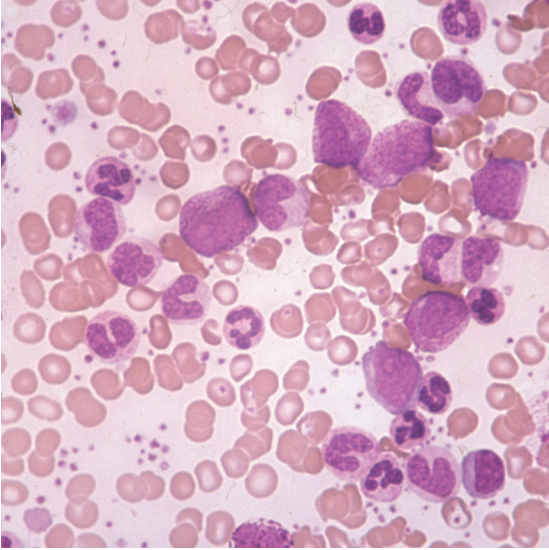


**Fig. 4.8** Bone marrow: lymphoblasts in ALL L1 (↻ see Fig. 4.8 p.141).

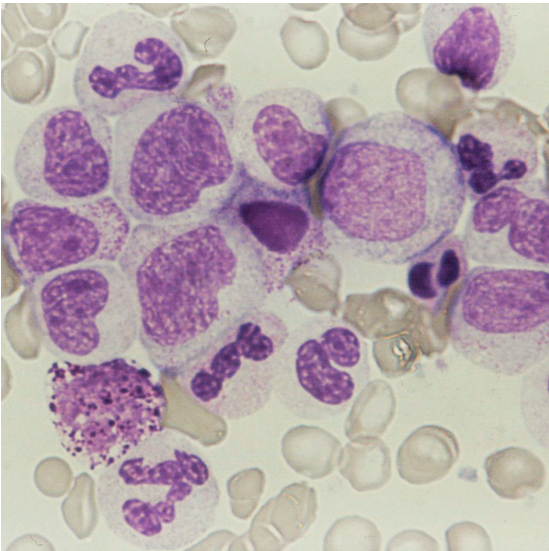


**Fig. 4.9** Bone marrow: T-cell ALL showing numerous lymphoblasts (↻ see Fig. 4.9 p.142).

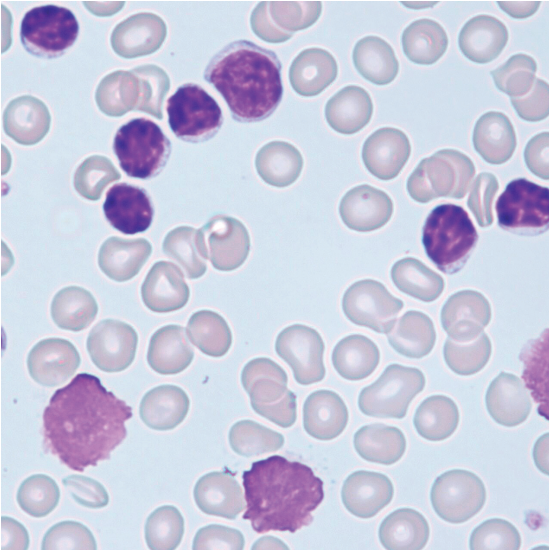




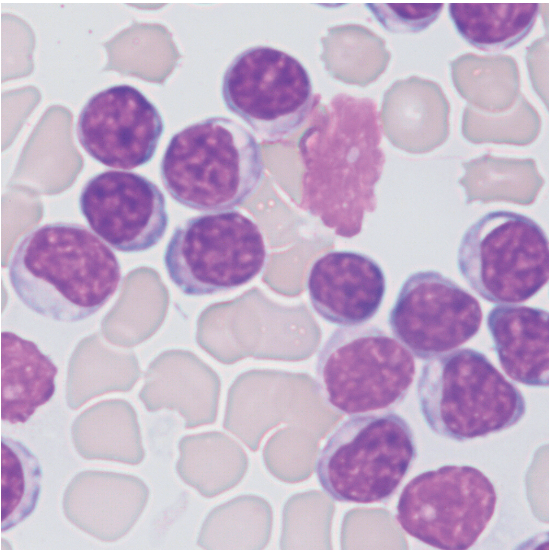
**Fig. 4.10** Peripheral blood film in CML: note large numbers of granulocytic cells at all stages of differentiation (low power) (↻ see Fig. 4.10 p.149).



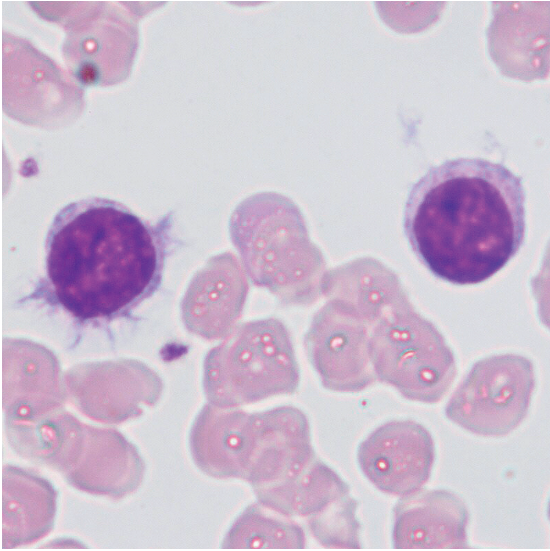
**Fig. 4.11** Peripheral blood film in CML: high power (↻ see Fig. 4.11 p.150).



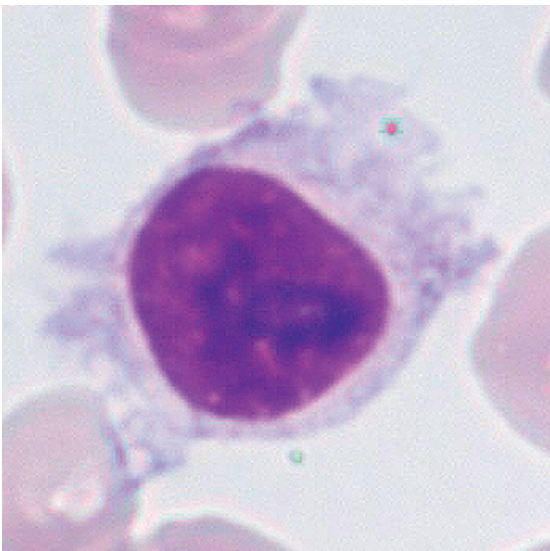
**Fig. 4.12** Blood film in CLL showing smear cells (bottom of field) (↻ see Fig. 4.12 p.159).



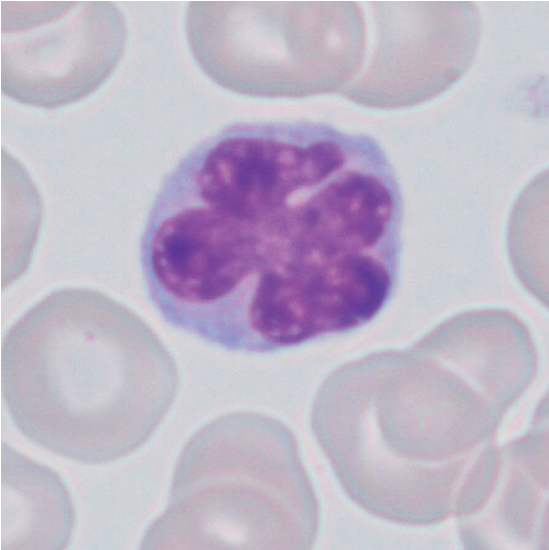
**Fig. 4.15** Blood film in PLL: cells are larger than those seen in CLL have large prominent nucleoli and moderate chromatin condensation (↻ see Fig. 4.15 p.169).



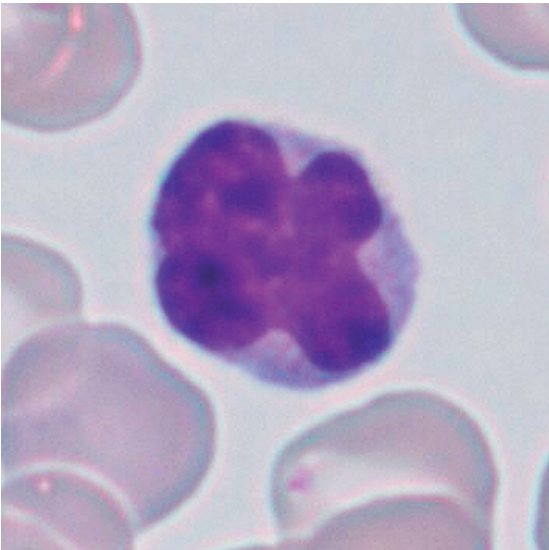
**Fig. 4.16** Blood film in HCL showing typical 'hairy' lymphocytes (medium power) (↻ see Fig. 4.16 p.171).



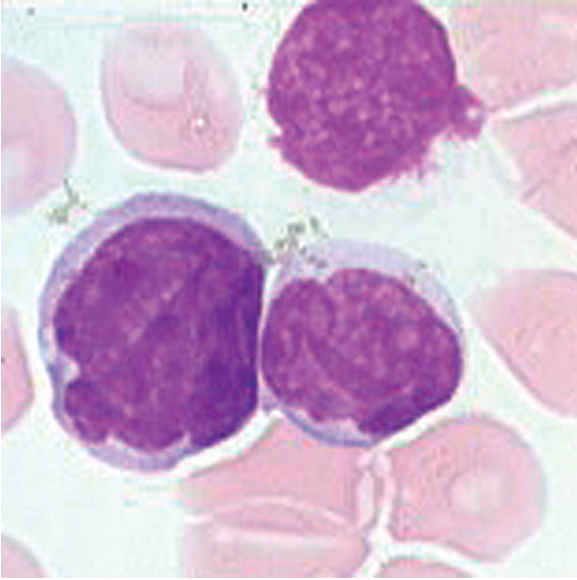
**Fig. 4.17** Blood film in HCL showing typical 'hairy' lymphocytes (high power) (↻ see Fig. 4.17 p.171).



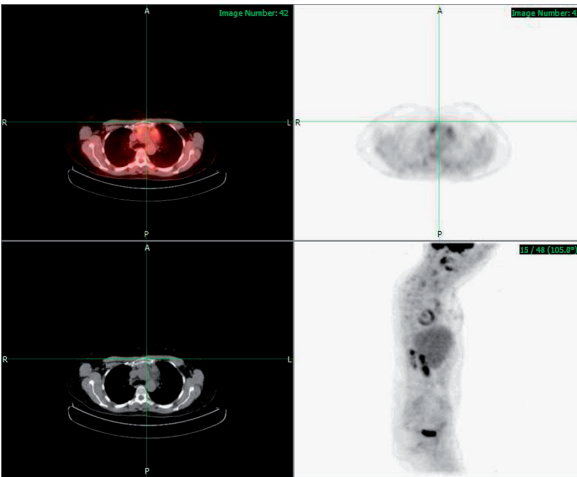
**Fig. 4.19(a)** Blood films showing typical ATL cell with lobulated 'clover leaf' nucleus' (↻ see Fig. 4.19(a) p.179).



**Fig. 4.19(b)** Blood films showing typical ATL cell with lobulated 'clover leaf' nucleus' (↻ see Fig. 4.19(b) p.179).

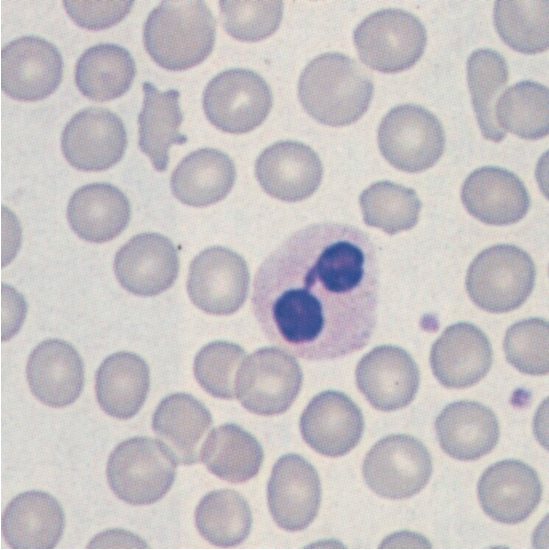


**Fig. 5.1** Blood film in Sézary syndrome showing typical cerebriform nuclei (↻ see Fig. 5.1 (a) p.200).

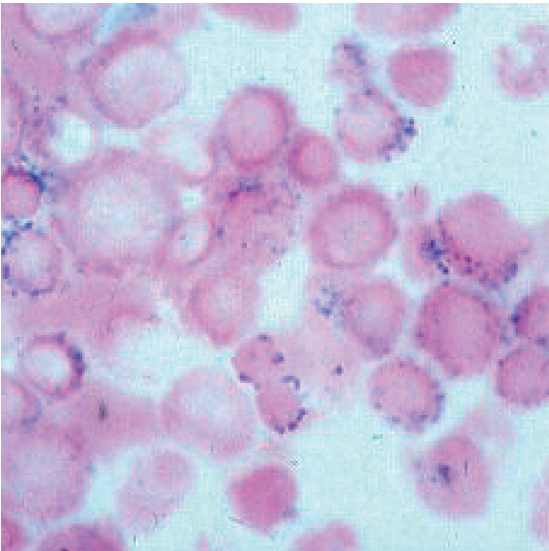


**Fig. 5.2** PET and CT scans in patient with nodular sclerosing Hodgkin lymphoma demonstrating active disease (moderate FDG uptake on PET) in residual anterior mediastinal mass on CT after ABVD chemotherapy (↻ see Fig. 5.2 p.218).

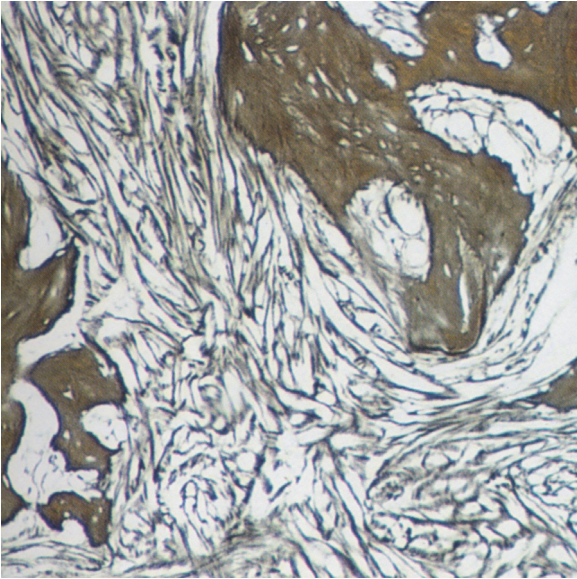




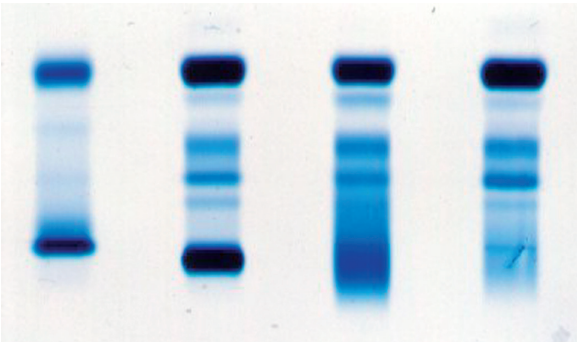
**Fig. 6.1** Blood film in MDS showing bilobed pseudo-Pelger neutrophil (➔ see Fig. 6.1 p.236).



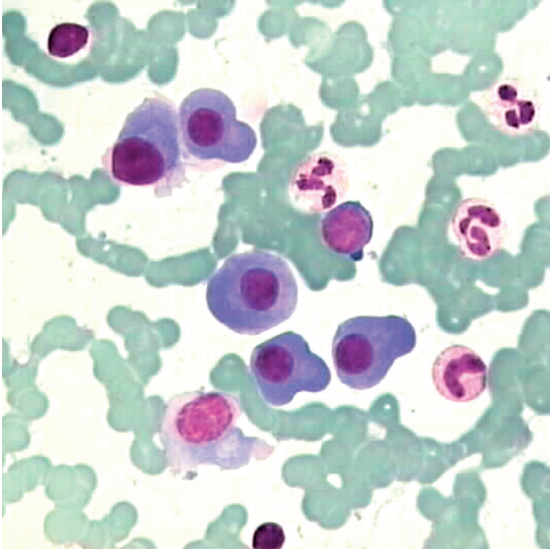
**Fig. 6.2** Bone marrow in RARS stained for iron: note iron granules round the nucleus of the erythroblast (➔ see Fig. 6.2 p.243).



**Fig. 7.1** Bone marrow trephine in myelofibrosis: note streaming effect caused by intense fibrosis (↻ see Fig. 7.1 p.294).



**Fig. 8.1** Electrophoresis: from L → R: urine with BJP and generalized proteinuria (albumin band at top of strip, BJP near foot); serum M band in myeloma; polyclonal gammopathy; normal sample, showing albumin,  $\alpha$ ,  $\beta$ , and  $\gamma$  globulins (↻ see Fig. 8.1 p.338).

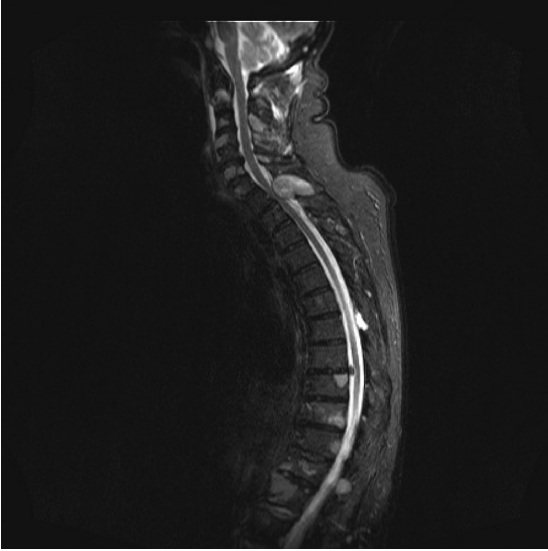


**Fig. 8.2** Bone marrow aspirate in myeloma showing numerous plasma cells (➔ see Fig. 8.2 p.341).

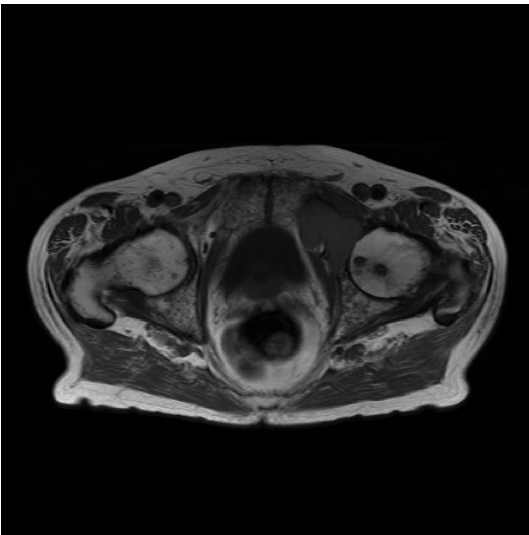


**Plate 1** Radiograph of pelvis in a patient with multiple myeloma showing abnormal low density bone texture in the left superior pubic ramus and ischium (➔ see p.338).





**Plate 2** MRI of cervical and thoracic spine in a patient with multiple myeloma: substantial extra-osseous mass at T1 displacing the spinal cord anteriorly and causing some cord compression (➡ see Investigations and diagnosis p.338).



**Plate 3** MRI of pelvis in a patient with multiple myeloma (same as Plate 29 ) showing corresponding soft tissue mass replacing bone & numerous widespread foci of signal change throughout the bony pelvis representing myeloma deposits (➡ see p.338).



**Plate 4** T1 sagittal sequence MRI of thoraco-lumbar spine in a patient with multiple myeloma showing multiple myeloma deposits and partial collapse of one vertebral body (➤ see p.338).